Human Neurons Express the Polyspecific Cation Transporter hOCT2, Which Translocates Monoamine Neurotransmitters, Amantadine, and Memantine

ANDREAS E. BUSCH, ULRICH KARBACH, DAGMAR MISKA, VALENTIN GORBOULEV, AIDA AKHOUNDOVA, CHRISTOPHER VOLK, PETRA ARNDT, JOCHEN C. ULZHEIMER, MARK S. SONDERS, CARMEN BAUMANN, SIEGFRIED WALDEGGER, FLORIAN LANG, and HERMANN KOEPSELL


Received October 6, 1997; Accepted May 8, 1998 This paper is available online at http://www.molpharm.org

ABSTRACT

Recently, we cloned the human cation transporter hOCT2, a member of a new family of polyspecific transporters from kidney, and demonstrated electric uptake of tetraethylammonium, choline, N\textsubscript{1}-methylnicotinamide, and 1-methyl-4-phenylpyridinium. Using polymerase chain reaction amplification, cDNA sequencing, in situ hybridization, and immunohistochemistry, we now show that hOCT2 message and protein are expressed in neurons of the cerebral cortex and in various subcortical nuclei. In Xenopus laevis oocytes expressing hOCT2, electrogenic transport of norepinephrine, histamine, dopamine, serotonin, and the antiparkinsonian drugs memantine and amantadine was demonstrated by tracer influx, tracer efflux, electrical measurements, or a combination. Apparent \( K_m \) values of 1.9 \( \pm 0.6 \) \( \mu \)M (norepinephrine), 1.3 \( \pm 0.3 \) \( \mu \)M (histamine), 0.39 \( \pm 0.16 \) \( \mu \)M (dopamine), 80 \( \pm 20 \) \( \mu \)M (serotonin), 34 \( \pm 5 \) \( \mu \)M (memantine), and 27 \( \pm 3 \) \( \mu \)M (amantadine) were estimated. Measurement of trans-effects in depolarized oocytes and human embryonic kidney cells expressing hOCT2 suggests that there were different rates and specificities for cation influx and efflux. The hypothesis is raised that hOCT2 plays a physiological role in the central nervous system by regulating interstitial concentrations of monoamine neurotransmitters that have evaded high affinity uptake mechanisms. We show that amantadine does not interact with the expressed human Na\textsuperscript{+}/Cl\textsuperscript{−} dopamine cotransporter. However, concentrations of amantadine that are effective for the treatment of Parkinson’s disease may increase the interstitial concentrations of dopamine and other aminergic neurotransmitters by competitive inhibition of hOCT2.

In 1994, we cloned from rat kidney the cation transporter rOCT1 and demonstrated that it translocates small organic cations with diverse structures, including choline and some monoamine neurotransmitters, into epithelial cells in an electrogenic fashion (Gründemann et al., 1994; Busch et al., 1996a, 1996b; Koepsell, 1998). In distinction from other cloned Na\textsuperscript{+}/Cl\textsuperscript{−}-dependent transporters for norepinephrine, dopamine, and serotonin (Rudnick and Clark, 1993), the OCT-mediated transport of monoamines and other cations is independent of a transmembrane Na\textsuperscript{+} gradient. rOCT1 was the first member of a rapidly growing transporter family that contains highly homologous electrogenic cation transporters (Busch et al., 1996b; Okuda et al., 1996; Schweifer and Barlow, 1996; Gorboulev et al., 1997; Gründemann et al., 1997; Zhang et al., 1997a, 1997b; Terashita et al., 1998) and the polyspecific organic anion transporter OAT1 (Sekine et al., 1997). The OCT family belongs to a superfamily that includes multidrug resistance proteins, facilitative diffusion systems, and proton symporters (Marger and Saier, 1993). Recently, the highly homologous OCT subtype rOCT2 was isolated from rat kidney (Busch et al., 1996b; Okuda et al., 1996), and homologous transporters were identified from pig (pOCT2; Gründemann et al., 1997) and human (hOCT2; Gorboulev et al., 1997). Significant species differences in tissue distribution and function of the OCT1 and OCT2 transporters were observed. For example, recent data suggest that in different species, OCT2 transporters may be localized in luminal or basal membranes of renal epithelial cells (Gorboulev et al., 1997; Karbach U and Koepsell H, unpublished observations). Using PCRs, mRNAs with homology to rOCT2 and hOCT2 were detected in brain of rat and human (Gorboulev et al., 1997, Gründemann et al., 1997). Because Na\textsuperscript{+}-independent

ABBREVIATIONS: PCR, polymerase chain reaction; PBS, phosphate-buffered saline; SSC, standard saline citrate; MPP, 1-methyl-4-phenylpyridinium; hDAT, human Na\textsuperscript{+}/Cl\textsuperscript{−} dopamine cotransporter; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HEK, human embryonic kidney; TEA, tetraethylammonium; MES, 3-(N-morpholino)propanesulfonic acid.
transport of choline, thiamine, or norepinephrine has been detected in brain and glial cells (Yamamura and Snyder, 1973; Paterson and Hertz, 1989; Streich et al., 1996), we hypothesized that the cation transporters of the OCT family could serve these functions. In the current study, we show that the polyspecific organic cation transporter hOCT2 is transcribed in neurons of human brain and transports the monoamine neurotransmitters dopamine, norepinephrine, serotonin, and histamine and the antiparkinsonian drugs amantadine and memantine, which are known to increase interstitial monoamine neurotransmitter concentrations.

**Experimental Procedures**

PCRs, DNA sequencing, and generation of cRNA probes. Total RNA was isolated from a small piece of human frontal cortex obtained during removal of a subcortical tumor, as approved by the local ethics committee (Gorboulev et al., 1997). To remove traces of genomic DNA, the RNA was treated with RNase-free DNase (Stratagene, Heidelberg, Germany). cDNA was reverse transcribed and subjected to PCR analysis (RT-PCR) using hOCT1- and hOCT2-specific primers and the Expand Long Template PCR System (Boehringer-Mannheim, Mannheim, Germany). For amplification of the specific primers and the Expand Long Template PCR System (Boehringer-Mannheim) with an alkaline phosphatase-coupled anti-digoxigenin antibody from goat and visualized by incubation with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Gorboulev et al., 1997).

**Immunohistochemistry.** An antibody was raised in rabbits against a peptide representing residues 317–332 of hOCT2. The peptide was coupled to ovalbumin and the immunization was performed as described (Poppe et al., 1997). Next, 5-μm-thick cryosections were fixed with 4% (w/v) paraformaldehyde dissolved in 137 mM NaCl, 2.7 mM KCl, 5 mM Na₂HPO₄, and 1.6 mM KH₂PO₄ (PBS) and blocked with PBS containing 2% (w/v) skim milk powder and 0.05% (w/v) Triton X-100 (PBS-MT). For the antibody reaction, the sections were incubated for 20 hr (4°) with the antisem, which was diluted 1:200 in PBS-MT. After washing with PBS-MT, the sections were incubated for 2 hr at room temperature with peroxidase-labeled goat anti-rabbit IgG, which was diluted 1:50 in PBS-MT. The immune reaction was visualized using diaminobenzidine (Graham and Karnovsky, 1966). The specificity of the antibody reaction was verified by negative controls on parallel sections. The controls that had been incubated with preimmune serum or with the antisem that had been blocked with the antigenic peptide.

**Expression of hOCT2 in oocytes of Xenopus laevis and transport measurements.** The cDNA of hOCT2 was subcloned into a pPO2 vector containing untranslated regions of the Xenopus β-globin gene and linearized with NcoI, and cRNA was synthesized (Gorboulev et al., 1997). X. laevis oocytes were collected, defollicu-

**Northern blots and in situ hybridization.** Northern blots were performed with poly(A)⁺ RNA from different areas of human brain that was obtained from Clontech (Heidelberg, Germany). Then, 2 μg of RNA was applied per lane to a denaturating formaldehyde 1.2% agarose gel, transferred to a charged modified nylon membrane, and the hybridization of RNA was applied per lane to a denaturating formaldehyde 1.2% agarose gel, transferred to a charged modified nylon membrane, and of RNA was applied per lane to a denaturating formaldehyde 1.2% agarose gel, transferred to a charged modified nylon membrane, and

**Electrophysiology.** For electrical measurements, oocytes were superfused with 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES-Tris, pH 7.4, or with 98 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES-Tris, pH 7.4 (~ 3 ml/min, 22–24°C). Measurements of the membrane potential and two-electrode voltage-clamp recordings were performed as described previously (Busch et al., 1996b; Nagel et al., 1997). The cation-induced change of the membrane potential and the size of cation-induced currents varied significantly, depending on the bath of oocytes. Data are shown for sets of experiments that were each obtained on the same day. The experiments were repeated with two or three batches of oocytes, and qualitatively similar results were obtained. For the determination of current-voltage relations, steady state current was measured during the last 100 msec of 500-msec rectangular voltage pulses to different potentials. The pulses were applied from a holding potential of ~50 mV at a frequency of 6.4 Hz. For measurements of membrane poten-
tials, HEK 293 cells were grown on glass coverslips near confluence and mounted as the bottom of a perfusion chamber on the stage of an inverted microscope. The cells were perfused constantly with buffers without and with K+, and the membrane voltages were measured using the slow-whole cell patch-clamp method. To gain electrical access to the cells, the pipette solutions contained 100 mg/liter nystatin.

**Expression of hOCT2 in HEK 293 cells and transport measurements.** hOCT2 was subcloned into the expression vector pRc-CMV (InVitrogen, Leek, The Netherlands), and the construct was used to transfect HEK 293 cells (CRL-1573; American Type Culture Collection, Rockville, MD), which were grown in Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal calf serum (Sigma, Deisenhofen, Germany). The transfected cells were selected for neomycin resistance by growing them in the presence of increasing geneticin (G418) concentrations (0.2–0.8 mg/ml), and single constantly transfected clones were isolated. The transport measurements were performed with suspended cells derived from a single clone. When the cells became confluent 4–5 days after passage, they were washed with PBS and suspended by shaking. The cells were collected by 10-min centrifugation at 1000 g and suspended at 37°C in K buffer (139.7 mM KCl, 8 mM K2HPO4, and 1.6 mM KH2PO4, pH 7.4). In some experiments, the cells were preloaded with cations by incubating them for 30 min at 37°C with PBS or K buffer containing radioactively labeled or nonradioactive organic cations. After this time period, equilibrium was reached (Fig. 10a). Then, the cells were spun down at 1000 × g, suspended in ice-cold PBS or K buffer, and washed twice at 0°C with the respective buffer. For uptake measurements, preloaded or nonpreloaded cells were warmed (40 sec at 37°C) and suspended in PBS or K buffer (37°C) that contained different concentrations of [3H]MPP, [3H]dopamine, or [3H]choline with or without 200 μM cyamine 863 (Busch et al., 1996a). Efflux measurements from cells preloaded with radioactively cations were performed by incubating 20 μl of ice-cold cell suspensions with 200 μl of PBS or K buffer (37°C). Uptake and efflux reactions were stopped with ice-cold PBS containing 100 μM quinine.

**Materials.** [3H]Dopamine (1.8 TBq/mmol), [3H]choline (2.6 TBq/mmol), [3H]serotonin (0.7 TBq/mmol), [3H]norepinephrine (0.4 TBq/mmol), and [3H]histamine (1.9 TBq/mmol) were obtained from Amersham Buchler (Braunschweig, Germany). 1-[3H]methyl-4-phenylpyridinium (3.1 TBq/mmol) was from Du Pont de Nemours (Deireich, Germany). [3H]Memantine (5.9 GBq/mmol) was a kind gift of Merz (Frankfurt, Germany). Amantadine, memantine, and peroxidase-coupled goat anti-rabbit IgG antiserum were purchased from Sigma. All other chemicals were obtained as described previously (Busch et al., 1996b).

**Results**

The transcription of hOCT1 and hOCT2 in human brain was investigated. Previously, we amplified a cDNA fragment from human brain that was identical to nucleotides 1648–1845 of hOCT1 (Gorboulev et al., 1997). Using 5’ and 3’ primers from noncoding cDNA regions of hOCT1 for RT-PCR experiments (see Experimental Procedures), the full-length clone could be amplified from liver but not from brain. This indicates that hOCT1 is not transcribed in human brain and that the previously detected carboxyl-terminal cDNA fragment belongs to an unknown gene product with a domain identical to hOCT1. Fig. 1 shows a Northern blot with mRNAs of different brain areas that was hybridized with an hOCT2-specific cRNA probe. In brain, much smaller hybridization signals were obtained than in the kidney (<5%, data not shown). In brain, the main hybridization was observed at ~4.4 kb, but two distinct hybridization bands at ~2.5 and ~4.4.5 kb were obtained in kidney (Gorboulev et al., 1997). The hybridization in hippocampus and various subcortical nuclei such as thalamus, nucleus subthalamicus, nucleus caudatus, and nucleus amygdaloideus was stronger than that in substantia nigra (Fig. 1). To verify whether hOCT2, rather than a highly homologous gene, is expressed in human brain, PCR’s were performed with reverse-transcribed mRNA from human frontal cortex. A series of overlapping hOCT2-specific primers were used, including the primers K11’ and K7’ (see Experimental Procedures), which were derived from the 5’- and 3’- noncoding region of hOCT2 mRNA, respectively. DNA sequencing of the overlapping PCR fragments showed that full-length mRNA of hOCT2 is transcribed in human brain. The cDNA sequence was identical with that of hOCT2 from kidney (Gorboulev et al., 1997).

Previously, an Na+-independent polyspecific organic cation transport system was characterized in the myocardial, smooth muscle, or glandular cells that translocates norepinephrine and was called extraneuronal norepinephrine uptake2 system (Iversen, 1967; Trendelenburg, 1988). Because recent uptake studies with the human glioma cell line SKMG-1 suggested that this norepinephrine uptake2 system also is expressed in brain (Streich et al., 1996), we performed RT-PCR experiments to elucidate whether hOCT1 and hOCT2 are transcribed in SK-MG-1 cells. hOCT2 cDNA could not be amplified. With primers from the 5’- and 3’- noncoding regions of hOCT1, a 2.5-kb fragment with partial sequence identity to hOCT1 rather than the expected 1.8-kb fragment of hOCT1 was amplified. This suggests that a splice variant of hOCT1 is expressed in glial cells that could be identical to the norepinephrine uptake2 system.

To determine the cellular localization of hOCT2 message, in situ hybridization was performed with an hOCT2-specific cRNA probe using sections of human cerebral cortex and...
hippocampus. Fig. 2 shows that \textit{hOCT2} is transcribed in pyramidal cells of cerebral cortex (area 18) and in pyramidal cells of hippocampus. The mRNA of \textit{hOCT2} was detected in the somata and in dendrites of neurons (Fig. 2e). Subsequently, an \textit{hOCT2}-specific peptide antibody was raised to examine \textit{hOCT2} protein expression in hippocampus. Using this antibody, the same localization of \textit{hOCT2} in human kidney was observed as described previously (Gorboulev \textit{et al.}, 1997). Fig. 3 shows that the pyramidal cells in the hippocampus were stained with the antiserum and that the staining of neurons could be blocked with the antigenic peptide. The relatively poor preservation of the postmortem tissue did not allow a subcellular localization of \textit{hOCT2}.

Previously, we showed that \textit{hOCT2} mediates electrogenic Na$^+$-independent transport of cations with diverse structures such as TEA, MPP, N$^+$-methyl nicotinamide, and choline (Gorboulev \textit{et al.}, 1997). For \textit{rOCT1}, we also demonstrated transport of monoamine neurotransmitters (Busch \textit{et al.}, 1996a). Both transporters do not translocate basic amino acids and are inhibited by various cations, including quinine and cyanine 863 (Busch \textit{et al.}, 1996b; Nagel \textit{et al.}, 1997). To evaluate the prospective neuronal functions of \textit{hOCT2} in addition to choline uptake, we investigated whether \textit{hOCT2} is capable of transporting monoamine neurotransmitters and the antiparkinsonian drugs amantadine and memantine. Transport expressed by \textit{hOCT2} was investigated by tracer influx and efflux of radioactively labeled cations and by electrical measurements with voltage-clamped \textit{X. laevis} oocytes. Tracer uptake and efflux experiments also were performed in stably transfected human HEK 293 cells. Fig. 4 shows a tracer uptake experiment in which the cyanine 863 inhibitable uptake rates of 90 $\mu$M norepinephrine, serotonin, histamine, and dopamine and of 60 $\mu$M memantine measured with Ori buffer in the bath were compared in water-injected and \textit{hOCT2} mRNA-injected oocytes. The data show that \textit{hOCT2} is capable of translocating each of these neurotransmitters and memantine. In subsequent experiments performed in Ori buffer, the apparent $K_m$ values of 1.9 $\pm$ 0.6 mM (norepinephrine), 1.3 $\pm$ 0.3 mM (histamine), 0.39 $\pm$ 0.16 mM (dopamine), and 0.08 $\pm$ 0.02 mM (serotonin) were obtained for the neurotransmitters. The values indicate low affinities for neu-

![Fig. 2. In situ hybridization of cortex and hippocampus from the human with \textit{hOCT2}-specific cRNA. Cryosections through area 18 of the cerebral cortex (a and b) and through the hippocampus (c, d, and e) were fixed and hybridized with an antisense (a, c, and e) and a sense (b and d) fragment of cRNA as described in Experimental Procedures. Specific hybridizations were observed in pyramidal cells of the cerebral cortex and hippocampus. Arrow in e, hybridization with mRNA in a dendrite. Scale bars: a–d 250 $\mu$m; e, 25 $\mu$m.](image1)

![Fig. 3. Light microscopic immunohistochemical localization of \textit{hOCT2} in pyramidal cells of human hippocampus. a, Cryosections through human hippocampus were stained with a rabbit antiserum against an \textit{hOCT2}-specific peptide. b, Staining of the pyramidal cells was observed, which was blocked when the antiserum was preincubated with the antigenic peptide. Scale bars, 50 $\mu$m.](image2)
rotransporter transport by hOCT2. Fig. 5a shows the concentra-
tion dependence of the cyanine-sensitive dopamine uptake
expressed by hOCT2. Comparing the $V_{\text{max}}$ values of ex-
pressed neurotransmitter uptake with the uptake of choline
and MPP in one batch of oocytes, the following values given
in nmol/oocyte/hr were obtained: $0.42 \pm 0.11$ (norepineph-
rine), $0.30 \pm 0.04$ (histamine), $0.59 \pm 0.13$ (dopamine), $0.36 \pm$
$0.05$ (serotonin), $0.58 \pm 0.13$ (choline), and $0.08 \pm 0.01$
(MPP).

The antiparkinsonian drug amantadine has been shown to
be a noncompetitive N-methyl-D-aspartate receptor antago-
nist and to increase the interstitial dopamine concentration
in brain (Symchowicz et al., 1973; Kornhuber et al., 1995).
Here, we investigated whether amantadine interacts directly
with hOCT2. Fig. 5b shows high affinity inhibition of dopa-
mine uptake in hOCT2-expressing oocytes by amantadine.
An IC$_{50}$ value of 23
$\pm 4 \mu M$ was determined. In Fig. 5c, we
tested the capability of amantadine to
trans-stimulate hOCT2-mediated efflux from oocytes preloaded
with [$^{3}$H]MPP. These efflux measurements were performed as
previously with roCT1 (Busch et al., 1996b). The oocytes had
been stored in the presence of 1 mM choline, and the efflux
measurements were performed with Ori buffer in the bath.
Under these conditions, the membrane potential was $\sim -70$
MV (see below). In water-injected control oocytes, a slow MPP
efflux was observed that was identical when no cations, 0.2
mM MPP, 1.5 mM TEA, or 0.5 mM amantadine was in the bath
(initial efflux rates, $0.09 \pm 0.01$ pmol/oocyte/hr; 12 oocytes).
In hOCT2-injected oocytes, the initial efflux rate of MPP was
significantly ($p < 0.01$) increased to $0.25 \pm 0.03$ pmol/
oocyte/hr (three oocytes). This shows that hOCT2 mediates
MPP efflux under trans-zero conditions. When 0.2 mM MPP,
1.5 mM TEA, or 0.5 mM amantadine was added to the bath,
the hOCT2-mediated MPP efflux was significantly increased
over that measured under trans-zero conditions (trans-MPP,
$0.42 \pm 0.05$ pmol/oocyte/hr; trans-TEA, $0.51 \pm 0.12$ pmol/
oocyte/hr; trans-amantadine, $0.57 \pm 0.10$ pmol/oocyte/hr; six
oocytes; $p < 0.05$).

![Fig. 4. Transport expression by hOCT2 in X. laevis oocytes of mono-
amine neurotransmitters and memantine. X. laevis oocytes were injected
with 50 nl of water without or with 10 ng of hOCT2 cRNA and incubated
for 3 days. Uptake of 90 $\mu$M of [$^{3}$H]norepinephrine, [$^{3}$H]serotonin, [$^{3}$H]his-
tamine, or [$^{3}$H]dopamine and of 60 $\mu$M memantine was measured in
the absence or presence of the hOCT2 inhibitor cyanine 863 (36 $\mu$M). The
cyanine-inhibited uptake is indicated. Median $\pm$ standard error values
from 10 parallel measurements are given.

For a further characterization, electrophysiological meth-
ods were used. When hOCT2 cRNA-injected X. laevis oocytes
were superfused with 5 mM dopamine, 50 $\mu$M memantine, or
50 $\mu$M amantadine, a reversible decrease in the membrane
potential was observed, whereas water-injected control
oocytes did not show a significant response (Fig. 6a). In the
voltage-clamp configuration, inward currents were induced
by dopamine, memantine, and amantadine (Fig. 6b) that
could be inhibited by cyanine 863 (not shown). These cur-
rents showed substrate saturation and were dependent on
the membrane potential (Fig. 7). With hOCT2-expressing
oocytes clamped at $-50$ mV, half-maximal currents were
induced at $0.52 \pm 0.16$ nm dopamine, $34 \pm 5$ nm memantine,
and $27 \pm 3$ nm amantadine. These values are comparable to
the apparent $K_{m}$ values obtained from the uptake measure-
ments described above. With the rat organic cation transporter rOCT1, we recently observed that nontransported inhibitors like quinine and cyanine 863 may induce inward currents by inhibiting electrogenic efflux of choline (Nagel et al., 1997). The trans-inhibition of choline efflux by inhibitory cations and the electrogenic cation influx of transported cations can be distinguished because only the inward currents induced by transported cations become larger as the potential becomes more negative inside. Fig. 7b shows that the inward currents induced by 5 mM dopamine, 50 μM memantine, and 50 μM amantadine increased with increasing inside negative membrane potentials. This is consistent with the interpretation that the observed currents are caused by electrogenic translocation of dopamine, memantine, and amantadine.

Next, we investigated whether the trans-stimulation of MPP efflux in the experiments of Fig. 5c is due to a depolarization of the oocytes by organic cations in the bath. We performed trans-experiments with depolarized oocytes in which Na⁺ in the bath was replaced by K⁺. Electrical measurements with water-injected control oocytes showed a depolarization from −52 ± 7 to −7 ± 2 mV (>10 oocytes) after replacement of Na⁺ in the bath by K⁺. After preincubation of control oocytes with 1 mM choline, 0.2 mM MPP, 4 mM dopamine, or 1 mM amantadine, the membrane potential was not changed significantly. In hOCT2-expressing oocytes, membrane potentials between −40 and −50 mV were measured with Na⁺ in the bath (Fig. 8). The membrane potential dropped to −10 mV when Na⁺ was replaced by K⁺. When these oocytes were preincubated with 1 mM choline, the membrane potential was increased to −72 ± 8 mV in the presence of Na⁺ and to −45 ± 14 mV in the presence of K⁺ (Fig. 8). At variance, no significant increase of the membrane potential was observed after preincubation with 0.2 mM MPP, 1 mM amantadine, or 4 mM dopamine. This indicates a much smaller electrogenic efflux of these cations than choline. In Fig. 9a, we tested choline, MPP, and amantadine for trans-effects on MPP efflux in depolarized oocytes. This figure also shows that the efflux of [³H]MPP in the presence of Na⁺ was decreased when the oocytes were preincubated with choline (compare columns 1 and 3 in Fig. 9a) and that MPP efflux was increased when Na⁺ in the bath was replaced by K⁺.

---

**Fig. 6.** Electrical measurements with *X. laevis* oocytes that were injected with hOCT2-cRNA or water. For expression, the oocytes were incubated for 3 days in Ori buffer. For the measurements, the oocytes with superfused with Ori buffer or with Ori containing 50 μM amantadine (■), 50 μM memantine (□), or 5 mM dopamine (▲). a, The membrane potential was measured in the absence and presence of the indicated cations. b, The oocytes were clamped to the indicated membrane potentials, and the currents were determined that were induced by superfusion of hOCT2-expressing oocytes with 50 μM amantadine, 50 μM memantine, or 5 mM dopamine.

**Fig. 7.** Induction of currents in hOCT2-expressing oocytes clamped to different membrane potentials after superfusion with different concentrations of amantadine, memantine, or dopamine. a, hOCT2-cRNA-injected oocytes were clamped at −50 mV and superfused for 30 sec with the indicated cation concentrations. Mean ± standard error values of 8–10 measurements of induced currents in different oocytes are presented. The values from different oocytes were normalized against the currents induced by 1 mM TEA. b, The potential dependence of the currents induced by 5 mM dopamine or 50 μM amantadine in hOCT2-expressing oocytes is presented (mean ± standard error).
With depolarized oocytes, the MPP efflux was slightly trans-inhibited by the transported cations choline (1 mM), MPP (0.2 mM), or amantadine (1 mM) rather than being trans-stimulated, as in polarized oocytes (Fig. 5c). Electrical measurements showed that the addition of 1 mM choline, 0.2 mM MPP, or 1 mM amantadine to depolarized hOCT2-expressing oocytes did not lead to membrane depolarizations of >3 mV (data not shown). With the nontransported inhibitor cyanine 863 on the trans-side, the hOCT2-mediated MPP efflux was inhibited by ~90% (Fig. 9a). The data indicate that the trans-stimulation of MPP efflux observed at high membrane potentials is due to a cation-induced membrane depolarization. The trans-inhibition of efflux in depolarized oocytes suggests a slower out-to-in reorientation of the loaded compared with the unloaded transporter.

In Fig. 9b, we investigated trans-effects of organic cations on the hOCT2-mediated influx of 0.1 μM [3H]MPP in depolarized oocytes. By replacement of Na+ in the bath with K+, the hOCT2-mediated MPP influx was reduced by ~50%. Preloading of the oocytes with 1 mM choline or 0.2 mM MPP led to a significant increase in MPP uptake. At variance, the MPP uptake was slightly reduced when the oocytes were preloaded with 1 mM amantadine or 4 mM dopamine. These data suggest different affinities or transport activities of hOCT2 for the influx and efflux of some organic cations. The trans-stimulation of MPP influx by choline may be partially explained by the choline-induced membrane hyperpolarization, but the trans-stimulation by MPP may indicate a more rapid in-to-out orientation of loaded compared with unloaded transporter forms.

In an effort to exclude possible artifacts particular to the oocyte expression system, uptake experiments were performed with HEK cells stably transfected with hOCT2. Fig. 10a shows the time course of MPP uptake in the hOCT2-transfected HEK 293 cells measured in the absence and presence of cyanine 863. In the absence of cyanine 863, a rapid initial MPP uptake was observed within 2 sec, followed by a slow further uptake that reached equilibrium within 5 min. The nonspecific uptake of MPP measured in the presence of 200 μM cyanine 863 was much slower. After a 1-hr incubation, about the same equilibrium was obtained as in the absence of cyanine 863. The effect of the membrane potential on the initial MPP uptake is investigated in Fig. 10b. After 1 sec with Na+ in the bath, an uptake of 0.4 ± 0.1 pmol/mg of protein was determined, which was inhibited by 75 μM cyanine 863 to 0.03 ± 0.01 pmol/mg of protein (three measurements). In the presence of Na+ (PBS buffer), membrane voltages of ~44 ± 2 mV (six measurements) were determined. After replacement of Na+ by K+ (K buffer), the membrane potentials were ~6 ± 1 mV (six measurements). With K+ in the bath, the uptake after 1 sec was reduced to 0.14 ± 0.04 pmol/mg of protein (three measurements). This was expected for potential-dependent transport. The concentration dependence of the cyanine-sensitive uptake of MPP

![Fig. 8. Membrane potentials of hOCT2-expressing oocytes without and with preincubation with organic cations that were measured with Na+ or K+ in the bath. hOCT2-cRNA-injected oocytes were preincubated for 12 hr without organic cations or with the indicated concentrations of choline, MPP, amantadine, and dopamine. The membrane potential was measured with Ori buffer in the bath (□) or after replacement of Na+ in the Ori buffer by K+ (□). Mean ± standard deviation values from six oocytes are shown.](image-url)

![Fig. 9. The trans-effects of organic cations on MPP efflux (a) and MPP influx (b) in depolarized oocytes. Water-injected control oocytes or oocytes expressing hOCT2 were injected with 0.1 pmol of [3H]MPP (efflux) or with different amounts of nonradioactive cations (preloading for uptake measurements). The indicated intracellular cation concentrations were estimated from the injected amounts of cations by assuming an intracellular aqueous space of 0.5 μl. a, Initial [3H]MPP efflux rates were estimated by measuring the efflux of washed oocytes between 10- and 70-sec incubations in Ori buffer (Na+, trans-zero) or in K oocyte buffer without or with the indicated cations (depolarized oocytes: K+ trans-zero or K+ cations). b, trans-zero condition in Fig. 5c in which the oocytes had been stored in the presence of 1 mM choline, b. The initial MPP uptake rates were estimated from the uptake after a 5-min incubation in Ori buffer (Na+ without preloading) or in K oocyte buffer (K+ without preloading or K+ cation concentration) containing 0.1 μM [3H]MPP. Mean ± standard error values from 8–10 oocytes are presented.](image-url)
and dopamine in hOCT2-transfected and in nontransfected HEK 293 cells is shown in Fig. 11. Only in the transfected cells was significant cyanine-inhibitable cation uptake observed. The apparent \( K_m \) values for hOCT2-mediated influx of MPP (16 ± 3 \( \mu M \)) and dopamine (0.33 ± 0.13 mM) were not significantly different from the values obtained after expression of hOCT2 in oocytes (see above and Gorboulev et al., 1997). Because the expression of cation transport by hOCT2 in stably transfected HEK 293 cells varied during the cultivation, the \( V_{\text{max}} \) values of MPP, choline, and dopamine were compared within one batch of cells. In such an experiment, \( V_{\text{max}} \) values of 2.7 ± 0.4 (MPP), 16.8 ± 1.5 (choline), and 19.4 ± 3.0 (dopamine) \( \text{nmol/mg of protein/min} \) were determined. As already observed with oocytes, similar \( V_{\text{max}} \) values were obtained for dopamine and choline, whereas the \( V_{\text{max}} \) value for MPP was much smaller.

To further investigate hOCT2 for symmetry, we compared the influx and efflux rates of choline and MPP in depolarized HEK 293 cells at their respective \( K_m \) concentrations (Gorboulev et al., 1997). Again, the depolarization was achieved by raising the extracellular K\(^+\) concentration. Fig. 12 shows efflux of 19 \( \mu M \) \[^3H\]MPP and 210 \( \mu M \) \[^3H\]choline in hOCT2-transfected and in nontransfected HEK 293 cells measured in the presence of Na\(^+\) or K\(^+\). In the control cells, small initial efflux rates were determined. In the hOCT2-transfected HEK 293 cells, the efflux of choline and MPP was significantly higher and was increased after depolarization of the cells (shown for MPP). In the depolarized cells, initial efflux rates of 2.3 ± 0.2 and 5.2 ± 1.3 pmol/mg of protein/sec were estimated for 19 \( \mu M \) \[^3H\]MPP and 210 \( \mu M \) \[^3H\]choline, respectively. For uptake of 19 \( \mu M \) \[^3H\]MPP and 210 \( \mu M \) \[^3H\]cho-
To evaluate the possible relevance of hOCT2 regarding the therapeutic actions of amantadine in brain during treatment of Parkinson’s disease, we tested whether amantadine also affects the hDAT that mediates high affinity uptake of dopamine into dopaminergic neurons (Giros et al., 1992; Sonders et al., 1997). To examine the action by amantadine on transport by hDAT, X. laevis oocytes expressing hDAT were superfused with 10 μM dopamine, 50 μM amantadine, or 10 μM dopamine plus 50 μM amantadine. Drug-elicited currents were measured in voltage-clamped oocytes during a series of jumps to a range of membrane potentials (Fig. 14). Amantadine itself did not elicit transport-associated current at hDAT, and in combination with dopamine, it showed no significant inhibition of the transport-associated current of dopamine. These data indicate that at a concentration equivalent to its apparent $K_m$ value for hOCT2, amantadine has no discernible effect on hDAT.

**Discussion**

hOCT2 is a polyspecific electrogenic cation transporter originally shown to be expressed in kidney that translocates small organic cations with diverse structures, including choline and MPP (Gorboulev et al., 1997). We report that hOCT2 also is expressed in neurons of various brain areas, including cerebral cortex, hippocampus, corpus striatum, nucleus amygdaloideus, and thalamus. hOCT2 is not identical to the Na$^+$-independent corticosterone-sensitive extraneuronal norepinephrine uptake 2 transporter from myocardial cells (Iversen, 1967; Trendelenburg, 1988), which also is expressed in the human glioma cell line SK-MG-1 (Streich et al., 1996). hOCT2 has a $\times 100$-fold lower sensitivity to corticosterone (data not shown) and is not transcribed in SK-MG-1 cells. Evidence is presented that hOCT2 also mediates low affinity transport of the monoamine neurotransmitters dopamine, norepinephrine, serotonin, and histamine. In distinction to the Na$^+$/Cl$^-$-dependent high affinity transporters for norepinephrine, dopamine, and serotonin whose expression is largely restricted to the neurons that synthesize these neurotransmitters, expression of hOCT2 is relatively widespread across regions of the human brain. In light of the broad substrate specificity of hOCT2, this polyspecific cation transporter may serve the function in brain of limiting the action of aminergic neurotransmitters that have evaded high affinity uptake mechanisms. Mental or psychiatric alterations or diseases such as depression and schizophrenia have been linked to disturbances in dopamine transport, but there are no links to the high affinity Na$^+$/Cl$^-$-dependent dopamine transporters (Seeman and Niznik, 1990; Gelernter et al., 1995; Maier et al., 1996). It therefore will be interesting to analyze the putative role of hOCT2 in such disorders.
hOCT2 transports the neurotoxin MPP with a similar affinity as hDAT (Giros et al., 1992). MPP is a cerebral oxidation product of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine that passes the blood-brain barrier. In humans and in several animal models, an injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine causes a selective degeneration of dopaminergic neurons that project from the substantia nigra to the corpus striatum, which leads to Parkinson’s disease (Chiuheh et al., 1985; Snyder and D’Amato, 1986; Kinemuchi et al., 1987; Tipton and Singer, 1993). The selective degeneration of these neurons by MPP probably is due to MPP uptake via hDAT in the nerve terminals or to other factors as the intracellular binding to neurotoxin rather than to MPP uptake via hOCT2. However, hOCT2 may be involved indirectly because neuronal uptake by this transporter will reduce the overall concentration of MPP or of environmental MPP-like toxins that may induce Parkinson’s disease (Calne and Langston, 1983). Recently, an autosomal recessive form of juvenile parkinsonism was localized to a fragment of chromosome 6q25.2–27 (Matsumine et al., 1997). We have localized the gene of hOCT2 to chromosome 6q26 (Koehler et al., 1997), which represents an intriguing proximity to the gene defect of juvenile parkinsonism.

An intriguing finding was that the antiparkinsonian drugs amantadine and memantine are transport substrates and competitive inhibitors of hOCT2. It is noteworthy that the affinity of amantadine to hOCT2 is in the same range as the amantadine concentrations in the serum or cerebrospinal fluid (4–17 μM) that were effective for the symptomatic treatment of Parkinson’s disease (Kornhuber et al., 1995). Although the pharmacological action of amantadine may also involve noncompetitive N-methyl-D-aspartate receptor antagonism, muscarinic mechanisms, and dopamine release from neuronal storage sites (Grelak et al., 1970; Parkes, 1974; Kornhuber et al., 1991; Chen et al., 1992), the current data provide the first evidence that this compound may increase extracellular neurotransmitter concentration by inhibition of dopamine uptake via hOCT2. No other neurotransmitter transporter has been identified as a target for these compounds; moreover, we report that the high affinity dopamine transporter hDAT is insensitive to therapeutically relevant concentrations of amantadine, as suggested previously (Sonders et al., 1997). hOCT2-mediated uptake of amantadine into neurons also could explain the accumulation of amantadine in brain observed during the first days of treatment (Kornhuber et al., 1995).

Here, we further characterize transport properties of hOCT2. In measurement of the trans-effects in depolarized oocytes and HEK 293 cells, a functional asymmetry of hOCT2 is noted. The affinity of hOCT2 for MPP is considerably higher than that for dopamine and amantadine, whereas the transporter may operate symmetrically for others. In depolarized cells, choline, MPP, dopamine, and amantadine slightly inhibited MPP efflux. At variance, the influx of MPP was trans-inhibited by dopamine and amantadine and trans-stimulated by choline and MPP. The data suggest that the in-to-out reorientation of transporter forms is accelerated after loading with MPP or choline from the inside but remains unchanged or is slightly decreased after loading with dopamine or amantadine. The out-to-in orientation of transporter forms may be generally slowed down after loading with cations from the outside. This suggests different carrier specificities for cation influx and efflux. Combining tracer flux and electrical measurements, we showed that hOCT2-mediated cation influx and efflux rates in polarized cells are significantly determined by the membrane potential. For example, it could be demonstrated that the trans-stimulation of cation efflux in polarized cells measured for rOCT1 (Busch et al., 1996b) and hOCT2 (current report) is a consequence of the membrane depolarization by transportations cations on the outside.

The detailed physiological role of hOCT2 for the cerebral homeostasis of choline, cationic drugs, and xenobiotics in relation to Na+-dependent choline and monoamine transporters can only be determined by measurements with human brain slices or synaptosomes. This is a demanding task because well preserved shock-frozen human brain tissue is required, and it may be difficult to quantify the activities of different transporters in different cell types. Currently, the reported localization and functional characteristics of hOCT2 suggest that this transporter affects interstitial and neuronal concentrations of organic cations in brain. The observed trans-effects of intracellular choline on cation uptake indicate that the capacity of hOCT2 to translocate cationic drugs and xenobiotics from brain interstitium can be modulated by the intraneuronal choline concentrations. In summary, our data demonstrate the expression of hOCT2 in human brain and suggest that it could represent a “background transporter” for monoamine neurotransmitters. hOCT2 inhibition by the antiparkinsonian drugs amantadine and memantine, at clinically relevant concentrations, provides the first evidence for the mechanism by which these compounds increase extracellular dopamine concentrations.

Acknowledgments

We thank J. C. Tonn (Neurosurgical Department, University of Würzburg, Würzburg, Germany) and P. Riederer (Department of Psychiatry, University of Würzburg, Würzburg, Germany) for supplying us with tissue samples from human brain, G. Quack (Merz, Frankfurt, Germany) for providing [14C]memantine, and E. Schlatter (Department of Internal Medicine, University of Münster, Münster, Germany) for performing the electrical measurements in HEK 293 cells. The figures were prepared by M. Christof.

References
