Research Article

The Poorly Membrane Permeable Antipsychotic Drugs Amisulpride and Sulpiride Are Substrates of the Organic Cation Transporters from the SLC22 Family

Joao N. Dos Santos Pereira,¹ Sina Tadjerpisheh,¹ Manar Abu Abed,¹ Ali R. Saadatmand,¹ Babette Weksler,² Ignacio A. Romero,³ Pierre-Olivier Couraud,^{4,5,6} Jürgen Brockmüller,¹ and Mladen V. Tzvetkov^{1,7}

Received 21 February 2014; accepted 18 July 2014; published online 26 August 2014

Abstract. Variations in influx transport at the blood-brain barrier might affect the concentration of psychotropic drugs at their site of action and as a consequence might alter therapy response. Furthermore, influx transporters in organs such as the gut, liver and kidney may influence absorption, distribution, and elimination. Here, we analyzed 30 commonly used psychotropic drugs using a parallel artificial membrane permeability assay. Amisulpride and sulpiride showed the lowest membrane permeability ($P_e < 1.5 \times 10^{-6}$ cm/s) and will require influx transport to penetrate the blood-brain barrier and other physiological barriers. We then studied the uptake of amisulpride and sulpiride by the organic cation transporters of the SLC22 family OCT1, OCT2, OCT3, OCTN1, and OCTN2. Amisulpride was found to be transported by all five transporters studied. In contrast, sulpiride was only transported by OCT1 and OCT2. OCT1 showed the highest transport ability both for amisulpride ($CL_{int} = 1.9$ ml/min/mg protein) and sulpiride ($CL_{int} = 4.2$ ml/min/mg protein) and polymorphisms in *OCT1* significantly reduced the uptake of both drugs. Furthermore, we observed carrier-mediated uptake that was inhibitable by known OCT inhibitors in the immortalized human brain microvascular endothelial cell line hCMEC/D3. In conclusion, this study demonstrates that amisulpride and sulpiride are substrates of organic cation transporters of the SLC22 family. SLC22 transporters may play an important role in the distribution of amisulpride and sulpiride, including their ability to penetrate the blood-brain barrier.

KEY WORDS: amisulpride; blood-brain barrier; membrane permeability; organic cation transporters; sulpiride.

INTRODUCTION

In order to reach their therapeutic target, psychotropic drugs have to cross the blood-brain barrier. The paracellular diffusion of compounds through the blood-brain barrier is restricted due to the presence of tight junctions between the endothelial cells that constitute the brain capillaries. The transcellular diffusion of compounds

is limited by insufficient membrane permeability or by the presence of efflux transporters. Some small molecules, for example nutrients, overcome this limitation by using influx transporters to enter the brain (1). Membrane influx transporters are known to be important for the distribution of drugs both in the brain and in other organs relevant for drug metabolism and elimination (2). However, the current knowledge about influx transport of psychotropic drugs is very limited.

Most psychotropic drugs have a relatively high $\text{LogD}_{7.4}$. Therefore, according to the Biopharmaceutics Drug Disposition Classification System (3), their cellular uptake should not depend on carrier-mediated transport. However, psychotropic drugs that have low membrane permeability will likely require a membrane transporter to enter the brain. Indeed, drugs with lower membrane permeability have a lower brain-to-plasma ratio of the unbound drug. Amisulpride and sulpiride have a brain-to-plasma ratio below 0.1, in contrast to highly lipophilic psychotropic drugs, which have a brain-to-plasma ratio close to 1 (4,5).

Psychotropic drugs are mainly weak organic bases. They are positively charged at the physiological pH of 7.4, and

Electronic supplementary material The online version of this article (doi:10.1208/s12248-014-9649-9) contains supplementary material, which is available to authorized users.

¹ Institute for Clinical Pharmacology, University Medical Center Göttingen, Georg-August University, Robert-Koch-Str. 40, 37075, Göttingen, Germany.

² Weill Cornell Medical College, New York, New York 10065, USA.

³ Department of Life Sciences, The Open University, Walton Hall, Milton Keynes, UK.

⁴ Inserm, U1016, Institut Cochin, Paris, France.

⁵ CNRS, UMR8104, Paris, France.

⁶ Université Paris Descartes, Sorbonne Paris Cité, Paris, France.

⁷ To whom correspondence should be addressed. (e-mail: mtzvetk@gwdg.de)

some of them have been reported to inhibit organic cation transporters of the SLC22 family (6,7). Therefore, organic cation transporters from this family may also mediate the penetration of psychotropic drugs through the blood-brain barrier.

The SLC22 family is a large group of polyspecific transporters that includes the five organic cation transporters: OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), OCTN1 (SLC22A5), and OCTN2 (SLC22A5) (2,8). OCT1 is strongly expressed in the sinusoidal membrane of human hepatocytes and is responsible for the hepatic uptake of several weak basic drugs (9–13). OCT2 is mainly expressed on the basolateral membrane of epithelial cells in the kidney proximal tubules, being responsible for the renal elimination of several drugs (14). OCT1 and OCT2 have been both proposed to be expressed at the blood-brain barrier (15). The anticonvulsant lamotrigine has been shown to be a substrate for OCT1 in immortalized human brain endothelial cells (16). Additionally, several psychotropic drugs have been shown to be inhibitors of OCT1 and OCT2 (6,7). OCT3, also known as extraneuronal monoamine transporter (EMT), is expressed ubiquitously and is known to transport monoamines as well as other organic cations (9,17,18). OCTN1 and OCTN2 are expressed in several tissues important for drug distribution, including the kidney (both OCTN1 and OCTN2). Importantly, OCTN2 is also expressed at the blood-brain barrier (17). OCTN1 and OCTN2 transport zwitterionic substrates like ergothioneine and L-carnitine, respectively, but are also able to transport organic cations (18–20).

In this study we used an artificial membrane assay (parallel artificial membrane permeability assay (PAMPA)) to identify psychotropic drugs with low membrane permeability. These drugs may require influx transporters to penetrate the blood-brain barrier. Therefore, we used transporter-overexpressing HEK293 cells to characterize in detail the ability of organic cation transporters from the SLC22 family to transport the poorly permeable psychotropic drugs amisulpride, sulpiride, tiapride, and sultopride. Finally, we studied the uptake of amisulpride and sulpiride in hCMC/D3 cells, an immortalized human brain microvascular endothelial cell line commonly used as a model for the blood-brain barrier.

MATERIALS AND METHODS

Materials

Radiolabeled ^3H -Sulpiride, with a specific activity of 80.6 Ci/mmol, was obtained from Hartmann Analytic (Braunschweig, Germany). Acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Sultopride and the internal standard sumatriptan-d6 were obtained from Santa Cruz Biotechnology (Heidelberg, Germany). All other non-radiolabeled chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany). PAMPA 96-well plates were obtained from BD Biosciences (Heidelberg, Germany). The 96-well Costar® UV plates were obtained from Corning (NY, USA). HEK293 T-REx™ cells, the Flp-In™ system, the pcDNA5.1 plasmid, the pCR-XL-TOPO plasmid, Hank's buffered salt solution (HBSS), media, and cell

culture additives were obtained from Life Technologies (Darmstadt, Germany). Cultrex® rat collagen I was obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany), and EndoGRO™-MV (SMCE-004) cell culture media was obtained from Merck. Restriction enzymes were obtained from Thermo Fischer Scientific (Waltham, MA, USA).

Parallel Artificial Membrane Permeability Assay

The PAMPA was performed using PAMPA 96-well plates (BD Biosciences) according to the manufacturer's instructions. Briefly, 300 μl of phosphate buffered saline (PBS, pH 7.4) solution with increasing drug concentrations was pipetted into the donor wells and 200 μl of PBS (pH 7.4) into the acceptor wells. Each concentration was measured in duplicate. After incubation at room temperature for 5 h, the drug concentrations from both the donor and acceptor wells were determined by UV absorption in a TECAN Ultra Microplate Reader (TECAN, Crailsheim, Germany). The following wavelengths were used: 230 nm for amitriptyline, citalopram, clomipramine, doxepine, fluoxetine, haloperidol, methylphenidate, nortriptyline, and paroxetine; 260 nm for desipramine, fluphenazine, imipramine, lamotrigine, melperon, milnacipram, perphenazine, and tranylcypromine; and 280 nm for amisulpride, clozapine, duloxetine, flupentixol, mirtazapine, olanzapine, paliperidone, perazine, promethazine, quetiapine, risperidone, sulpiride, sultopride, and tiapride.

Membrane permeability (P_e) was calculated as follows:

$$P_e = -\ln[1 - C_A / (1 - (0.3 \cdot C_D + 0.2 \cdot C_A) / 0.3 \cdot C_0)] / [A \times (1/V_D + 1/V_A) \times t]$$

where C_0 is the initial concentration in the donor well and C_D and C_A are the end concentrations in the donor and acceptor wells, respectively. A represents the area of each well (0.3 cm^2), V_D is the volume of the donor well (0.3 ml), V_A is the volume of the acceptor well (0.2 ml) and t is the time of incubation in seconds (18,000 s = 5 h). The assays were performed using at least two different concentrations for each drug and average P_e values were calculated.

HEK293 Cell Lines Overexpressing OCT1, OCT2, OCT3, OCTN1, and OCTN2

HEK293 cells overexpressing the human OCT1 gene and its variants have been generated from HEK293 T-REx™ cells using the Flp-In™ system as described in detail before (21). HEK293 cells overexpressing human OCT3, and the prcCMV::OCT2 plasmid, were kind gifts from Prof. Koepsell and Dr. Gorboulev (University of Würzburg, Germany). HEK293 cell lines overexpressing the human OCT2, OCTN1, and OCTN2 genes were generated using the Flp-In™ system following the procedure used for OCT1. Briefly, the OCT2 gene was cut from the prcCMV::OCT2 plasmid using *Bam*HI and *Not*I, and subsequently ligated into the pcDNA5.1 plasmid, which had been cut with the same enzymes. The OCTN1 and OCTN2 open reading frames were amplified from human kidney cDNA by PCR. The cDNA was synthesized from total kidney RNA as previously described

(11). The primers used for the amplification of OCTN1 were 5'-AAGTTTCGGATCCGCAAGTGGGAAGCATGC GGGACTA-3' and 5'-ATTTCAGCGGCCGCAACGAA TTTCTCCACAGG-3', and for the amplification of OCTN2 were 5'-GCTCTGTAAGCTTCTGAGGGCGGCATGCG GGACTA-3' and 5'-AGCTGGCGATATCTGGCAAGACA GTCTTTCC-3'. Artificial restriction sites for *Bam*HI, *Not*I, *Eco*RV, and *Hind*III were integrated into the primers (underlined in the primer sequences). The OCTN1 fragment was originally cloned into pCR-XL-TOPO and then re-cloned into the pcDNA5.1 plasmid using the *Bam*HI and *Not*I sites. The OCTN2 fragment was cut with *Eco*RV and *Hind*III and ligated into the pcDNA5.1 plasmid, which was previously cut with the same restriction enzymes. The obtained constructs were verified by sequencing of the whole reading frame. OCTN1 carried the major leucine₅₀₃ allele of the common Leu503Phe polymorphism. The OCT2, OCTN1, and OCTN2 constructs were chromosomally integrated into HEK293 T-REx™ cells using the Flp-In™ system, according to the manufacturer's instructions. Genomic integration was confirmed by PCR and sequencing, and transporter overexpression was validated by real-time reverse transcriptase-PCR. The activity of the HEK293 cell lines overexpressing OCT2, OCTN1, and OCTN2 was tested using the model substrates MPP⁺ and TEA⁺ (data available on request).

Uptake Measurements in SLC22-Overexpressing HEK293 Cells

To measure the uptake of sulpiride, 5×10^5 HEK293 cells were cultured in a single well of 12-well plates precoated with poly-D-lysine (1–4 kDa) for 3 days to reach complete confluence. All uptake measurements were performed at pH 7.4. The cells were washed with 1 ml 37°C HBSS. The reaction was started by adding 400 μ l 37°C HBSS containing sulpiride. The concentrations of sulpiride used varied between 5 and 500 μ M. A mixture of ³H-labeled and non-radiolabeled sulpiride in ratio of 1:4,000 was used. The reaction was stopped after exactly 2 min by adding 2 ml ice-cold HBSS. The uptake was performed for 2 min for all cell lines in order to compare the uptake among the cell lines expressing different transporters. The time was chosen in accordance with previous studies on the organic cation transporters of the SLC22 family (10,21–26). The cells were washed twice with 2 ml ice-cold HBSS and lysed with 500 μ l of a 0.1 N NaOH solution containing 0.1% SDS. Four hundred microliters of the cell lysates were supplemented with 9 ml liquid scintillator (AQUASAFE 500 Plus™ from Zinsser Analytics, Frankfurt-am-Main, Germany) and the accumulated substrate was quantified in a liquid scintillation counter (Beckman LS5000TD from Beckman Coulter, Krefeld, Germany). Counts per minute were normalized to the total protein amount, as determined using the bicinchoninic acid assay (BCA method, (27)).

To measure the uptake of amisulpride, Petri dishes with a diameter of 100 mm (BD Falcon, Heidelberg, Germany) were precoated with poly-D-lysine, 9×10^6 cells were plated and incubated for 48 h to reach confluence. Prior to the uptake measurements, cells were washed with 10 ml 37°C HBSS. The uptake was initiated by adding 5 ml 37°C HBSS supplemented with amisulpride. The concentrations of

amisulpride used varied between 5 and 200 μ M. After 2 min, the reaction was stopped by adding 20 ml ice-cold HBSS and the cells were washed twice with 20 ml ice-cold HBSS. The cells were transferred to a 2 ml tube and an aliquot was kept for protein quantification. The remaining cells were lysed in 1 ml of a mixture of 80% acetonitrile and 20% 30 mM potassium dihydrogen phosphate buffer (pH 6.5). The cell debris was pelleted by centrifugation (10 min at 16,000 g, 4°C). The supernatant was evaporated to dryness under a nitrogen stream at 40°C. The dry residue was reconstituted in 200 μ l of 30 mM dihydrogen phosphate buffer (pH 5.0) and used for the HPLC quantification as described below. Intracellularly accumulated amisulpride was quantified by HPLC and normalized to the total protein content of the sample determined by the BCA method.

To measure the uptake of sultopride and tiapride, 5×10^5 HEK293 cells were cultured in a single well of 12-well plates for 3 days to reach complete confluence. The cells were washed with 1 ml 37°C HBSS. The uptake was started by adding 400 μ l 37°C HBSS containing 5 μ M of sultopride or tiapride and stopped after exactly 2 min by adding 2 ml ice-cold HBSS. The cells were washed twice with 2 ml ice-cold HBSS and lysed with 500 μ l of a lysis solution (80% acetonitrile, 20% water). The cell lysate was centrifuged (16,000 g, 10 min) and the supernatant was diluted 1:10 in 0.1% formic acid. The intracellular amounts of sultopride and tiapride were quantified by LC-MS/MS.

Uptake Measurements in HCMEC/D3 Cells

The immortalized human brain endothelial cell line hCMEC/D3 (28,29) was propagated in EndoGRO™-MV medium on surfaces coated with rat collagen I. For uptake measurements, 10^6 HCMEC/D3 cells were plated in a single well of a 6-well plate and cultured for 2 days to reach complete confluence. The cells were washed with 2 ml 37°C HBSS.

To measure the uptake of sulpiride, the reaction was started by adding 1 ml 37°C HBSS containing 1, 5, or 25 μ M sulpiride. We used a mixture of ³H-labeled and non-radiolabeled sulpiride in a molar ratio of 1:400. The reaction was stopped after 2 min by adding 5 ml ice-cold HBSS. The cells were washed twice with 5 ml ice-cold HBSS and lysed with 1 ml of a 0.1 N NaOH solution containing 0.1% SDS. Eight hundred microliters of the cell lysates was mixed with 15 ml liquid scintillator (AQUASAFE 500 Plus™ from Zinsser Analytics). The amount of sulpiride was quantified in a liquid scintillation counter (Beckman LS5000TD from Beckman Coulter) and normalized to the total protein content of the sample.

To measure the uptake of amisulpride, the reaction was started by adding 1 ml 37°C HBSS containing 1, 5, or 25 μ M of amisulpride. The reaction was stopped after exactly 2 min by adding 5 ml ice-cold HBSS. The cells were washed twice with 5 ml ice-cold HBSS and lysed with 1 ml of a lysis solution (80% acetonitrile, 20% water) containing 10 ng/ml of sumatriptan-d6, which was used as internal standard. The cell lysate was centrifuged (16,000 g, 10 min) and the supernatant was diluted 1:10

in 0.1% formic acid and used for the quantification of amisulpride by LC-MS/MS.

The uptake measurements at 4°C were performed after preincubating the cells for 15 min on ice and the uptake reaction was carried out in ice-cold HBSS buffer.

HPLC Quantification of Amisulpride

Amisulpride was quantified as described by Kudris *et al.* with modifications (30). A LaChrom system (Merck Hitachi, Darmstadt, Germany) was used with an interface (D-7000, Merck Hitachi), a pump (L-7100, Merck Hitachi), an autosampler (L-7200, Merck Hitachi), a fluorescence detector (L-7400, Merck Hitachi), and a degasser (L-7614, Merck Hitachi). Separation was carried out at 40°C on a LiChrospher 100 RP-18e (5 µm, 4×12.50 mm) with a LiChrospher100 CN guard column (5 µm, both from Merck). Isocratic elution was performed with a mobile phase of 88% 30 mM potassium dihydrogen phosphate buffer (pH 6.5, adjusted with triethylamine) and 12% acetonitrile at a flow rate of 1.0 ml/min. Metoclopramide was used as internal standard. Fluorescence detection was performed using excitation and emission wavelengths of 274 and 370 nm, respectively. The peaks of amisulpride and metoclopramide were detected with retention times of 9 and 12 min, respectively, and were quantified using the peak areas. We used calibrator concentrations of 1, 5, 10, 25, and 50 ng/ml, and the limit of quantification of amisulpride was 1 ng/ml.

LC-MS/MS Quantification of Tiapride, Sultopride, and Amisulpride

The LC-MS/MS quantification of tiapride, sultopride, and amisulpride was performed based on previously described methods (31,32) with modifications. Briefly, 10 µl of the sample were injected into a chromatographic system comprised of a PerkinElmer/Sciex HPLC coupled with a API4000 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany). The separation was achieved in a Brownlee SPP RP-Amide column (4.6×100 mm, 2.7 µm particle size; Perkin Elmer, Waltham, MA, USA) with a Phenomenex SecurityGuard Standard precolumn (C18, ODS, 4 mm×2 mm, KJO-4282, Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 0.1% formic acid, 6.9% acetonitrile, and 1.1% methanol. Under these conditions, the retention times of tiapride, sultopride, and amisulpride were 6.6, 10.7, and 16.0 min, respectively. The mass transitions used for the quantification of tiapride, sultopride, and amisulpride were m/z 329.4>256.1, 355.5>227.3, and 370.2>242.1, respectively.

Prediction of Drug Chemical Properties, Data, and Statistical Analyses

LogD_{7.4} and pKa values of the drugs analyzed were estimated based on their chemical structures using ADMET Predictor 5.5 (Simulations Plus, Lancaster, CA, USA). The Michaelis-Menten constant (K_M) and maximal transport rates (V_{max}) was determined by a non-linear regression fitted to the

Michaelis-Menten equation using SigmaPlot 12 (Systat Software Inc., Erkrath, Germany).

The Student's *t* test was used for two independent group comparisons. ANOVA was used for multiple group comparisons followed by post hoc pair-wise comparisons using Tukey's HSD test. Spearman's correlation was used to analyze the correlation between the estimated LogD_{7.4} and measured membrane permeability (P_e) values. The statistical analyses were performed with IBM SPSS Statistics version 21.0 (IBM Corporation, Ehningen, Germany).

RESULTS

Membrane Permeability of Psychotropic Drugs

Most psychotropic drugs are weak bases and positively charged at physiological pH. However, a fraction of the drug exists in a non-charged form and can permeate the cell membrane. We have studied the membrane permeability of 30 psychotropic drugs at pH 7.4 using a PAMPA assay (Fig. 1) Amisulpride and sulpiride were the least membrane permeable of the drugs tested ($P_e=0.36\pm0.1\times10^{-6}$ cm/s and $P_e=1.19\pm0.3\times10^{-6}$ cm/s, respectively). In comparison, the most membrane permeable drug was doxepine, with P_e of $24.9\pm1.6\times10^{-6}$ cm/s. Only 26% of the variability in the drug membrane permeabilities could be explained by variation in the *in silico* predicted logD_{7.4} ($r^2=0.26$, $p=0.004$, Fig. 1).

Amisulpride and sulpiride are weak bases with pKa of 9.0 and 8.8, respectively (Fig. 2). At the physiological pH of 7.4, 98% of the amisulpride and 96% of the sulpiride molecules will be positively charged. The low membrane permeability and the weak basic chemical properties of amisulpride and sulpiride suggest that both drugs may require carrier-mediated influx transport to penetrate cell membranes and may be good substrates for the organic cation transporters from the SLC22 family.

Amisulpride and Sulpiride as Substrates of SLC22 Cation Transporters

We compared the ability of the organic cation transporters from the SLC22 family to mediate the cellular uptake of amisulpride and sulpiride. First, we incubated amisulpride at a single concentration of 5 µM with HEK293 cells overexpressing OCT1, OCT2, OCT3, OCTN1, or OCTN2 (Fig. 3a). The experiments were also performed in the presence of known OCT inhibitors. TBA⁺ was used to inhibit OCT1 and OCT2 uptake (9), irinotecan was used to inhibit OCT3 uptake (33), and L-carnitine was used to inhibit OCTN1 and OCTN2 uptake (34).

Amisulpride was transported by all the SLC22 transporters tested, and the inhibitors used were able to significantly inhibit amisulpride transport (Fig. 3a). The strongest transport was observed in cells overexpressing OCT1, followed by OCTN2 (7.1-fold and 5.9-fold increase compared to the control cells transfected with empty pcDNA5, respectively). In cells overexpressing OCTN1, OCT3, and OCT2, the increase was 3.2-fold, 2.9-fold, and 2.2-fold, respectively. The transport by OCT1 and OCT2 was completely inhibited by TBA⁺ ($p<0.01$, Student's *t* test, Fig. 3a), and the transport by OCT3 was completely inhibited by irinotecan ($p<0.05$,

Fig. 1. Comparison of the carrier-independent membrane permeabilities of commonly used psychotropic drugs. The membrane permeability (P_e) was measured using a PAMPA assay (BD Biosciences). Shown are means and standard error of the means of at least two independent experiments. In each experiment two different drug concentrations were measured and each measurement was performed in duplicate, resulting in eight or more data points per drug. *Upper left hand panel* shows the correlation between experimentally determined P_e and the predicted $\log D_{7.4}$ of the drugs. The $\log D_{7.4}$ values were estimated based on the chemical structures using ADMET Predictor version 5.5 (Simulations Plus, Lancaster, CA, USA)

Student's t test, Fig. 3a). The transport of amisulpride by OCTN1 and OCTN2 was inhibitable by L-carnitine ($p < 0.05$, Student's t test, Fig. 3a). None of the inhibitors used showed an influence on the uptake of amisulpride in the control cells.

Next, we determined the concentration dependence of the amisulpride uptake using concentrations ranging between 5 and 200 μM . In all the cells overexpressing a transporter, the concentration-dependent uptake of amisulpride was higher than in the control cells (Fig. 3b, c). In order to

measure only the transporter-mediated amisulpride uptake, the uptake in the control cells was subtracted from the uptake in the cells overexpressing a transporter (Fig. 3d, e).

The transporter-mediated amisulpride uptake showed typical Michaelis-Menten kinetics (Fig. 3d, e). OCT1 showed the highest affinity (K_M of $31.3 \pm 5.4 \mu\text{M}$; Table I), while the remaining transporters showed comparatively moderate affinities for the uptake of amisulpride ($K_M > 150 \mu\text{M}$). All transporters showed similarly low capacity for amisulpride

Fig. 2. Chemical structures of amisulpride and sulpiride. The pK_a values were estimated using ADMET Predictor version 5.5 (Simulations Plus, Lancaster, CA, USA)

Fig. 3. Transport of amisulpride by the organic cation transporters of the SLC22 family. **a** Cellular uptake of amisulpride at a single concentration of 5 μM in cells overexpressing OCT1, OCT2, OCT3, OCTN1, OCTN2, and the control cell line (transfected with the empty expression vector). The uptake was inhibited either by 1 mM TBA⁺ (OCT1 and OCT2), 250 μM irinotecan (OCT3), or 500 μM L-carnitine (OCTN1 and OCTN2). **b** and **c** Concentration dependence of amisulpride uptake by OCT1, OCT2, and OCT3 (**b**) and by OCTN1 and OCTN2 (**c**). The results are separated between panels **b** and **c** for clarity of presentation. **d** and **e** Transporter-mediated uptake of amisulpride. The transporter-mediated component of the total cellular uptake was estimated by subtracting the uptake measured in the control cell lines from the uptake in cell lines overexpressing the corresponding transporter. Shown are the means and standard error of the means of three independent experiments. * $p < 0.05$ and ** $p < 0.01$, as calculated by the Student's *t* test

uptake ($V_{\text{max}} < 200 \text{ pmol min}^{-1} \text{ mg protein}^{-1}$). Therefore, with the highest intrinsic clearance of $1.9 \text{ ml min}^{-1} \text{ mg protein}^{-1}$, OCT1 was the best transporter for amisulpride within the organic cation transporters from the SLC22 family.

Sulpiride uptake was increased by 2.5-fold in cells overexpressing OCT1, and 1.8-fold in cells overexpressing OCT2, compared to the control cells (Fig. 4a). The uptake increase was completely inhibited by TBA⁺ ($p < 0.01$,

Student's *t* test). No increase in sulpiride uptake in relation to the control was observed in cells overexpressing OCT3, OCTN1, and OCTN2.

A concentration-dependent increase in the uptake of sulpiride was observed in the OCT1 and OCT2 overexpressing cell lines, in comparison to the control cells (Fig. 4b). Similarly to amisulpride, the transport of sulpiride showed Michaelis-Menten kinetics (Fig. 4c). Both OCT1 and OCT2

Table I. Kinetics of Amisulpride and Sulpiride Transport by Organic Cation Transporters of the SLC22 Family

	K_M [μ M]	V_{max} [pmol min ⁻¹ mg _{protein} ⁻¹]	CL_{int} (V_{max}/K_M) [ml min ⁻¹ mg _{protein} ⁻¹]
Amisulpride			
OCT1	31.3±5.4	59.6±6.4	1.9
OCT2	167.9±32.1	99.2±18.8	0.6
OCT3	191.9±6.1	162.4±28.6	0.8
OCTN1	179.9±20.1	78.8±13.6	0.4
OCTN2	185.3±68.0	168.8±34.8	0.9
Sulpiride			
OCT1	259.7±5.4	1081.4±188	4.2
OCT2	187.2±21.6	439±4.7	2.3

showed low affinity ($K_M>150\text{ }\mu\text{M}$), but high capacity for the uptake of sulpiride ($V_{max}>400\text{ pmol min}^{-1}\text{ mg protein}^{-1}$; Table I). The calculated sulpiride intrinsic clearance by OCT1 and OCT2 were 4.3 and 2.4 ml min⁻¹ mg protein⁻¹, respectively. These values are higher than the values obtained for the transport of amisulpride by OCT1 and OCT2, suggesting higher capacity of OCT1 and OCT2 to transport sulpiride than amisulpride.

Effect of OCT1 Polymorphism on the Uptake of Amisulpride and Sulpiride by OCT1

The OCT1 gene SLC22A1 is highly polymorphic. Approximately 10% of Caucasians are homozygous carriers and 40% are heterozygous carriers of a loss-of-function OCT1 allele (12). Our results show that among the organic cation transporters of the SLC22 family, OCT1 is the

Fig. 4. Transport of sulpiride by the organic cation transporters of the SLC22 family. **a** Cellular uptake of sulpiride at a single concentration of 5 μM in cells overexpressing OCT1, OCT2, OCT3, OCTN1, OCTN2, and the control cell line (transfected with the empty expression vector). The uptake was inhibited either by 1 mM TBA⁺ (OCT1 and OCT2), 250 μM irinotecan (OCT3), or 500 μM L-carnitine (OCTN1 and OCTN2). **b** Concentration dependence of sulpiride uptake by OCT1 and OCT2. **c** Transporter-mediated uptake of sulpiride. The transporter-mediated component of the total cellular uptake was estimated by subtracting the uptake measured in the control cell lines from the uptake in cell lines overexpressing the corresponding transporter. Shown are the means and standard error of the means of three independent experiments. * $p < 0.05$ and ** $p < 0.01$, as calculated by the Student's t test

transporter with the highest intrinsic clearance for both amisulpride and sulpiride (Table 1). Therefore, it is important to study how genetic variants in the OCT1 gene affect amisulpride and sulpiride transport. We measured the uptake of amisulpride and sulpiride by OCT1 in cells overexpressing the known common functional variants of OCT1. The uptake of both drugs was significantly lower in cells expressing the loss-of-function OCT1 variants OCT1*2 to *6, in comparison to the wild-type OCT1*1 variant (post hoc Turkey-HSD test $p < 0.001$; Fig. 5). These results indicate that uptake of amisulpride and sulpiride, in organs where OCT1 plays a role in the distribution, may be reduced in individuals carrying loss-of-function OCT1 polymorphisms.

Uptake of Amisulpride and Sulpiride in the hCMEC/D3 Endothelial Cell Line

Next, we analyzed the uptake of amisulpride and sulpiride in brain microvascular endothelial cells. We used hCMEC/D3 cells, a well-characterized immortalized human brain microvascular endothelial cell line (29). We observed concentration- and temperature-dependent uptake of amisulpride and sulpiride (Fig. 6a, b). This indicates that a carrier-mediated uptake mechanism is present for both drugs.

Furthermore, the uptake of amisulpride and sulpiride was inhibitable by MPP⁺ (Fig. 6c, d), a model organic cation and competitive inhibitor of OCT1, OCT2, and OCT3 (8).

MPP⁺ inhibited 45% of the temperature-dependent fraction of the amisulpride uptake (the difference between the uptake at 37 and 4°C) and 71% of the temperature-dependent fraction of the sulpiride uptake. Verapamil inhibited the temperature-dependent uptake of sulpiride by 63%, but did not affect the uptake of amisulpride. These experiments strongly suggest that the uptake of amisulpride and sulpiride in hCMEC/D3 cells is a carrier-mediated process partially mediated by organic cation transporters of the SLC22 family.

Tiaprside and Sultopride are not Transported by the Cation Transporters of the SLC22 Family

Tiaprside and sultopride, two psychotropic drugs with high structural similarities to amisulpride and sulpiride, also showed low membrane permeability in the PAMPA assays ($P_e < 1.5 \times 10^{-6}$ cm/s, Fig. 1). We compared the ability of the organic cation transporters from the SLC22 family to mediate the cellular uptake of tiaprside and sultopride. In contrast to amisulpride and sulpiride, the uptake of tiaprside and sultopride was not increased in any of the HEK293 cells overexpressing the organic cation transporter and was not decreased by inhibitors specific for the different transporters tested (Fig. 7). Therefore, we concluded that tiaprside and sultopride are not substrates of the organic cation transporters of the SLC22 family.

Fig. 5. Loss-of-function OCT1 polymorphisms and their effect on the uptake of amisulpride and sulpiride. **a** The haplotype combinations of the five most frequent loss-of-function polymorphisms and their frequencies in Caucasians. The haplotype combinations were obtained from Tzvetkov *et al.* (11). **b** and **c** HEK293 cells overexpressing the wild-type OCT1*1 (black bars), the loss-of-function variants OCT1*2-*6 (gray bars) and the control cells transfected with the empty vector (white bars) were incubated for 2 min with 5 μ M of amisulpride (**b**) or sulpiride (**c**). Uptake is shown as a percentage of the uptake in OCT1*1. The means and standard error of the means of three independent experiments are shown. *** $p < 0.001$ for comparing the variant OCT1 alleles with the OCT1*1 allele (post hoc Turkey-HSD after significant ANOVA test for differences among the groups)

Fig. 6. Uptake of amisulpride and sulpiride in human brain microvascular endothelial cells. Uptake of amisulpride (**a**, **b**) and sulpiride (**c**, **d**) was measured in the immortalized human brain microvascular endothelial cell line hCMEC/D3. Panels **a** and **b** show the concentration-dependent uptake of amisulpride and sulpiride. Uptake of 1, 5, and 25 μM of amisulpride (**a**) and sulpiride (**b**) was measured at 37°C (closed circles) and 4°C (open circles) for 2 min. Panels **c** and **d** show the inhibition of the uptake of amisulpride and sulpiride. The uptake of 5 μM amisulpride (**c**) or 5 μM sulpiride (**d**) was inhibited with 1 mM of MPP⁺ or verapamil. Shown are the means and standard error of the means of two or more independent experiments. * $p < 0.05$ and *** $p < 0.001$ for comparison with the uptake without inhibitors at 37°C (post hoc Turkey-HSD after significant ANOVA test for differences among the groups)

DISCUSSION

We have identified amisulpride and sulpiride as drugs with low membrane permeability that will need carrier-mediated transport to enter cells and penetrate the blood-brain barrier. We demonstrated that amisulpride can be transported by OCT1, OCT2, OCT3, OCTN1, and OCTN2, while sulpiride can only be transported by OCT1 and OCT2. SLC22 transporters are expressed at the blood-brain barrier and at other physiological barriers important for drug absorption, distribution, metabolism, and elimination (8,9,35). Therefore, SLC22 cation transporters may be important for the distribution of amisulpride and sulpiride, including their ability to reach their targets in the brain.

Amisulpride and sulpiride are psychotropic drugs in the class of substituted benzamides that are used to treat schizophrenia (36–38). Both drugs are selective dopamine receptor D2 blockers and need to pass the blood-brain barrier in order to reach their site of action. Although amisulpride and sulpiride are low permeability drugs, previous to this study, it was not known how they penetrate the blood-brain barrier. Here, we clearly demonstrate that amisulpride is a

substrate of the organic cation transporters of the SLC22 family. To our best knowledge, this is the first study analyzing influx transport of amisulpride.

Sulpiride has been previously suggested as a substrate for PEPT1 (39). However, PEPT1 is not expressed at the blood-brain barrier (35,40). Here, we showed that sulpiride is a substrate of OCT1 and OCT2. Our data is in line with previously reported ability of sulpiride to inhibit OCT2 (7) and suggested involvement of OCTs in the uptake of sulpiride in Caco-2 cells (39).

Previous studies have reported mRNA expression of OCT1, OCT2, OCT3, OCTN1, and OCTN2 (15,16,19,35) and protein expression of OCT1, OCT2, and OCT3 (15,35) at the blood-brain barrier. Furthermore, OCT1 and OCT2 have been suggested to be able to take up lamotrigine, MPTP, and MPP⁺ in the brain (15,16). Taken together with the ability of OCTs to transport amisulpride and sulpiride reported here, it could be suggested that OCT1, OCT2, and other organic cation transporters from the same family may be substantially involved in the transport of amisulpride and sulpiride in the brain.

We also performed transport measurements in the human brain microvascular endothelial cell line hCMEC/D3,

Fig. 7. Transport of tiapride and sultopride by the organic cation transporters of the SLC22 family. Cellular uptake of tiapride (**a**) and sultopride (**b**) at a single concentration of 5 μ M in cells overexpressing OCT1, OCT2, OCT3, OCTN1, OCTN2, and the *control cell line* (transfected with the empty expression vector). The uptake was inhibited either by 1 mM TBA⁺ (OCT1 and OCT2), 250 μ M irinotecan (OCT3), or 500 μ M L-carnitine (OCTN1 and OCTN2)

a well-characterized model of the blood-brain barrier (28,29). We observed concentration- and temperature-dependent uptake for both amisulpride and sulpiride (Fig. 6), suggesting the presence of carrier-mediated uptake in human brain endothelial cells. Furthermore, the uptake of both amisulpride and sulpiride was inhibited by MPP⁺, a well-known inhibitor of OCT1, OCT2, and OCT3 (8). Taken together with the previously reported functional expression of OCT1 in the hCMEC/D3 cells (16), our data strongly supports the involvement of OCT1 or another organic cation transporter from the SLC22 family in the uptake of amisulpride and sulpiride through the blood-brain barrier.

In contrast to MPP⁺, verapamil, another known inhibitor of OCT1 and OCT2, did not change the rates of amisulpride uptake into hCMEC/D3 cells (Fig. 6c). This may be a result of simultaneous inhibition of the OCT-mediated influx and MDR1-mediated efflux by verapamil (41). MDR1 is known to transport amisulpride (42) and is functionally expressed in hCMEC/D3 cells (43–45). Therefore, verapamil may inhibit both the uptake and the efflux of amisulpride in hCMEC/D3 cells, resulting in a lack of a net change in the intracellular amisulpride levels (Fig. 6d). In contrast, sulpiride is not a substrate of MDR1 (46). Therefore, verapamil may only inhibit the influx of sulpiride and lead to reduced intracellular sulpiride in hCMEC/D3 cells, as we observed (Fig. 6d). These results indicate possible interplay between influx and efflux processes on uptake through the blood-brain barrier. However, one should account that the levels of expression of OCTs and MDR1 may vary between hCMEC/D3 cells and the HBMECs constituting the blood-brain barrier (20,43). Therefore, the net uptake observed in hCMEC/D3 cells cannot be directly extrapolated to the human blood-brain barrier.

The intrinsic clearance of amisulpride by OCT1 is 2.5-fold higher than the intrinsic clearance by OCTN2, suggesting a dominant role of OCT1 in transport through the blood-brain barrier (Table I). However, one should account for the higher expression of OCTN2 at the blood-brain barrier (35). Furthermore, OCT1 and OCTN2 may be localized on the different sides of the blood brain barrier. OCT1 and OCT2 have been previously shown to be localized at the luminal side

of the brain microvascular endothelial cells (15) and may mediate the uptake of both amisulpride and sulpiride from the blood into the endothelial cells. In bovine BMECs, OCTN2 is expressed at the basolateral membrane of the endothelium (47). OCTN2 can transport drugs in both directions across the plasma membrane (8). Therefore, OCT1 and OCT2 may both be involved in the uptake from the blood, and OCTN2 may be involved in the basolateral efflux transport of amisulpride from the intracellular space of the capillary endothelial cells into the brain.

The chemical structures of amisulpride and sulpiride are similar (Fig. 2). However, the small structural differences between them seem to account for distinct substrate specificity and transport kinetics (Table I). Specifically, our experiments show that amisulpride is a substrate of OCT1, OCT2, OCT3, OCTN1, and OCTN2, and sulpiride is a substrate of OCT1 and OCT2 only (Figs. 3 and 4 and Table I).

It is interesting to observe that although OCTs transport amisulpride and sulpiride, none of the OCTs transport tiapride or sultopride (Fig. 6). These results suggested that structural differences between these drugs may play a role in substrate recognition by the organic cation transporters of the SLC22 family. One such difference may be the amine moieties near the aromatic ring, which are present in sulpiride and amisulpride, but missing in tiapride and sultopride (Supplemental figure 1).

The maximal plasma concentrations after oral administration of 50 mg amisulpride or 100 mg sulpiride are 0.13 and 0.29 μ M, respectively (48,49). These values are much lower than the K_M for the uptake of amisulpride and sulpiride by the transporters studied (Table I), meaning that transport *in vivo* will not be saturated.

Besides the distribution to the brain, SLC22-mediated transport may allow amisulpride and sulpiride to cross several other pharmacologically relevant barriers. The renal clearances of amisulpride and sulpiride are 330 and 223 ml/min, respectively (48,49) indicating that tubular secretion plays a substantial role in their renal elimination. OCT2 is strongly expressed in proximal tubules (8), and it is probable that OCT2 is responsible for the significant tubular secretion of both drugs. Interestingly, in the prescribing information of amisulpride and sulpiride, there are several known drug-drug

interactions listed, including quinidine, verapamil, or imipramine. All these drugs are known inhibitors of OCT2 (9). OCT1 is proposed to be expressed on the apical membrane in the gut and, in addition to PEPT1, might play a role in sulpiride absorption (50). OCT1 is the major organic cation transporter in human hepatocytes (11,26). Amisulpride and sulpiride are not metabolized in the liver, but biliary excretion may account for up to 20% of their clearance (48,49). Hence, influx transport by SLC22 transporters in kidney and intestine (and potentially in liver) may be an important determinant of the plasma concentrations of both drugs.

OCT1 is highly genetic polymorphic. Five of the six common OCT1 alleles are known to lead to reduced and even absent activity in close to 9% of Caucasians. Here, we present *in vitro* data demonstrating a strong reduction of the OCT1-mediated uptake of amisulpride and sulpiride by the five most common OCT1 loss-of-function alleles (Fig. 5). This adds amisulpride and sulpiride to the relatively limited number of clinically relevant drugs shown to be substrates of OCT1 and whose pharmacokinetics may be affected by common OCT1 polymorphisms (10–13,51,52). The role of OCT1 in the transport of amisulpride and sulpiride through the blood-brain barrier and in other organs needs confirmation in *in vivo* models before concluding about the importance of OCT1 polymorphisms in the treatment of psychiatric patients with amisulpride or sulpiride.

An involvement of other influx transporters in the uptake of amisulpride and sulpiride into the brain cannot be excluded. Among the other members of the SLC22 family, SLC22A17 and SLC22A23 were reported to be expressed at the blood-brain barrier (35,53). However, the rat isoforms of both transporters are not able to transport typical organic cations (53) and data about the human isoforms is still missing. On the other hand, SLC22A16 (OCT6) is known to transport organic cations, but data on its expression at the blood-brain barrier is missing (18). Beyond the SLC22 family, PMAT, an organic cation transporter from the SLC29 family (SLC29A4), is also expressed in the brain and in immortalized rat BMEC cells (54) and may be involved in the uptake of amisulpride and sulpiride.

CONCLUSION

Our study shows that the psychotropic drugs amisulpride and sulpiride have only limited membrane permeability and require carrier-mediated influx transport to reach the brain. In addition, we could show carrier-mediated transport of amisulpride and sulpiride in an immortalized HBMEC cell line that was inhibitable by the OCT inhibitor MPP⁺. According to our *in vitro* data, amisulpride is a substrate of all organic cation transporters of the SLC22 family tested. Therefore, amisulpride may penetrate the blood-brain barrier *via* transport by OCT1, OCTN2, and possibly OCTN1, OCT2, and OCT3. In comparison, sulpiride may only use OCT1 and possibly OCT2 to penetrate the blood-brain barrier. OCT2 may also be important for the renal elimination of both drugs. Further work is required to clarify the clinical implications of these findings, with respect to therapy efficacy, genetic variation, and potential drug-drug interactions.

ACKNOWLEDGMENTS

We acknowledge Tobias Schmidt for support during the generation and characterization of the OCTN1 and OCTN2 overexpressing cell lines. This study was financially supported by the German Research Foundation (DFG) *via* the Clinical Research Group “Genotype-phenotype relationships and neurobiology of the longitudinal course of psychosis” in work package 3 (<http://www.kfo241.de>; grant number BR 2471/1-1), to JB and MVT.

REFERENCES

1. Strazielle N, Gherzi-Egea JF. Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules. *Mol Pharm*. 2013;10(5):1473–91.
2. Zamek-Gliszczyński MJ, Hoffmaster KA, Tweedie DJ, Giacomini KM, Hillgren KM. Highlights from the international transporter consortium second workshop. *Clin Pharmacol Ther*. 2012;92(5):553–6.
3. Benet L, Broccatelli F, Oprea T. BDDCS applied to over 900 drugs. *AAPS J*. 2011;13(4):519–47.
4. Culot M, Fabulas-da Costa A, Sevin E, Szorath E, Martinsson S, Renftel M, *et al*. A simple method for assessing free brain/free plasma ratios using an *in vitro* model of the blood brain barrier. *PLoS ONE*. 2013;8(12):e80634.
5. Dufour A, De Santi C. Pharmacokinetics and metabolism of amisulpride (In French). *Ann Psychiatr*. 1988;3:7.
6. Ahlin G, Karlsson J, Pedersen JM, Gustavsson L, Larsson R, Matsson P, *et al*. Structural requirements for drug inhibition of the liver specific human organic cation transport protein 1. *J Med Chem*. 2008;51(19):5932–42.
7. Kido Y, Matsson P, Giacomini KM. Profiling of a prescription drug library for potential renal drug-drug interactions mediated by the Organic Cation Transporter 2. *J Med Chem*. 2011;54(13):4548–58.
8. Koepsell H, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res*. 2007;24(7):1227–51.
9. Nies A, Koepsell H, Damme K, Schwab M. Organic cation transporters (OCTs, MATes), *in vitro* and *in vivo* evidence for the importance in drug therapy. In: Fromm MF, Kim RB, editors. *Drug transporters. Handbook of experimental pharmacology*. Berlin: 201: Springer; 2011. p. 105–67.
10. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, *et al*. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest*. 2007;117(5):1422–31.
11. Tzvetkov MV, dos Santos Pereira JN, Meineke I, Saadatmand AR, Stingl JC, Brockmüller J. Morphine is a substrate of the organic cation transporter OCT1 and polymorphisms in OCT1 gene affect morphine pharmacokinetics after codeine administration. *Biochemical Pharmacology*. 2013(0).
12. Tzvetkov MV, Saadatmand AR, Bokelmann K, Meineke I, Kaiser R, Brockmüller J. Effects of OCT1 polymorphisms on the cellular uptake, plasma concentrations and efficacy of the 5-HT₃ antagonists tropisetron and ondansetron. *Pharmacogenomics J*. 2012;12(1):22–9.
13. Tzvetkov MV, Saadatmand AR, Lotsch J, Tegeder I, Stingl JC, Brockmüller J. Genetically polymorphic OCT1: another piece in the puzzle of the variable pharmacokinetics and pharmacodynamics of the opioidergic drug tramadol. *Clin Pharmacol Ther*. 2011;90(1):143–50.
14. Morrissey KM, Stocker SL, Wittwer MB, Xu L, Giacomini KM. Renal transporters in drug development. *Annu Rev Pharmacol Toxicol*. 2013;53(1):503–29.
15. Lin C-J, Tai Y, Huang M-T, Tsai Y-F, Hsu H-J, Tzen K-Y, *et al*. Cellular localization of the organic cation transporters, OCT1 and OCT2, in brain microvessel endothelial cells and its

- implication for MPTP transport across the blood–brain barrier and MPTP-induced dopaminergic toxicity in rodents. *J Neurochem.* 2010;114(3):717–27.
16. Dickens D, Owen A, Alfirevic A, Giannoudis A, Davies A, Weksler B, *et al.* Lamotrigine is a substrate for OCT1 in brain endothelial cells. *Biochem Pharmacol.* 2012;83(6):805–14.
 17. Grundemann D, Schechinger B, Rappold G, Schomig E. Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat Neurosci.* 1998;1(5):349–51.
 18. Koepsell H. The SLC22 family with transporters of organic cations, anions and zwitterions. *Mol Asp Med.* 2013;34(2–3):413–35.
 19. Kido Y, Tamai I, Ohnari A, Sai Y, Kagami T, Nezu J-i, *et al.* Functional relevance of carnitine transporter OCTN2 to brain distribution of L-carnitine and acetyl-L-carnitine across the blood–brain barrier. *J Neurochem.* 2001;79(5):959–69.
 20. Okura T, Kato S, Deguchi Y. Functional expression of organic cation/carnitine transporter 2 (OCTN2/SLC22A5) in human brain capillary endothelial cell line hCMEC/D3, a human blood–brain barrier model. *Drug Metab Pharmacokinet.* 2014;29(1):5.
 21. Saadatmand AR, Tadjerpisheh S, Brockmöller J, Tzvetkov MV. The prototypic pharmacogenetic drug debrisoquine is a substrate of the genetically polymorphic organic cation transporter OCT1. *Biochem Pharmacol.* 2012;83(10):1427–34.
 22. Wu X, Huang W, Prasad PD, Seth P, Rajan DP, Leibach FH, *et al.* Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. *J Pharmacol Exp Ther.* 1999;290(3):1482–92.
 23. Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, *et al.* Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol.* 1997;16(7):10.
 24. Wu X, Kekuda R, Huang W, Fei Y-J, Leibach FH, Chen J, *et al.* Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake2) and evidence for the expression of the transporter in the brain. *J Biol Chem.* 1998;273(49):32776–86.
 25. Gründemann D, Harfinger S, Golz S, Geerts A, Lazar A, Berkels R, *et al.* Discovery of the ergothioneine transporter. *Proc Natl Acad Sci U S A.* 2005;102(14):5256–61.
 26. Nies AT, Koepsell H, Winter S, Burk O, Klein K, Kerb R, *et al.* Expression of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) is affected by genetic factors and cholestasis in human liver. *Hepatology.* 2009;50(4):1227–40.
 27. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, *et al.* Measurement of protein using bicinchoninic acid. *Anal Biochem.* 1985;150(1):76–85.
 28. Weksler B, Romero I, Couraud P-O. The hCMEC/D3 cell line as a model of the human blood brain barrier. *Fluids Barriers CNS.* 2013;10(1):16.
 29. Weksler BB, Subileau EA, Perrière N, Charneau P, Holloway K, Leveque M, *et al.* Blood–brain barrier-specific properties of a human adult brain endothelial cell line. *FASEB J.* 2005;19(13):1872–4.
 30. Kudris I, Skakun N, Orlova I, Libina V, Kulikov A. Analysis of amisulpride in human plasma by SPE and LC with fluorescence detection. *Chromatographia.* 2011;73(1–2):67–74.
 31. Fisher DS, Partridge SJ, Handley SA, Couchman L, Morgan PE, Flanagan RJ. LC–MS/MS of some atypical antipsychotics in human plasma, serum, oral fluid and haemolysed whole blood. *Forensic Sci Int.* 2013;229(1–3):145–50.
 32. Moon Y, Paek B, Kim H-H, Ji H, Lee H, Park H-G, *et al.* Determination of tiapride in human plasma using hydrophilic interaction liquid chromatography–tandem mass spectrometry. *Arch Pharm Res.* 2004;27(9):901–5.
 33. Shnitsar V, Eckardt R, Gupta S, Grottker J, Müller GA, Koepsell H, *et al.* Expression of human organic cation transporter 3 in kidney carcinoma cell lines increases chemosensitivity to melphalan, irinotecan, and vincristine. *Cancer Res.* 2009;69(4):1494–501.
 34. Stocker SL, Emami Riedmaier A, Schwab M, Giacomini KM. OCT (SLC22A) and OCTN family. *Pharmacogenomics of Human Drug Transporters: John Wiley & Sons, Inc.;* 2013. p. 171–208.
 35. Geier EG, Chen EC, Webb A, Papp AC, Yee SW, Sadee W, *et al.* Profiling solute carrier transporters in the human blood–brain barrier. *Clin Pharmacol Ther.* 2013;94(6):636–9.
 36. Pani L, Gessa GL. The substituted benzamides and their clinical potential on dysthymia and on the negative symptoms of schizophrenia. *Mol Psychiatry.* 2002;7(3):247–53.
 37. Rama Rao VA, Bailey J, Bishop M, Coppen A. A clinical and pharmacodynamic evaluation of sulpiride. *Psychopharmacology.* 1981;73(1):77–80.
 38. Komossa K, Rummel-Kluge C, Hunger H, Schmid F, Schwarz S, Silveira da Mota Neto Joaquim I, *et al.* Amisulpride versus other atypical antipsychotics for schizophrenia. *Cochrane Database of Systematic Reviews* [Internet]. 2010; (1). Available from: <http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD006624.pub2/abstract>.
 39. Watanabe K, Sawano T, Endo T, Sakata M, Sato J. Studies on intestinal absorption of sulpiride (2): transepithelial transport of sulpiride across the human intestinal cell line caco-2. *Biol Pharm Bull.* 2002;25(10):1345–50.
 40. Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, *et al.* Quantitative targeted absolute proteomics of human blood–brain barrier transporters and receptors. *J Neurochem.* 2011;117(2):333–45.
 41. Pauli-Magnus C, von Richter O, Burk O, Ziegler A, Mettang T, Eichelbaum M, *et al.* Characterization of the major metabolites of verapamil as substrates and inhibitors of P-glycoprotein. *J Pharmacol Exp Ther.* 2000;293(2):376–82.
 42. Schmitt U, Abou El-Ela A, Guo LJ, Glavinas H, Krajcsi P, Baron JM, *et al.* Cyclosporine A (CsA) affects the pharmacodynamics and pharmacokinetics of the atypical antipsychotic amisulpride probably via inhibition of P-glycoprotein (P-gp). *J Neural Transm.* 2006;113(7):787–801.
 43. Dauchy S, Miller F, Couraud P-O, Weaver RJ, Weksler B, Romero I-A, *et al.* Expression and transcriptional regulation of ABC transporters and cytochromes P450 in hCMEC/D3 human cerebral microvascular endothelial cells. *Biochem Pharmacol.* 2009;77(5):897–909.
 44. Poller B, Gutmann H, Krähenbühl S, Weksler B, Romero I, Couraud P-O, *et al.* The human brain endothelial cell line hCMEC/D3 as a human blood–brain barrier model for drug transport studies. *J Neurochem.* 2008;107(5):1358–68.
 45. Ohtsuki S, Ikeda C, Uchida Y, Sakamoto Y, Miller F, Glacial F, *et al.* Quantitative targeted absolute proteomic analysis of transporters, receptors and junction proteins for validation of human cerebral microvascular endothelial cell line hCMEC/D3 as a human blood–brain barrier model. *Mol Pharm.* 2012;10(1):289–96.
 46. Feng B, Mills JB, Davidson RE, Mireles RJ, Janiszewski JS, Troutman MD, *et al.* In vitro P-glycoprotein assays to predict the in vivo interactions of P-glycoprotein with drugs in the central nervous system. *Drug Metab Dispos.* 2008;36(2):268–75.
 47. Miecz D, Januszewicz E, Czeredys M, Hinton BT, Berezowski V, Cecchelli R, *et al.* Localization of organic cation/carnitine transporter (OCTN2) in cells forming the blood–brain barrier. *J Neurochem.* 2008;104(1):113–23.
 48. Wiesel FA, Alfredsson G, Ehrnebo M, Sedvall G. The pharmacokinetics of intravenous and oral sulpiride in healthy human subjects. *Eur J Clin Pharmacol.* 1980;17(5):385–91.
 49. Rosenzweig P, Canal M, Patat A, Bergougnan L, Zieleniuk I, Bianchetti G. A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. *Hum Psychopharmacol Clin Exp.* 2002;17(1):1–13.
 50. Han T, Everett RS, Proctor WR, Ng CM, Costales CL, Brouwer KLR, *et al.* Organic cation transporter 1 (OCT1/mOct1) is localized in the apical membrane of caco-2 cell monolayers and enterocytes. *Mol Pharmacol.* 2013;84(2):182–9.
 51. Swift B, Nebot N, Lee JK, Han T, Proctor WR, Thakker DR, *et al.* Sorafenib Hepatobiliary disposition: mechanisms of hepatic uptake and disposition of generated metabolites. *Drug Metab Dispos.* 2013;41(6):1179–86.
 52. Jung N, Lehmann C, Rubbert A, Knispel M, Hartmann P, van Lunzen J, *et al.* Relevance of the organic cation transporters 1 and 2 for antiretroviral drug therapy in human immunodeficiency virus infection. *Drug Metab Dispos.* 2008;36(8):1616–23.
 53. Bennett K, Liu J, Hoelting C, Stoll J. Expression and analysis of two novel rat organic cation transporter homologs, SLC22A17 and SLC22A23. *Mol Cell Biochem.* 2011;352(1–2):143–54.
 54. Okura T, Kato S, Takano Y, Sato T, Yamashita A, Morimoto R, *et al.* Functional characterization of rat plasma membrane monoamine transporter in the blood–brain and blood–cerebrospinal fluid barriers. *J Pharm Sci.* 2011;100(9):3924–38.