Functional expression of a high affinity mammalian hepatic choline/organic cation transporter

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Abstract Uptake by the liver of the organic cation and essential nutrient choline is required for the hepatic synthesis of phosphatidylcholine. Uptake of other organic cations is also important for the metabolism and secretion of numerous endobiotics and drugs. Although a high affinity mammalian hepatic choline transporter has been genetically defined, it has not been previously identified. We have developed stable transfectants of BALB/3T3 cells, using a murine member of the organic cation transporter gene family (mOct1/Slc22a1), and used these cells to characterize the transport of the organic cation choline and model organic cation tetraethylammonium (TEA). Functional expression of mOct1/Slc22a1 in BALB/3T3 cells confers the saturable, temperature-dependent uptake of choline with a $K_m$ of 42 $\mu$M, and uptake of TEA with a $K_m$ of 43 $\mu$M. We subsequently used our cell culture uptake system to kinetically define in HepG2 cells a high affinity choline uptake process, which transports choline with a $K_m$ similar to that of mOct1/Slc22a1 protein. We also demonstrated that organic cation transport by mOct1/Slc22a1 is inhibited by several organic cations, and that the gene is expressed in the perinatal period, at a time when phosphatidylcholine synthesis increases.¶ We conclude that mOct1/Slc22a1 encodes a high affinity mammalian hepatic choline/organic cation transporter. This transporter may be important for hepatic phosphatidylcholine synthesis, and for the metabolism and secretion of many organic cationic drugs.—Sinclair, C. J., K. D. Chi, V. Subramanian, K. L. Ward, and R. M. Green. Functional expression of a high affinity mammalian hepatic choline/organic cation transporter. J. Lipid Res. 2000. 41: 1841–1848.

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The liver has a critical role in the synthesis and metabolism of phospholipids, as well as in the secretion of many endobiotics and xenobiotics into blood and bile. The organic cation choline is an essential nutrient and precursor for the hepatic synthesis of phospholipids (1). Hepatic choline levels are approximately 100-fold greater than blood concentrations, and de novo choline synthesis is minimal, suggesting the existence of a transport mecha-

nism for hepatic choline uptake (2). In addition, kinetic data from the isolated perfused rat liver and from hepatic plasma membrane uptake studies demonstrate the presence of a high affinity choline transporter. This transporter, however, has not yet been previously identified (3–5).

Many commonly used drugs are organic cationic amines, which are taken up into the liver prior to metabolism and secretion, and several hepatic plasma membrane organic cation transporters have been kinetically defined. Functional studies of rat liver plasma membrane vesicles have demonstrated the presence of basolateral membrane transporters for both choline and other organic cations (4–9). Although several members of the organic cation transporter gene family have been identified, only organic cation transporter 1 gene (OCT1) appears to be expressed in the liver (10–14). We have identified a murine liver cDNA (mOct1/Slc22a1) homologous to OCT1 and functionally expressed it in Xenopus oocytes (13). Although we were previously able to demonstrate the uptake of choline by oocytes injected with mOct1/Slc22a1 cRNA, the oocyte expression system lacked the sensitivity to detect the saturable, high affinity choline uptake previously kinetically defined in liver plasma membranes and the isolated perfused rat liver (3, 5, 13). Similarly, studies expressing human or rat OCT1 in Xenopus oocytes also could not demonstrate the high affinity transport of choline. We hypothesized that functional expression of mOct1/Slc22a1 in a mammalian culture system that lacks high affinity choline trans-

Abbreviations: ANOVA, analysis of variance; bLPM, basolateral liver plasma membrane; BSEP, bile salt export pump; CDP-choline, cytidine diphosphate-choline; DMEM, Dulbecco’s modified Eagle’s medium; NMN, N-methyl nicotinamide (1-NMN); N’MN, N’-methyl nicotinamide (6-NMN); Ntcp, Na+/taurocholate cotransport polypeptide; OCT1, organic cation transporter 1; PEMT, phosphatidylethanolamine N-methyltransferase; Spgp, sister of Pglycoprotein; TBS, Tris-buffered saline; TEA, tetraethylammonium; TMA, tetramethylammonium.

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port would provide a sensitive system to kinetically define choline transport by mOct1/Slc22a1.

In the present study, we have used BALB/3T3 cells, which lack significant high affinity choline transport, to develop clonal cell lines that are stably transfected with mOct1/Slc22a1. We have subsequently used these cells to functionally characterize the transport of both choline and the model organic cation tetraethylammonium bromide (TEA). These data indicate that the hepatic mOct1/Slc22a1 cDNA codes for a high affinity mammalian choline/organic cation transporter with kinetics similar to those detected in the liver. This transporter may be important for hepatic phospholipid synthesis and for the metabolism and secretion of organic cation drugs.

**EXPERIMENTAL PROCEDURES**

**Materials**

14C-labeled TEA (2.4 mCi/mmol) and 3H-labeled choline chloride (3H]choline, 2.8 TBq/mmol) were obtained from New England Nuclear (Boston, MA). Plasmid maxipreps were from Qiagen (Valencia, CA) and the molecular biology enzymes were purchased from Boehringer Mannheim (Mannheim, Germany). Vector pcDNA3 was from Invitrogen (Carlsbad, CA), Cellfectin, G418, and tissue culture media were purchased from Life Technologies (Grand Island, NY). All other chemicals and reagents were purchased from Sigma (St. Louis, MO). C57Bl/6j mice were purchased from Jackson Laboratories (Bar Harbor, ME) and choline-deficient and control Chow were obtained from ICN (Costa Mesa, CA).

**Construction of plasmids**

Mouse expressed sequence tag 581,126 (mOct1/Slc22a1) was purchased from Research Genetics (Huntsville, AL). The cDNA was digested with NotI and BamHI and ligated into the expression vector pcDNA3, which contains ampicillin and neomycin resistance cassettes. The correct orientation was verified by restriction digestion and sequencing. Competent Escherichia coli DH5α cells were then transformed with the pcDNA3-mOct1/Slc22a1 recombinant plasmid and selected with ampicillin resistance. The plasmid was purified with the Qiagen maxi kit and verified by sequencing.

**Cell culture and stable transfection**

Mammalian BALB/3T3 fibroblast cells [American Type Culture Collection, Manassas, VA] were cultured in 75-cm² flasks, containing Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum. The pcDNA3-mOct1/Slc22a1 recombinant plasmid or vector alone was lipofected with Cellfectin (GIBCO-BRL, Grand Island, NY), according to the instructions of the manufacturer. The medium was supplemented with G418 (400 µg/ml), allowing stable transfected clonal cell lines to be selected by G418 resistance. All cells were cultured in a humidified environment (37°C and 5% CO2). The cells were split with trypsin (1:5) and added to 35-mm² sterile plates with 3 ml of DMEM containing G418 at 400 µg/ml.

**Transport studies**

Eleven transfected clonal cell lines were initially assayed for TEA uptake, using 50 µM [14C]TEA for 15 min at 37 or 4°C. Untransfected cells and cells transfected with vector alone were used as negative controls and results were comparable in both groups. [14C]tetraethylammonium bromide and [3H]choline chloride uptake by mOct1/Slc22a1-transfected or control BALB/3T3 cells was performed at TEA concentrations ranging from 6.25 to 500 µM, and at choline chloride concentrations ranging from 6.25 to 200 µM, in uptake solution (Hanks’ balanced salt solution-10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid buffer with respective organic cation, pH 7.4). Each assay was performed with cells grown to 70–80% confluence in a 35-mm² dish, and all cells were initially washed with 3 ml of Tris-buffered saline (TBS, pH 7.4) prior to uptake. Uptake was performed at either 37 or 4°C over 15 min in 750 µl of uptake solution, a volume adequate to cover all cells on the dish. Uptake was terminated with 3 ml of TEA stop solution (TBS-1 mM TEA (pH 7.4) at 4°C) and cells were washed twice with 3 ml of stop solution. [3H]choline transport experiments were performed similarly, except that uptake was performed for 30 min and terminated with four washes of choline stop solution (TBS-1 mM choline chloride (pH 7.4) at 4°C). The cells were solubilized with 0.1 M NaOH for 5 min, followed by neutralization with 0.1 M HCl and 1 M Tris (pH 7.4). Radioactivity was measured by liquid scintillation counting, using 10 ml of Scinert (Fisher Scientific) scintillation cocktail. Proteins were measured in duplicate by the method of Bradford (Bio-Rad, Hercules, CA), with bovine serum albumin as a standard. Competition experiments were conducted with 50 µM radioligand and varying concentrations of nonradiolabeled thiamine, N'-methylnicotinamide (N'MN), N'-methylnicotinamide (NMN), tetramethylammonium (TMA), choline, and quinidine.

**Effect of pH gradients on initial uptake of [14C]TEA**

The cells were washed once with 3 ml of TBS, and uptake was performed in buffers with pH ranging from 6.3 to 8.3 for 7.5 min at 37 and 4°C. Cell viability after uptake was measured in parallel experiments under identical conditions, using trypan blue staining, and was >99%.

**Northern analysis**

Total mRNA was isolated by phenol-chloroform extraction and 15 µg of RNA was loaded per lane. Northern blotting was performed as previously described, using a full-length mOct1/Slc22a1 cDNA as a template for synthesis of a radiolabeled probe (15, 16). All blots were stripped and reprobed with ubiquitin to document the integrity of the RNA.

**Data analysis**

The data were analyzed by a paired Student’s t-test and analysis of variance (ANOVA). Differences were deemed to be statistically significant at P < 0.05. Saturation curve data were analyzed by a multiple regression-modeling program. Statistical analysis and computer modeling were performed with Sigma Plot and TableCurve 2D, (Jandel Scientific, San Rafael, CA) and Excel (Microsoft, Redmond, WA).

**RESULTS**

**Plasmid construction and cell transfection**

To develop cell lines functionally expressing mOct1/Slc22a1, the cDNA was ligated into vector pcDNA3. BALB/3T3 fibroblast cells were lipofected with the recombinant vector and stable transfectants were selected by G418 resistance. Eleven clonal cell lines transfected with mOct1/Slc22a1 were isolated and initially assayed for organic cation transport by measuring uptake of the model organic cation TEA (50 µM [14C]TEA) at 37 and 4°C. BALB/3T3 cells and stable transfectants expressing pcDNA3 vector
without insert were used as controls. As seen in Fig. 1, transfection of BALB/3T3 cells with mOct1/Slc22a1 resulted in uptake of radiolabeled TEA at 37°C in all 11 clonal cell lines. TEA uptake was significantly increased in the mOct1/Slc22a1-transfected cells, compared with controls (cell line 12) \( (P < 0.001) \). Uptake was temperature dependent and minimal at 4°C. In contrast, uptake of radiolabeled TEA by control BALB/3T3 cells was minimal at both 4 and 37°C. TEA uptake by mOct1/Slc22a1 was thus defined as the difference in TEA transport at 37°C between mOct1/Slc22a1-transfected and control BALB/3T3 cells, and was identical to uptake of mOct1/Slc22a1-transfected BALB/3T3 cells at 37°C minus transport at 4°C. To confirm the presence of the mOct1/Slc22a1 transcript in transfected cells expressing transport function, Northern blotting was performed with BALB/3T3 cells transfected with either mOct1/Slc22a1 or with vector alone. The 1.8-kb mOct1/Slc22a1 transcript was abundantly expressed in the mOct1/Slc22a1-transfected cells, whereas the transcript was not evident in controls (Fig. 2).

**Fig. 1.** Uptake of \([^{14}C]\)TEA by mOct1/Slc22a1-transfected and control BALB/3T3 cells. Uptake of 50 μM \([^{14}C]\)TEA was assayed over 15 min at 37°C (solid columns) and 4°C (open columns) in BALB/3T3 cells stably transfected with mOct1/Slc22a1 (cell lines 1–11, \( n = 2 \)) or with vector alone (cell line 12, \( n = 8 \)). TEA uptake was demonstrable in all mOct1/Slc22a1-transfected cell lines.

**Fig. 2.** Northern blot analysis of mOct1/Slc22a1-transfected and control-transfected BALB/3T3 cells. Northern blotting was performed on RNA (15 μg/lane) isolated from BALB/3T3 cells transfected with mOct1/Slc22a1 or with vector alone. The 1.8-kb transcript is abundantly expressed in cells transfected with mOct1/Slc22a1 (left lane), but is absent in the control cells transfected with vector alone (right lane).

**Fig. 3.** \([^{14}C]\)TEA uptake by mOct1/Slc22a1. (A) Uptake of 50 μM \([^{14}C]\)TEA by mOct1/Slc22a1-transfected BALB/3T3 cells was assayed over 60 min and is linear for at least 30 min at 37°C (solid circles) while it is minimal at 4°C (solid squares). (B) \([^{14}C]\)TEA uptake by mOct1/Slc22a1-transfected BALB/3T3 cells was assayed for 15 min over a range of substrate concentrations (6.25–500 μM). Uptake by mOct1/Slc22a1 was saturable with respect to concentration, with a \( K_m \) of 43 μM and a \( V_{max} \) of 1.03 pmol of TEA/mg protein per minute (Fig. 3B). The ability of mOct1/Slc22a1-transfected cells to transport TEA is consistent with results from expression studies performed in Xenopus oocytes (13).

We subsequently examined the effect of adding exogenous organic cations on TEA uptake in mOct1/Slc22a1-transfected cells at 37°C. Figure 4 demonstrates that TEA
uptake is inhibited by the organic cation 1-NMN, and there is a trend toward inhibition by the relatively inactive related 6-NMN (N9MN) compound at concentrations of 5–10 mM. TEA uptake by mOct1/Slc22a1 was also inhibited by the organic cations thiamine, TMA, and quinidine.

We also investigated the effects of proton gradients on organic cation uptake by mOct1/Slc22a1. mOct1/Slc22a1-transfected and untransfected cells were assayed for TEA uptake in external buffers ranging from pH 6.3 to 8.3 (Fig. 5). The presence of outside:inside directed proton gradients (external pH 6.3 and 6.5) results in significantly lower organic cation uptake, and inside:outside directed proton gradients (external pH 8.3) results in a slight trend toward increased uptake, compared with control buffer (pH 7.4). Using ANOVA, the effect of proton gradients on organic cation uptake was significant (P < 0.01).

Viability assessed by trypan blue exclusion was >99% in all buffers. Unfortunately, attempts to measure electrogenic uptake by using buffers of varying K+ concentrations resulted in diminished trypan blue exclusion, consistent with cellular cytotoxicity.

Previous data in the isolated perfused rat liver demonstrate the presence of saturable choline uptake, with a \( K_m \) in the range of physiological choline concentrations (in addition to a second, nonsaturable uptake process) (3). Thus, we investigated whether mOct1/Slc22a1 codes for a high affinity mammalian hepatic choline transporter. BALB/3T3 cells transfected with mOct1/Slc22a1 transported choline in a time- and temperature-dependent manner. Choline uptake was linear for 30 min at 37°C, and minimal at 4°C. Figure 6 is a saturation curve demonstrating the uptake of choline by mOct1/Slc22a1 over a range of choline concentrations. Choline uptake by mOct1/Slc22a1 is saturable, and when modeled to the Michaelis-Menten equation demonstrates a \( K_m \) of 42 \( \mu \)M and a \( V_{max} \) of 8.08 pmol/mg protein per minute.

To determine whether both choline and TEA uptake was mediated by mOct1/Slc22a1, we examined the effect of excess choline on \([^{14}C]\)TEA transport, and the effect of excess TEA on \([^3H]\)choline transport. Uptake by mOct1/Slc22a1 of 12.5 \( \mu \)M \([^3H]\)choline in the presence or absence of 10 mM nonradiolabeled TEA, and uptake of 25 \( \mu \)M \([^{14}C]\)TEA in the presence and absence of 1 mM nonradiolabeled choline were assayed. In both sets of experiments, uptake of the respective organic cations was inhibited by 70% (P < 0.001), consistent with uptake occurring via the same transporter.

Furthermore, we used our in vitro cell culture uptake system to measure choline uptake in HepG2 cells, and kinetically identified a high affinity choline uptake process in this liver-derived cell line. \([^3H]\)choline uptake by HepG2 cells was time and temperature dependent. Figure 7 demonstrates choline uptake by HepG2 cells over a range of concentrations.
concentrations (6.25–2,000 μM) at 37°C. Michaelis-Menten modeling revealed the presence of high affinity (K_m = 11 μM) and low affinity (K_m = 347 μM) choline transport processes (P < 0.01, using an F-statistic and comparing to a single transporter model). Data represent means ± SEM.

Finally, the hepatic phosphatidylcholine increase that occurs during the perinatal period is likely due to an increased synthesis of cytidine diphosphate-choline (CDP-choline) (17). This necessitates a quantitative increase in hepatic choline uptake during the perinatal period. Thus, we subsequently examined the ontogenic expression of mOct1/Slc22a1. Figure 8 demonstrates that mOct1/Slc22a1 gene expression in C57BL/6J mice is absent in the late embryonic and early neonatal period, but becomes abundantly expressed by 3 days after birth. These data are consistent with the increased requirement for hepatic choline uptake after birth. Experiments in which C57BL/6J mice were fed choline-deficient diets for 3 and 8 weeks, however, failed to demonstrate any changes in mOct1/Slc22a1 expression.

**DISCUSSION**

An essential function of the liver is the uptake, metabolism, and secretion of numerous xenobiotic and endobiotic substrates. The major synthetic pathway for phosphatidylcholine biosynthesis required by all mammalian cells is the CDP-choline (Kennedy) pathway (18). Although the liver also possesses an additional synthetic phosphatidylethanolamine N-methyltransferase (PEMT) pathway, the CDP-choline pathway remains quantitatively most important (18). The liver provides the phospholipids, which are secreted into blood and bile, and it has been estimated that the mouse secretes into bile every 24 h the equivalent of its entire hepatic pool of phosphatidylcholine (19). In fact, PEMT-deficient mice die of hepatic failure within 72 h of the administration of a choline-deficient diet (20). Therefore, the liver has an increased requirement for the uptake of the organic cation choline, which serves as an essential substrate for hepatocellular phosphatidylcholine synthesis (1, 18). Although several mammalian hepatic organic cation transporters have been kinetically characterized, the electrogenic organic cation transporter OCT1 remains the only hepatic member of this class of transporters that has been isolated or cloned (10–14). Other mammalian members of the organic cation gene family are expressed in either kidney or neuronal tissue, but are not present in adult liver (21–32). Previous studies in which OCT1 has been expressed in oocytes have demonstrated transport of choline, but have been unable to demonstrate that it functions as a high affinity choline transporter, with a K_m for choline in a physiologic concentration (10, 12, 13, 21, 22).

Our study demonstrates that uptake of both choline and TEA by mOct1/Slc22a1 occurs in a time-, temperature-, and concentration-dependent manner, all characteristic of a carrier-mediated transport process. The K_m of mOct1/Slc22a1 for TEA has a slightly higher affinity...
(lower $K_m$) than either the rOCT1 or hOCT1 transporter (10, 12). While the kinetic data for rOCT and hOCT1 were derived by expression in *Xenopus laevis* oocytes, rather than in mammalian expression systems, the $K_m$ for TEA that we noted in this study was also similar to that detected in *mOct1/Slc22a1* cRNA-injected *Xenopus* oocytes (13). Nonetheless, although TEA is a model organic cation that is well characterized in membrane transport studies, it is not a physiologically relevant substrate in vivo, and therefore cannot represent the natural ligand for mOct1/Slc22a1.

In contrast to TEA, choline is an essential organic cationic nutrient that is taken up from the portal circulation by the liver, and used as a precursor for phosphatidylcholine synthesis (1, 18). Our data demonstrate that mOct1/Slc22a1 transports choline into the cell in a time- and temperature-dependent manner. Plasma concentrations of choline in normal individuals, and in rodents and humans fed choline-containing compounds, range between 10 and 80 µM, although levels in the portal circulation after choline ingestion may be higher (3, 33–35). The $K_m$ of mOct1/Slc22a1 for choline transport is 42 µM, allowing this transporter to work efficiently under physiologic choline concentrations. Although choline transport by rOCT1 expressed in *Xenopus* oocytes has been reported, the $K_m$ of 1.1 mM (21) is far in excess of physiologic choline concentrations. We cannot exclude the possibility that both rOCT1 and hOCT1 are also physiologically important choline transporters, and the apparent high $K_m$ is due to expression in the *Xenopus* oocyte system.

All nucleated mammalian cells require choline uptake in order to synthesize phosphatidylcholine via the CDP-choline pathway (18, 20). Therefore, because expression of mouse, rat, and human *OCT1* appears limited to the liver, kidney, and gut, other choline transport mechanisms must exist (and are likely to be members of the organic cation transporter gene family). In fact, we demonstrate that *mOct1/Slc22a1* is not expressed in the liver in the late embryonic or early neonatal period, necessitating the existence of other hepatic choline transport processes. Hepatic phosphatidylcholine increases in the perinatal period, likely as a consequence of CTP:phosphocholine cytidylyltransferase translocation to the endoplasmic reticulum, and a resultant increased synthesis of CDP-choline (17). Therefore, there is an increased requirement for choline uptake by the liver in the perinatal period, and *mOct1/Slc22a1* expression in the early neonatal period may allow the hepatocyte an increased capacity for choline uptake. In addition, bile salts stimulate biliary phosphatidylcholine secretion, and expression of bile salt transporters [Na+/taurocholate cotransport polypeptide (Ntcp), sister of P-glycoprotein (Spgp)/bile salt export pump (BSEP)] does not occur prior to birth (36). Therefore, the ontogenic expression of *mOct1/Slc22a1* occurs at a time when increases in hepatic choline uptake are required. In addition, serum choline levels in newborn rodents are high, and decline as the rodent matures (37, 38). Therefore, lower affinity choline transport processes may be functional in the liver of the neonatal rodent, but high affinity transporters are likely required as the animal matures. In addition, experiments in which mice are fed choline-deficient diets for 3 and 8 weeks failed to demonstrate any changes in *mOct1/Slc22a1* gene expression, as well as several other enzymes/transporters involved in hepatocellular lipid metabolism/secretion (PEMT, CTP:phosphocholine cytidylyltransf erase, Spgp/BSEP, Ntcp). These data indicate that phospholipid metabolism and secretion in the mouse may differ from that in the rat (39).

Studies of the isolated perfused rat liver demonstrate the presence of a high affinity, saturable uptake process for choline, and a second, nonsaturable transport process that occurs at higher choline concentrations (3). We have used the liver-derived HepG2 cell line and our in vitro choline uptake assay system to detect both a high affinity transporter and a low affinity transport process for choline. Previous studies in liver and placental plasma membrane vesicles performed at high choline concentrations have also detected a low affinity choline transport process (4, 5, 40). Our data indicate that *mOct1/Slc22a1* codes for the high affinity hepatocellular choline transporter, but do not provide evidence that it codes for the low affinity transporter.

Organic cation uptake by *mOct1/Slc22a1* was inhibited by several organic cations including NMN, with a trend toward inhibition at high concentrations of the relatively inactive 6-NMN. Previous studies in *Xenopus* oocytes, using the latter compound, caused a paradoxical increase in TEA uptake (although the $K_m$ was increased), an effect that was likely due to changes in membrane potential (41). These studies indicate that *mOct1/Slc22a1* codes for a hepatic transporter that is inhibited by NMN, although there remain significant kinetic differences in choline and TEA uptake between *mOct1/Slc22a1* and the rat or human orthologs. In agreement with previous studies of TEA uptake in basolateral liver plasma membrane vesicles, the inhibitors thiamine, TMA, and quinidine all significantly inhibited TEA uptake (6–9). Finally, organic cation uptake by *mOct1/Slc22a1* is significantly inhibited by outside:inside directed proton gradients and may be stimulated by inside:outside gradients, albeit to a relatively small degree, potentially indicative of organic cation/H+ counterexchange. These data are in agreement with transporters that have been kinetically defined in basolateral liver plasma membrane (bLPM) uptake studies, but differ from the kinetics of rat OCT1 (9, 14). Finally, our data indicate that *mOct1/Slc22a1* transports both choline and the model organic cation TEA with high affinity. There is significant inhibition of [3H]choline uptake in the presence of TEA, and inhibition of [14C]TEA by choline, indicating that *mOct1/Slc22a1* is directly responsible for the uptake of both substrates.

When *mOct1/Slc22a1* was expressed in *Xenopus* oocytes, we were unable to detect high affinity choline transport (13). Therefore, in the present study we used a BALB/3T3 mammalian expression system, which provided data consistent with previous studies in the intact rat liver, isolated perfused rat liver, and bLPM vesicles kinetically defining hepatic choline and organic cation transporters (3–5).
mOct1/Slc22a1 is 95 and 80% identical to OCT1 and hOCT1, respectively. Because prior studies, which have demonstrated low affinity choline transport by these mOct1/Slc22a1 orthologs, have been performed in *Xenopus* oocytes, it is possible that they also serve as high affinity choline transporters, and the high $K_m$ (low affinity) for choline transport was due to expression in a *Xenopus* oocyte system (21). Although we cannot exclude the possibility that the low affinity choline transport process evident in the HepG2 cells was due to mOct1/Slc22a1, and was not evident in the mOct1/Slc22a1-transfected BALB/3T3 cells because they lack liver-specific posttranscriptional processing, it is more likely that mOct1/Slc22a1 represents only one of several choline transporters.

In conclusion, we have used BALB/3T3 cells to functionally express mOct1/Sl22a1 and determine that it encodes a high affinity hepatic choline transporter. Although high affinity hepatic choline transporters have been kinetically defined, these data are the first demonstration of a mammalian transporter for choline that functions in the physiological concentrations occurring in the portal circulation. We believe that mOct1/Slc22a1 is important for both phospholipid synthesis and homeostasis, as well as for regulating organic cation metabolism and secretion.

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