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Perspectives in Pharmacology

Pharmacological and physiological functions of the polyspecific organic cation transporters OCT1, 2 and 3 (SLC22A1-3)

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Abbreviations:

OCT, organic cation transporter; SLC, Solute carrier superfamily; EMT, extra-neuronal monoamine transporter; TMD, transmembrane domain; ABC, ATP-binding cassette; TEA, tetraethylammonium; MPP^+, 1-methyl-4-phenylpyridinium

Abstract

For the elimination of environmental toxins and metabolic waste products, the body is equipped with a range of broad-specificity transporters, that are generally present in the liver, kidney and intestine. The polyspecific organic cation transporters, OCT1, 2 and 3 (SLC22A1-3), mediate the facilitated transport of a wide variety of structurally diverse organic cations, including many drugs, toxins and endogenous compounds. OCT1 and OCT2 are found in the basolateral membrane of hepatocytes, enterocytes and renal proximal tubular cells. OCT3 has a more widespread tissue distribution and is considered to be the major component of the extraneuronal monoamine transport system (EMT or uptake-2), which is responsible for the peripheral elimination of monoamine neurotransmitters. Studies with knockout mouse models have directly demonstrated that these transporters can have a major impact on the pharmacological behavior of various substrate organic cations. The recent identification of polymorphic genetic variants of human OCT1 and OCT2 that severely affect transport activity thus suggests that some of the interpatient differences in response and sensitivity to cationic drugs may be caused by variable activity of these transporters.

The body is continuously exposed to a wide variety of environmental toxins and metabolic waste products. To rid itself of these compounds, it is equipped with various detoxification mechanisms such as metabolizing enzymes and transport proteins mediating their inactivation and excretion. For excretion, a plethora of transmembrane transport proteins is present in the major excretory organs, liver, kidney and intestine. The Solute Carrier (SLC) superfamily is by far the largest superfamily of transporters, consisting of about 225 members in humans (for reference: Human Genome Organization (HUGO) at http://www.gene.ucl.ac.uk/nomenclature).

Whereas most of these transporters are highly specialized, mediating facilitated transport of essential nutrients (*e.g.* glucose, amino acids, nucleosides and fatty acids), some members are more generalized. Due to their broad substrate specificity, the latter are also termed polyspecific transporters. They play a major role in the elimination of, and protection against, noxious compounds.

Among the SLC superfamily, two families (SLC21 and 22) with polyspecific members have been identified, together mediating the transport of a wide variety of structurally diverse organic anions, cations and uncharged compounds. The SLC21 family of organic anion transporting polypeptides (OATPs) currently consists of 9 members in humans, transporting a range of relatively large (usually >450 Da), mostly anionic amphipathic compounds, including bile salts, eicosanoids, steroid hormones and their conjugates (reviewed by Hagenbuch and Meier, 2003). The SLC22 family currently consists of 12 members in humans, encompassing organic cation transporters (OCTs), the carnitine transporter (OCTN2/SLC22A5) (Wu et al., 1998), the urate anion-exchanger (URAT1/SLC22A12) (Enomoto et al., 2002) and several organic anion transporters (OATs, reviewed by Sweet et al., 2001). In this review we focus on the polyspecific organic cation transporters (OCT1, 2 and 3) that belong to the family SLC22 (Koepsell et al., 2003; Koepsell and Endou, 2003).

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Cloning and functional characteristics

The first identified member of the organic cation transporter family, OCT1 (SLC22A1), was isolated by expression cloning from rat kidney (Gründemann et al., 1994). In this initial study it was shown that rOCT1 had functional characteristics similar to the previously described organic cation transport process in the basolateral membrane of renal proximal tubules and hepatocytes. rOCT1 encodes a 556 amino acid protein and has a proposed secondary structure displaying 12 transmembrane domains (TMD). It contains a large extracellular loop, located between the first and second TMD, with three predicted *N*-linked glycosylation sites (Meyer-Wentrup et al., 1998) that may be involved in protein stability, intracellular routing or in protection from extracellular proteases (Figure 1). Currently, mammalian orthologs of OCT1 have been cloned from mouse (Schweifer and Barlow, 1996), human (Gorboulev et al., 1997; Zhang et al., 1997), and rabbit (Terashita et al., 1998).

By homology screening, a second member of the organic cation transporter family, designated OCT2 (SLC22A2), was isolated from rat kidney (Okuda et al. 1996) and later also cloned from human (Gorboulev et al., 1997), pig (Gründemann et al., 1997) and mouse (Mooslehner et al., 1999). rOCT2 encodes a 593 amino acid protein with a calculated molecular mass of 66 kDa and 67% identity with rOCT1. The third member of the organic cation transporter family, designated OCT3 (SLC22A3), was independently cloned and identified as the extraneuronal monoamine transporter (EMT, see section catecholamine transport) by two different groups (Kekuda et al., 1998; Wu et al., 1998; Gründemann et al., 1998) and later also cloned from mouse (Verhaagh et al., 1999). rOCT3 encodes a 551 amino acid protein with a predicted molecular mass of 61 kDa and 48% identity with rOCT1 (Kekuda et al., 1998).

The functional characteristics of these transporters have been extensively investigated using cRNA injected *Xenopus laevis* oocytes and transfected mammalian cell lines. OCT1, 2 and 3 all mediate the facilitated transport of a broad range of structurally diverse organic cations and they

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have extensively overlapping substrate specificities. In general, the OCTs mediate the (bidirectional) transport of small hydrophilic compounds, ranging in size from about 60-350 Da, with at least one positively charged amine moiety at physiological pH. Although many compounds have been shown to inhibit or modulate transport activity of the OCTs, not all of them are transported substrates. Substrates for which transport has been directly demonstrated include the model substrate tetraethylammonium (TEA), the parkinsonian neurotoxin 1-methyl-4-phenylpyridinium (MPP^+), clinically used drugs such as antiparkinsonians (amantadine and memantine), antidiabetics (biguanides) and the H_2-receptor agonist cimetidine, biogenic amines (dopamine, norepinephrine) and several other endogenous compounds (choline and creatinine). In addition to organic cations, it has also been demonstrated that hOCT1 and hOCT2 mediate the transport of some anionic prostaglandins (Kimura et al., 2002), indicating that a positive charge is no absolute prerequisite for OCT substrates (Table 1).

Expression and subcellular localization

Northern analysis and RNA *in situ* hybridization demonstrated that in rats, rOCT1 mRNA is expressed in liver, kidney and intestine (Gründemann et al., 1994). In humans, hOCT1 is primarily expressed in the liver, indicating a difference in tissue distribution of OCT1 between humans and rodents (Gorboulev et al., 1997; Zhang et al., 1997). OCT2 mRNA was detected predominantly in the kidney in rats and humans (Okuda et al., 1996; Gorboulev et al., 1997). The tissue distribution and subcellular localization of OCT1 and OCT2 have been analyzed by immunohistochemistry in rats and humans (Meyer-Wentrup et al., 1998; Karbach et al., 2000; Sugawara-Yokoo et al., 2000; Motohashi et al., 2002). In the liver, rOCT1 was detected in sinusoidal membranes of hepatocytes around the central veins of the hepatic lobuli. In the kidney, rOCT1 was mainly observed in the pars convoluta (S1) and cortical pars recta (S2) of the proximal tubules, with lower expression in the medullary pars recta (S3), whereas rOCT2 was mainly expressed in the S2 and S3 segments (Meyer-

Wentrup et al., 1998; Karbach et al., 2000). By Western analysis, using isolated renal basolateral and brush-border (apical) membranes of proximal tubules, it was shown that both rOCT1 and rOCT2 are localized basolaterally (Urakami et al., 1998; Karbach et al., 2000) (Figure 2).

In addition to the kidney, OCT2 is also expressed in various regions of the brain. By RT-PCR, rOCT2 was detected in dopamine-rich areas of the brain: substantia nigra, nucleus accumbens and striatum (Gründemann et al., 1997). In humans, hOCT2 was detected by *in situ* hybridization and immunohistochemistry in the pyramidal cells of the cerebral cortex and hippocamus. In the brain, OCT2 might represent a "background" transporter for the removal of monoamine neurotransmitters that have escaped re-uptake by high-affinity monoamine transporters, which are not members of the OCT family (Busch et al., 1998). In contrast to what has been reported by Gründemann et al. (1997), rOCT2 (and rOCT3) was also detected by RT-PCR in the choroid plexus (Sweet et al., 2001). Transfection of intact choroid plexus with an rOCT2-GFP fusion protein construct resulted in strong apical membrane fluorescence with no detectable signal in the basal and lateral membranes, suggesting that rOCT2 mediates transport across the ventricular membrane of the choroid plexus (Sweet et al., 2001).

Expression of OCT2 in the kidney is gender-dependent (Urakami et al., 1999; 2000; Slitt et al., 2002). Starting from the observation that the uptake of TEA by renal cortical slices of male rats is greater than that by females, Urakami et al. (1999) found significantly higher levels of rOCT2 mRNA and protein in the kidney of males compared to females. Moreover, the expression of OCT2 in the kidney was demonstrated to be age-dependent. Whereas in young rats, levels of OCT2 mRNA between males and females were similar, they increased dramatically in males at the age of 30 days (Slitt et al., 2002). Treatment of male and female rats with testosterone significantly increased the expression of rOCT2 in the kidney and stimulated the uptake of TEA in renal slices. Estradiol treatment on the other hand caused a decrease in the expression of rOCT2 and in the uptake of TEA in renal slices, but only in male rats (Urakami et al., 2000). The roles of these steroid hormones

were confirmed in gonadectomized rats, where the levels of rOCT2 in the kidney became similar between males and females (Slitt et al., 2002). Together, these studies suggest that rOCT2 is responsible for the gender differences in the renal elimination of organic cations. Although the physiological function of this sexual dimorphism is unknown, it might be responsible for some of the gender-based differences in drug response and sensitivity in patients.

In contrast to OCT1 and OCT2, which are predominantly expressed in the major excretory organs, OCT3 has a much more widespread tissue distribution. Initially, in a limited study, hOCT3 was detected by RT-PCR analysis in brain cortex, heart and liver (Gründemann et al., 1998). In a more extensive study, hOCT3 mRNA was detected at high levels in aorta, skeletal muscle, prostate, adrenal gland, salivary gland, liver, term placenta and fetal lung (Verhaagh et al., 1999). In rats, expression of rOCT3 as analyzed by Northern analysis was highest in intestine and placenta, intermediate in brain and heart, low in kidney and lung but undetectable in liver (Kekuda et al., 1998). By *in situ* hybridization rOCT3 was detected in different regions of the brain, especially in the cerebellum, hippocampus, pontine nucleus and cerebral cortex, whereas by immunocytochemical staining, hOCT3 was detected in normal human astrocytes (Wu et al., 1998; Inazu et al., 2003). Rajan et al. (2000) found that in mice mOCT3 mRNA is also expressed in the retinal pigment epithelium of the eye, suggesting involvement of OCT3 in the clearance of dopamine and histamine from the subretinal spaces and possibly in the disposition of neurotoxins such as MPP^+. As yet, there is not conclusive published evidence showing whether OCT3 is localized in the basolateral or apical membrane of polarized cells.

Catecholamine transport

Besides a possible role of the OCT transporters in the clearance of xenobiotics, they have also been implicated in the elimination of endogenous cationic compounds such as the biogenic amine neurotransmitters. The biogenic amines are a class of neurotransmitters including the tyrosine-

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derived catecholamines (dopamine, epinephrine and norepinephrine), serotonin (5-hydroxytryptamine, 5-HT) and histamine. They are released by sympathetic neurons via exocytosis into the synaptic cleft where they act as neurotransmitters. Efficient removal of the neurotransmitters after release is critical to well-regulated synaptic transmission and is mediated by re-uptake, enzymatic degradation and diffusion. The re-uptake of released catecholamines is mediated by high-affinity, Na^+- and Cl^--dependent transporters present in the outer membrane of the presynaptic nerve endings. This transport system is also known as uptake-1 and consists of the norepinephrine transporter (NET, SLC6A2) and the dopamine transporter (DAT, SLC6A3), both members of the Solute Carrier superfamily of transporters. In addition to uptake-1, a second transport system is present in the peripheral non-neuronal tissues, designated uptake-2 or extraneuronal monoamine transport system (EMT). EMT was first identified by Iversen (1965) as a low-affinity uptake system for catecholamines in isolated rat heart. It was independently cloned by two different groups and was designated EMT/OCT3 (Kekuda et al., 1998; Wu et al., 1998; Gründemann et al., 1998). In addition to EMT/OCT3, OCT1 and OCT2 have also been shown to mediate the transport of catecholamines and other biogenic amines (Breidert et al., 1998; Gründemann et al., 1998). Neuronal re-uptake by uptake-1 is quantitatively most important for the clearance of released catecholamines, accounting for about 90% of their removal at the nerve endings. Although OCT3 has been proposed to be the classical EMT, the three OCTs together are thought to be responsible for the (predominantly) extraneuronal clearance of catecholamines that have escaped from re-uptake by uptake-1 (reviewed by Eisenhofer, 2001).

Alternative splicing of OCTs

Eukaryotic cells have the capability to increase the coding capacity of their genes via alternative splicing, which is predicted to occur in more than half of the human genes (Kan et al., 2001). Alternative splicing allows a single gene to produce a variety of proteins with altered tissue

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distribution, localization, regulation or function. Zhang et al. (1997) identified an alternatively spliced isoform of OCT1 in rat kidney, named rOCT1A, that is generated by skipping of exon 2. As a consequence of this deletion, the rOCT1A transcript encodes a truncated protein of 430 residues (rOCT1 has 556 residues) lacking the first two N-terminal TMDs and the large extracellular loop. Surprisingly, the absence of the first two TMDs has no significant effect on the transport of TEA as tested in RNA-injected oocytes, indicating that they are not essential, at least for the transport of this compound. The tissue distribution of rOCT1A RNA was similar to that of rOCT1, but the physiological function of this splice variant, if any, is as yet unknown (Zhang et al., 1997).

Hayer et al. (1999) identified several alternatively spliced isoforms of OCT1 in human cells. By RT-PCR, four different transcripts representing variants of hOCT1 were identified in the human glioma cell line SK-MG-1 and two of these were also found in human liver. Further analysis showed that these transcripts represented the full length hOCT1 and three shorter alternatively spliced isoforms, two of which are lacking the last two C-terminal TMDs and one the last 6 TMDs. Functional analysis by measuring the uptake of MPP^+ in transfected HEK293 cells indicated that neither one of the hOCT1 splice variants exhibited significant decynium-22 sensitive uptake of MPP^+. Whether the transport of other substrates is affected in these isoforms is not known.

For OCT2, an alternatively spliced variant was isolated from human kidney (Urakami et al., 2002). This variant, named hOCT2-A, contains a premature stop codon due to an intron splicing error, failing to remove the intron between exon 7 and 8. The open reading frame of this transcript encodes a 483-amino acid protein with 81% amino acid identity with hOCT2 (which consists of 555 amino acids). As a consequence of the premature termination of translation, hOCT2-A consists of only 9 TMDs instead of 12, lacking the last three C-terminal TMDs. The tissue distribution of hOCT2 and hOCT2-A RNAs were somewhat different; hOCT2-A was found primarily in kidney, but mRNA was also detected in brain, liver, colon, skeletal muscle, testis and placenta. In contrast, hOCT2 was detected in kidney, brain, testis and placenta. Functional analysis using transfected

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HEK293 cells demonstrated that hOCT2-A still exhibited transport activity of TEA similar to that of hOCT2, but transport of MPP^+ and cimetidine were decreased and that of guanidine was completely abolished. Whether the distinct substrate specificity and tissue distribution of hOCT2-A have any physiological or pharmacological relevance is unclear.

Genetic variations in OCTs

A major complication in drug therapy is the high interindividual variability in response and sensitivity to drugs. With the development of high-throughput screening methods for the detection of polymorphisms, it is now becoming increasingly clear that genetic variations in proteins affecting the pharmacokinetics of drugs are important determinants in this variability. Recently, several groups have reported polymorphic genetic variations in members of the organic cation transporter family (Kerb et al., 2002; Saito et al., 2002; Leabman et al., 2002; 2003; Shu et al., 2003; Lazar et al., 2003). For OCT1, Kerb et al. (2002) identified among a population of 57 healthy Caucasians, 25 genetic variants, 8 of which resulted in amino acid substitutions. Functional characterization, by measuring the uptake of various OCT1 substrates in RNA-injected oocytes, demonstrated reduced transport activity in three of these variants (Arg61Cys, Cys88Arg and Gly401Ser). Among 247 subjects of diverse ethnic backgrounds, Leabman et al. (2003) identified 15 nonsynonymous polymorphisms in OCT1, four (Arg61Cys, Gly220Val, Gly401Ser, Gly465Arg) resulting in reduced, and one (Ser14Phe) in increased uptake of MPP^+ (Shu et al., 2003).

Within the same population, 28 polymorphisms were detected in OCT2, 8 of which resulted in nonsynonymous amino acid substitutions (Leabman et al., 2002). Two of these variants, each with an allelic frequency of more than 1% in the African-American population (Met165Ile and Arg400Cys), displayed a significantly reduced transport activity. The variant with the highest allelic frequency (Ala270Ser), had a prevalence of about 12.7% among all different ethnic groups but had a more subtle effect on hOCT2 function. Overall, the frequency of synonymous changes was higher

than that of non-synonymous changes, suggesting an evolutionarily preserved function of hOCT2 (Leabman et al., 2002).

The positions of the mutations and their effects on transport activity and specificity may provide insight into the molecular mechanism by which substrates are recognized and transported by the organic cation transporters. Arg61, Cys88 (hOCT1) and Met165 (hOCT2) are localized in the first large extracellular loop and TMD-2 respectively. The severe effect of these mutations on transport activity is surprising, since complete absence of TMD-1 and -2 has previously been shown to have no significant consequences for the transport of TEA by rOCT1 (Zhang et al., 1997). Possibly, this part of the protein plays a role in the substrate selectivity but is not essential for basic transport activity. Gly401 (hOCT1) and Arg400 (hOCT2) belong to a stretch of five amino acids that is highly conserved within the superfamily of facilitated transporters, indicating that this sequence might be essential for basal transport activity (Gorboulev et al., 1997).

Based on the allelic frequencies of the above-mentioned variants with (severe) effects on OCT function (about 1%), one would expect that homozygotes for these alleles would arise in the human population with a frequency of about 1 per 10.000 individuals. Interestingly, OCT2 and OCT3 in mice are only expressed from the maternal allele. Together with the Igf2r gene, they form a gene cluster that has been shown to be paternally imprinted in mice (Zwart et al., 2001). It is not known whether OCT2 and OCT3 are also imprinted in humans, but if so, this implicates that the actual frequency of humans with a partial or complete deficiency in OCT2 might be much higher. The possible clinical and physiological consequences of a genetic deficiency in the organic cation transporters are discussed in the next section.

Knockout mouse models

Given their broad substrate specificity and possible physiological and pharmacological relevance, a clear understanding of the *in vivo* functions of the OCT transporters will be of great importance. For

that purpose, knockout mouse models have been generated for all three organic cation transporters (Jonker et al., 2001; 2003; Zwart et al., 2001).

Oct1 deficient mice are viable and healthy, and display no obvious phenotypical abnormalities, indicating that Oct1 is not essential for normal physiological functioning in mice. However, absence of Oct1 does have a pronounced effect on the pharmacokinetics of substrate organic cations. In *Oct1*$^{-/-}$ mice, the accumulation in liver of intravenously administered TEA was dramatically reduced (by 85% and 77% after 20 and 60 min, respectively) compared to wild-type mice, indicating that Oct1 is the main sinusoidal uptake system for TEA in the liver. In addition, direct small intestinal excretion of TEA in *Oct1*$^{-/-}$ mice was reduced by about 50%. This indicated that Oct1 also mediates the basolateral uptake of TEA into enterocytes which is necessary for subsequent excretion into the gut lumen (Jonker et al., 2001). Together, these findings may be of clinical importance as they might help predicting and analyzing the consequences of a deficiency in OCT1 in humans. A genetic deficiency in OCT1 could have both positive and negative consequences for drug therapy. On the one hand, reduced hepatic uptake of drugs may result in a decreased therapeutic efficacy of drugs that have their desired pharmacodynamic action (or metabolic activation) in the liver. On the other hand, reduced hepatic uptake of drugs could be beneficial for drugs that have adverse side effects in the liver. The latter is exemplified by the biguanide metformin, an antidiabetic drug which displays reduced hepatic uptake and toxicity in *Oct1*$^{-/-}$ mice (Wang et al., 2002; 2003). Biguanides form a class of drugs widely used for the treatment of hyperglycemia in patients with type 2 diabetes mellitus. The biguanides metformin, buformin and phenformin are excellent substrates of OCT1 as demonstrated *in vitro* in rOCT1-transfected CHO cells (Wang et al., 2002). A severe, sometimes lethal toxic side effect of the biguanides is lactic acidosis (*i.e.* accumulation of lactate in plasma), which is thought to result from interference with processes in the liver. For this reason phenformin was withdrawn from clinical use in many countries in the late 1970s. In *Oct1*$^{-/-}$ mice the accumulation of metformin into the liver was more than 30-fold reduced as compared to wild-type

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mice (Wang et al., 2002). Coincident with this decreased hepatic accumulation, metformin-induced levels of blood lactate were significantly decreased in *Oct1*$^{-/-}$ mice, indicating the involvement of Oct1 in processes leading to lactic acidosis (Wang et al., 2003).

The absence of Oct1 from the liver also resulted in a shift in the elimination of transported drugs from hepatobiliary towards renal elimination. Consequently, the urinary excretion of drugs in *Oct1*$^{-/-}$ mice was increased instead of decreased, contrary to what was initially expected (Jonker et al., 2001; Wang et al., 2002). The analysis of the role of Oct1 in renal drug secretion in mice was also complicated by its functional redundancy with Oct2. Therefore, to further analyze the role of these transporters in the renal secretion, we generated *Oct2* single knockout and *Oct1/2* double knockout mice (Jonker et al., 2003). Like for Oct1, a deficiency in Oct2 and a combined deficiency in Oct1 and Oct2 had no obvious effect on the physiology of mice. Absence of Oct2 in itself had little effect on the pharmacokinetics of TEA, but in *Oct1/2*$^{-/-}$ mice, the renal secretion of this compound was completely abolished, leaving only glomerular filtration as an excretion mechanism for TEA. As a consequence, plasma levels of TEA were substantially elevated in *Oct1/2*$^{-/-}$ mice (Jonker et al., 2003). Unlike rodents that express both Oct1 and Oct2 in the kidney, humans express only OCT2 in kidney (Gründemann et al., 1998). Therefore, it is likely that the *Oct1/2*$^{-/-}$ mouse model better reflects the effect of an OCT2 deficiency on kidney function in humans than the *Oct2*$^{-/-}$ mouse model. Thus, based on our findings, we expect that humans with a deficiency in OCT2 will have an impaired renal secretion of some drugs, which may result in increased drug exposure.

Oct3 deficient mice have been generated by Zwart et al. (2001). *Oct3*$^{-/-}$ mice are viable and fertile and show no apparent neural or physiological defect or imbalance of the monoamine neurotransmitters norepinephrine and dopamine. A clear effect, however, was observed on the uptake of MPP^+ into the heart. The heart was originally identified as one of the main uptake-2 sites and it expresses high levels of OCT3 (Iversen, 1965; Gründemann et al., 1998). After intravenous administration, accumulation of MPP^+ into the heart was reduced by 72% in *Oct3*$^{-/-}$ mice compared

to wild-type mice. In addition, the placenta was identified as a novel uptake-2 site. In pregnant females of an *Oct3* heterozygous cross, the accumulation of intravenously administered MPP^+ into *Oct3*$^{-/-}$ fetuses was three-fold reduced compared to wild-type fetuses. In the placenta, Oct3 co-localizes with the monoamine degrading enzyme monoamine oxidase A (MAO-A), suggesting that together they might form a functional elimination pathway for monoamines produced in the embryo (Zwart et al., 2001). The lack of effect of absence of OCT3 from other sites, such as the kidney, is most likely due to the functional redundancy between the different OCTs and might be further analyzed in combined knockouts of these transporters.

Conclusions and perspectives

Since the identification of the first member of the OCT family, almost a decade ago, our knowledge of the organic cation transporters has considerably improved. Functional expression of the cloned transporters in oocytes and mammalian cell lines has allowed a detailed analysis of their transport properties and substrate specificities *in vitro*. The recent development of mouse models with deficiencies in each of these transporters has added an extra dimension to their functional characterization. Although the studies with the Oct knockout mice are still limited, they have already clearly demonstrated that the organic cation transporters play an important role in the pharmacokinetics of substrate drugs, mediating hepatic uptake and direct intestinal secretion (OCT1), renal secretion (OCT1 and OCT2) and uptake into the heart and transport across the placenta (OCT3).

The relevance of these transporters for the transport and elimination of endogenous compounds on the other hand is still unclear. It should be realized, however, that the homeostasis of endogenous compounds such as the monoamine neurotransmitters and other hormones is strictly regulated at the levels of production, degradation and clearance, and an imbalance at one of these levels is likely to be readily compensated. In addition, as we observed for OCT1 and OCT2 in the mouse kidney,

considerable functional redundancies may exist between the different transporters. For this reason, it would be of interest to study the effects of a combined deficiency in OCT1, OCT2 and OCT3 in mice as well as that of complementary apical transporters. We would like to add that the relatively safe and sanitized conditions under which laboratory mice are usually housed may not be optimal for identifying all physiological and toxicological functions. Physiological aberrations can be subtle or become only apparent under specific conditions and might be easily missed unless specifically looked for.

With the identification of functionally important genetic polymorphic variants in the human OCT1 and OCT2 genes, it will be of great interest to determine whether these polymorphisms also correlate with altered drug response and sensitivity in patients. If indeed the findings in the knockout mice can be extrapolated to humans, these mouse models will provide powerful tools for predicting and explaining differences in drug (hyper-)sensitivity and toxicity between patients. We expect that in the future, the increased understanding of the function of the organic cation transporters may lead to improvement of the design and use of drugs and drug therapies.

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Footnotes

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Legends

Figure 1. Predicted secondary structure of OCT1. The protein is thought to contain 12 transmembrane domains with both N- and C-terminus located intracellularly. The first large extracellular loop contains three putative *N*-linked glycosylation sites (indicated by branches). Cytoplasmic (IN) and extracellular (OUT) orientation are indicated.

Figure 2. Localization of OCT1 and OCT2 in kidney and liver. In the kidney, OCT1 (in rodents but not in humans) and OCT2 are present in the basolateral membrane of the proximal tubule. In a schematic representation of a cross-section of a proximal tubule, the localization of OCT1 and OCT2 is indicated by a ball with arrows. In the liver, OCT1 is present in the basolateral (sinusoidal) membrane of hepatocytes. Partly reprinted from Junqueira et al., Basic Histology, copyright 1998, with permission from The McGraw-Hill Companies. Partly reprinted from Bloom and Fawcett, A textbook of histology, copyright 1994, Chapman & Hall; Reproduced by permission of Hodder Arnold.

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Table 1

Transported substrates of the rat and human OCTs.

Substrate	rOCT 1	hOCT 1	rOCT 2	hOCT 2	rOCT 3	hOCT 3	References
Model compounds							
Tetraethylammonium (TEA)	+	+	+	+	?	?	Gorboulev et al., 1997 Gründemann et al., 1994; 1998b; 1999 Kekuda et al., 1998 Wu et al., 1998
1-methyl-4-phenylpyridinium (MPP^+)	+	+	+	+		+	Busch et al., 1996 Gorboulev et al., 1997 Gründemann et al., 1998b; 1999
N-methyl-nicotinamide (NMN)	+	+		+			Busch et al., 1996 Gorboulev et al., 1997
Guanidine	+/-		+		+	-	Gründemann et al., 1999 Kekuda et al., 1998
Tributylmethylammonium (TBuMA)		+					Van Montfoort et al., 2001
N-methyl-quinine		+					Van Montfoort et al., 2001
N-methyl-quinidine		+					Van Montfoort et al., 2001
Azidoprocainamide methoiodide		+					Van Montfoort et al., 2001
Drugs							
Amantadine	+		+	+			Busch et al., 1998 Goralski et al., 2002
Memantine				+			Busch et al., 1998
Cimetidine	+/-	-	+			+	Gründemann et al., 1999 Zhang et al., 1998

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Metformin	+						Wang et al., 2002
Buformin	+						Wang et al., 2002
Phenformin	+						Wang et al., 2002
Biogenic amines							
Agmatine	+/-	+/-	+	+	+	+	Gründemann et al., 2003
Dopamine	+		+	+	+	-	Busch et al., 1996; 1998 Gründemann et al., 1998a; 1998b Wu et al., 1998
Epinephrine	+		+			+	Breidert et al., 1998 Gründemann et al., 1998a; 1998b
Histamine	-		+	+	+		Busch et al., 1998 Gründemann et al., 1998a; 1999
Norepinephrine	+		+	+		+	Breidert et al., 1998 Busch et al., 1998 Gründemann et al., 1998a; 1998b
Serotonin (5-hydroxytryptamine)	+		+	+		+	Breidert et al., 1998 Busch et al., 1998 Gründemann et al., 1998a; 1998b
Tyramine	+					+	Breidert et al., 1998 Gründemann et al., 1998b
Other endogenous compounds							
Choline	+		?	+	-		Busch et al., 1996 Gorboulev et al., 1997 Gründemann et al., 1998b Sweet et al., 2002
Prostaglandin E2		+		+			Kimura et al., 2002
Prostaglandin F2α		+		+			Kimura et al., 2002
Creatinine	+/-		+			-	Gründemann et al., 1999

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Nucleosides							
2-deoxytubercidin (dTub)	+		-				Chen and Nelson, 2000
Cytosine arabinoside	+		-				Chen and Nelson, 2000
2-chlorodeoxyadenosine	+/-		-				Chen and Nelson, 2000
Azidothymidine (AZT)	+/-		-				Chen and Nelson, 2000

Only substrates are listed for which transport has been directly demonstrated in cRNA injected *Xenopus laevis* oocytes or transfected cell lines. +, transported; +/-, poorly transported; -, no transport detected; ?, controversial.

Figure 1

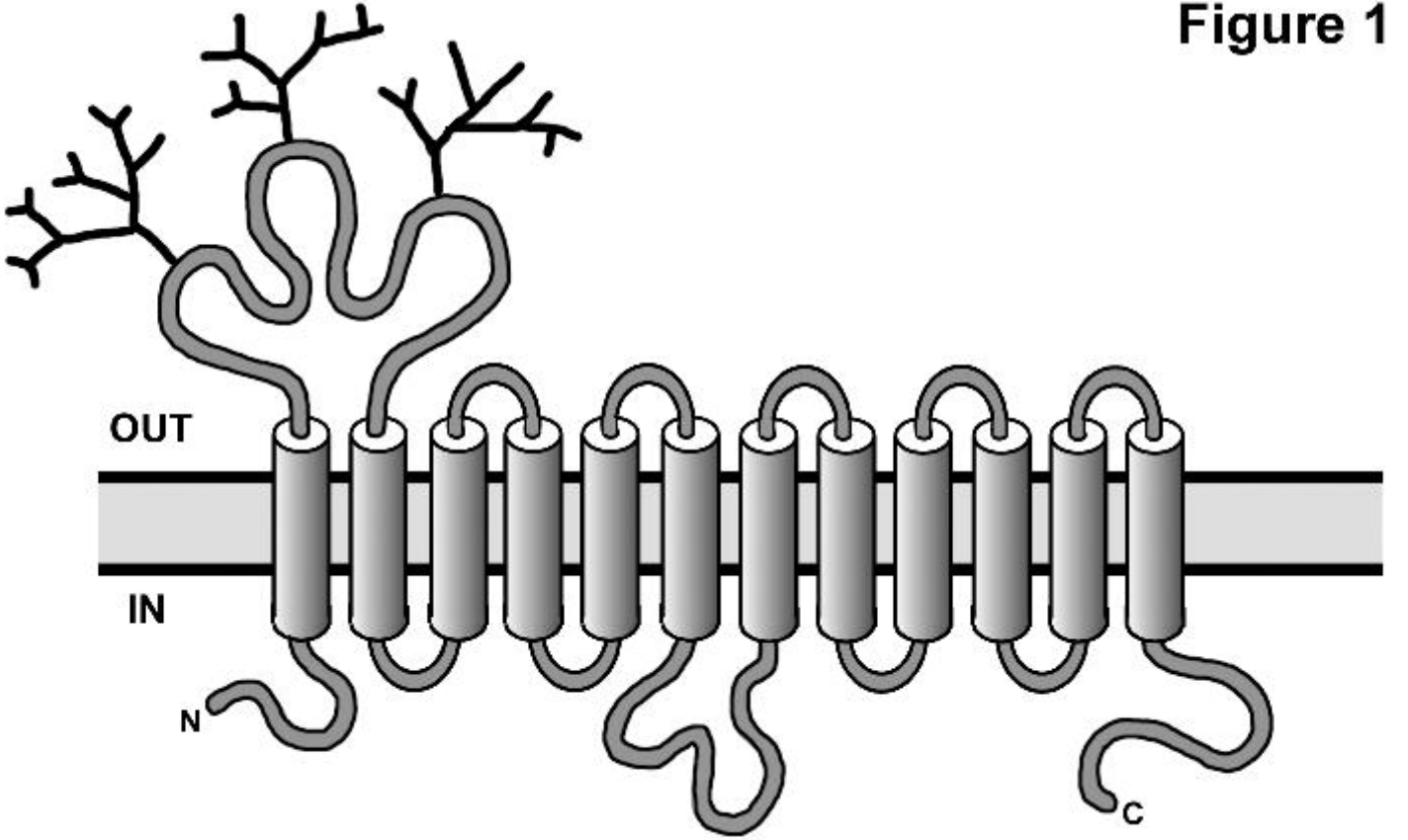

Figure 2