Kinetic and Selectivity Differences between Rodent, Rabbit,
and Human Organic Cation Transporters (OCT1)

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ABSTRACT

Organic cation transporters play an important role in the absorption, distribution, and elimination of clinical agents, toxic substances, and endogenous compounds. In kidney preparations, significant differences in functional characteristics of organic cation transport between various species have been reported. However, the underlying molecular mechanisms responsible for these interspecies differences are not known. The goal of this study was to determine the kinetics and substrate selectivities of organic cation transporter (OCT1) homologs from mouse, rat, rabbit, and human that may contribute to interspecies differences in the renal and hepatic handling of organic cations. With a series of n-tetraalkylammonium (nTAA) compounds, a correlation between increasing alkyl chain length and affinity for the four OCT1 homologs was observed. However, the apparent affinity constants ($K_i$) differed among the species homologs. For the mouse homolog mOCT1, apparent $K_i$ values ranged from 7 μM for tetrabutylammonium to 2000 μM for tetramethylammonium. In contrast, the human homolog hOCT1 exhibited weaker interactions with the nTAA compounds. Trans-stimulation studies and current measurements in voltage-clamped oocytes demonstrated that larger nTAA compounds were transported at greater rates in oocytes expressing hOCT1, whereas smaller nTAA were transported at greater rates in oocytes expressing mOCT1 or rOCT1. The rabbit homolog rbOCT1 exhibited intermediate properties in its interactions with nTAA compared with its rodent and human counterparts. This report demonstrates that the human OCT1 homolog has functional properties distinct from those of the rodent and rabbit OCT1 homologs. The study underscores potential difficulties in extrapolating data from preclinical studies in animal models to humans.

Renal and hepatic secretion of organic cations is a major pathway of xenobiotic elimination from the systemic circulation. It is now known that numerous clinically used drugs as well as toxic and endogenous compounds are substrates for secretory transporters in the kidney and liver (Muller and Jansen, 1997; Zhang et al., 1998a). These transport mechanisms have been studied in detail over the past two decades in a variety of tissue and membrane preparations and in cell culture models as well (Gisclon et al., 1987; Wright and Wunz, 1988; Sokol and McKinney, 1990; Wright and Wunz, 1995). Electrotransport of organic compounds have been characterized in kidney and liver basolateral membranes (BLMs) that move organic cations down the electrochemical gradient from the blood into tubular cells and hepatocytes. In contrast, in kidney and liver brush border membranes (BBMs) several organic cation/proton exchange mechanisms have been described that are distinct from the BLM transport systems (Moseley et al., 1990; Pritchard and Miller, 1993, 1996; Moseley et al., 1997; Muller and Jansen, 1997). Together, the BLM and BBM transporters responsible for the transcellular flux of OCs from the blood to the tubule fluid or bile and hence play an important role in removing many compounds from the systemic circulation.

Although this two-step model of transcellular flux of organic cations is similar among species, several interspecies variations in the characteristics of renal organic cation transport have been reported. For example, recent studies of the effects of n-tetraalkylammonium (nTAA) compounds on organic cation transport in the rabbit and rat kidney suggest a marked interspecies variation in the renal handling of organic cations (Üllrich et al., 1991; Groves et al., 1994).

The goal of the current study was to compare the functional characteristics of the organic cation transporter 1 (OCT1) from mouse, rat, rabbit, and human. With a series of nTAA compounds with varying alkyl chain lengths, we found a correlation between increasing alkyl chain length and affinity for OCT1 regardless of the species. However, notable differences in the nTAA affinities and turnover rates were found among species. In particular, the rodent transporters

ABBREVIATIONS: BLM, basolateral membrane; BBM, brush border membrane; nTAA, n-tetraalkylammonium; OCT, organic cation transporter; MPP+, 1-methyl-4-phenylpyridinium; TMA, tetramethylammonium; TEA, tetraethylammonium; TPrA, tetrapropylammonium; TBA, tetrabutylammonium; TPeeA, tetrapienlylammonium.
had a higher affinity for the nTAAAs compared with the human transporter, and the human transporter had comparatively higher turnover rates for larger nTAAAs than the rodent homologs. This is the first report of kinetic and selectivity differences among organic cation transporter homologs. Such differences may have important consequences for species comparisons of in vivo disposition of organic cations.

**Experimental Procedures**

**cRNA Transcription and Xenopus Oocyte Expression.** Oocytes were harvested from oocyte positive *Xenopus laevis* (Nasco, Fort Atkinson, WI) and were dissected and treated with collagenase D (Boehringer-Mannheim Biochemicals, Indianapolis, IN) in a calcium-free ORII solution as previously described (Chun et al., 1997). Oocytes were maintained at 18°C in modified Barth’s medium. Healthy stage V and VI oocytes were injected with capped cRNA (1 μg/μl). Capped cRNA was transcribed in vitro with T7 polymerase (mCAP RNA capping kit; Stratagene, La Jolla, CA) from SpeI linearized plasmids containing mouse (mOCT1), rat (rOCT1), rabbit (rbOCT1), or human (hOCT1) transporter cDNAs (Grundemann et al., 1994; Schweifer and Barlow, 1996; Zhang et al., 1997; Terashita et al., 1998).

**Tracer Uptake Measurements.** Transport of [3H]1-methyl-4-phenylpyridinium (MPP+) (82 Ci/mmol, DuPont/New England Nuclear, Boston, MA) in oocytes was measured 2 to 7 days after injection as described previously (Zhang et al., 1997; Terashita et al., 1998). MPP+ uptakes were carried out as follows: groups of seven to nine oocytes were incubated in Na+ buffer (100 mM NaCl, 2 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES/Tris, pH 7.2) containing [3H]MPP+ (1 μM: 0.1 μM [3H]MPP+, and 0.9 μM unlabeled MPP+) for 1 h. Uptake was stopped by washing the oocytes five times with 3 ml of ice-cold Na+ buffer. The radioactivity associated with each oocyte was then determined by scintillation counting. For inhibition studies, unlabeled nTAA compounds were added to the reaction solutions as needed.

In trans-stimulation studies, groups of five to seven OCT1 cRNA- or uninjected oocytes were washed three times with K+ buffer (2 mM NaCl, 100 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 10 mM HEPES/Tris, pH 7.2). The oocytes were then quickly injected with 50 nl of a K+ solution containing an unlabeled nTAA compound. The concentration and injection volume were chosen based on an average oocyte intracellular volume of 500 nl to produce intracellular nTAA concentrations that would be at saturating levels (i.e., >10-fold the K_i value). Oocytes injected with 50 nl of K+ buffer were used for controls in the trans-stimulation studies. After injections, the oocytes were quickly transferred to a small disposable borosilicate glass culture dish containing an unlabeled nTAA compound. The concentration and injection volume were chosen based on an average oocyte intracellular volume of 500 nl to produce intracellular nTAA concentrations that would be at saturating levels (i.e., >10-fold the K_i value). Oocytes injected with 50 nl of K+ buffer were used for controls in the trans-stimulation studies. After injections, the oocytes were quickly transferred to a small disposable borosilicate glass culture dish containing an unlabeled nTAA compound. The concentration and injection volume were chosen based on an average oocyte intracellular volume of 500 nl to produce intracellular nTAA concentrations that would be at saturating levels (i.e., >10-fold the K_i value).

**Electrophysiology Studies.** Electrophysiology experiments were performed at room temperature (21–23°C) 5 to 10 days post-injection. Steady-state ligand-induced currents were measured with

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>mOCT1</th>
<th>rOCT1</th>
<th>rbOCT1</th>
<th>hOCT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA</td>
<td>2040 ± 75</td>
<td>905 ± 63</td>
<td>5780 ± 1070</td>
<td>12400 ± 1280</td>
</tr>
<tr>
<td>TEA</td>
<td>128 ± 17.1</td>
<td>100 ± 10.7</td>
<td>93.6 ± 6.0</td>
<td>158 ± 40.5</td>
</tr>
<tr>
<td>TPrA</td>
<td>19.8 ± 2.1</td>
<td>21.0 ± 7.8</td>
<td>35.5 ± 6.0</td>
<td>102 ± 13.0</td>
</tr>
<tr>
<td>TBA</td>
<td>7.3 ± 0.2</td>
<td>16.9 ± 6.0</td>
<td>25.1 ± 7.3</td>
<td>29.6 ± 3.6</td>
</tr>
</tbody>
</table>

**Fig. 1.** Effect of nTAA compounds (50 μM) of increasing chain length on [3H]MPP+ (1 μM) uptake (1 h) by OCT1-expressing oocytes. The uptake of [3H]MPP+ (1-h incubation) in Na+ uptake buffer was measured in oocytes expressing mOCT1 (●), rOCT1 (■), rbOCT1 (○), or hOCT1 (□). Each data point represents the mean rate of uptake as a percentage of the control in which no inhibitor was present. Data represent mean ± S.E. (n = 6–9) obtained from two to four experiments in separate batches of oocytes.

**Fig. 2.** Inhibition of [3H]MPP+ uptake (1 h) by nTAA compounds in oocytes expressing mOCT1. Mean values of the uptake relative to control (no inhibitor present) are shown. Data normalized to control (no inhibitor) represent mean values from 7 to 9 oocytes in a representative experiment and were fit by nonlinear regression as described previously (Zhang et al., 1999). The K_i values for TMA (●), TEA (■), TPrA (○), and TBA (□) are listed in Table 1.
two-electrode voltage clamp. Oocytes were voltage clamped at -50 mV and superfused with Na⁺ buffer 2 min before and after 30 s ligand superfusions. Recordings were obtained in a 25-μl recording chamber at flow rates of -3 ml/min. The nTAA-induced current was the difference between the values measured in the presence of nTAA and the average of values recorded in Na⁺ buffer alone before and after nTAA superfusion. Uninjected oocytes were used as controls. In addition, the nontransported inhibitor quinidine (100 μM) was used as a negative control to ensure that the observed inwardly directed currents were not due to inhibition of cation efflux from the oocyte (data not shown) (Nagel et al., 1997).

**Data Analysis.** Values are expressed as mean ± S.E. or mean ± S.D. as indicated in the legends. Six to nine oocytes were used to generate a data point in each experiment. All experiments were repeated at least once with different batches of oocytes, unless indicated otherwise in the legends. Apparent \( K_i \) values were determined as described previously (Zhang et al., 1998b, 1999).

**Materials.** The nTAA compounds were purchased from Sigma Chemical Co. (St. Louis, MO): tetramethylammonium (TMA, 5 M solution), tetrethylammonium (TEA), tetrapropylammonium (TPrA), and tetratetramethylammonium (TPeA). All other reagents were purchased from either Sigma Chemical Co. or Fisher Scientific (Pittsburgh, PA) or as indicated. [3H]MPP⁺ (82 Ci/mmol) was purchased from DuPont/New England Nuclear.

**Results**

**Inhibition of OCT1-Mediated Uptake by nTAA Compounds.** Inhibition studies with nTAA compounds of increasing chain length were carried out to determine the relationship between alkyl chain length and inhibition potency. The inhibition potency of the nTAA compounds (50 μM) for the OCT1 transporters from mouse, rat, rabbit, and human increased with alkyl chain length (Fig. 1). The concentration dependence of the nTAA compounds in inhibiting [3H]MPP⁺ (1 μM) uptake was determined. The data were fit as described previously to determine the apparent \( K_i \) values (Zhang et al., 1998b, 1999). Representative inhibition curves for TMA, TEA, TPrA, and TBA are shown in Fig. 2 for mOCT1. The apparent \( K_i \) values decreased with increasing chain length from >2000 μM for TMA to ~7 μM for TBA (Table 1). Similar trends between increasing chain length and inhibition potency were found for all species. However, notable potency differences were found among species (Table 1). Most apparent were potency differences between the human and rodent transporters. The rbOCT1 homolog exhibited intermediate sensitivity to the nTAA compounds. One interesting and notable exception was TEA; TEA appeared to have a similar affinity for all four OCT1 homologs.

**Efflux of nTAAs from Oocytes Expressing OCT1.** Trans-stimulation studies are often used to test whether a compound is an actual substrate (permeant) for a transporter (Dantztler et al., 1991; Moseley et al., 1996; Wright and Wunz, 1998; Zhang et al., 1999). Trans-stimulation studies were carried out under depolarized conditions (i.e., in K⁺-containing buffer) to minimize the effects of membrane potential on [3H]MPP⁺ (1 μM) uptake. In oocytes expressing hOCT1, [3H]MPP⁺ influx was trans-stimulated the most by TEA and TPrA. TMA, TBA, or TPeA neither trans-inhibited nor trans-stimulated hOCT1-mediated [3H]MPP⁺ influx (Fig. 3). In marked contrast, in oocytes expressing mOCT1 or rOCT1, the smaller nTAA compounds, TMA and TEA, trans-stimulated [3H]MPP⁺ influx, whereas the larger nTAA compounds trans-inhibited [3H]MPP⁺ influx (Fig. 3). The rabbit homolog appeared to have a somewhat intermediate function between its rodent and human counterparts. The influx of [3H]MPP⁺ was trans-stimulated the most by TEA, weakly by TPrA, but not by TMA in oocytes expressing rbOCT1. The larger nTAA compounds TBA and TPeA weakly trans-inhibited [3H]MPP⁺ influx (Fig. 3). It is possible that apparent trans-inhibition may result from cis-inhibition due to rapid efflux of com-
pounds from the oocytes. This possibility may not be excluded; however, it is unlikely because of the large volume into which the compounds would efflux (i.e., 100 μl), which would substantially reduce their concentrations on the cis-side.

**nTAA-Dependent Currents in Voltage-Clamped Oocytes.** The translocation of the nTAA compounds was further studied by measuring nTAA-dependent currents under voltage-clamped conditions in oocytes expressing OCT1. Figure 4 shows representative current traces in oocytes superfused with saturating concentrations of nTAA compounds for 30 s (gray bars at the top). Figure 5 shows the nTAA-dependent currents normalized with the currents from 1 mM TEA superfusion. TEA was chosen because it has a similar affinity for the four OCT1 homologs. In oocytes expressing hOCT1, TEA and TPrA induced the largest currents, followed by the larger nTAA compound TBA, and then the smallest nTAA compound TMA. In rbOCT1 cRNA-injected oocytes, TEA induced the largest currents followed by TMA, TPrA, and TBA, respectively. In contrast, in the rodent OCT1-expressing oocytes, the small nTAA compounds TMA and TEA induced the largest currents and the larger nTAA compounds induced very small currents.

**Discussion**

Organic cation transporters in the kidney and liver play an important role in the removal of potentially toxic drugs and their metabolites from the systemic circulation (Zhang et al., 1998a). The fundamental mechanisms involved in the sequential flux of organic cations across renal and hepatic epithelia are reported to be similar among mammalian species (Pritchard and Miller, 1993, 1996; Zhang et al., 1998a). However, the extent of interspecies differences in kinetic characteristics and selectivities of organic cation transporters is not well understood. Our understanding of organic cation transport mechanisms is largely based on studies performed with tissue preparations from the rabbit kidney and rat liver (Wright, 1985; Gisclon et al., 1987; Dantzler et al., 1991; Moseley et al., 1992, 1996, 1997; Martel et al., 1996). Relatively few studies have examined renal or hepatic transport mechanisms in humans (Ott et al., 1991; Chun et al., 1997; Muller and Jansen, 1997). There have been several reports of species differences in the substrate selectivity of renal organic cation transport systems (Ullrich et al., 1991; Groves et al., 1994). This suggests that there could be kinetic and selectivity differences in organic cation transporters among species, which could result in substantial differences in the in vivo handling of drugs and toxins.

Marked species differences in the substrate selectivity of the renal organic cation transport system were found when studying the interactions of nTAA compounds with the BLM organic cation transport system in rabbit and rat kidneys (Ullrich et al., 1991; Groves et al., 1994). A correlation between alkyl chain length (from methyl to pentyl) and increasing affinity for the rabbit renal organic cation transport systems in the basolateral membrane was found (Groves et al., 1994). In contrast, increasing the alkyl chain length beyond two (ethyl) did not improve the affinity for the BLM transport system in the rat (Ullrich et al., 1991). These data can be rationalized in several ways. For example, it is possible that different OCT isoforms with different affinities for nTAA compounds are expressed in the rabbit and rat kidney. In fact, marked differences in the tissue distribution of organic cation transporter mRNA isoforms have been reported (Gorboulev et al., 1997; Zhang et al., 1997; Terashita et al., 1998). Alternatively, the same OCT isoform could be expressed in rat and rabbit kidney, but the rat and rabbit isoforms might differ in their kinetic properties and selectivities.

The goal of this study was to compare the kinetics and selectivities of nTAA compounds with the OCT1 isoforms from mouse, rat, rabbit, and human. OCT1 is expressed in the livers and kidneys of these species. Therefore, intrinsic functional differences between OCT1 species homologs may
explain the observed interspecies differences in the transport of nTAA compounds across the basolateral membrane of the nephron.

Cis-inhibition of [3H]MPP+ (1 μM) uptake in OCT1-expressing oocytes by nTAA (50 μM) demonstrated a correlation between increasing alkyl chain length and increasing affinity for OCT1, regardless of the species. The finding that the nTAA did not alter current in water-injected oocytes (Fig. 4) suggests that the nTAA were not affecting MPP+ uptake via indirect effects on membrane potential; rather, these compounds were directly inhibiting MPP+ transport. This relationship was found in studies of organic cation transporters (Fig. 6).

Multiple sequence alignment of four OCT1 homologs from mouse (mOCT1), rat (rOCT1), rabbit (rbOCT1), and human (hOCT1). The alignment was generated with the PILEUP program in the Genetics Computer Group software package. Amino acids conserved in at least three of the four transporters are shaded; 109 amino acid differences are found between the human and rodent transporters. Of these, 68 residues are only found in hOCT1. These amino acid residue differences are distributed throughout the sequence and are not clustered in any obvious regions.
transport in isolated rabbit renal tissue preparations and previously for the cloned human transporter hOCT1 expressed in HeLa cells (Wright and Wunz, 1988; Zhang et al., 1999). This result does not explain the previous finding that the inhibitory potency of nTAAAs decreased with increasing alkyl chain length in rat kidney for nTAA compounds with side chains greater than ethyl (Ullrich et al., 1991). It is possible that another transporter, such as rOCT2, plays a greater role in the BLM transport of OCs in the rat kidney (Okuda et al., 1996). The inhibition potencies of the nTAA compounds with the OCT1 from mouse, rat, rabbit, and human differed considerably. In general, the nTAA compounds interacted less potently with the human transporter compared with its rodent and rabbit counterparts (Table 1).

The substrate selectivities of the transporters were investigated with both trans-stimulation studies and ligand-induced current measurements in voltage-clamped oocytes. The findings generally support the following conclusions: 1) The smallest nTAA, TMA, is transported by mOCT1 and rOCT1, but to a lesser extent by rbOCT1 and hOCT1. 2) TEA is transported well (is a substrate) by all four species homologs. 3) TPrA exhibits striking interspecies differences in its transport characteristics. That is, TPrA trans-stimulated hOCT1 [3H]MPP+ influx greatly and rbOCT1 weakly, whereas it potently trans-inhibited mOCT1 and rOCT1 [3H]MPP+ influx. In fact, of the nTAA compounds tested, TPrA was the most potent trans-inhibitor of the rodent transporters. Voltage-clamp studies showed that the rodent transporters were able to translocate TPrA, albeit at much slower rates than TMA and TEA. In contrast, hOCT1 and to a lesser extent rbOCT1 took up TPrA at comparatively faster rates relative to TEA. 4) hOCT1 and rbOCT1 transported TBA at a slightly slower rate than TPrA. Because saturating concentrations of TPrA and TBA were used in these experiments, the marked interspecies differences in transport are most likely a result of differences in the turnover rates.

The molecular mechanism responsible for the observed interspecies differences in OCT1 function is unknown. It is possible that the permeability pathway in the mouse and rat OCT1 has a smaller diameter and hence is more restrictive in the size of the substrates. Another explanation is that the rodent transporters have a more hydrophobic binding pocket, making subsequent debinding steps slower. Currently, very little is known about the OCT1 molecular structure involved in substrate binding and translocation. Sequence analysis suggests that OCT1 homologs share a very similar secondary structure consisting of 12 membrane-spanning helices. However, because hOCT1 is 80, 78, and 81% identical with the mouse, rat, and rabbit homologs, respectively, there have been numerous amino acid changes during the evolution of OCT1 (Fig. 6). Changes in tertiary structure of the permeation pathway due to amino acid residue(s) changes must account for the functional differences described in this study; 109 amino acid differences are found between the human and rodent OCT1 proteins. Of those, 68 are unique to hOCT1 (i.e., only found in hOCT1). Many of these residues (57%) have different chemical properties from corresponding residues of the other OCT1s (e.g., polar versus nonpolar), which could potentially have dramatic effects on function. Recently, it has been shown that even a single amino acid change in a transporter protein can lead to substantial changes in the transporter’s function (Wang and Giacomini, 1999). Chimera and mutation studies could provide insight into the amino acids involved in substrate binding and translocation of OCT1.

In summary, our results show that the human OCT1 is functionally distinct from other mammalian OCT1 homologs. In general, hOCT1 interacts with nTAA compounds with a lower affinity compared with the rodent and rabbit homologs. hOCT1 also is able to translocate larger nTAAAs at comparatively faster rates. Based on these results, it is reasonable to expect that kinetic and selectivity differences exist among the OCT1 species homologs in their interactions with drugs and toxins. Currently, assays to study OC and other xenobiotic transporters are being developed to screen for drug-drug interactions. These assays can potentially be used to estimate pharmacokinetic parameters based on in vitro data. The results reported in this study underscore the importance of using the human clones for preclinical drug evaluation.

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