Common Drugs Inhibit Human Organic Cation Transporter 1 (OCT1)-Mediated Neurotransmitter Uptake

Kelli H. Boxberger, Bruno Hagenbuch, and Jed N. Lampe

Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center (K.H.B., B.H., J.N.L.), The University of Kansas Cancer Center (B.H.), and The University of Kansas Liver Center (B.H., J.N.L.), Kansas City, Kansas

Received September 20, 2013; accepted March 31, 2014

ABSTRACT

The human organic cation transporter 1 (OCT1) is a polycspecific transporter involved in the uptake of positively charged and neutral small molecules in the liver. To date, few endogenous compounds have been identified as OCT1 substrates; more importantly, the effect of drugs on endogenous substrate transport has not been examined. In this study, we established monooamine neurotransmitters as substrates for OCT1, specifically characterizing serotonin transport in human embryonic kidney 293 cells. Kinetic analysis yielded a $K_m$ of 197 micromolar and a $V_{max}$ of 561 pmol/mg protein/minute for serotonin. Furthermore, we demonstrated that serotonin uptake was inhibited by diphenhydramine, fluoxetine, imatinib, and verapamil, with IC$_{50}$ values in the low micromolar range. These results were recapitulated in primary human hepatocytes, suggesting that OCT1 plays a significant role in hepatic elimination of serotonin and that xenobiotics may alter the elimination of endogenous compounds as a result of interactions at the transporter level.

Introduction

It is widely accepted that the liver plays an essential role in removing drugs, toxins, and other xenobiotics from circulation in the human body. The liver is also involved in the clearance of several endogenous compounds, including circulate monooamine neurotransmitters (Chu et al., 1999; Eisenhofer et al., 2004). As early as 1967, it was demonstrated that the liver is capable of removing more than 70% of the serotonin in portal blood by filtration and metabolism (Thomas and Vane, 1967; Tyce, 1990). Several endogenous compounds, particularly the monoamines, are positively charged at physiologic pH and therefore require transport proteins to facilitate crossing the plasma membrane into hepatocytes. However, the high-affinity dopamine, norepinephrine, and serotonin (SERT) transporters are not expressed in the liver (Ramaamoorthy et al., 1993; Eisenhofer, 2001), leaving open to question the transporter(s) responsible for monooamine clearance in the human liver.

Organic cation transporters (OCTs), a subset of the solute carrier SLC22 superfamily of transporters, are polycspecific transporters that mediate the uptake of a wide variety of positively and neutrally charged compounds (Koepsell, 2013). Recently, broad substrate specificity combined with tissue localization, primarily in detoxifying organs, has coupled OCTs to the elimination of several drugs and toxins as well as endogenous compounds (Koepsell et al., 2007; Nies et al., 2011). Expressed primarily in the liver, OCT1 is involved in the hepatic elimination of numerous small molecules and has been linked to the transport of biogenic amines. Previously, the rat organic cation transporter, rOCT1, was shown to transport catecholamines (Busch et al., 1996; Breidert et al., 1998; Jonker and Schinkel, 2004). Additionally, human OCT1 has been associated with neurotransmitter transport; however, there is some controversy in the literature as to substrate specificity and transport efficiency (Kerb et al., 2002; Lips et al., 2005; Amphoux et al., 2006).

Moreover, the interference of drugs with endogenous neurotransmitter clearance, particularly at the transporter level, has not been investigated. Although it is becoming increasingly necessary to identify transporter-mediated drug-drug interactions in the modern age of polypharmacy, little is currently known about the effect of therapeutics on the transport and elimination of endogenous substrates. To elucidate the interactions of common medications and other xenobiotics with endogenous substrates of human OCT1, transport and inhibition of the biogenic amines dopamine, norepinephrine, and serotonin were characterized in both human embryonic kidney 293 (HEK293) cells and primary human hepatocytes.

Materials and Methods

Radiolabeled [3H]-1-methyl-4-phenylpyridinium iodide (MPP+, 85.0 Ci/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Radiolabeled [3H]-dihydroxyphenylethylamine (dopamine, 46.0 Ci/mmol), [3H]-norepinephrine hydrochloride (14.9 Ci/mmol), and [3H]-hydroxytryptamine creatinine sulfate (serotonin, 28.3 Ci/mmol) were purchased from Perkin Elmer (Boston, MA). Sodium chloride was purchased from Amresco (Solon, OH). HEPES sodium salt (NaCl) and potassium chloride (KCl) were purchased from Fisher Scientific (Fair Lawn, NJ). Imatinib (Gleevec) was purchased from Sigma-Aldrich (St. Louis, MO).

ABBREVIATIONS: MPP+, 1-methyl-4-phenylpyridinium; OCT, organic cation transporter; PMAT, plasma membrane monoamine transporter; rOCT, rat organic cation transporter; SERT, serotonin reuptake transporter.
Cell Culture. HEK293 cells were grown at 37°C under humidified 5% CO₂ in Dulbecco’s modified Eagle’s medium (ATCC, Manassas, VA) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA). Cells were seeded on 24-well plates coated with 0.1 mg/ml poly-n-lysin (Invitrogen) at a density of 175,000 cells/well. Twenty-four hours after plating, cells were transiently transfected with pcDNAs/FRT (“empty vector,” Invitrogen) or pcDNAs/FRT-hOCT1 (OCT1) using the FuGENE HD transfection reagent (Promega, Madison, WI). Transfection mixtures contained 0.25 μg plasmid cDNA, 0.75 μl FuGENE HD, and Opti-MEM 1+ GlutaMAX-I (Invitrogen) to a final volume of 25 μl/well. Transfected cells were incubated at 37°C as already described for 24 hours before use.

Freshly isolated human hepatocytes seeded on collagen-coated 24-well plates at a density of 350,000 cells/well were obtained from the KUMC Department of Pharmacology, Toxicology and Therapeutics Cell Isolation Core Laboratory. Hepatocytes were isolated from livers of male and female patients, aged 19–57.

Western Blotting. Cultured cells and hepatocytes were lysed in hypotonic homogenization solution (1 mM NaCl, 5 mM Tris-HCl, pH 7.5) with protease inhibitor cocktail (Roche, Indianapolis, IN) using a tissue homogenizer on ice. Lysates were subjected to centrifugation at 900 g for 10 minutes, after which supernatant was collected and subjected to further centrifugation at 10,000 g for 20 minutes. The resulting pellets containing protein-enriched plasma membrane were resuspended in hypotonic homogenization solution including protease inhibitor, and protein concentration was determined by BCA Protein Assay (Pierce, Rockford, IL). Proteins (0.5 μg for HEK lysates, 50 μg for hepatocyte lysates) were resolved by SDS-PAGE on 4–15% Mini-PROTEAN TGX polyacrylamide gels (Bio-Rad, Hercules, CA). Subsequently, proteins were transferred to nitrocellulose membrane. Immunoblotting was performed using standard procedures, with anti-OCT1 primary antibody (Novus Biologicals, Littleton, CO) at a concentration of 1:2000, and HRP-conjugated secondary antibody at 1:10,000. Proteins were detected with ECL substrate (Pierce).

Transport Assays. HEK293 uptake assays were performed 24 hours post-transfection at 37°C. Media were aspirated, and cells were washed three times with warm (37°C) uptake buffer (116 mM NaCl, 5.3 mM KC1, 1 mM Na₂HPO₄, 0.8 mM MgSO₄, 5.5 mM d-glucose, and 20 mM HEPES sodium salt, pH 7.4). After washing, cells were incubated with 200 μl of uptake buffer containing radiolabeled substrate and sufficient unlabeled substrate to reach specified substrate concentration, as well as putative inhibitors (drugs) where indicated, for the specified amount of time. Transport for all substrates was measured within the initial linear time range (at 30 seconds for MPP⁺ and at 5 minutes for all other substrates). Uptake was terminated by washing four times with ice-cold uptake buffer. To quantify uptake, cells were lysed with 300 μl/well 1% TX-100 in phosphate-buffered saline, of which 200 μl was transferred to 24-well scintillation plates (Perkin Elmer, Waltham, MA) and mixed with 750 μl of Optiphase Supermix scintillation cocktail (Perkin Elmer). Radioactivity was measured using a MicroBeta Trilux liquid scintillation counter (Perkin Elmer). The remaining cell lysate was used for protein determination for normalization by BCA Protein Assay with bovine serum albumin standards (Pierce). Transporter-specific uptake (net uptake) was determined by subtracting uptake into empty vector cells from that of OCT1-expressing cells (Fig. 1A). These monoamines was significantly higher in OCT1-expressing cells than in empty vector cells for all three neurotransmitters (Fig. 1A). OCT1-mediated transport (net uptake) was obtained by subtracting the uptake into empty vector cells from that of OCT1-expressing cells (Fig. 1B). At a time point of 5 minutes, serotonin appeared to be the most efficiently transported substrate. Dopamine uptake was approximately 50% that of serotonin, and norepinephrine uptake was approximately 20% compared with serotonin. The observed norepinephrine

Results

Functional Characterization of Human OCT1 in Transiently Transfected HEK293 Cells. To establish that our model of transient expression of OCT1 in HEK293 cells was functional, transport of the model cation [³H]-1-methyl-4-phenylpyridinium (MPP⁺) was characterized. Initial time dependencies at low (0.5 μM) and high (100 μM) concentrations demonstrated uptake of MPP⁺ to be linear through 1 minute, and kinetics experiments yielded a Kₘ of 35 ± 7 μM and a V_max of 500 ± 36 pmol/mg of protein/min (data not shown). This compares well with previously published values (Gründemann et al., 2003; Umehara et al., 2007) and suggested that our model of OCT1 transport was functional and suitable for further experiments.

OCT1-Mediated Neurotransmitter Transport. To study the effect of drugs on OCT1-mediated uptake of endogenous substrates, it was first necessary to identify suitable endogenous substrates. To establish monoamine neurotransmitters as substrates of OCT1, transport of 100 μM (0.8 μCi/ml) [³H]-dopamine, [³H]-norepinephrine, and [³H]-serotonin was measured in pcDNAs/FRT- (empty vector) and OCT1-transfected HEK293 cells at 37°C for 5 minutes. Uptake of these monoamines was significantly higher in OCT1-expressing cells than in empty vector cells for all three neurotransmitters (Fig. 1A). OCT1-mediated transport (net uptake) was obtained by subtracting the uptake into empty vector cells from that of OCT1-expressing cells (Fig. 1B). At a time point of 5 minutes, serotonin appeared to be the most efficiently transported substrate. Dopamine uptake was approximately 50% that of serotonin, and norepinephrine uptake was approximately 20% compared with serotonin. The observed norepinephrine
uptake was minimal and therefore unlikely to be physiologically relevant. Dopamine uptake by OCT1, although significant, is again unlikely to be germane to the liver because of the low circulating levels of this neurotransmitter. Serotonin, however, is found at high concentrations in the gut; consequently, portal blood levels are significantly higher than arterial blood, reportedly as much as 3-fold (Toh 1954; Gershon and Tack, 2007), making it the best candidate of the three neurotransmitters to be transported by OCT1 in vivo. Because of this, and the data suggesting that serotonin was the superior neurotransmitter substrate for OCT1, serotonin was selected as the model endogenous substrate for further study.

Kinetic Characterization of Serotonin Uptake by OCT1. To further characterize serotonin transport, uptake kinetics assessment were performed. Serotonin influx was assessed in empty vector- and OCT1-expressing cells after incubation with increasing concentrations of [3H]-serotonin, from 50 μM to 2 mM (0.8–2.0 μCi/ml), for 5 minutes at 37°C. Net uptake was fit to the Michaelis-Menten equation to yield a $K_m$ of $197 \pm 42 \mu M$ and $V_{max}$ of $561 \pm 36$ pmol/mg protein/min (Fig. 2). These data suggest that serotonin is transported by OCT1 with affinity and capacity comparable to other OCT1 substrates.

Inhibition of Serotonin Transport. Because OCT1 has moderate affinity for serotonin, it is feasible to hypothesize that OCT1-mediated uptake of serotonin could be inhibited by drugs or other xenobiotics. To evaluate this possibility, the following eight compounds were selected: acyclovir, cimetidine, diphenhydramine, fluoxetine, imatinib, metformin, tyramine, and verapamil. These potential inhibitors were selected based on previous reports that showed interactions with OCT1 or serotonin-transporting proteins (Sitte et al., 1998; Dresser et al., 2001; Nies et al., 2011). Uptake of 200 μM [3H]-serotonin, from 50 μM to 2 mM (0.8–2.0 μCi/ml), for 5 minutes at 37°C. Dimethylsulfoxide controls were included at concentrations equivalent to other OCT1 substrates.

Inhibition of serotonin transport by common drugs. Transport of 200 μM serotonin (1.2 μCi/ml) was measured in HEK293 cells transfected with empty vector or OCT1 plasmid cDNA in the presence of 10 μM or 100 μM drug for 5 minutes at 37°C. Dimethylsulfoxide controls were included at concentrations equivalent to those of imatinib preparations. Mean ± S.D. of net uptake is shown. *P < 0.05.

Discussion

This study demonstrates that serotonin is a substrate of human OCT1 and, more importantly, that OCT1-mediated serotonin transport can be inhibited by several commonly prescribed drugs. Taken together, these findings suggest that hepatic clearance of endogenous...
substrates, including biogenic amines, can be affected by small molecule therapeutics at the transporter level. Our results illustrate that diphenhydramine, fluoxetine, imatinib, and verapamil inhibit serotonin uptake in OCT1-expressing HEK293 cells and in primary human hepatocytes.

The liver has been established as a key organ in the elimination of endogenous compounds, including monoamine neurotransmitters, from the body. As previously mentioned, it is unlikely that OCT1 plays a major role in the uptake of dopamine or norepinephrine in the liver because of both low circulating concentrations and relatively low transport, as documented in Fig. 1. However, the same cannot be said for serotonin. Approximately 95% of the body’s serotonin is synthesized and stored in the gut, where it is released to initiate peristalsis and activate secretory reflexes (Gershon and Tack, 2007). While the SERT is expressed in the gut and functions in the reabsorption of released serotonin, a significant portion of serotonin reaches portal circulation; in fact, serotonin concentrations in portal blood can be as much as threefold higher than in arterial blood (Toh, 1954). Additionally, the liver is responsible for the removal of up to 70% of the serotonin from portal blood (Thomas and Vane, 1967; Tyce, 1990). Given that SERT is not expressed in the liver (Ramamoorthy et al., 1993), we hypothesized that OCT1 may be one transporter involved in serotonin uptake in the liver. Previous studies have investigated serotonin transport by OCT1, although with conflicting results. Kerb et al. (2002) demonstrated serotonin transport by human OCT1 as a test probe for comparison of wild-type and polymorphic transporters. Conversely, Amphoux et al. (2006) reported that human OCT1 showed very little specific transport of serotonin, among other neurotransmitters; they also failed to show OCT3-mediated transport of several monoamines known to be OCT3 substrates, and those that were transported yielded $K_m$ values much higher than established elsewhere (Duan and Wang, 2010), suggesting a potential flaw in the expression system or other methods used. In any case, we have demonstrated OCT1-mediated serotonin transport in both HEK293 cells and in hepatocytes, confirming that serotonin is indeed a substrate of OCT1, and our results indicate that OCT1 is an important element in the elimination of serotonin from portal blood.

Although this study suggests that OCT1 is a key component in hepatic elimination of serotonin, we cannot completely rule out minor contributions of other cation transporters that have also been reported to transport biogenic amines. Organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) are both high-capacity neurotransmitter transporters and together constitute the “uptake2” mechanism for monoamine clearance in the brain (Wu et al., 1998; Zhou et al., 2007). Studies have shown that, in addition to OCT1, both OCT3 and PMAT are expressed in the liver, though at very low levels. PMAT mRNA levels were nearly undetectable in the liver (Engel et al., 2004), suggesting its function pertains primarily to the brain, and hepatic OCT3 mRNA levels were shown to be between 6% and 30% that of OCT1 (Nies et al., 2009; Chen et al., 2010). Additionally, the affinities of PMAT and OCT3 for serotonin are relatively low (Duan and Wang, 2010) compared with that established in this study for OCT1 (Fig. 2). Combined, the low expression levels and relative transport affinities of OCT3 and PMAT suggest that they are likely minor components of serotonin uptake in hepatocytes, further solidifying the role of OCT1 in serotonin elimination in the liver.

Because hepatic clearance of serotonin may rely heavily on OCT1 transport, it is important to understand the effects that drug interaction with OCT1 has on serotonin uptake in the liver. The inhibition screen
comparison of the IC\textsubscript{50} values obtained in the present study with previously reported values obtained using other substrates might give some insight into how different substrates are handled by OCT1. Previously, diphenhydramine and fluoxetine inhibited MPP\textsuperscript{+} uptake with IC\textsubscript{50} values of 3.4 \textmu M and 2.8 \textmu M, respectively (Müller et al., 2005; Haenisch et al., 2012), and verapamil inhibited TEA\textsuperscript{+} transport with an IC\textsubscript{50} of 2.9 \textmu M (Zhang et al., 1998). These values are comparable to the IC\textsubscript{50} values we obtained for OCT1-mediated uptake of serotonin, which suggests that serotonin and the two model substrates MPP\textsuperscript{+} and TEA\textsuperscript{+} are transported in a similar way by OCT1. In contrast, previous reports demonstrated that imatinib inhibited OCT1-mediated metformin uptake with an IC\textsubscript{50} value of 1.5 \textmu M (Minematsu and Giacomini, 2011), whereas in our study imatinib inhibited serotonin uptake with an IC\textsubscript{50} value of 10.2 \mu M. This confirms the substrate-dependent inhibition seen with other organic cation transporters (Belzer et al., 2013; Martínez-Guerrero and Wright, 2013) and suggests that these transporters have complex binding pockets, with different interaction sites for different substrates. Regardless, these novel results are strong evidence that xenobiotics may inhibit serotonin uptake in the liver, potentially hindering proper hepatic clearance of serotonin in vivo, and it is plausible that these same effects would be seen with other endogenous substrates as well.

Undoubtedly, drug-mediated inhibition of serotonin transport would be dependent on drug concentrations achieved in vivo. Peak plasma drug concentrations have been shown to reach 66 ng/ml (0.3 \mu M) for diphenhydramine, 302 ng/ml (1 \mu M) for fluoxetine, 3380 ng/ml (6.8 \mu M) for imatinib, and 400 ng/ml (0.9 \mu M) for verapamil (Blyden et al., 1986; Peng et al., 2004; http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=d534867a-e4ef-46ce-b61d-857387ce450a#section-12.3; http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=8834bd7d-c7ae-41e0-9d54-017876885ae#nml34090-1). These plasma levels are not necessarily high enough to elicit significant OCT1 inhibition. However, in this case, because OCT1 is localized to the liver, portal blood drug concentrations are likely more relevant. Although drug concentrations have not been measured in portal blood, it is likely that portal drug concentrations are significantly higher than those measured in plasma, given that all of the drugs tested are dosed orally. This suggests that these drugs may indeed affect serotonin transport in the liver.

Inhibition of serotonin uptake in the liver may bear several implications, including a potential increase in circulating serotonin levels as well as locally increased extracellular serotonin concentrations in the liver. Increased serotonin levels in the circulation have the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established. Work completed by Pierre-Alain Clavien and others exposed a critical limitation of the rat liver perfusion model: the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established. Work completed by Pierre-Alain Clavien and others exposed a critical limitation of the rat liver perfusion model: the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established. Work completed by Pierre-Alain Clavien and others exposed a critical limitation of the rat liver perfusion model: the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established. Work completed by Pierre-Alain Clavien and others exposed a critical limitation of the rat liver perfusion model: the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established. Work completed by Pierre-Alain Clavien and others exposed a critical limitation of the rat liver perfusion model: the potential to lead to certain specific toxicities, including alterations in the blood coagulation cascade. When activated, platelets degranulate, releasing a variety of factors, including serotonin, to initiate coagulation (Troxlé et al., 2007). Recently, SSRIs have been shown to exert an anticoagulative effect as a result of decreases in serotonin levels in the blood (Bottlender et al., 1998). Conversely, drug-mediated inhibition of serotonin uptake in the liver could increase blood serotonin levels, potentially resulting in hypercoagulopathy. In addition, increases in circulating serotonin have the potential to cause acute changes in blood pressure due to its vasoactive properties (Rapport, 1949; Page and McCubbin, 1953). Furthermore, serotonin has been implicated in changes in renal blood flow (Blackshear et al., 1986), which might suggest a role for increased circulating serotonin levels in kidney dysfunction. Additionally, the importance of serotonin in both liver injury and regeneration has recently been established.

Fig. 5. Serotonin transport in primary human hepatocytes. (A) Between 20 and 24 hours after plating, freshly isolated primary human hepatocytes were incubated for 5 minutes with 1.2 \textmu Ci/ml (radiolabeled only, 42 nM) serotonin. Carrier-mediated transport was inhibited by the presence of 1 mM unlabeled serotonin (right). (B) OCT1 expression was confirmed in hepatocytes used to measure serotonin uptake. 50 \mu g of protein isolated from hepatocytes was resolved by SDS-PAGE and blotted with anti-OCT1 antibody; 0.5 \mu g of protein isolated from HEK293 cells transfected with empty vector (HEK-EV) and OCT1 (HEK-OCT1) was included as control. (C) Drug-mediated inhibition of serotonin transport was conducted as in (A) in the presence of 10 \mu M and 100 \mu M diphenhydramine, fluoxetine, imatinib, or verapamil. Dimethylsulfoxide controls were included at sufficient concentrations to match those in imatinib preparations. Net uptake is represented as the mean ± S.D. of three independent experiments.*\textit{P} < 0.05.
by OCT1 could potentiate the pathogenesis of nonalcoholic steatohepatitis. In contrast, increased serotonin levels in resorption patients could be beneficial for liver regeneration. In any case, drug-mediated inhibition of serotonin uptake by OCT1 may well have important physiologic consequences. In conclusion, we have established that serotonin is a viable substrate for human OCT1 and, more importantly, that commonly prescribed drugs inhibit its uptake. Diphenhydramine, fluoroxetine, imatinib, and verapamil significantly inhibited serotonin transport in both HEK293 cells and in primary human hepatocytes. Moreover, these compounds appear to be fairly potent inhibitors of serotonin uptake, as IC₅₀ values were determined to be in the low micromolar range for all four drugs. The implications of serotonin uptake inhibition in the liver may be several, and the results of this study bring new insights to the potential for drugs and other xenobiotics to interfere with endogenous substrate transport and elimination.

Acknowledgments
The authors thank Dr. Kathy Giacomini (University of California, San Francisco, San Francisco, CA) for the kind gift of the hOCT1 expression vector and Dr. Sylvie Kandel (Xenotech, LLC, Lenexa, KS) for helpful scientific discussions. The hepatocytes used in this study were derived from samples collected and provided by the KUMC Department of Pharmacology, Toxicology and Therapeutics Hepatocyte Core laboratory and the Kansas University Liver Center.

Authorship Contributions
Participated in research design: Boxberger, Hagenbuch, Lampe.
Conducted experiments: Boxberger.
Performed data analysis: Boxberger, Hagenbuch, Lampe.
Wrote or contributed to the writing of the manuscript: Boxberger, Hagenbuch, Lampe.

References