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Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta

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Abstract

This report describes the structure, function, and tissue distribution pattern of rat OCTN1 (novel organic cation transporter 1). The rat OCTN1 cDNA was isolated from a rat placental cDNA library. The cDNA is 2258 bp long and codes for a protein of 553 amino acids. Its amino acid sequence bears high homology to human OCTN1 (85% identity) and rat OCTN2 (74% identity). When expressed heterologously in mammalian cells, rat OCTN1 mediates Na^+-independent and pH-dependent transport of the prototypical organic cation tetraethylammonium. The transporter interacts with a variety of structurally diverse organic cations such as desipramine, dimethylamiloride, cimetidine, procainamide, and verapamil. Carnitine, a zwitterion, interacts with rat OCTN1 with a very low affinity. However, the transport of carnitine via rat OCTN1 is not evident in the presence or absence of Na^+. We conclude that rat OCTN1 is a multispecific organic cation transporter. OCTN1-specific mRNA transcripts are present in a wide variety of tissues in the rat, principally in the liver, intestine, kidney, brain, heart and placenta. In situ hybridization shows the distribution pattern of the transcripts in the brain (cerebellum, hippocampus and cortex), kidney (cortex and medulla with relatively more abundance in the cortical-medullary junction), heart (myocardium and valves) and placenta (labyrinthine zone).

Keywords: Organic cation transport; OCTN1; Carnitine; Drug disposition; In situ hybridization; (Rat)

1. Introduction

Ionic compounds, which are present endogenously or obtained exogenously (xenobiotics), present a complex problem to the body's mechanisms of clearance and metabolism because of the diverse structure of these compounds. Recent studies have defined a family of multispecific organic ion transporters responsible for the carrier-mediated clearance of a wide range of structurally diverse xenobiotics and

Abbreviations: OCT, organic cation transporter; OCTN, novel organic cation transporter; rOCTN, rat OCTN; hOCTN, human OCTN; TEA, tetraethylammonium; HRPE, human retinal pigment epithelial; RT-PCR, reverse transcriptase-polymerase chain reaction; SSPE, saline–sodium phosphate–EDTA; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline; DMA, 5-(*N*,*N*-dimethyl)amiloride; MPP, 1-methyl-4-phenylpyridinium

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endobiotics [1,2]. Most of these transporters are expressed primarily in the kidney, liver and intestine, the tissues involved in drug elimination. The members of this family include the organic cation transporter (OCT)1 [3], OCT2 [4], OCT3 [5], novel organic cation transporter (OCTN)1 [6], OCTN2 [7], and the organic anion transporter (OAT)1 [8,9], OAT2 [10], OAT3 [11] and OAT4 [12].

OCTN1 was originally cloned from human fetal kidney [6]. In contrast to OCTs and OATs that exhibit a restricted tissue distribution pattern, OCTN1 is expressed in a wide variety of tissues and cell lines of human origin [6]. The transport characteristics of hOCTN1 include multispecific, bidirectional organic cation transport in a pH-dependent manner [13]. It has been hypothesized that this transporter is responsible for the efflux of organic cations across the renal brush border membrane via the organic cation/H^+ antiport mechanism [13]. This is interesting because OCT1–3 are potential-sensitive transporters. H^+ does not play any direct role in the transport mechanism of these transporters. hOCTN1 exhibits the highest homology in amino acid sequence to hOCTN2 [7] and relatively much lower homology to OCTs and OATs. Interestingly, OCTN2 is an Na^+-dependent transporter for the zwitterions carnitine and acylcarnitines and an Na^+-independent transporter for several organic cations [14,15]. Thus, OCTN1 appears to be unique among the members of the organic ion transporter family due to its postulated function as an organic cation/H^+ antiporter [13].

Our laboratory has been interested for several years in the transport function of placenta, an organ that plays an obligatory role in the development of the fetus. Placenta expresses several transporters that mediate the delivery of essential nutrients from the mother to the fetus. In addition, this tissue is also involved in drug elimination, a function that is very important to protect the developing fetus from pharmacologically active drugs that might be present in the maternal circulation. Therefore, identification and characterization of the drug transport systems expressed in the placenta is clinically very relevant. We have recently cloned OCT3 and OCTN2 from placenta or placental cell lines [5,7]. Here we report the cloning and functional characterization of OCTN1 from rat placenta. We also show that OCTN1 mRNA is expressed in a wide variety of tissues in the rat as assessed by Northern blot analysis and by in situ hybridization.

2. Materials and methods

2.1. Materials

[*ethyl*-1-^{14}C]Tetraethylammonium (TEA) bromide (specific activity 55 mCi/mmol) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). L-[^{3}H]Carnitine (specific activity 65 Ci/mmol) was obtained from Moravek Biochemicals, Inc. (Brea, CA, USA). Unlabeled organic cations and carnitine were obtained from Research Biochemicals International (Natick, MA, USA) or Sigma. Cell culture media and lipofectin were obtained from Life Technologies, Inc. (Gaithersburg, MD, USA). Restriction enzymes were obtained from Promega (Madison, WI, USA). Magna nylon transfer membranes were purchased from Micron Separations, Inc. (Westboro, MA, USA). Human retinal pigment epithelial (HRPE) cells were originally provided by Dr. M.A. Del Monte (University of Michigan, Ann Arbor, MI, USA) and have been in use in our laboratory for several years [16]. The Ready-to-go oligolabeling kit used in the preparation of cDNA probes for library screening was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA).

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) and probe preparation

Total RNA was isolated from JAR cells (a human placental trophoblast cell line) with Trizol reagent (Life Technologies) according to manufacturer's protocol. Poly(A)$^+$ RNA was then prepared by affinity chromatography on an oligo dT-cellulose column (Life Technologies). The integrity of the RNA sample was checked by formaldehyde–agarose gel electrophoresis. A pair of PCR primers specific for the hOCTN1 cDNA was designed: 5′-caccacctccctgttctt-3′ (forward primer) and 5′-atgatgctgcccactctg-3′ (reverse primer). Primers were based upon the published sequence of hOCTN1 and correspond to nucleotide positions 578–595 and 1550–1567 in hOCTN1 cDNA [6]. RT-PCR was conducted using

JAR cell poly(A)$^+$ RNA with Geneamp RNA-PCR kit (Perkin Elmer). The RT-PCR reaction product was run on a 1% agarose gel for determination of the size of the product. A ~1.0 kbp product, expected on the basis of the positions of the primers, was obtained. This product was excised from the gel and gene-cleaned. The product was then cloned into pGEM-T vector (Promega) and the cDNA insert was sequenced by the dideoxynucleotide chain termination method for confirmation of its identity.

2.3. Screening of cDNA library

The Superscript Plasmid System (Life Technologies) was used to establish the directional cDNA library using the poly(A)$^+$ RNA isolated from rat placenta. The ~1.0 kbp fragment of hOCTN1 cDNA, obtained by RT-PCR, was used to screen the rat placental cDNA library [17,18]. The probe was labeled with [α-^{32}P]dCTP using the Ready-to-go oligolabeling kit. The cDNA library was screened at low stringency conditions as described [17,18]. Hybridization was carried out for 20 h at 60°C in a solution containing 5×saline–sodium phosphate–EDTA (SSPE) (1×SSPE = 0.15 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA), 5×Denhardt's solution, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Posthybridization washing involved extensive washes with 3×SSPE/0.5% sodium dodecyl sulfate (SDS) at room temperature. Positive clones were identified, and the colonies were purified by secondary screening.

2.4. DNA sequencing

Sense and antisense strands of the cDNAs were both 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.

2.5. Functional expression of rat OCTN1 cDNA in HRPE cells

The cloned rat OCTN1 cDNA was oriented in the pSPORT plasmid in such a way that its expression was under the control of the T7 promoter. The cDNA was heterologously expressed in HRPE cells by the vaccinia virus expression system as described [5,7]. Transport measurements were made at room temperature using [^{14}C]TEA or [^{3}H]carnitine as substrates. The transport buffer was composed of 25 mM Tris/HEPES (pH 8) or 25 mM HEPES/Tris (pH 7.5) supplemented with 140 mM NaCl (or 140 mM *N*-methyl-D-glucamine chloride), 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. After incubation for 30 min at 37°C, transport was terminated by aspiration of the buffer followed by two washes with 2 ml of ice-cold transport buffer. The cells were then solubilized with 0.5 ml of 1% SDS in 0.2 NaOH and transferred to vials for quantitation of the radioactivity associated with the cells. HRPE cells transfected with vector alone under similar conditions served as control.

2.6. In situ hybridization

Four weeks old male rat kidney, heart and whole brain were collected and immediately frozen in liquid nitrogen. Additionally, rat term placentas were also collected and frozen. Unfixed 12 μm serial sections were prepared on a cryostat, mounted on 2% 3-aminopropyltriethyloxysilane-coated slides, air dried for 10 min, and stored at −70°C until required. Nonradioactive in situ hybridization using digoxigenin UTP-labeled probes was performed on tissue sections as described previously [15,19]. Cryostat sections were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.3) at 4°C for 20 min, washed, and permeabilized with proteinase K (10 μg/ml in PBS containing 0.1% Tween 20) for 10 min at room temperature. Sections were then refixed in 4% paraformaldehyde in PBS, prehybridized for 1 h at 65°C in 50% formamide, and incubated with sense and antisense riboprobes for 16 h at 65°C. For immunologic detection of the probe, sections were incubated for 2 h with alkaline phosphatase-coupled anti-digoxigenin antibody and color reaction was developed using a commercially available chromogenic kit (Boehringer-Mannheim, Indianapolis, IN, USA). For the preparation of the rOCTN1-specific riboprobes, a 0.85 kbp fragment of the rOCTN1 cDNA, obtained through the digestion of pSPORT-rOCTN1 cDNA by *Sty*I, was subcloned into the pBluescript vector. The orientation of the cDNA in-

sert in the pBluescript vector was determined by sequencing. Antisense and sense riboprobes were synthesized with T7 RNA polymerase or T3 RNA polymerase after linearization of the plasmid with appropriate restriction enzymes. The riboprobes were labeled using a digoxigenin-labeling kit (Boehringer-Mannheim, Indianapolis, IN, USA).

2.7. Northern blot analysis

A hybridization ready multiple tissue blot containing size-fractionated poly(A)$^+$ RNA from 12 different rat tissues was purchased from Origene (Rockville, MD, USA). This blot was probed sequentially under high stringency conditions using ^{32}P-labeled rat OCTN1 cDNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA as probes.

2.8. Data analysis

Each uptake experiment was done in duplicate or triplicate and repeated two or three times. Uptake values given are means ± S.E.M. of these replicates. The dose–response data for various inhibitors were analyzed by a non-linear regression method using the computer program Sigma Plot (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Structural features of rOCTN1

Using a ∼1.0 kbp fragment of human OCTN1 cDNA obtained by RT-PCR, we screened a rat placental cDNA library. Several positive clones were obtained. The cDNA inserts of these clones were initially characterized by restriction fragment analysis and nucleotide sequencing of the 5′-ends. These analyses showed that the positive clones consisted of two different cDNA inserts. One of these was found to be the rat homolog of human OCTN1 and the other to be rat homolog of human OCTN2 [15]. The rat OCTN1 clones contained cDNA inserts of

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GACTCCTGCAATCGCCGGACCCCTTTCTCCCAACCCACCTCCCTCGTGCA
AGTTGAGGAGAGGCGGGAAGCATGAGGGACTATGACGAGGTGATAGCCTT
                      M  R  D  Y  D  E  V  I  A  F
CCTGGGCGATTGGGGGCCCTTCCAGCGCCTTATCTTCTTCCTGCTCAGCG
 L  G  D  W  G  P  F  Q  R  L  I  F  F  L  L  S  A
CCAGCATCATCCCCAATGGCTTCAATGGTATGTCAGTCGTGTTCCTGGCG
  S  I  I  P  N  G  F  N  G  M  S  V  V  F  L  A
GGGACCCCGGAGCATCGTTGCCTTGTGCCTCACACCGTGAACTTGAGCAG
G  T  P  E  H  R  C  L  V  P  H  T  V  N  L  S  S
CGCGTGGCGCAACCACAGCATCCCCTTGGAAACGAAGGACGGACGACAGG
 A  W  R  N  H  S  I  P  L  E  T  K  D  G  R  Q  V
TGCCTCAAAGCTGCCGCCGCTACCGACTGGCCACCATCGCCAACTTCTCT
  P  Q  S  C  R  R  Y  R  L  A  T  I  A  N  F  S
GCGCTGGGGCTGGAGCCCGGACTGGATGTAGATCTGGAGCAGCTGGAGCA
A  L  G  L  E  P  G  L  D  V  D  L  E  Q  L  E  Q
AGAGAGCTGCCTGGATGGCTGGGAGTACAGCAAGGACGTCTTCCTGTCCA
 E  S  C  L  D  G  W  E  Y  S  K  D  V  F  L  S  T
CCATCGTGACAGAGTGGAATCTGGTGTGTGAGGATGACTGGAAGACACCC
  I  V  T  E  W  N  L  V  C  E  D  D  W  K  T  P
CTCACCACCTCCCTGTTCTTCGTAGGCGTTCTCTGTGGCTCCTTCGTGTC
L  T  T  S  L  F  F  V  G  V  L  C  G  S  F  V  S
TGGGCAGCTGTCAGACAGGTTCGGCAGGAAGAAAGTCCTCTTTGCCACCA
 G  Q  L  S  D  R  F  G  R  K  K  V  L  F  A  T  M
TGGCTGTGCAGACTGGATTCAGCTTCGTGCAGATTTTCTCAACCAACTGG
  A  V  Q  T  G  F  S  F  V  Q  I  F  S  T  N  W
GAGATGTTTACTGTGTTATTTGCCATCGTGGGCATGGGCCAAATCTCCAA
E  M  F  T  V  L  F  A  I  V  G  M  G  Q  I  S  N
CTATGTGGTGGCCTTCATACTCGGGACCGAAATCCTGAGCAAGTCGGTTC
 Y  V  V  A  F  I  L  G  T  E  I  L  S  K  S  V  R
GCATCCTCTTCTCCACGTTGGGAGTCTGTACATTTTTTGCCATCGGCTAC
  I  L  F  S  T  L  G  V  C  T  F  F  A  I  G  Y
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M  V  L  P  L  F  A  Y  F  I  R  D  W  R  M  L  L
GCTGGCACTGACACTGCCTGGGCTGTTCTGTGTCCCCCTGTGGTGGTTTA
 L  A  L  T  L  P  G  L  F  C  V  P  L  W  W  F  I
TTCCAGAGTCTCCCCGGTGGCTGATATCCCAGAGGAGATTTGAAGAGGCA
  P  E  S  P  R  W  L  I  S  Q  R  R  F  E  E  A
GAACAGATCATCCAGAAAGCCGCAAAGATGAATGGCATCATGGCACCAGC
E  Q  I  I  Q  K  A  A  K  M  N  G  I  M  A  P  A
AGTGATATTTGATCCTCTGGAGCTACAGGAGCTAAACTCCTTGAAGCAGC
 V  I  F  D  P  L  E  L  Q  E  L  N  S  L  K  Q  Q
AGAAAGTCTTCATTCTGGACCTGTTCAAGACTCGGAACATTGCCACAATA
  K  V  F  I  L  D  L  F  K  T  R  N  I  A  T  I
ACAGTGATGTCTGTGATGCTGTGGATGCTAACCTCAGTGGGTTACTTTGC
T  V  M  S  V  M  L  W  M  L  T  S  V  G  Y  F  A
TCTGTCTCTCAATGTTCCTAATTTGCACGGAGATGTCTACCTGAACTGCT
 L  S  L  N  V  P  N  L  H  G  D  V  Y  L  N  C  F
TCCTCTCTGGCCTGATTGAAGTTCCAGCTTATTTCACAGCCTGGCTGCTA
  L  S  G  L  I  E  V  P  A  Y  F  T  A  W  L  L
CTTCGAACCCTGCCTCGGAGATATATTATAGCTGGGGTGCTGTTCTGGGG
L  R  T  L  P  R  R  Y  I  I  A  G  V  L  F  W  G
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 G  G  V  L  L  L  V  Q  V  V  P  E  D  Y  N  F  V
TGTCCATTGGCCTGGTGATGCTGGGGAAATTTGGGGTCACCTCTGCCTTC
  S  I  G  L  V  M  L  G  K  F  G  V  T  S  A  F
TCCATGCTGTACGTCTTCACAGCGGAGCTCTACCCAACCCTGGTCAGGAA
S  M  L  Y  V  F  T  A  E  L  Y  P  T  L  V  R  N
CATGGCTGTGGGCATCACCTCCATGGCCTCGAGGGTGGGCAGCATCATTG
 M  A  V  G  I  T  S  M  A  S  R  V  G  S  I  I  A
CCCCCTATTTCGTGTACCTGGGTGCTTATAACCGACTCCTGCCCTACATC
  P  Y  F  V  Y  L  G  A  Y  N  R  L  L  P  Y  I
CTCATGGGCAGTCTGACTGTCCTCATTGGAATCATCACACTTTTTTTCCC
L  M  G  S  L  T  V  L  I  G  I  I  T  L  F  F  P
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 E  S  F  G  V  T  L  P  E  N  L  E  Q  M  Q  K  V
TCAGAGGGTTCAGATGTGGGAAAAAATCAACAGTCTCCATGGACAGAGAA
  R  G  F  R  C  G  K  K  S  T  V  S  M  D  R  E
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E  N  P  K  V  L  I  T  A  F
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CAGCCTGAAAGCCTGCCCTCTTGGTATGGGGACAGGAGGATCGAGAAGTC
AAGGTCATCATTGACTATGTCAGGAGTGTGAGACCAGCCTGTCTCACCAC
AAGCAGCCCTGTCTGAACAAAACAAAACAAAACAAAACAAAACAAAACAA
AACAAAACAAAAGCCTTTCCGCTGAAAGGTACTAATAGAAACAATGAGCA
CCAAACTGGACTTGTGGAGAAATACACAATATCTCATAAATTCTGGGCCA
CTCTTCCAGATGGTCTTGTTTTTAAGAACCAACGTTTCTAAACAGTCTTG
ACTGCTAACTATTCCATGAAATAGATTTGTAAGAATTTTTTTTTTGAAAA
TGTGTTAGTCAAGGACTGGCAAAATCCATATGAAGATGATCACACATTTT
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AAAAAAAA
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Fig. 1. Nucleotide sequence of rOCTN1 cDNA and predicted amino acid sequence.

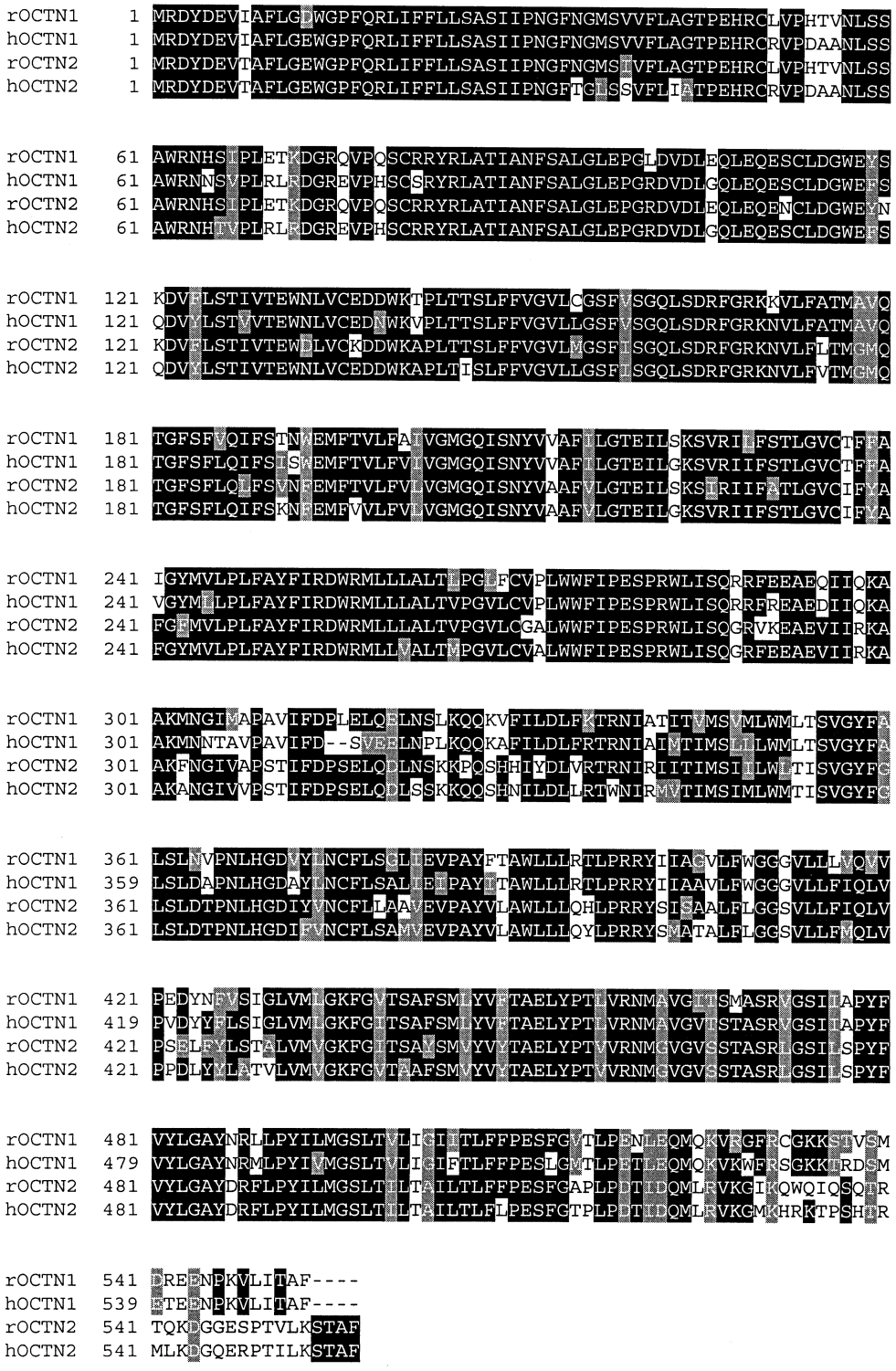


Fig. 2. Comparison of amino acid sequences among rat OCTN1, human OCTN1, rat OCTN2, and human OCTN2. Identical amino acids are shown in black boxes and conservative substitutions are shown in gray boxes.

varying size. The clone containing the longest cDNA insert was selected for structural and functional analysis. The nucleotide sequence (GenBank accession No. AF169831) and the deduced amino acid sequence of rOCTN1 cDNA are given in Fig. 1. The cDNA is 2258 bp long with a 1662 bp long open reading frame (including the termination codon). The 5′- and 3′-untranslated regions are 71 bp and 525 bp long, respectively. The 3′-untranslated region contains the poly(A)$^+$ tail and the polyadenylation signal (AATAA). The predicted protein consists of 553 amino acids and hydropathy analysis of the amino acid sequence predicts 12 transmembrane domains. When modeled as the previously known members of the mammalian organic cation/anion transporter family, both the amino terminus and the carboxy terminus of the rOCTN1 protein are located on the cytoplasmic side of the membrane. The extracellular loop between transmembrane domains 1 and 2 consists of 109 amino acids and contains three putative *N*-glycosylation sites (Asn-57, Asn-64, and Asn-91). The protein also contains a single cAMP-dependent phosphorylation site in the cytosolic C-terminal tail (Ser-536) and five protein kinase C-dependent phosphorylation sites located in the first, second, and third intracellular loops (Ser-164, Ser-225, Ser-280, Ser-286, and Ser-323). The predicted molecular mass of the protein is 62.4 kDa.

Within the family of organic cation/anion transporters, there exists significant homology among different members of the family. Comparative analysis of amino acid sequences indicates rat OCTN1 to have significant homology to all members (Fig. 2). Rat OCTN1 exhibits significant homology to human OCTN1 (~85% identity, ~91% similarity) and human OCTN2 (~71% identity, ~82% similarity). Rat OCTN1 is also homologous to rat OCTN2, exhibiting ~74% identity and ~85% similarity. Comparison of rat OCTN1 with rat OCTs shows the following homology: OCT1 (34% identity, 50% similarity), OCT2 (32% identity, 49% similarity) and OCT3 (28% identity, 48% similarity).

3.2. Functional characteristics of rOCTN1

The functional characteristics of rOCTN1 were studied in a mammalian cell heterologous expression system using the vaccinia virus expression technique.

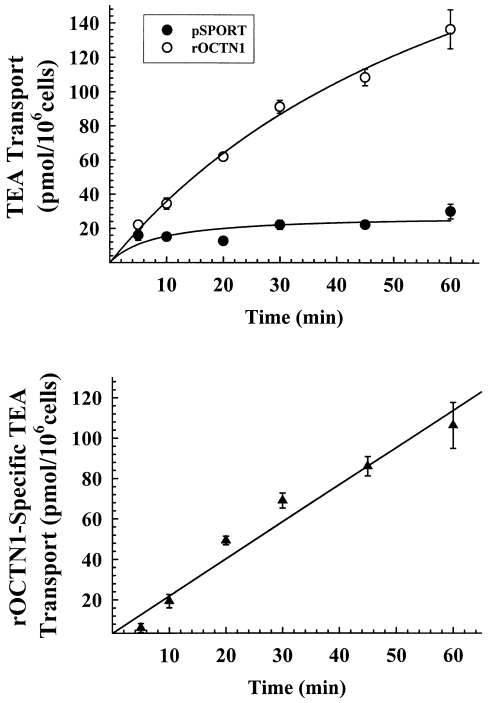


Fig. 3. Time course of TEA transport in HRPE cells transfected with either rOCTN1 cDNA (○) or pSPORT vector (●). Transport of 20 μM [^{14}C]TEA was measured at pH 8.0 in an Na^+-free uptake medium (NMDG chloride was used to replace NaCl iso-osmotically). TEA transport mediated specifically by rOCTN1 (▲) was calculated by subtracting transport measured in pSPORT-transfected cells from transport measured in rOCTN1 cDNA-transfected cells.

rOCTN1 cDNA was functionally expressed in HRPE cells using this technique and the transport function was monitored by measuring the cellular uptake of TEA, a prototypical organic cation. Endogenous TEA uptake was assessed under identical conditions using cells transfected with vector alone. Uptake was measured in an Na^+-free medium at pH 7.5. Uptake of TEA (20 μM) was found to be several-fold higher in rOCTN1 cDNA-transfected cells than in vector-transfected cells (Fig. 3, upper panel). When the cDNA-specific uptake alone was considered, the uptake was linear at least up to 60 min (Fig. 3, lower panel). At 60 min, the increase in uptake in cDNA-transfected cells over vector-transfected cells was 4.5-fold. The cDNA-specific uptake was found to be influenced significantly by the pH of the uptake medium. The uptake increased gradually when the pH of the medium was changed from 6.5 to 8.0 (Fig. 4). When the pH was increased further to 8.5,

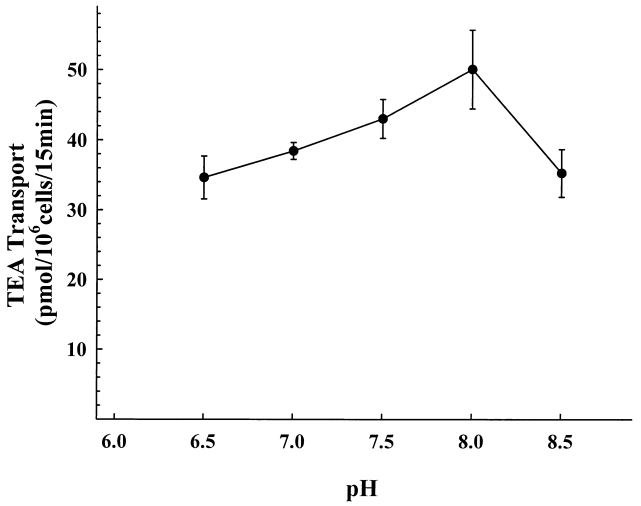


Fig. 4. Influence of pH on rOCTN1-mediated TEA transport. Transport of 20 μM [^{14}C]TEA was measured in rOCTN1 cDNA-transfected HRPE cells and in pSPORT vector-transfected HRPE cells in parallel at different pH in the absence of Na^+ for 15 min. Data represent rOCTN1-specific transport, calculated by subtracting transport in vector-transfected cells from transport in cDNA-transfected cells.

Table 1
Inhibition of rOCTN1-mediated TEA uptake by various organic cations

Inhibitor	[^{14}C]TEA transport	
	pmol/10^6 cells/15 min	%
Control	65.41 ± 6.13	100
Choline (5 mM)	63.10 ± 1.73	97
Cimetidine (5 mM)	10.91 ± 5.57	17
Guanidine (5 mM)	64.30 ± 9.12	98
MPP^+ (5 mM)	40.61 ± 5.63	62
Nicotine (5 mM)	19.12 ± 2.76	29
Procainamide (5 mM)	12.87 ± 6.68	20
Verapamil (5 mM)	13.25 ± 4.22	20
Amiloride (1 mM)	51.56 ± 3.32	79
Imipramine (1 mM)	19.92 ± 0.41	31
Desipramine (1 mM)	10.49 ± 1.13	16
DMA (1 mM)	4.04 ± 0.37	6

HRPE cells were transfected with rOCTN1 cDNA. [^{14}C]TEA (20 μM) transport in these cells was measured for 15 min in the absence of Na^+ and in the absence or presence of indicated concentrations of various unlabeled organic cations. Transport measured in vector-transfected cells was subtracted from transport in cDNA-transfected cells to calculate rOCTN1-specific transport.

the uptake decreased. Based on these data, subsequent uptake measurements were made at pH 8.0 using a 15 min incubation period.

The specificity for organic cations for the transport process mediated by rOCTN1 was studied using competition experiments in which the ability of various unlabeled organic cations (1 or 5 mM) to compete with [^{14}C]TEA for the uptake process was assessed (Table 1). 5-(*N*,*N*-dimethyl)amiloride (DMA), desipramine, cimetidine, procainamide and verapamil were found to be the most potent inhibitors, causing 80–90% inhibition. Nicotine, imipramine and 1-methyl-4-phenylpyridinium (MPP) showed moderate inhibition (40–70%), whereas choline, guanidine and amiloride were the least effective inhibitors (3–20%). These data indicate a broad specificity for rOCTN1 in the transport of organic cations. We then studied the dose–response relationship for the inhibition of rOCTN1-mediated TEA uptake by the potent inhibitors DMA, desipramine, cimetidine and procainamide (Fig. 5). The order of inhibitory potency was: desipramine > DMA > procainamide > cimetidine. The IC_{50} values (concentration of compounds necessary to cause 50% inhibition of TEA

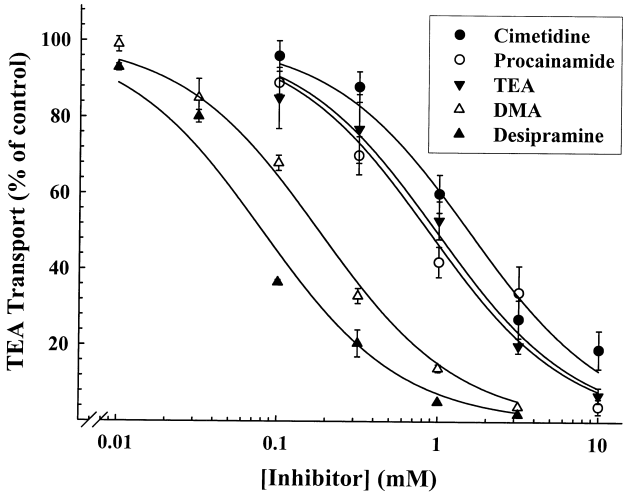


Fig. 5. Dose–response relationship for the inhibition of rOCTN1-mediated transport of [^{14}C]TEA by various organic cations. Transport of 20 μM [^{14}C]TEA was measured in rOCTN1-expressing HRPE cells at pH 8.0 in an Na^+-free medium for 15 min in the absence or presence of increasing concentrations of desipramine (▲), DMA (△), procainamide (○), TEA (▼), and cimetidine (●). Transport was adjusted for endogenous transport activity (i.e. transport in vector-transfected cells) to determine rOCTN1-specific transport. Transport in the absence of inhibitors was taken as control (100%).

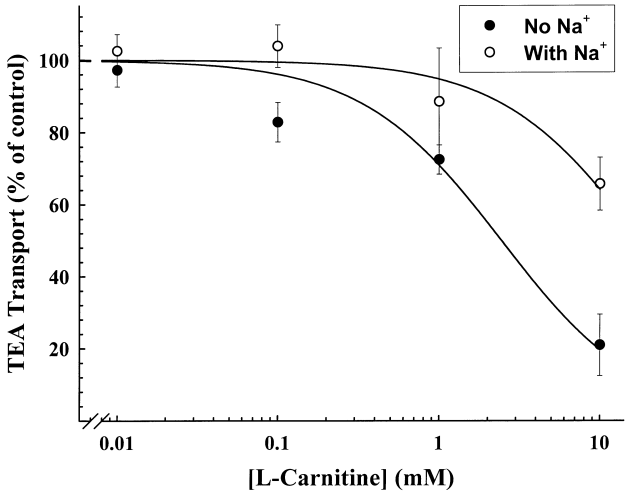


Fig. 6. Influence of carnitine on rOCTN1-mediated TEA transport in the presence or absence of Na^+. Transport of 20 μM [^{14}C]TEA was measured in rOCTN1-expressing HRPE cells at pH 8.0 in a medium containing NaCl (○) or in a medium in which NaCl was replaced iso-osmotically with NMDG chloride (●) for 15 min. Measurements were made in the absence or presence of increasing concentrations of carnitine. Transport was adjusted for endogenous transport activity (i.e. transport in vector-transfected cells) to determine rOCTN1-specific transport. Transport in the absence of carnitine was taken as control (100%).

uptake) for these compounds were 0.08 ± 0.01, 0.18 ± 0.01, 0.86 ± 0.16, and 1.54 ± 0.20 mM, respectively. The dose–response relationship was also studied for TEA by assessing the inhibition of the uptake of [^{14}C]TEA by increasing concentrations of unlabeled TEA. Unlabeled TEA was found to compete with [^{14}C]TEA for the uptake process with an IC_{50} value of 0.96 ± 0.10 mM.

3.3. Interaction of carnitine with rat OCTN1

The functional studies described thus far show convincingly that rOCTN1 is an organic cation transporter. However, OCTN1 is structurally related closer to OCTN2 than to OCT1, OCT2 and OCT3. While OCT1, OCT2 and OCT3 function only as Na^+-independent organic cation transporters, OCTN2 functions as a Na^+-independent organic cation transporter as well as an Na^+-dependent carnitine transporter [7,14,15,20]. This is true with OCTN2 cloned from different animal species (human, rat, and mouse) [15,20]. Therefore, we investigated in the present study the interaction of carnitine with rat OCTN1. The uptake of [^{14}C]TEA mediated by rat OCTN1 was measured in the presence and absence of Na^+ and the influence of carnitine on this uptake was assessed (Fig. 6). In the absence of Na^+, carnitine was found to inhibit rOCTN1-mediated TEA uptake to a significant extent, but only at concentrations >1 mM. Interestingly, the inhibitory potency was weaker in the presence of Na^+. Thus, carnitine does appear to interact with rOCTN1, though with a low affinity, but the interaction does not require Na^+. These data are different from those obtained with OCTN2 in our previous studies [15,20]. OCTN2 transports TEA in the presence as well as in the absence of Na^+ as does rOCTN1, but the ability of carnitine to inhibit OCTN2-mediated TEA uptake is markedly potentiated in the presence of Na^+, indicating that the interaction of carnitine with OCTN2 is Na^+-dependent. In contrast, the interaction of carnitine with rOCTN1 is Na^+-independent. We then investigated the ability of rOCTN1 to transport carnitine (Table 2). The uptake of car-

Table 2
Comparison of carnitine transport in vector-transfected HRPE cells and rOCTN1 cDNA-transfected HRPE cells

Carnitine concentration	Carnitine transport (pmol/10^6 cells/15 min)			
	Na^+-free medium		Na^+-containing medium	
	pSPORT	rOCTN1 cDNA	pSPORT	rOCTN1 cDNA
2.5 μM	11 ± 1	11 ± 1	23 ± 1	22 ± 2
0.5 mM	2697 ± 107	2897 ± 107	2690 ± 216	3207 ± 78
1 mM	4610 ± 341	4480 ± 169	4887 ± 560	4073 ± 463

HRPE cells were transfected with either pSPORT vector alone or rOCTN1 cDNA. Carnitine transport in these cells was measured for 15 min in the presence and absence of Na^+. Uptake buffers contained indicated concentrations of carnitine along with 20 nM [^{3}H]carnitine.

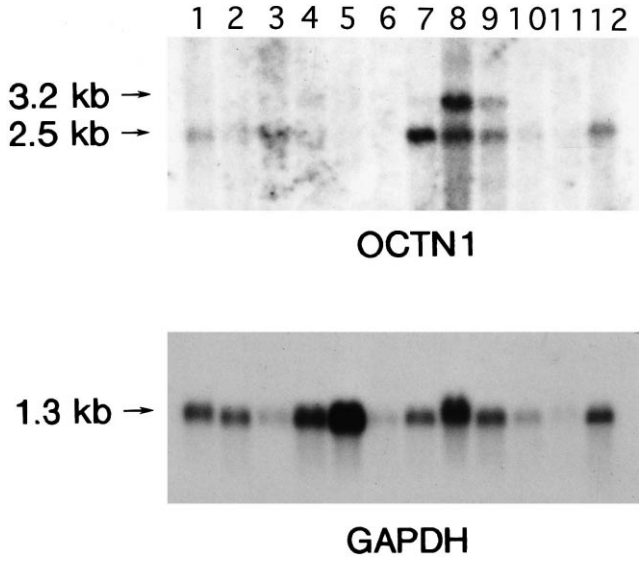


Fig. 7. Northern blot analysis of rOCTN1-specific transcripts in rat tissues. The upper panel is the hybridization signals obtained with the rOCTN1 cDNA probe and the lower panel is the hybridization signals obtained with the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe. Lanes 1–12 represent brain, thymus, lung, heart, skeletal muscle, stomach, small intestine, liver, kidney, spleen, testis, and skin, respectively.

nitine in control cells and in rOCTN1-expressing cells was compared in the presence and absence of Na^+ at three different concentrations of carnitine. At all three concentrations (2.5 μM, 0.5 mM, and 1 mM), there was no detectable level of carnitine transport that was mediated by rOCTN1.

Taken collectively, these results show that rOCTN1 is an organic cation transporter and that, though structurally related to OCTN2 very closely, rOCTN1 does not mediate Na^+-coupled carnitine transport to any significant level.

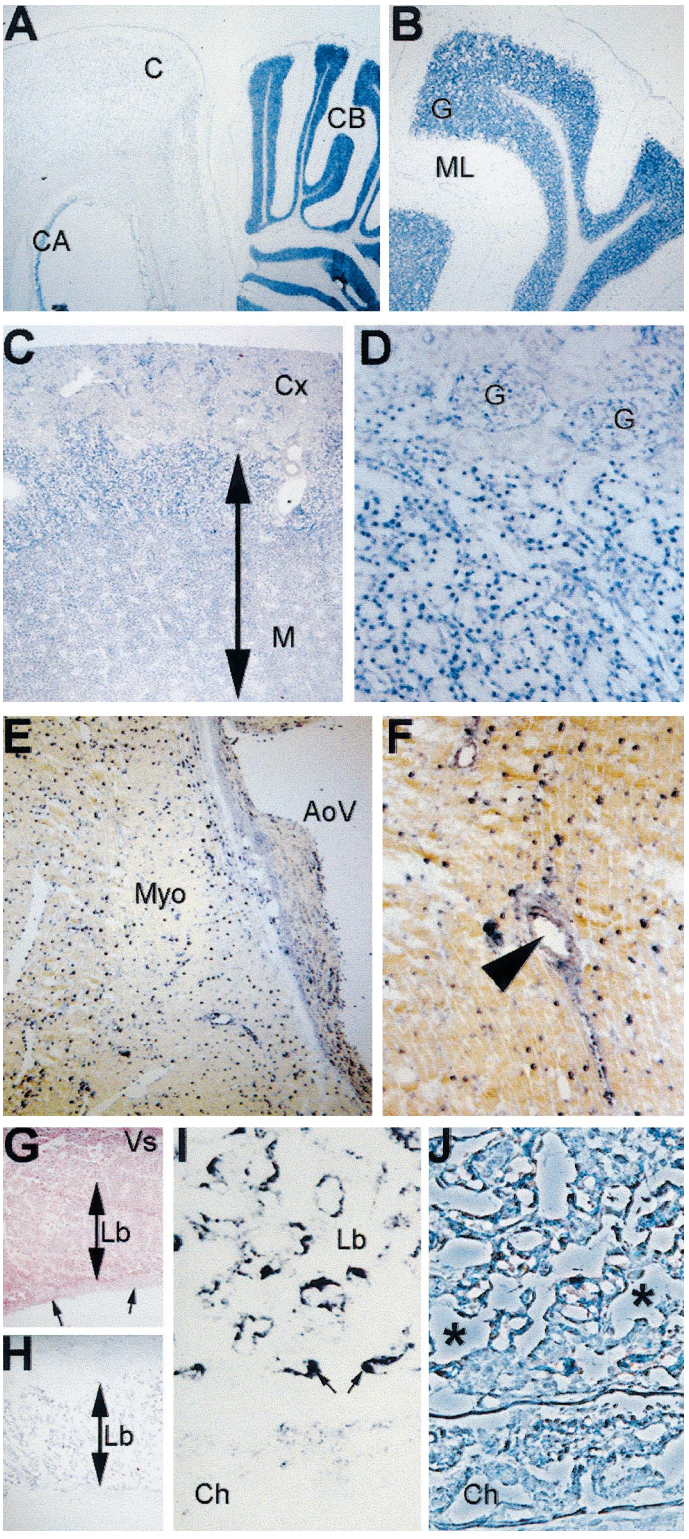
3.4. *Tissue distribution pattern of OCTN1 mRNA in the rat*

Northern blot analysis was carried out with poly-$(A)^+$ RNA isolated from 12 different tissues from the rat to determine the tissue distribution pattern of OCTN1 in rat (Fig. 7). The OCTN1-specific transcripts are detectable in a wide variety of tissues with abundant expression in the intestine, liver, and kidney. Comparatively lower levels of expression are evident in the brain, thymus, lung, heart, spleen, and skin. The transcripts are below detectable levels in the skeletal muscle, stomach, and testis. There are two distinct transcripts that are 2.5 kb and 3.2 kb in size. The 2.5 kb transcript is present in all of the positive tissues whereas the levels of expression of the 3.2 kb transcript vary among these tissues. Maximal expression of the 3.2 kb transcript is seen in the liver.

We then performed in situ hybridization with tissue sections from rat brain, kidney, heart, and placenta using a rat OCTN1-specific antisense riboprobe to determine the regional distribution of OCTN1 mRNA in these tissues (Fig. 8). In the brain, OCTN1 mRNA is most abundantly expressed in cerebellum, especially in the granular layer. Lesser, but detectable, levels of expression are also seen in the molecular layer of the cerebellum. The expression at low levels is also detectable throughout the cerebral cortex and in the cornu ammonis pyramidal neurons of the hippocampus. In the kidney, the expression is evident in the cortical layer as well as in the medullary layer, but the expression is most abundant in the outer stripe of the outer medulla. The glomeruli are also positive for OCTN1 expression. Even though the mRNA is detectable in the absorptive cells of both proximal and distal tubules, the expression is more prominent in the straight portion of the proximal tubules. In the heart, OCTN1 mRNA expression is seen in the myocardium, lamina fibrosa of the heart valves and the blood vessels. In the placenta, expression is detectable throughout the labyrinthine zone where it is found in the villous structures surrounding the lacuna filled with maternal blood. Very low levels of expression are also seen in the visceral yolk sac and chorionic plate. There were no detectable hybridization signals in any of these tissues when a rat OCTN1-specific sense riboprobe was used instead of the antisense riboprobe (data not shown).

4. Discussion

OCTN1 was originally cloned from human liver [6]. The human OCTN1 was found to be structurally related to the known members of the organic cation transporter family, but the homology was much less compared to the homology found among the known members of the family. This led the investigators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who cloned the transporter to name OCTN1 as a novel organic cation transporter. Human OCTN1 served as a probe in the isolation of a second human novel organic cation transporter OCTN2 [7]. hOCTN1 and hOCTN2 are very closely related to each other in primary structure. Thus, these two members constitute a distinct subfamily within the group of organic cation transporters. The remaining members (OCT1, OCT2, and OCT3) form another subfamily based on the primary structure.

The transport function of hOCTN1 has been studied in detail [6,13]. It mediates the transport of a wide variety of organic cations in a pH-dependent manner. The transport process is electroneutral as evidenced from the lack of any detectable effect of K^+-induced membrane depolarization on hOCTN1-mediated transport of organic cations in *Xenopus laevis* oocytes expressing hOCTN1 heterologously [13]. It is believed that OCTN1 may be the organic cation/H^+ antiporter that has been described in the kidney brush border membrane vesicles [13]. Interestingly, hOCTN1 also transports carnitine, a zwitterion, but whether this transport occurs via an Na^+-coupled process or the cation species of carnitine is preferentially transported has not been investigated [13].

The present investigation was undertaken to elucidate the structure, function, and tissue distribution pattern of the rat homolog of OCTN1. To date, OCTN1 has not been cloned from any animal species other than human. Since most of the information available in the literature on the characteristics of organic cation transport in the kidney or other tissues has been derived using the rat as the animal model, elucidation of the structure, function, and tissue distribution of OCTN1 in the rat is of importance for comparative purposes. The results of the present investigation show that rat OCTN1 is structurally very close to human OCTN1 and that rat OCTN1 interacts with a wide variety of organic cations as does human OCTN1. However, there are some significant differences between rat and human OCTN1 in substrate specificity. Rat OCTN1 does not interact with choline whereas human OCTN1 does. Verapamil exhibits much higher affinity for human OCTN1 than for rat OCTN1. Similarly, there are also differences in the interaction of carnitine with these two transporter homologs. The transport of TEA mediated by human OCTN1 is inhibited by carnitine with an IC_{50} (i.e. concentration needed for 50% inhibition) of <1 mM in the presence of Na^+. Moreover, human OCTN1 recognizes carnitine as a transportable substrate. In contrast, rat OCTN1 interacts with carnitine with a very low affinity. In the presence of Na^+, carnitine at a concentration of 1 mM does not inhibit rat OCTN1-mediated TEA transport. A significant inhibition is observed however at carnitine concentrations >1 mM. Interestingly, the carnitine-induced inhibition is greater in the absence of Na^+ than in the presence of Na^+. This shows that the interaction of rat OCTN1 with carnitine is not Na^+-dependent. In addition, even though carnitine interacts with rat OCTN1 at high concentrations as assessed by the ability of carnitine to inhibit TEA transport mediated by rat OCTN1, carnitine is not a transportable substrate for this transporter. In the presence as well as in the absence of Na^+, there was no detectable carnitine transport via rat OCTN1. This was not due to the low affinity of the transporter for carnitine because the transport measurements were made at a wide range of carnitine concentrations. We conclude that rat OCTN1 does

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Fig. 8. Detection of OCTN1 mRNA transcripts in rat tissues by in situ hybridization with rOCTN1-specific digoxigenin-labeled antisense riboprobe. A and B (low power and high power, respectively) represent hybridization signals in the brain. Positive signals are evident in cortex (C), cornu ammonis pyramidal neurons of hippocampus (CA) and granular (G) and molecular (ML) layers of cerebellum (CB). C and D (low power and high power, respectively) represent hybridization signals in the kidney. Expression is positive in cortex (Cx) and medulla (M), with highest levels of expression in the outer stripe of the outer medulla. Positive signals are present in glomeruli (G) and in proximal and distal tubules. E and F (low power and high power, respectively) represent hybridization signals in the heart. Positive signals are seen in myocardium (Myo) and in lamina fibrosa of heart valves (AoV). Expression is evident throughout the myocardium as well as in intermuscular arterioles (arrowhead). H, I, and J represent hybridization signals in the placenta and G represents hematoxylin-eosin staining of the placenta. Positive signals are detectable in the labyrinthine zone (Lb) and the signals are comparatively much weaker in the venous sinusoids (Vs) and in the chorionic plate (Ch). Expression is abundant in the villous structures surrounding the lacuna filled with maternal blood (arrows in I and asterisk in J).

not transport carnitine and that it does exhibit significant differences in substrate specificity with human OCTN1.

The present studies describe, for the first time, the tissue distribution pattern of OCTN1. The signals obtained with the rat OCTN1 probes in Northern blot analysis and in in situ hybridization analysis are specific and do not represent cross-hybridization with OCTN2 mRNA due to sequence homology. This is supported by the following reasonings and observations. First, hybridization was carried out under high stringency conditions and therefore it is unlikely that the probes cross-hybridize with OCTN2 mRNA. Second, the predominant signal in Northern blot analysis is 2.5 kb in size in the case of OCTN1 in contrast to the 3.0 kb predominant signal for rat OCTN2 [21]. Furthermore, the tissue distribution pattern is quite different for OCTN1 (present study) and OCTN2 [21] in rat tissues. OCTN1 is expressed most abundantly in the intestine, liver, and kidney whereas OCTN2 is expressed most abundantly in the testis, colon, kidney and liver. Only a weak expression is noted in the intestine for OCTN2. Third, there are significant differences in the regional distribution pattern of OCTN1 mRNA and OCTN2 mRNA in rat tissues. The present study shows that OCTN1 mRNA is expressed in the rat kidney more abundantly in the cortico-medullary junction than in the cortex, suggesting a preferential expression in the proximal straight tubules. On the other hand, OCTN2 mRNA is expressed primarily in the cortical region, suggesting a preferential expression in the proximal and distal convoluted tubules [15]. In addition, OCTN2 mRNA is present at much higher levels in the Purkinje cell bodies than other regions in the cerebellum [15] while no such differential expression is noted in the Purkinje cell bodies for OCTN1 mRNA (present study).

OCTN1 is expressed very abundantly in the intestine, kidney and liver where it is likely to play a role in the handling of xenobiotics. The observed findings that OCTN1 is also expressed in the brain and the placenta are of pharmacological importance because of the ability of OCTN1 to interact with a broad spectrum of pharmacologically active drugs. This suggests that OCTN1 might influence the disposition of various drugs in these tissues. It is not clear whether OCTN1 participates in the influx of drugs into the cells or in the efflux of drugs out of the cells. If OCTN1 is indeed an organic cation/H^+ antiporter as has been suggested for human OCTN1, this transporter is likely to participate in the efflux of drugs. It is however to be noted that such an efflux process is expected to be located in the brush border membrane of the placental trophoblast. But, our previous studies have failed to detect an organic cation/H^+ antiport process for TEA, an OCTN1 substrate, in human placental brush border membrane vesicles [22,23]. Furthermore, Northern blot analysis and in situ hybridization studies show that OCTN1 is expressed widely in different organs. But, the organic cation/H^+ antiport process has been demonstrated primarily in the kidney, liver, intestine and placenta [24,25]. An additional finding relevant to this issue is the expression of OCTN1 predominantly in the junctional zone between the medulla and the cortex in the kidney. Drug elimination via the organic cation/H^+ antiport process occurs predominantly in the proximal convoluted tubules that are located in the cortex [24,25]. More studies are needed to establish unequivocally whether or not OCTN1 is an organic cation/H^+ antiporter.

OCTN2, which is very closely related to OCTN1 in amino acid sequence, is an Na^+-independent organic cation transporter as well as an Na^+-dependent carnitine transporter. It is clear that OCTN1 is an Na^+-independent organic cation transporter. Whether or not OCTN1 is an Na^+-dependent transporter for any physiological substrate remains to be seen. Interestingly, the tissue distribution pattern of OCTN1 and OCTN2 is strikingly similar with some notable exceptions. While the distribution pattern of mRNA is exactly the same for both transporters in the placenta and heart, there are significant differences in the regional distribution of mRNA for these transporters in the kidney and brain ([15], present study). The physiological and pharmacological implications of the observed similarities and differences in the tissue distribution pattern and substrate specificity of the two closely related transporters OCTN1 and OCTN2 remain to be seen.

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