A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane

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König, Jörg, Yunhai Cui, Anne T. Nies, and Dietrich Keppler. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G156-G164, 2000.—We cloned and expressed a new organic anion transporting polypeptide (OATP), termed human OATP2, (OATP-C, LST-1; symbol SLC21A6), involved in the uptake of various lipophilic anions into human liver. The cDNA encoding OATP2 comprised 2073 base pairs, corresponding to a protein of 691 amino acids, which were 44% identical to the known human OATP. An antibody directed against the carboxy terminus localized OATP2 to the basolateral membrane of human hepatocytes. Northern blot analysis indicated a strong expression of OATP2 only in human liver. Transport mediated by recombinant OATP2 and its localization were studied in stably transfected Madin-Darby canine kidney strain II (MDCKII) and HEK293 cells. Confocal microscopy localized recombinant OATP2 protein to the lateral membrane of MDCKII cells. Substrates included 17β-glucuronosyl estradiol, monoglucuronosyl bilirubin, dehydroepiandrosterone sulfate, and cholytaurine. 17β-Glucuronosyl estradiol was a preferred substrate, with a Michaelis-Menten constant value of 8.2 µM; its uptake was Na⁺ independent and was inhibited by sulfobromophthalein, with an inhibition constant value of 44 nM. Our results indicate that OATP2 is important for the uptake of organic anions, including bilirubin conjugates and sulfobromophthalein, in human liver.

bilirubin conjugates; 17β-glucuronosyl estradiol; hepatic transport; SLC21A6; sulfobromophthalein

The removal of endogenous and xenobiotic substances from blood is one major function of the liver, and a number of different uptake and export systems are involved in this process. In contrast to the canalicular membrane domain, where mostly export pumps are located (22, 41), the basolateral membrane exhibits a variety of uptake transporters (34), in addition to some export pumps (24, 25). On the basis of transport properties and sequence similarities, at least two different families of uptake transporters are present in the basolateral hepatocyte membrane. The Na⁺-taurocholate cotransporting polypeptides cloned from human (12) and rat (13) mediate the uptake of bile salts in a Na⁺-dependent manner. In contrast to the Na⁺-taurocholate cotransporting polypeptides, members of the organic anion transporting polypeptide (OATP)/prostaglandin transporter family differ much more with respect to their substrate specificity and tissue distribution. Until now, eight members of this family have been cloned: human OATP (28), rat OATP1 (15), rat OATP2 (35), rat OATP3 (1), the prostaglandin transporters human PGT (31, 32) and rat PGT (21), and the two kidney organic anion transporters OAT-K1 (36) and OAT-K2 (33). All of these Na⁺-independent carrier systems exhibit a broad substrate specificity. As determined in several expression systems, transported substrates include 17β-glucuronosyl estradiol (E₂17βG) (7, 20), sulfobromophthalein (BSP) (15, 19, 28), estrone 3-sulfate, ochratoxin A (23), dehydroepiandrosterone sulfate (DHEAS; 27), and cholytaurine (19, 28). From all members, rat OATP1 and human OATP are the ones best characterized with respect to their transport properties using the Xenopus laevis oocyte expression models and stably transfected mammalian cells. Rat OATP1 (originally termed OATP) has been localized to the basolateral membrane of hepatocytes as well as to the apical membrane domain of the S3 segment of the kidney proximal tubule epithelia (5). In addition, OATP1 is present in the choroid plexus of rat brain (4). Human OATP is highly expressed in brain, in addition to lung, liver, kidney, and testis (28). Although human OATP was cloned on the basis of sequence information obtained from rat OATP1 and both proteins share a similar substrate specificity, marked differences were found with respect to transport rates and apparent Michaelis-Menten constant (Kₘ) values for different substrates (7, 34). Together with the different expression pattern, these findings suggested that human OATP is related but not orthologous to rat OATP1. Therefore, we searched the expressed sequence tag (EST) library to obtain sequence information on as yet unknown members of the OATP family. We cloned the full-length cDNA encoding a new human OATP-related protein and localized it to the basolateral membrane of human hepatocytes. Two stably transfected mammalian cell lines served to determine transport characteristics of the recombinant protein. On the basis of amino acid sequence identity and the identified substrates, we named this new member of the human organic anion transporter family OATP2 (SLC21A6).

MATERIALS AND METHODS

Materials. Pepstatin, leupeptin, aprotinin, agar, FCS, and the protein standard mixture (relative molecular weight of 26,600–180,000) for the SDS-PAGE were from Sigma (Deisenhofen, Germany). Lysozyme and ampicillin were from Boehringer Mannheim (Mannheim, Germany); agarose was from...
Rothe (Karlsruhe, Germany). RNase inhibitor (RNAGuard), Stratsat Tissue Northern blot (MTN) and β-actin primers were from Clontech (Heidelberg, Germany). Marathon-Ready cDNA, Advantage cDNA polymerase mix, and the human 12-lane multiple tissue Northern blot (MTN) and β-actin primers were from Clontech (Heidelberg, Germany). Marathon-Ready cDNA, Advantage cDNA polymerase mix, and the human 12-lane multiple tissue Northern blot (MTN) and β-actin primers were from Clontech. 

**Antibodies.** The ESL antibody was raised in rabbits against the 21 amino acids at the carboxy terminus of the deduced OATP2 cDNA sequence (ESLNKKNKHFVPSAGADSETHC). The peptide was synthesized automatically, coupled to maleimide-activated keyhole limpet hemocyanin, and the rabbits were immunized with this conjugate. The mouse monoclonal antibody OKT9 against the human transferrin receptor (42) was purchased from Progen (Heidelberg, Germany). The mouse monoclonal antibody against desmoplakin (cocktail) was purchased from Dianova (Hamburg, Germany).

**Cloning of the human OATP2 cDNA.** On the basis of the sequence information of a 392-bp cDNA clone (EMBL/ GenBank accession number H62893), which exhibits 70.4% sequence identity to human OATP over 240 aligned base pairs, one reverse primer was designed and subjected to a 5’ rapid amplification of cDNA ends (RACE) reaction using the Marathon-Ready cDNA kit (Clontech). In detail, a gene-specific primer was designed (oOATP2.rev 5'-CCATGAAGAAATGTG-CATGGCATGG-GCAAGAGC-3') and the PCR was performed in a volume of 50 µl containing 5 µl Marathon-Ready cDNA, 0.2 µM sense primer AP1 (delivered with the kit), 0.2 µM anti-sense primer oOATP2.rev, 5 µl of 10× PCR buffer (400 mM tricine-KOH, pH 9.2, 150 mM potassium acetate, 750 mg/ml BSA), 0.2 mM deoxynucleoside triphosphates, and 1 µl of Advantage cDNA polymerase mix (Clontech), 50 mM NaCl, 10 mM Tris·HCl, pH 7.5, 50 mM KCl, 25 mM (NH₄)₂SO₄, 0.1 mM EDTA, 5 mM β-mercaptoethanol, 0.25% Thesit, 11 µg/ml TaqStart antibody, and Klen-Taq DNA polymerase] under the following PCR cycling conditions: 2 min denaturation at 94°C followed by 5 cycles of 10-s denaturation at 94°C, annealing/elongation for 3 min at 70°C, 5 cycles with 10-s denaturation at 94°C, 3 min annealing/ elongation at 68°C, and, subsequently, 25 cycles with 10-s denaturation at 94°C and annealing/elongation for 3 min at 66°C. The reaction was finished by 10 min at 72°C. The amplified fragment was subcloned into pCR2.1 TOPO (Invitrogen, BV, Groningen, The Netherlands) and sequenced. On the basis of this sequence information, a forward primer was designed in the 5’-untranslated region of the human OATP2 cDNA clone and used in a 3’-RACE reaction to amplify the complete OATP2 cDNA. This 3’-RACE reaction was also performed using the Marathon-Ready cDNA kit according to the manufacturer’s instructions with the gene-specific OATP2 primer OATP2.5’ for (5’-TGTTGTTCATGAGCATCAACAC-3’) under the same PCR cycling conditions as described for the 5’-RACE. The amplified fragment of ~2.7 kb was subcloned into the vector pCR2.1 TOPO (Invitrogen), resulting in the plasmid pOATP2.TOPO, which was sequenced by 4Base Lab (Reutlingen, Germany). For subcloning of the OATP2 cDNA into the expression vector pcDNA3.1 (+) (Invitrogen), the plasmid pOATP2.TOPO was digested with BsrXI and the OATP2 fragment was cloned into the BstX I-digested and dephosphorylated vector pcDNA3.1 (+), resulting in the plasmid pOATP2.31. On completion of the plasmid, the correctness of the restriction sites and the orientation of the cDNA were verified by sequencing.

**Northern blot analysis.** The Northern blot analyses were performed using the commercial human 12-lane MTN blot (Clontech). For the β-actin control, a human β-actin cDNA fragment, supplied with the Northern blot, and for OATP2, the 659-bp Hind III restriction fragment (bp 214–872) were used as probes. The membrane was prehybridized for 2 h at 42°C in 10 ml of hybridization buffer [6× sodium chloride-sodium citrate (SSC), 0.5% SDS, 5× Denhardt’s solution, 50% formamide, 100 µg/ml denatured salmon sperm DNA] without and hybridized with the labeled DNA fragments for 18 h under the same conditions. Nick translation was performed by use of the Rediprime DNA labeling system (Amersham-Pharmacia, Freiburg, Germany) according to the manufacturer’s instructions. The labeled DNA fragments were purified using NucTrap probe purification columns (Stratagene). After hybridization, the membrane was washed once in 2× SSC-0.1% SDS for 20 min at 42°C, once in 1× SSC-0.1% SDS, and once in 0.5× SSC-0.1% SDS. Both washing steps were carried out for 20 min at 55°C. The blot was air dried, and autoradiography was performed at −80°C with an intensifying screen for 24 h (OATP2) and 18 h (β-actin).

**DNA sequencing.** With the use of the T7 sequencing kit from Amersham-Pharmacia and [γ-32P]dATP, the cDNA clones were sequenced according to the deoxynucleotide chain termination method of Sanger et al. (37). Dried gels were exposed to Kodak BioMax MR-1 films (Sigma).

**Computer analysis.** The HUSAR program (38), based on the Wisconsin Genetics Computer Group program package (10), was used during this study for restriction mapping, sequence analyses, and sequence alignments.

**Cell culture and transfection studies.** HEK293 (human embryonic kidney) and Madin-Darby canine kidney strain II (MDCKII) cells were cultured in minimum essential medium (Sigma), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin, at 37°C and 5% CO₂.

Cells were transfected using the polybrene (hexadimethrine bromide) method (8). Briefly, exponentially growing cells were incubated in a 10-cm petri dish with 10 µg plasmid DNA and 30 µg polybrene in 3 ml of complete medium for 8 h under normal culture conditions. Cells were then incubated with 5 ml of 30% DMSO in complete medium at room temperature for 5 min. The DMSO mixture was then removed, and cells were washed twice with complete medium and cultured overnight before starting Geneticin (G418) selection. After 3 wk of G418 selection (600 µg/ml), single colonies were screened for OATP2 expression by immunoblot analysis and immunofluorescence microscopy. Expression of recombinant OATP2 was further enhanced by culturing transfected cells with 10 µM sodium butyrate (9).

Preparation of membrane vesicles. Sinusoidal membrane vesicles from human liver (6) and crude membrane fractions from cultured cells were prepared as described earlier (9).

Tissue samples and immunofluorescence studies. Tissue samples for immunofluorescence studies were obtained peroperatively as described recently (25). Moreover, frozen sections for immunofluorescence microscopy were prepared as described in detail (25). All antibodies were diluted with PBS (140 mM NaCl, 10 mM phosphate, pH 7.4) supplemented with 5% FCS at the following dilutions: ESL at 1:100, OKT9 and CD26 at 1:50, anti-desmoplakin (cocktail) at 1:20, and Cy2-conjugated anti-rabbit IgG and Cy3-conjugated anti-rabbit IgG and Cy3-conjugated anti-mouse antibodies were from Dianova (Hamburg, Germany).
mouse IgG at 1:400. Fluorescence microscopy was performed on an Axiovert S100TV microscope (Carl Zeiss, Jena, Germany) equipped with a video camera (Hamamatsu Photonics, Hamamatsu, Japan). Captured files were analyzed with the Openlab imaging software (Improvision, Coventry, UK).

Immunoblot analysis. Membrane fractions were diluted with sample buffer and incubated at 37°C for 30 min before separation on 4% stacking and 10% resolving SDS polyacrylamide gels. Immunoblotting was performed using a tank blotting system from Bio-Rad (Munich, Germany) and enhanced chemiluminescence detection (NEN Life Science Products, Boston, MA). Primary antibody (ESL) was diluted 1:5,000 in 10 mM Tris·HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween 20. The secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) used at a 1:2,000 dilution.

Deglycosylation. Membrane proteins (30 µg) were denatured by incubation with 1% SDS in a total volume of 10 µl at 37°C for 30 min. The denatured proteins were added to 100 µl of digestion buffer (17 mM NaH2PO4, 33 mM Na2HPO4, 0.2 mM Na4EDTA, and 1.5% N-octylglucoside at pH 7.5) in the presence or absence of 5 units of peptide N-glycosidase F (EC 3.5.1.52). After overnight incubation at 37°C, the samples were analyzed by immunoblot analysis.

Immunofluorescence microscopy of transfected cells. Transfected HEK293 or MDCK cells were grown polarized on Transwell membrane inserts (pore size of 3 µm; Costar, Cambridge, MA). Sodium butyrate was added to the culture medium 24 h after the transfection (9). After fixation with 4% paraformaldehyde in PBS for 10 min and permeabilization in 1% Triton X-100 in PBS for 10 min, cells were incubated with the polyclonal antibody ESL (diluted 1:50 in PBS) for 30 min at room temperature. Cells were then washed three times with PBS and incubated with Cy2-conjugated goat anti-rabbit IgG (diluted 1:200 in PBS) for 30 min at room temperature. Nuclei were stained with propidium iodide (0.2 µg/ml) added into the dilution of the secondary antibody. Membranes were cut from the inserts and mounted onto slides with 50% glycerol in PBS. Confocal laser-scanning immunofluorescence microscopy was performed using a LSM-410 apparatus from Carl Zeiss as described previously (9).

Transport assays. Transfected HEK293 cells were seeded in six-well plates (coated with 0.1 mg/ml poly-d-lysine) at a density of 10⁶ cells per well and cultured with 10 mM sodium butyrate for 24 h. For uptake studies, cells were first washed with uptake buffer (142 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 1 mM KH2PO4, 1.2 mM MgSO4, 1.5 mM CaCl2, 5 mM glucose, and 12.5 mM HEPES, pH 7.3) and then incubated with 1 µ1 of uptake buffer containing the tritium-labeled substrate. For inhibition studies, inhibitors were included in the uptake buffer at different concentrations. After incubation for 3 min at 37°C, the substrate was removed, and cells were washed three times with cold uptake buffer before they were lysed with 1 ml of 0.1% SDS in water. The cell-associated radioactivity was determined by transferring 250-µl aliquots of the lysate to scintillation vials and counting radioactivity using a Beckman scintillator (LS 6000IC, Beckman Instruments, Munich, Germany). Protein content was determined according to Lowry using 100 µl of lysate. Uptake was measured at concentrations between 1 nM and 100 µM E217G for determination of Km and maximal velocity (Vmax) values.

RESULTS

Cloning of the cDNA encoding human OATP2 (SLC21A6). A GenBank search against the human OATP cDNA sequence revealed an EST sequence (EMBL/GenBank accession number H62893) that exhibits 70.4% identity over 240 aligned base pairs. On the basis of this sequence information, the complete cDNA encoding the human OATP2 protein was cloned by a combination of a 5’-RACE and a 3’-RACE PCR. The coding region covers 2073 bp, and the region around the ATG translational start codon fulfilled the Kozak consensus sequence (26) with an invariant A at position −4 and an invariant T at position −2. The nucleotide base pair identities of the coding region to human OATP (28), rat OATP1 (15), rat OATP2 (35), and rat OATP3 (1) were 60.7%, 60.1%, 57.6%, and 58.8%, respectively, calculated under default parameters of the BESTFIT program of the HUSAR program package (38).

Analysis of the deduced amino acid sequence of OATP2. The open reading frame of 2073 bp encodes a deduced protein of 691 amino acids with a calculated molecular mass of 76,446 Da. The amino acid identity to the previously known human OATP isoform, calculated under default parameters of the BESTFIT program of the HUSAR program package (38), is 44%, and is 44%, 46%, and 46% to the rat orthologs OATP1, OATP2, and OATP3, respectively. An alignment of these four peptide sequences is shown in Fig. 1. All OATP isoforms represent glycoproteins with native molecular masses of ~80 kDa. A computer-aided transmembrane (TMHMM) analysis (40) based on a CLUSTAL alignment of all OATPs demonstrates that they consist of 12 predicted transmembrane domains (Fig. 2) with both ends of the protein located intracellularly. From 11 potential N-glycosylation sites in the predicted OATP2 amino acid sequence, six are located outside in predicted extracellular loops. In comparison, human OATP exhibits eight potential N-glycosylation sites (28) with seven of them located in extracellular loops and three of them at identical positions as the predicted N-glycosylation sites of human OATP2.

Tissue distribution of human OATP2. The tissue distribution of human OATP2 was studied by Northern blotting using the human 12-lane MTN blot and an OATP2 cDNA fragment under stringent hybridization and washing conditions. This cDNA fragment shows only 65% identity to the known human OATP, excluding cross-reactivity with this mRNA. In contrast to human OATP (or OATP1), which is highly expressed in human brain, kidney, liver, and testis (28), strong signals for human OATP2 were only detected in liver (Fig. 3). The length of the detected mRNA species was 2.8 kb, likely to correspond to the fully spliced OATP2 mRNA, and 4.5 kb, probably corresponding to a partially or unspliced mRNA. Prolonged exposure of the blot for up to 96 h revealed no additional signals in other tissues, suggesting that human OATP2 is almost exclusively expressed in human liver. Additional tissues not tested in our Northern blotting but expressing OATP2 may still be identified.

Stable expression of recombinant OATP2 in mammalian cells. OATP2 cDNA was transfected into HEK293 and MDCKII cells, and sodium butyrate was used to enhance the expression of recombinant OATP2 in these
Expression of OATP2 was verified by immunoblot analysis. As a positive control, we used a preparation of human liver basolateral membranes. The polyclonal antibody ESL detected two major bands in human liver samples (Fig. 4), one of \(84\) kDa and one of \(58\) kDa. The specificity of the ESL antibody was demonstrated by the comparison of OATP2-transfected cells with vector-transfected cells (Fig. 4). Immunoreactive bands with a molecular mass of \(84\) kDa were observed using the ESL antibody in both MDCKII and HEK293 cells transfected with OATP2 cDNA. No specific signals were observed in vector-transfected cells (Fig. 4). Deglycosylation experiments using peptide N-glycosidase F demonstrated that the relative molecular mass of the recombinant protein was reduced to \(58\) kDa, indicating that the lower band detected in human liver corresponds to the unglycosylated protein and that the \(84\)-kDa OATP2 is indeed a glycoprotein.

**Immunolocalization of OATP2 in liver and transfectants.** Incubation of cryosections from human liver with the ESL antibody revealed fluorescent staining of the lateral and the basal membrane domains of hepatocytes (Fig. 5, A and D). No expression of OATP2 was detectable in bile ductular cells (not shown), as examined by comparative localization of cytokeratin 19 as a marker for bile ductular cells (3). Strong staining was observed in all liver specimens studied. Localization of OATP2 in the basolateral membrane was confirmed by double labeling of cryosections with anti-desmoplakin (Fig. 5, E and F) and anti-transferrin receptor antibody.

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**Fig. 1.** Alignment of human organic anion transporting polypeptide OATP2 (symbol SLC21A6; EMBL/GenBank accession number AJ132573), human OATP1 (previously termed OATP; SLC21A3; U21943), rat OATP1 (L19031), rat OATP2 (U88036), and rat OATP3 (AF041105). Sequences were aligned by the CLUSTAL program from the HUSAR program package; identity scores relative to OATP2 are calculated by the BESTFIT program out of the same package. Areas highlighted in black indicate the amino acids that are identical to the amino acid of the deduced OATP2 sequence at this position. Numbers indicate the amino acid position corresponding to OATP2. Peptide sequence recognized by the polyclonal antibody ESL is indicated by the dashed line.
ies (not shown) as markers for the lateral and basal domains, respectively. OATP2 was absent from the canalicular domain (Fig. 5, A–C) as shown by double labeling of liver sections with the ESL antibody and an antibody directed against dipeptidylpeptidase IV, which is localized to the apical membrane of hepatocytes (14). No plasma membrane staining was observed when cryosections were incubated with the pre-ESL immune serum.

The cellular localization of recombinant OATP2 was studied using confocal immunofluorescence laser scanning microscopy. In both MDCKII and HEK293 cells, OATP2 was sorted to the plasma membrane (Fig. 6). In polarized MDCKII cells, OATP2 localization was restricted to the lateral membrane, consistent with its basolateral localization in hepatocytes (Fig. 5). No staining could be observed in vector-transfected MDCKII and HEK293 cells. The transfected HEK293 cells were used for the functional characterization of OATP2 because of the higher expression level compared with the transfected MDCKII cells.

**Functional characterization of OATP2.** Several candidate substrates were tested using transfected cells in whole cell uptake assays. E217βG, MGB, cholyltaurine, and DHEAS were characterized as substrates for OATP2 (Table 1). Uptake of E217βG mediated by OATP2 was Na⁺ independent. No significant reduction of the uptake rate could be observed when Na⁺ was replaced by choline. Uptake of E217βG was temperature dependent. At 4°C, the uptake rate of E217βG was reduced to 7% of that measured at 37°C. OATP2-mediated uptake of E217βG was time dependent (linear up to 5 min) and saturable (Fig. 7). Apparent $K_m$ values (8.2 µM) and $V_{max}$ values (48 pmol·mg protein$^{-1}$·min$^{-1}$) were determined by double-reciprocal plots according to Lineweaver and Burk (30).

Inhibition of OATP2-mediated E217βG uptake. Cis inhibition studies of E217βG uptake are summarized in Table 2. Extracellular glutathione and 2-oxoglutarate (tested as exchange substrates at concentrations up to 5 mM) had no significant effect on E217βG uptake. The inhibitor with the highest potency detected so far was BSP, with an IC$_{50}$ value of 50 nM. Complete inhibition was observed at 100 nM BSP (Table 2). As shown in Fig. 7B, the inhibition of E217βG uptake by BSP was competitive and exhibited an inhibition constant value of 44 nM.

**DISCUSSION**

Our study describes a new member of the OATP gene family, which was cloned on the basis of sequence similarities to human OATP. The protein encoded by this gene, now termed human OATP2, symbol SLC21A6, shows between 44% and 46% amino acid identity to the
previously known members of the OATP family: human OATP, rat OATP1, rat OATP2, and rat OATP3 (Fig. 1). On the nucleotide level, the identity score increases to between 58% and 60%, as calculated for the coding region of the gene. Analysis of the deduced amino acid sequence revealed several structural similarities between OATP2 and the other members of the OATP family. Human OATP1 (previously termed OATP) has 10–12 putative membrane-spanning domains (28). We compared the putative transmembrane domain organization of human OATP1 and OATP2, calculated by the TMHMM program package (40) (Fig. 2). This analysis revealed a very similar membrane domain organization for both transporters with 12 predicted membrane-spanning domains for each. On the basis of this putative membrane domain organization, OATP1 exhibits seven extracellular N-glycosylation sites and OATP2 exhibits six N-glycosylation sites with three of them at
Table 1. Substrate specificity of human OATP2 (SLC21A6).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Uptake, pmol·min⁻¹·mg protein⁻¹</th>
<th>Uptake Ratio of OATP2 to Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Glucuronosyl estradiol, 5 µM</td>
<td>24.6 ± 1.5</td>
<td>6.1*</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulfate, 5 µM</td>
<td>6.4 ± 0.5</td>
<td>3.2*</td>
</tr>
<tr>
<td>Cholyltaurine, 5 µM</td>
<td>2.6 ± 0.3</td>
<td>4.3*</td>
</tr>
<tr>
<td>Monoglucuronosyl bilirubin, 80 nM</td>
<td>0.8 ± 0.2</td>
<td>2.7*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 3; for uptake of monoglucuronosyl bilirubin, n = 4. Uptake of [³H]-labeled 17β-glucuronosyl estradiol, dehydroepiandrosterone sulfate, and cholyltaurine was measured at 5 µM substrate concentration. Uptake of [³H]-labeled monoglucuronosyl bilirubin was measured at 80 nM. All uptake experiments were performed at 37°C. Significance of differences between organic anion transporting polypeptide OATP2-transfected and control vector (Co)-transfected HEK293 (HEK) cells was determined by unpaired student’s t-test. *P < 0.01.

Table 2. Cis inhibition of 17β-glucuronosyl estradiol uptake in human OATP2-transfected HEK293 cells.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>17β-Glucuronosyl Estradiol Uptake, % control</th>
</tr>
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<tbody>
<tr>
<td>None (control)</td>
<td>100</td>
</tr>
<tr>
<td>Glutathione</td>
<td>111 ± 1</td>
</tr>
<tr>
<td>1 mM</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>5 mM</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>Cholate</td>
<td></td>
</tr>
<tr>
<td>20 µM</td>
<td>37 ± 3*</td>
</tr>
<tr>
<td>100 µM</td>
<td>14 ± 4*</td>
</tr>
<tr>
<td>DIDS</td>
<td></td>
</tr>
<tr>
<td>10 µM</td>
<td>30 ± 1*</td>
</tr>
<tr>
<td>50 µM</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>Sulfochromophthalein</td>
<td></td>
</tr>
<tr>
<td>50 nM</td>
<td>49 ± 1*</td>
</tr>
<tr>
<td>100 nM</td>
<td>19 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SD from 3 experiments, each performed in duplicate. Uptake of [³H]-labeled 17β-glucuronosyl estradiol was measured at 1 µM substrate concentration in the presence of inhibitors at the concentrations indicated. *P < 0.01.

Fig. 7. OATP2-mediated 17β-glucuronosyl estradiol (E17βG) uptake. A: time course of E17βG uptake. For uptake measurement, HEK 293 cells transfected with OATP2 (HEK-OATP2) or vector (HEK-Co) were incubated with 1 µM E17βG at 37°C. At the indicated time points, intracellular radioactivity was determined as described in MATERIALS AND METHODS. B: Kinetics of OATP2-mediated E17βG uptake was measured for 3 min at the concentrations indicated. Net OATP2-mediated uptake was calculated by subtracting values obtained with HEK-Co cells from those obtained with HEK-OATP2 cells. For determination of the inhibition constant value for sulfobromophthalein (BSP), the same experiment was performed in the presence of 50 nM BSP. Michaelis-Menten constant and maximal velocity values were determined by double-reciprocal plots according to Lineweaver and Burk (30). All data represent means ± SD of 2 experiments performed in triplicate.
On the basis of the deduced amino acid sequence, we designed an antibody, termed ESL, directed against the carboxy terminus of the protein (Fig. 1). The specificity of this antibody was tested by immunoblot analysis (Fig. 4). This antibody served to localize the protein in human liver as well as the recombinant protein expressed in transfected cells. Immunofluorescence microscopy demonstrated that the OATP2 protein is exclusively localized to the basolateral membrane of hepatocytes (Fig. 5). This localization is consistent with the localization described for the other OATP family members (11, 17, 29) except rat OATP1, which also exhibits an additional apical localization in kidney (5) and brain (4).

We used our transfectants for further characterization of the recombinant protein and for its comparison with the native protein with respect to localization and transport properties. Several studies have addressed the transport function and substrate specificity of OATP family members on the basis of transport experiments in Xenopus laevis oocytes (1, 28, 35). In our study, we used mammalian cells stably transfected with the OATP2 cDNA. The use of Xenopus laevis oocytes may be more artificial than studies using mammalian cells expressing the recombinant protein, and kinetic studies carried out in Xenopus laevis oocytes may be affected by the different protein and lipid environments. In OATP2 cDNA-transfected HEK293 cells, the protein was localized to the plasma membrane, whereas, in polarized MDCKII cells transfected with the OATP2 cDNA, this plasma membrane localization was restricted to the lateral membrane (Fig. 6). This localization is in accordance with the localization found in human liver (Fig. 5) and established for other OATPs including rat OATP1 (11). Apical staining was observed neither in human hepatocytes nor in the polarized MDCKII transfectants. No expression of OATP2 was detectable in bile ductular cells.

With the use of the HEK293 transfectants, several substances were tested as possible substrates for OATP2. As shown in Table 1, typical organic anions, transported by other members of the OATP family (for review, see Ref. 3), were also substrates for human OATP2. Substances transported by OATP2 included MGB and E17βG. The latter was the preferred substrate, with a K_m value of 8.2 µM (Fig. 7). This K_m value was in the same range as the one determined for rat OATP1 after stable transfection (11). Other important physiological organic anions such as cholate and BSP wereISK inhibitors of E17βG uptake, suggesting that they are also substrates for human OATP2 (Table 2). More studies on additional transport characteristics of human OATP2 are needed.

After completion of our studies and during preparation of this manuscript, a paper was published that describes a new transport protein, termed LST-1, the liver-specific transporter 1 (2). Localization of LST-1 in liver was not determined, but the sequence comparison of OATP2 and LST-1 revealed 99.8% identity on the amino acid level. LST-1 was expressed in Xenopus laevis oocytes and shown to transport organic anions, including DHEAS, E17βG, and thyroid hormones (2).

In conclusion, we have cloned human OATP2 as a new member of the OATP family and localized it to the basolateral hepatocyte membrane. OATP2 mRNA was strongly expressed in liver, and no other tissue tested so far showed detectable OATP2 mRNA expression. The use of stably transfected human embryonic kidney (HEK293) and canine kidney (MDCKII) cells allowed us to study the localization of the recombinant protein and to determine the substrate specificity of this transporter. Transported substrates included typical organic anions such as E17βG, DHEAS, cholytaurine, and MGB, suggesting the involvement of OATP2 in the hepatocellular uptake of important endogenous organic anions. Further studies are needed to address the full range of substrates of this major hepatic transporter and to characterize the driving force of human OATP2.

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