Cloning and Functional Characterization of a Potential-sensitive, Polyspecific Organic Cation Transporter (OCT3) Most Abundantly Expressed in Placenta*

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We have isolated a cDNA from rat placenta which, when expressed heterologously, mediates the transport of a wide spectrum of organic cations. The cDNA codes for a protein of 551 amino acids containing 12 putative transmembrane domains. Northern blot analysis indicates that this transporter is expressed most abundantly in the placenta and moderately in the intestine, heart, and brain. The expression is comparatively low in the kidney and lung and is undetectable in the liver. This transporter is distinct from the previously cloned organic cation transporters (OCT1, OCT2, NKT, NLT, RST, and OCTN1). When expressed in HeLa cells, the cDNA induces the transport of tetraethylammonium and guanidine. Competition experiments indicate that this transport process recognizes a large number of organic cations, including the neurotoxin 1-methyl-4-phenylpyridinium, as substrates. The cDNA-induced transport is markedly influenced by extracellular pH. However, when expressed in Xenopus laevis oocytes, the cDNA-induced transport is electrogenic, associated with the transfer of positive charge into the oocytes. Under voltage clamp conditions, tetraethylammonium evokes inward currents that are concentration- and potential-dependent. This potential-sensitive organic cation transporter, designated as OCT3, represents a new member of the OCT gene family.

Specific transport systems exist in mammalian cells that are responsible for the influx of organic cations into the cells as well as their efflux out of the cells (1, 2). Several xenobiotics are positively charged at physiological pH, and therefore the organic cation transport systems play a critical role in the handling of these xenobiotics by mammalian tissues. Special attention has been given to the function of organic cation transport systems in two organs, the kidney and the liver, because of the role these organs are known to play in the elimination of xenobiotics. Functional studies have led to the identification of two distinct classes of organic cation transport systems, one driven by the transmembrane potential difference and the other driven by the transmembrane H⁺ gradient (1, 2).

The potential-sensitive transport system is likely to participate in the influx of organic cations, whereas the H⁺ gradient-dependent transport system is likely to participate in the efflux of organic cations. In the renal proximal tubular epithelial cells, the cationic xenobiotics enter the cell across the basolateral membrane and exit the cell across the brush border membrane. Thus, a concerted action of the potential-sensitive transport system in the basolateral membrane and the H⁺-dependent transport system in the brush border membrane effectively mediates the elimination of several xenobiotics by the kidney.

The placenta, which provides the sole link between the mother and the developing fetus, functions as a protective barrier for the fetus by participating in the clearance of xenobiotics from the fetal circulation. The syncytiotrophoblast, which is the functional unit of the placenta, is a polarized cell layer consisting of a basal membrane in contact with the fetal circulation and a brush border membrane in contact with the maternal blood. As has been shown in the renal proximal tubule, it is likely that the two classes of organic cation transport systems operate in the placental syncytiotrophoblast in the elimination of cationic xenobiotics from the fetus. We have shown previously, using purified human placental brush border membrane vesicles, that a H⁺ gradient-dependent organic cation transport system is expressed in the placenta and that this system is likely to be responsible for the removal of cationic xenobiotics from the syncytiotrophoblast (3, 4). The presence of a potential-sensitive organic cation transport system has not been demonstrated in normal placenta, but such a system is present in the JAR human placental choriocarcinoma cell line (5).

In recent years, several members belonging to a common gene family of organic cation transporters have been cloned and characterized. The first member to be cloned was rat OCT1 which is a potential-sensitive transporter and is expressed primarily in the kidney and liver (6). Subsequently, rat and porcine OCT2 were cloned (7, 8). OCT2 is also a potential-sensitive transporter (7, 9) even though its function is influenced to some extent by H⁺ (8). Human homologs of OCT1 and OCT2 have also been cloned and functionally characterized (9, 10). Three additional members of the gene family have been identified (11–13). These are NKT (cloned from mouse kidney) (11), NLT (cloned from rat liver) (12), and RST (cloned from mouse kidney) (13). NKT, NLT, and RST are included in the gene family of organic cation transporters solely based on amino acid sequence similarity. There is no information available on the transport function of these three clones. Based on amino acid sequence homology, NKT is likely to be the murine homolog of the rat organic anion transporter (14, 15), which also appears to be a member of the OCT gene family. Most recently, cloning of an additional member of the gene family
has been reported (16). This clone, called OCTN1, is functional and appears to be H\(^+\)-dependent. Interestingly, except for OCTN1 which is expressed in several tissues in addition to the kidney, all other members of the family are expressed primarily in the kidney and/or liver. In this paper we report on the cloning of a new member of the potential-sensitive organic cation transporter gene family which is expressed most abundantly in placenta.

**EXPERIMENTAL PROCEDURES**

**Materials**—SuperScript Plasmid System for cDNA library construction and LipofectAMINE for transfection in mammalian cells were purchased from Invitrogen, Inc. Restriction enzymes were obtained from Promega. Magna nylon transfer membranes were purchased from Micron Separations (Westboro, MA). Unlabeled organic cations were from either Sigma or Research Biochemicals, Inc. (Natick, MA). The RNA transcription kit mMMESSAGE mMACHINE\textsuperscript{TM} for the synthesis of cRNA was obtained from Ambion. The Trizol reagent for the isolation of total RNA was from Life Technologies, Inc., and the ready-to-go oligo-labeling kit was from Amersham Pharmacia Biotech.

**Screening of the cDNA Library**—The cDNA probe used for screening was a kilobase fragment of rat OCT1 cDNA obtained by reverse transcriptase-polymerase chain reaction using rat intestinal mRNA. This probe corresponded to the nucleotide position 495–1388 in the base sequence. The plasmid cDNA library (17) was screened with this probe under low stringency conditions. Hybridization was carried out for 20 h at 60 °C in a solution containing 5× SSPE (1× SSPE = 0.15 M NaCl, 10 mM NaH\(_2\)PO\(_4\) and 1 mM EDTA), 5× Denhardt’s solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. Post-hybridization washing was done as described earlier (17) which involved extensive washes with 3× SSPE, 0.5% SDS at room temperature. Positive clones were identified, and the colonies purified by secondary screening.

**DNA Sequencing**—Both sense and antisense strands of the cDNA were sequenced by primer walking. Sequencing by the dideoxynucleotide chain termination method was performed by Taq DyeDeoxy terminator cycle sequencing with an automated Perkin-Elmer Applied Biosystems 377 Prism DNA Sequencer. The sequence was analyzed using the GCG sequence analysis software package GCG version 7.8 (Genetics Computer Group, Inc. Madison, WI). Data base searches were done using the GenBank\textsuperscript{TM} Program BLAST (18).

**Functional Expression of the cDNA in Mammalian Cells**—The cDNA was functionally expressed in HeLa cells by vaccinia virus expression system as described previously (19, 20). Transport measurements were made at room temperature using \[^{14}C\]guanidine and \[^{1}C\]tetrathylammonium. The transport buffer was composed of 25 mM Hepes (pH 8.5), supplemented with 290 mM mannitol, 5.4 mM KCl, 1.8 mM CaCl\(_2\), 0.8 mM MgSO\(_4\), and 5 mM glucose. When the influence of pH on transport was investigated, transport buffers of different pH values were prepared by varying the concentrations of Tris, Hepes, and Mes.\(^1\) The incubation time for transport measurements was 30 min. Transport was terminated by aspiration of the uptake buffer followed by two washes with 2 ml of ice-cold transport buffer. Following this, the cells were solubilized with 0.5 ml of 1% SDS in 0.2 N NaOH and transferred to vials for quantitation of the radioactivity associated with the cells. HeLa cells transfected with empty vector under similar conditions served as control. In experiments dealing with saturation kinetics, data were analyzed by nonlinear regression and confirmed by linear regression.

**cDNA Synthesis**—The pSPORT-cDNA construct was linearized with NcoI, and the cDNA insert was transcribed in vitro using T7 RNA polymerase in the presence of ribonuclease inhibitor and RNA cap analog. The mMMESSAGE mMACHINE\textsuperscript{TM} kit was employed for this purpose.

**Oocyte Expression and Electrophysiological Studies**—Mature (stages V–VI), defolliculated oocytes from Xenopus laevis (Nasco, Port Atkinson, WI) were selected and maintained at 18 °C in modified Barth’s medium (21) with 10 mg/ml gentamicin sulfate. Oocytes were injected 1 day after isolation with ~50 ng of cRNA and used for electrophysiological studies 5 days after cRNA injection. Water-injected oocytes were used as controls. A two-microelectrode voltage clamp system (22–24) was used to measure steady-state tetrathylammonium-evoked currents. Oocytes were superfused with a NaCl-containing buffer (pH 6.5, 7.5, or 8.5) (in mM: 96 NaCl, 2 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 Hepes/Mes/Tri) containing varying concentrations of tetrathylammonium. Membrane potential was held at ~50 mV. For studies involving the current-voltage (membrane potential) (I–V) relationship, step changes in membrane potential were applied for a duration of 100 ms in 20-mV increments. Kinetic parameters for the saturable transport of tetrathylammonium were calculated using the Michaelis-Menten equation. Data were analyzed by nonlinear regression and confirmed by linear regression.

**Uptake Measurements in Oocytes**—Uptake of \[^{14}C\]guanidine and \[^{1}C\]tetrathylammonium into oocytes was measured in a 24-well microtiter plate as described previously (25). The influence of membrane potential on the uptake was studied as described by Grundemann et al. (6) by changing the concentration of K\(^+\) in the uptake buffer or by adding Ba\(^{2+}\), a K\(^+\) channel blocker to the uptake buffer.

**RESULTS**

**Structural Features of the Rat Placental OCT3 cDNA**—Screening of the rat placental cDNA library using an ~0.9-kilobase pair fragment of the rat OCT1 cDNA as the probe led to the identification of three positive clones. Sequence analysis indicated that these clones were identical. One of these clones, designated as OCT3, was arbitrarily chosen for further structural analysis. The rat placental OCT3 cDNA (GenBank\textsuperscript{TM} accession number AF055286) is 3,502 base pairs long with an open reading frame of 1,656 base pairs (including termination codon), encoding a protein of 551 amino acids. The amino acid sequence of OCT3 is given in Fig. 1. The predicted molecular mass of the protein is 61 kDa. Hydrophobicity analysis using the algorithm of Kyte and Doolittle (26) and with 20–21 amino acid residues per membrane spanning domain indicated that the protein possesses 12 putative transmembrane domains. When rat OCT3 is modeled based on the published models of several cloned transporters and by the application of the inside-positive rule (27), both the amino terminus and the carboxyl terminus lie on the cytoplasmic side of the membrane. This model predicts a large extracellular loop (106 amino acids) between transmembrane domains 1 and 2 which contain three potential sites for N-linked glycosylation (NXT/S) at positions 72, 99, and 114. There is also an additional putative N-glycosylation site at position 199 in the short extracellular loop between the transmembrane domains 3 and 4. The predicted protein also contains three potential sites for protein kinase C-dependent phosphorylation (Ser-286, Thr-292, and Thr-459) and two potential sites for protein kinase A-dependent phosphorylation (Thr-346 and Thr-544) in putative intracellular domains. A comparison of the amino acid sequence of rat OCT3 with the protein sequence data base revealed that OCT3 is not identical to any of the previously cloned proteins. However, the OCT3 amino acid sequence bears significant homology to the known members of the organic cation transporter gene family. At the amino acid sequence level, the identity ranges between 30 and 51% and the similarity ranges between 54 and 73%. The greatest homology is seen with OCT1 and OCT2 (Fig. 1).

**Distributions of OCT3 Transcripts in Rat Tissues**—Poly(A\(^+\)) RNA prepared from several rat tissues was analyzed by Northern blot hybridization for the presence of mRNA transcripts for OCT3 (Fig. 2). A primary transcript, 3.5 kilobases in size, was detectable in the placenta, intestine, heart, brain, lung, and kidney. The transcript was not detectable in the liver. Among the positive tissues, the OCT3 transcript was most abundant in the placenta, followed by intestine, heart, and brain. The abundance of the transcript was low in the kidney and lung.
Functional Characterization of OCT3 Following Expression in HeLa Cells—In order to establish the functional identity of OCT3 as an organic cation transporter, we employed the vac-
cinia virus expression system to functionally express the OCT3 cDNA in HeLa cells. First, we used guanidinium as the organic
cation substrate for transport measurements in OCT3 cDNA-transfected cells. Cells transfected with empty vector
served as the control. Initial experiments indicated that the 
uptake of guanidinium in cDNA-transfected cells was higher than 
in control cells and that the cDNA-induced uptake was en-
hanced if the pH of the uptake medium was higher than 7.5 and
mannitol was substituted for NaCl. Fig. 3 describes the uptake of 
guanidinium in mannitol medium at varying pH. At pH 5.5, 
there was very little difference in guanidinium uptake between 
cDNA-transfected cells and control cells. However, when the 
pH of the uptake medium was raised above 5.5, the difference 
in uptake between the cDNA-transfected cells and control cells 
became evident. At pH 8.5, uptake of guanidinium (35 
M) was 
about 75% higher in cDNA-transfected cells compared with 
control cells. When the cDNA-induced uptake alone was ana-
lyzed, there was a marked pH dependence of the uptake activ-
ity. The uptake was found to be enhanced about 9-fold when the 
pH of the uptake medium was changed from 6.5 to 8.5. 
The substrate specificity of the cDNA-induced uptake activ-
ity was investigated by assessing the effect of various organic 
cations on the uptake of [14C]guanidinium in control and 
cDNA-transfected cells (Table I). The uptake of [14C]guanidinium 
in control cells was similar to that in cDNA-transfected cells and was 
inhibited by all organic cations tested. Similarly, the cDNA-specific 
uptake was also inhibited by several organic cations. It can be 
inferred from the data that HeLa cells express endogenously an 
organic cation transport system. When the inhibitory potency 
of various organic cations was compared between the endoge-
nous uptake and the cDNA-specific uptake, clear differences 
between the two uptake processes became apparent. Most no-
tably, choline and tetramethylammonium (TMA) did not have 
any significant effect on the cDNA-specific uptake, whereas 
compounds such as tetraethylammonium (TEA), cimetidine, and serotonin were found to be more potent 
in inhibiting the cDNA-specific uptake than in inhibiting the 
endogenous uptake. Thus, the cDNA-induced organic cation 
uptake process is clearly distinct from the organic cation up-
take process that is constitutively expressed in HeLa cells.

**Fig. 1.** Comparison of amino acid sequences of rat OCT3, rat OCT2, and rat OCT1. Regions of identity (dark shading) and similarity (light shading) are indicated.

**Fig. 2.** Northern blot analysis of OCT3 mRNA transcripts in rat tissues. Poly(A) RNA (5 μg/lane), prepared from different tissues, was size-fractionated and probed with OCT3 cDNA and glyceroldehyde 3-phosphate dehydrogenase (GAPDH) cDNA. The sizes of hybridizing bands were determined using RNA standards run in parallel in an adjacent lane. Lanes 1–7 represent mRNA samples from liver, kidney, intestine, placenta, lung, heart, and brain. The mobility of the placental mRNA is slightly retarded as evidenced from the hybridizing bands of the OCT3 mRNA as well as glyceroldehyde 3-phosphate dehydrogenase mRNA.

**Functional Characterization of OCT3 Following Expression in HeLa Cells**—In order to establish the functional identity of OCT3 as an organic cation transporter, we employed the vac-
cinia virus expression system to functionally express the OCT3 cDNA in HeLa cells. First, we used guanidinium as the organic cation substrate for transport measurements in OCT3 cDNA-transfected cells. Cells transfected with empty vector served as the control. Initial experiments indicated that the uptake of guanidinium in cDNA-transfected cells was higher than in control cells and that the cDNA-induced uptake was enhanced if the pH of the uptake medium was higher than 7.5 and...
TABLE I

Substrate specificity of the OCT3 cDNA-induced uptake

| Unlabeled compound         | [14C]Guanidine uptake |
|----------------------------|-----------------------|
|                            | pSPORT | pSPORT-cDNA | cDNA-specific |
|                            | nmol/10^6 cells/30 min | nmol/10^6 cells/30 min | nmol/10^6 cells/30 min |
| None                       | 0.46 ± 0.02 (100)     | 0.81 ± 0.04 (100)     | 0.35 (100) |
| Guanidine (12.5)           | 0.15 ± 0.01 (33)      | 0.25 ± 0.01 (31)      | 0.10 (29)  |
| TEA                        | 0.27 ± 0.01 (59)      | 0.52 ± 0.01 (41)      | 0.06 (17)  |
| TMA                        | 0.11 ± 0.02 (24)      | 0.48 ± 0.01 (59)      | 0.37 (106) |
| N^1-Methylnicotinamide     | 0.19 ± 0.01 (41)      | 0.27 ± 0.01 (33)      | 0.08 (23)  |
| Nicotine (8.0)             | 0.23 ± 0.01 (50)      | 0.42 ± 0.02 (52)      | 0.19 (54)  |
| Cimetidine (6.8)           | 0.28 ± 0.01 (61)      | 0.33 ± 0.01 (41)      | 0.05 (14)  |
| Choline                    | 0.09 ± 0.01 (20)      | 0.40 ± 0.01 (49)      | 0.31 (89)  |
| Desipramine (10.2)         | 0.01 ± 0.01 (2)       | 0.01 ± 0.01 (1)       | 0 (0)     |
| MPP                        | 0.13 ± 0.01 (28)      | 0.18 ± 0.01 (22)      | 0.05 (14)  |
| MPTP                       | 0.08 ± 0.01 (17)      | 0.11 ± 0.01 (14)      | 0.03 (9)   |
| Serotonin (9.1)            | 0.21 ± 0.01 (46)      | 0.24 ± 0.01 (30)      | 0.03 (9)   |
| Dimethylamiloride          | 0.10 ± 0.01 (22)      | 0.10 ± 0.01 (12)      | 0 (0)     |

With respect to the cDNA-induced uptake process, the most potent inhibitors were TEA, 1-methyl-4-phenylpyridinium (MPP), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, serotonin, dimethylamiloride, desipramine, and cimetidine. Moderate inhibition was observed with N^1-methylnicotinamide, guanidine, and nicotine. Choline and TMA showed very little or no inhibition. Among the inhibitors tested, TEA, TMA, N^1-methylnicotinamide, choline, and MPP are permanently cationic, whereas the remaining are cationic buffers.

TEA has been used as the prototypical organic cation in elucidating the functional characteristics of the previously cloned organic cation transporters (6, 7, 16). Since TEA was found to be a potent inhibitor of guanidine uptake mediated by OCT3, it appeared that TEA may be transported by this newly cloned organic cation transporter. Therefore we investigated the ability of OCT3 to transport TEA in HeLa cells (Fig. 4). The uptake of TEA was found to be higher in OCT3 cDNA-transfected cells than in control cells. The cDNA-specific uptake of TEA showed a marked pH dependence, a characteristic similar to that observed with the cDNA-induced guanidine uptake. The cDNA-specific TEA uptake was stimulated almost 4-fold when the pH of the uptake medium was changed from 6.5 to 8.5. However, an important difference was noticeable between guanidine and TEA when the uptake activity was compared in control cells and in cDNA-transfected cells. The endogenous uptake activity in control cells was severalfold lower for TEA than for guanidine. Because of this, the difference in the uptake activity between control cells and cDNA-transfected cells was greater for TEA than for guanidine. At pH 8.5, the uptake of guanidine increased only by about 75% (1.75-fold) as the result of OCT3 expression compared with control cells (Fig. 3). Under similar conditions, OCT3 expression led to an increase of 1100% (12-fold) in TEA uptake (Fig. 4).

FIG. 3. Influence of pH on OCT3 cDNA-induced guanidine uptake in HeLa cells. HeLa cells were transfected with either empty pSPORT vector (control) or pSPORT-OCT3 cDNA. Uptake of [14C]guanidine (35 μM) in these cells was measured for 30 min using NaCl-free uptake buffers of different pH. A, uptake in control cells and in cDNA-transfected cells. B, cDNA-specific uptake, calculated by subtracting the uptake in control cells from the uptake in cDNA-transfected cells. Values represent data from two separate transfection experiments, each done in triplicate.
was no significant difference in the rate of efflux between pH 8.5 and pH 6.5. This means that the influence of pH observed for OCT3-mediated influx of TEA was not due to the coupling of TEA influx to H$^+$ efflux.

**Functional Characterization of OCT3 following Expression in X. laevis Oocytes**—To determine the influence of membrane potential on OCT3-mediated transport, we investigated the transport function of OCT3 in X. laevis oocytes following microinjection of OCT3 cRNA. The X. laevis oocyte expression system is ideal to investigate the characteristics of electrogenic transport systems using electrophysiological approaches. As shown in Fig. 7, the uptake of radiolabeled guanidine and TEA at pH 7.5 was severalfold higher in cRNA-injected oocytes than in water-injected oocytes. We then studied the pH dependence of OCT3-mediated TEA uptake. The endogenous TEA uptake measured in water-injected oocytes was very low and was not influenced significantly by pH. On the other hand, TEA uptake in cRNA-injected oocytes was found to be influenced significantly by pH. The uptake was stimulated about 2-fold when the pH of the uptake buffer was changed from 5.5 to 7.5. The stimulation was 1.7-fold when the change in pH was from 6.5 to 8.5. These data show that OCT3-mediated TEA uptake in X. laevis oocytes is also influenced by pH. But the magnitude of stimulation is much less compared with the stimulation observed in HeLa cells.

To assess directly the influence of membrane potential on OCT3 transport activity, we measured TEA uptake in water-injected and OCT3 cRNA-injected oocytes under altered K$^+$ permeability conditions. The experimental approach employed in this study to alter K$^+$ permeability in oocytes was the same as the approach described by Grundemann et al. (6) in their study of the potential-sensitive organic cation transporter OCT1. In this approach, the oocyte membrane potential was depolarized either by increasing the concentration of K$^+$ in the uptake buffer (i.e. by decreasing the outwardly directed K$^+$ gradient across the membrane) or by adding Ba$^{2+}$ (an inhibitor of K$^+$ channel) to the uptake buffer. When we measured TEA uptake in OCT3 cRNA-injected oocytes, it was found that the cRNA-induced uptake was drastically reduced in the presence of K$^+$ (102 mM) in the uptake buffer (Fig. 8). Addition of Ba$^{2+}$ also decreased the cRNA-induced TEA uptake, although to a much lesser extent than observed with external K$^+$. These experiments provided the first compelling evidence that the transport activity of OCT3 is potential-sensitive.

A more direct approach to study the influence of membrane potential on a potential-sensitive transporter is by electrophysiological means using the two-microelectrode, voltage

![Fig. 4. Influence of pH on OCT3 cDNA-induced TEA uptake in HeLa cells.](image)

HeLa cells were transfected with either empty pSPORT vector (control) or pSPORT-OCT3 cDNA. Uptake of [14C]TEA (20 μM) in these cells was measured for 30 min using NaCl-free uptake buffers of different pH. A, uptake in control cells and in cDNA-transfected cells. B, cDNA-specific uptake, calculated by subtracting the uptake in control cells from the uptake in cDNA-transfected cells. Values represent data from two separate transfection experiments, each done in triplicate.

![Fig. 5. Saturation kinetics of OCT3 cDNA-induced TEA uptake in HeLa cells.](image)

Uptake of TEA was measured in control cells and cDNA-transfected cells at pH 8.5 over a TEA concentration range of 0.25–5 mM. The cDNA-specific uptake, calculated by subtracting the uptake in control cells from the uptake in cDNA-transfected cells, was used for kinetic analysis. Inset, Eadie-Hofstee plot. Values represent data from two separate transfection experiments, each done in triplicate.
clamp technique. If OCT3 is an organic cation-H\(^+\) antiporter, it is expected to be electroneutral, and therefore OCT3 function should not be associated with membrane currents under voltage clamp conditions. On the other hand, if OCT3 is a potential-sensitive organic cation transporter, it is expected to be electrorgenic, carrying net positive charge into the oocyte, and therefore OCT3 function should be associated with inward currents under voltage clamp conditions. The results of the electrophysiological experiments done with OCT3-expressing oocytes using TEA as the substrate are given in Fig. 9A. Perfusion of the oocytes with 10 mM TEA at pH 8.5 induced inward currents (>60 nA). When the pH of the perfusion buffer was altered to 6.5, there was no change in the inward currents induced by TEA, suggesting that OCT3 is electrorgenic and its function is not dependent on transmembrane H\(^+\) gradients. TEA failed to induce any noticeable currents at pH 8.5 or 6.5 in water-injected oocytes (Fig. 9B), showing that the TEA-induced currents are dependent on OCT3 expression. These data demonstrate unequivocally that OCT3 is a potential-sensitive organic cation transporter.

Fig. 10 details the electrophysiological properties of rat OCT3 with TEA as the substrate under voltage clamp conditions. The OCT3-induced inward currents were dependent on the testing membrane potential as well as on the concentration of TEA (Fig. 10A). The current increased in magnitude as the testing membrane potential became hyperpolarized, demonstrating that the transport activity of OCT3, measured by the magnitude of the substrate-induced inward currents, is stimu-
regression according to the Eadie-Hofstee method, comparable values were obtained (K_{0.5} = 5.7 ± 0.7 mM; I_{max} = 89 ± 4 nA) (Fig. 10D). The substrate-induced currents were found to be saturable at all testing membrane potentials. The kinetic constant K_{0.5}, a parameter indicative of the affinity of OCT3 for the substrate, decreased as the testing membrane potential became hyperpolarized (Fig. 10E). At a testing membrane potential of −50 mV, the K_{0.5} was 6.2 ± 1.8 mM, whereas the K_{0.5} decreased to 1.1 ± 0.2 mM when the testing membrane potential was changed to −150 mV. This suggests that the affinity of OCT3 for its substrate increases at hyperpolarizing membrane potentials. The kinetic constant I_{max}, a parameter indicative of the maximal velocity of the OCT3-mediated transport activity, increased as the testing membrane potential became hyperpolarized (Fig. 10F). This shows that OCT3 is activated by an inside-negative membrane potential.

**DISCUSSION**

We have reported here on the cloning and functional characterization of OCT3, a new member of the OCT gene family. Functional characterization of OCT3 was done in this study using a mammalian cell expression system as well as the X. laevis oocyte expression system. The use of both approaches enabled us to delineate thoroughly the influence of pH on OCT3 function. Studies involving the influence of pH on OCT3 function in mammalian cells, which were performed without clamping the membrane potential, actually suggested that OCT3 may be an organic cation-H^+ antiporter rather than a potential-sensitive organic cation transporter. However, when the OCT3 function was analyzed in X. laevis oocytes under voltage clamp conditions, the influence of pH on OCT3 function was found to be an indirect effect via pH-induced changes in membrane potential. Electrophysiological studies with OCT3-expressing oocytes carried out under voltage clamp conditions showed unequivocally that OCT3 is a potential-sensitive organic cation transporter and not an organic cation-H^+ antiporter. These findings are significant with respect to other members of OCT family. Grundemann et al. (8) have recently reported that the function of OCT2, studied using a mammalian cell expression system, is influenced by pH in a manner compatible with the organic cation-H^+ antiporter mechanism. But electrophysiological studies using X. laevis oocytes under voltage clamp conditions definitively showed that OCT2 is a potential-sensitive transporter and that its function is not influenced by pH (9). The most recently cloned OCTN1 has been claimed to be an organic cation-H^+ antiporter, solely based on the influence of pH on the transporter function in a mammalian cell expression system (16). Based on the data reported in the present paper, analysis of the transport function of OCTN1 under voltage clamp conditions may be needed to validate the organic cation-H^+ antiport nature of OCTN1.

There are some notable differences in substrate specificity among the cloned OCTs that have been shown to be functionally active. TEA is a prototypical organic cation that is recognized as a substrate by all of the functionally active OCTs (OCT1, OCT2, OCT3, and OCTN1) (6–9, 16, 28, 29, and present study). In contrast, TMA, which is a substrate for OCT1 and OCT2, is not recognized by OCT3. Similarly, choline has been shown to interact with OCT1 and OCT2 with appreciable affinity, but the interaction of this organic cation with OCT3 is very weak. Several neurotransmitters as well as the neurotransmitter MPP are transported by OCT1 as well as OCT2. Our present study has shown that OCT3 also interacts with serotonin, MPP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, and desipramine.

The tissue distribution of the OCT3 transcript is unique. Among the known members of the OCT gene family, transcripts are detectable only for OCTN1 in tissues other than the
liver and kidney (16). All other members of the family are expressed primarily in the kidney and/or liver (6–13). Even in the case of OCTN1, the transcript levels are much lower in the placenta than in the kidney. Thus, OCT3 represents the first member of the OCT family that is expressed abundantly in the placenta. This transporter is likely to play a critical role in the placental handling of cationic xenobiotics. The potential-sensitive OCT1 has been shown to localize to the renal basolateral membrane and thus is believed to participate in the entry of organic cations from the blood into the tubular epithelial cells, which is the first step in the renal elimination of cationic drugs. We speculate a similar role for OCT3, also a potential-sensitive organic cation transporter, in the placenta. It is likely that OCT3 is expressed in the fetal-facing basal membrane of the placenta where it functions in the entry of cationic drugs from the fetal circulation into the placental trophoblast. Once inside the trophoblast, these drugs can be eliminated into the maternal circulation across the maternal-facing brush border membrane, mediated by the organic cation-H⁺ antiport systems known to be present in this membrane. OCT3 may be responsible for the first step in the placental clearance of cationic xenobiotics from the fetus and may hence be a key player in the barrier function of the placenta to protect the developing fetus from possible deleterious effects of xenobiotics that may be present in the maternal circulation. OCT3 transcripts are found also in the kidney and intestine, two other organs known to be involved in the elimination of drugs. Interestingly, it is not expressed in the liver, also a major player in the metabolism and elimination of xenobiotics.

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