Localization and Genomic Organization of a New Hepatocellular Organic Anion Transporting Polypeptide*

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Based on sequence homology to the human organic anion transporting polypeptide 2 (OATP2; SLC21A6), we cloned a new member of the SLC21A superfamily of solute carriers, termed OATP8 (SLC21A8). The protein of 702 amino acids showed an amino acid identity of 80% with human OATP2. Based on Northern blotting, the expression of OATP8 was restricted to human liver. Cosmid clones containing the genes encoding human OATP1 (SLC21A3), OATP2 (SLC21A6), and OATP8 (SLC21A8) served to establish their genomic organization. All three genes contained 14 exons with 13 identical splice sites when transferred to the amino acid sequence. An antibody raised against the carboxyl terminus localized OATP8 to the basolateral membrane of human hepatocytes and the recombinant glycoprotein, expressed in MDCKII cells, to the lateral membrane. Transport properties of OATP8 were studied in stably transfected MDCKII and HEK293 cells. Organic anions transported by human OATP8 included sulfobromophthalein, with a \( K_m \) of 3.3 \( \mu \)m, and 17\( \beta \)-glucuronosyl estriadiol, with a \( K_m \) of 5.4 \( \mu \)m. Several bile salts were not substrates. Thus, human OATP8 is a new uptake transporter in the basolateral hepatocyte membrane with an overlapping but distinct substrate specificity as compared with OATP2, which is localized to the same membrane domain.

The gene superfamily of solute carriers (SLC)\(^1\) comprises a growing family of different types of transporters. Their substrates comprise a broad range of different structures ranging from inorganic compounds to organic cations and anions as well as amino acids and small peptides. Within this superfamily, the uptake transporters for organic anions are classified into the organic anion/prostaglandin transporter family SLC21A (Human Gene Nomenclature Committee Data Base). Best characterized within this gene family are the organic anion transporting polypeptides (OATPs)\(^1\). In humans, five different organic anion transporting polypeptides have been identified and cloned. Two of them, human OATP1 (SLC21A3)\(^2\) and OATP2 (SLC21A6)\(^3\)–\(^5\) have been characterized with respect to their transport characteristics, tissue distribution, and cellular localization. Human OATP1 (originally termed OATP\(^2\)) was cloned as an ortholog of the rat Oatp family member Oatp1 (6). As determined in several expression systems, transported substrates of human OATP1 include sulfobromophthalein (BSP)\(^2\), estrone-3-sulfate (9), dehydroepiandrosterone sulfate (10), and 17\( \beta \)-glucuronosyl estriadiol (11, 12). These substances were also identified as substrates or competitive inhibitors of the recently cloned second member of the human OATPs, OATP2 (SLC21A6)\(^5\). Despite the fact that both proteins share only 44% amino acid identity, they have a similar substrate specificity and a similar predicted topology\(^5\). However, they differ with respect to their tissue-specific expression. Human OATP1 was cloned from liver but exhibits the highest expression in brain\(^2\). In contrast, human OATP2 shows a high expression only in liver as tested by Northern blotting\(^3\)–\(^5\). Recently, the sequences of three additional members of the human OATP family designated OATP-B (SLC21A9), OATP-D (SLC21A11), and OATP-E (SLC21A12) were reported (see Table I for accession numbers). The amino acid sequences of these three transporters are quite distinct from human OATP1 and OATP2. Until now, the transporters OATP-B, -D, and -E were not further characterized.

So far, little is known about the genomic organization of the human OATP family members. The human OATP1 gene has been localized to chromosome 12p12\(^13\), and its promoter region as well as its regulatory properties have been studied\(^14\). In this study\(^14\), the length of the first exon and the first exon/intron splice site were determined, but the complete genomic organization of the OATP1 gene has not been reported. Moreover, no information is currently available on the genomic organization and the chromosomal localization of the other human OATP family members.

Therefore, we screened a genomic data base using the human OATP2 cDNA to identify a cosmid clone containing the genomic sequence of this transporter. We identified one cosmid clone containing cDNA sequences that were highly homologous to but distinct from OATP2 cDNA sequences. By combining these genomic DNA sequences, we identified a new OATP cDNA, which was termed OATP8 (SLC21A8). Based on this partial cDNA sequence, we cloned the full-length cDNA and analyzed the genomic organization of this transporter in comparison with the genomic organization of human OATP1 and human OATP2. Northern blot analysis revealed a liver-specific expression similar to that of OATP2. An OATP8-specific antibody raised against the carboxyl terminus served to detect the protein in the basolateral hepatocyte membrane of human liver. The recombinant protein was localized to the lateral membrane of stably transfected MDCKII cells. In addition to these MDCKII cells, stably transfected HEK293 cells served to determine transport characteristics of this new transporter OATP8.

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\(^1\) The abbreviations used are: SLC, solute carrier superfamily; BSP, sulfobromophthalein; E217G, 17\( \beta \)-glucuronosyl estriadiol; OATP, organic anion transporting polypeptide; PBS, phosphate-buffered saline; bp, base pairs; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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Experimental Procedures

Materials—Lysozyme and ampicillin were from Roche Molecular Biochemicals, agarose was from Roth (Karlsruhe, Germany), RNase inhibitor (RNAguard), and Stratscript Moloney murine leukemia virus reverse transcriptase, and restriction endonucleases were from Stratagene (Amsterdam, The Netherlands). Leupeptin, pepstatin, aprotinin, fetal calf serum, agar, and the protein standard mixture (relative molecular weight 40,000–150,000) were from Sigma. The Vector Blue kit (BRL) and the Vectastain ABC peroxidase kit (Vector Labs, Burlingame, CA) were purchased from Vector Laboratories (West Grove, PA).

Immunofluorescence Studies of Human Liver Sections—Normal liver tissue samples were obtained from patients who underwent liver resec-
tion because of hepatocellular carcinoma. Only healthy tissue, left after tumor surgery, was used for immunofluorescence studies. Liver tissue was deep-frozen in liquid nitrogen and mounted in Tissue-Tek (Miles, Elkhart, IN). Sections of 5 μm were prepared with a cryostate (Frigocut 1190, Jung, Braunschweig, Germany) and air-dried for 30 min in acetone (−20 °C) for 10 min. Sections were then incubated with the primary antibodies for 60 min at room temperature, washed three times with phosphate-buffered saline (PBS) (140 mM NaCl, 10 mM phosphate, pH 7.4), and subsequently incubated with the secondary antibodies. Finally, sections were washed twice in PBS, rinsed with distilled water, and mounted in Moviol ( Hoechst, Frankfurt, Germany). Antigen was rehydrated in PBS supplemented with 0.5% calf serum at the following dilutions: SKT and CD26 at 1:100; anti-desmoplakin at 1:20; anti-cytokeratin 19 at 1:5; and Cy2-conjugated anti-rabbit IgG and Cy3-conjugated anti-mouse IgG at 1:400. Fluorescence microscopy was performed on an Axioscan S100TV microscope (Carl Zeiss, Jena, Germany) as described (5).

Cloning of the Human OATP8 cDNA—On the basis of a GenBank™ search against the human OATP2 cDNA sequence (EBI/GenBank accession number AF132573) one cosmid clone was identified (EBI/GenBank accession number AC006552) that exhibited a high degree of base pair identity over several parts of the OATP2 cDNA. This clone contained several unordered partial genomic sequences derived from chimpanzee chromosome 12. An overlay of the OATP2 cDNA sequence and this genomic sequence revealed a partial cDNA sequence of 1726 bp that was identical to a partial OATP2 cDNA fragment (pore size, 5 μm; Costar, Cambridge, MA). Sodium butyrate was added to the culture for 3 days to induce the expression of the OATP2 cDNA fragment and to enhance the expression of the OATP2 protein. The amplified fragment was analyzed on an agarose gel and subcloned into the vector pCR2.1.TOPO (Invitrogen), resulting in the plasmid pOATP8.3. Based on these sequence informations, the full-length OATP8 cDNA was amplified under the same conditions using the liver Marathon-Ready cDNA-cloning kit and the primer pOATP8.3 forward primer oOATP8.3.for (5′-TATATAGTCATGACTTATCCAT-3′) located within the cloned ATG start region. The amplified fragment of 2241 bp was subcloned into the vector pCR2.1.TOPO (Invitrogen), resulting in the plasmid pOATP8.TOPO. For transfection studies, the OATP8-cDNA fragment was subcloned into the Bluescript di-gested and dephosphorylated vector pCDA3.1(+). The correctness of cloning and orientation was verified by sequencing.

DNA Sequencing—The cDNA clones were sequenced by use of α-[35S]dATP and the T7 sequencing kit (Amersham Pharmacia Biotech) according to the dideoxy nucleotide chain termination method of Sanger et al. (15). Dried gels were exposed to Kodak BioMax MR-1 films (Eastman Kodak). Sequence Analysis—Throughout this study the computer program Heidelberg Unix Sequence Analysis Resource (16), based on the Wisconsin Genetics Computer group program package (17), was used for restriction mapping, sequence analysis, and sequence alignments.

Northern Blot Analysis—The Northern blot analyses were performed using the commercial human 12-lane multiple tissue Northern blot from CLONTECH. For OATP8, the 589-bp EcoRI restriction fragment (bp 2059–2647) from clone pOATP8.3 and for the control a human β-actin cDNA fragment supplied with the kit were used as probes. The multiple tissue Northern blot membrane was prehydrized at 42 °C for 2 h in 10 ml of hybridization buffer (6× SSC, 0.5% SDS, 5× Denhardt’s solution, 50% formamide, 100 μg/ml denatured salmon sperm DNA). DNA fragment labeling was performed using the Rediprime DNA labeling system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. The labeled fragments were purified using NucTrap filtration columns from Stratagene. After hybridization, the membrane was washed once in 2× SSC, 0.1% SDS at 42 °C for 20 min followed by two washing steps at 60 °C for 20 min, one step with 1× SSC, 0.1% SDS, the following by 0.5× SSC, 0.1% SDS. The blot was dried and autoradiography was performed at −80 °C with an intensifying screen for 36 h (OATP8) and 18 h (β-actin).

Cell Culture and Transfection Studies—Human embryonic kidney (HEK293) and Madin-Darby canine kidney (MDCKII) cells were cultured in minimum essential medium (Sigma), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 95% humidity, and 5% CO2. Cells were transfected with the pcDNA3.1(+) vector (Invitrogen) and the commercial human 12-lane multiple tissue Northern blot from CLONTECH. For OATP8, the 589-bp EcoRI restriction fragment (bp 2059–2647) from clone pOATP8.3 for and for the control a human β-actin cDNA fragment supplied with the kit were used as probes. The multiple tissue Northern blot membrane was prehydrized at 42 °C for 2 h in 10 ml of hybridization buffer (6× SSC, 0.5% SDS, 5× Denhardt’s solution, 50% formamide, 100 μg/ml denatured salmon sperm DNA). DNA fragment labeling was performed using the Rediprime DNA labeling system (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. The labeled fragments were purified using NucTrap filtration columns from Stratagene. After hybridization, the membrane was washed once in 2× SSC, 0.1% SDS at 42 °C for 20 min followed by two washing steps at 60 °C for 20 min, one step with 1× SSC, 0.1% SDS, the following by 0.5× SSC, 0.1% SDS. The blot was dried and autoradiography was performed at −80 °C with an intensifying screen for 36 h (OATP8) and 18 h (β-actin).

Preparation of Membrane Vesicles—Basolateral membrane vesicles from human liver (19) and crude membrane vesicles from cultured cells were prepared as described earlier (18).

Immunoblot Analysis—Membrane proteins were diluted with sample buffer and incubated at 37 °C for 30 min prior to separation on 4% stacking and 10% resolving SDS-polyacrylamide gels. Immunoblotting was performed using a tank blotting system from Bio-Rad and an enhanced chemiluminescence detection kit (NEN Life Science Products). Primary antibody was diluted 1:5000 in TBS (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20). The secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) used at 1: 2000 dilution.

Deglycosylation and Tunicamycin Treatment—Basolateral membrane proteins from human liver were deglycosylated using peptide N-glyase (Calbiochem, 40 μg/ml) and 5 μg/ml tunicamycin. Deglycosylation of the recombinant protein, OATP8-transfected HEK293 cells were cultured with 1 μg/ml tunicamycin for 48 h. Crude membrane vesicles were then prepared from the treated cells and studied by immunoblot analysis.

Immunofluorescence Microscopy of Transfected Cells—Transfected HEK293 or MDCK cells were grown on Transwell membrane inserts (pore size, 5 μm; Costar, Cambridge, MA). Sodium butyrate was added

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to the culture medium 24 h before the experiment. HEK293 cells were fixed and permeabilized for 5 min in methanol (−20 °C), MDCK II cells were fixed for 10 min with 4% paraformaldehyde in PBS and permeabilized for 10 min in 1% Triton X-100 in PBS. Cells were thereafter incubated with the primary antibody SKT (diluted 1:50 in PBS) for 30 min at room temperature. After three washes with PBS, cells were 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 mg/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-510 apparatus from Carl Zeiss (Oberkochen, Germany).

**Transport Assays**—Transfected HEK293 cells were seeded in 6-well plates (coated with 0.1 mg/ml poly-d-lysine) at a density of 2 × 10^6 cells/well and cultured with 10 mM sodium butyrate for 24 h. For uptake studies cells were first washed with uptake buffer (142 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 1.2 mM MgSO4, 1.5 mM CaCl2, 5 mM glucose, and 12.5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, pH 7.3) and then incubated with 1 ml of uptake buffer containing tritium-labeled substrate at 37 °C. 3H-labeled substrates were obtained from NEN Life Science Products. 3H-labeled BSP was obtained through custom synthesis from Hartmann Andlytik (Köln, Germany). For *cis*-inhibition studies reduced glutathione (5 mM) was included into the uptake buffer. At different time points uptake was stopped by adding ice-cold buffer. After three washes with cold buffer, cells were lysed in 1 ml of 0.2% SDS in water. The cell-associated radioactivity was determined by transferring 250-μl aliquots of the lysate to scintillation vials and counting the radioactivity with a Beckman scintillation counter (LS 6000IC, Beckman Instruments GmbH, Munich, Germany). Protein content was determined according to Lowry using 100 μl of cell lysate.

**RESULTS**

**Cloning of the Human OATP8 cDNA**—A GenBank search using OATP2 (SLC21A6) cDNA as reference sequence revealed a chimpanzee genomic sequence (GenBank/EBI accession number AC006582) that exhibited a high percentage of identity over several parts of the cDNA. An overlay of the OATP2 cDNA sequence (GenBank/EBI accession number AJ132573) with this genomic DNA sequence, taking into consideration the conserved exon/intron splice junction sites, resulted in a new partial cDNA sequence of 1726 bp in length. This new cDNA sequence was located in the 5′-part of the human OATP2 cDNA and exhibited 87% base pair identity. Based on this information, the full-length human cDNA of this new member of the organic anion transporter family was cloned and further analyzed. The coding region covers 2106 bp and shows the highest degree of identity to human OATP2 (SLC21A6) of about 87% as calculated under the default parameters of the Bestfit program.
of the Heidelberg Unix Sequence Analysis Resource program package. The base pair identity of the coding region of the cDNA to human OATP1 (SLC21A3) is 61%. The calculation of the base pair identities of human OATPs to the other known human members of the family, namely OATP-B, OATP-D, and OATP-E, indicated that only short sequence stretches of the cDNAs were aligned, suggesting that these transporters are quite different on the nucleotide level. The new human OATP8 cDNA sequence is available under the GenBank/EBI accession number AJ251506.

Comparison of the Genomic Organization of the Human OATP8 (SLC21A8) Gene with the Human OATP2 (SLC21A6) and OATP1 (SLC21A3) Genes—After completion of cloning and sequencing of the OATP8 cDNA, a database search using the full-length cDNA revealed another genomic clone (GenBank/EBI accession number AC011604), that covers the complete full-length cDNA revealed another genomic clone (GenBank/EBI accession number AC022224 and AC022335, covering the human OATP1 (SLC21A3) and OATP2 (SLC21A6) gene, respectively. Like OATP8, the OATP1 gene is located on chromosome 12p12 (13). Using the OATP cDNAs and the identified genomic sequences, the organization of these OATP genes was determined (Fig. 1). All exons were flanked by the dinucleotide GT on the 5’donor site and AG on the 3’-acceptor site, which is consistent with the consensus sequences for splice junctions in eukaryotic genes (20). Despite the fact that OATP1, when compared with OATP2 and OATP8, had a different amino acid composition, the genomic organization was very similar (Fig. 1). All three genes contained 14 exons with an average exon size of about 150 bp. For OATP2 and OATP8, 13 exons were identical in length. A comparison of the genomic organization of human OATP8 and OATP1 revealed 9 exons identical in length. For all three OATP family members, the longest exon is exon 14 (OATP1, 220 bp plus 3’-untranslated region; OATP2, 211 bp plus 3’-untranslated region; and OATP8, 244 bp plus 3’-untranslated region). The shortest exon in all three transporter genes is exon 12 with 65 bp. A comparison of the splice junction sites of the OATP1, OATP2, and OATP8 genes on the amino acid level shows that all 13 splice sites are at identical positions (Fig. 1). Because the DNA sequences used for the analysis of the genomic organization comprised several pieces released in an unordered fashion, the length of the introns could not be determined.

Analysis of the Deduced Amino Acid Sequence of OATP8—The open reading frame of the OATP8 cDNA of 2106 bp encodes a protein of deduced 702 amino acids in length with a calculated molecular mass of 77,402 Da. The amino acid identity of OATP8 to other members of the organic anion transporter family were calculated (16) using the Heidelberg Unix Sequence Analysis Resource program (16). This analysis demonstrated that the OATP8 transporter (Fig. 1) shares the highest degree of amino acid identity with human OATP2 (SLC21A6) (3–5). The identity with other OATPs varied between 28% for human OATP-E and 40% for human OATP1 (Table I). Based on a CLUSTAL alignment a computer-aided transmembrane analysis using the TopPred II program (21, 22) demonstrates that OATP8 consists of 12 predicted transmembrane domains. This transmembrane organization is identical to the one predicted for the other known OATPs, (1, 5), underlining the relationship of human OATP8 with the other members of the OATP family. A comparison of OATP8 and OATP2 with respect to potential N-glycosylation sites revealed 11 glycosylation sites for both proteins with four of them located outside in predicted extracellular loops for OATP8 but with six predicted outside glycosylation sites for OATP2. Both proteins share predicted N-glycosylation of the fifth extracellular loop.

Tissue Distribution of Human OATP8—Tissue distribution of human OATP8 mRNA was studied by taking into account the high nucleotide identity between human OATP2 and OATP8. Therefore, an OATP8 cDNA restriction fragment was used as probe that shared only 78% identity to the human OATP2 cDNA. To prevent cross-hybridization of this OATP8 cDNA fragment with OATP2 mRNA, the Northern blot was performed under high stringency hybridization and washing conditions. As for OATP2 mRNA, a strong signal for OATP8 mRNA was only detected in liver (Fig. 2). Prolonged exposure of the blot for up to 84 h revealed no additional signals in other tissues, suggesting that human OATP8 (Fig. 2), like OATP2 (3–5), is highly and apparently exclusively expressed in human liver. The length of the detected mRNAs was 2.8 kb, likely corresponding to the fully spliced mRNA, and 4.5 kb, probably corresponding to unspliced or partially spliced mRNA.

Immunofluorescence Localization of OATP8 in Hepatocytes—Incubation of cryosections from human liver tissue with the SKT antibody yielded fluorescent staining of the basal and lateral plasma membrane domains of hepatocytes (Fig. 3, A, D, G, and H). Hepatocytes near the central vein showed stronger
staining than hepatocytes close to the portal vein (Fig. 3, G and H), indicating a lobular zonation of protein expression. Absence of OATP8 from the canalicular membrane was confirmed by double labeling with the SKT antibody and an antibody against the apical plasma membrane marker DPPIV (B). The merged picture is shown in C. Localization of OATP8 in the lateral membrane was confirmed by double labeling with the SKT antibody (D) and an antibody to desmoplakin (E), resulting in a yellow color (F) where both antigens colocalize. G–I give an overview of OATP8 expression in human liver. OATP8 is not detected in cholangiocytes (asterisks in H), which were identified in a consecutive section (I) by phase contrast and staining with an antibody to cytokeratin 19 (red fluorescence in I). Bar in A, 10 μm, same magnification for A–F; bar in G, 50 μm, same magnification for G–I. CV, central vein; PV, portal vein.

**Fig. 3.** Basolateral localization of OATP8 in human hepatocytes. Cryosections of normal human liver tissue (5 μm) were incubated with the SKT antibody raised against the carboxyl terminus of human OATP8 resulting in basolateral plasma membrane staining of hepatocytes (A, D, G, and H). OATP8 was not present in the apical plasma membrane domain as shown by double labeling with the SKT antibody and an antibody against the apical membrane marker DPPIV (B). The merged picture is shown in C. Localization of OATP8 in the lateral membrane was confirmed by double labeling with the SKT antibody (D) and an antibody to desmoplakin (E), resulting in a yellow color (F) where both antigens colocalize. G–I give an overview of OATP8 expression in human liver. OATP8 is not detected in cholangiocytes (asterisks in H), which were identified in a consecutive section (I) by phase contrast and staining with an antibody to cytokeratin 19 (red fluorescence in I). Bar in A, 10 μm, same magnification for A–F; bar in G, 50 μm, same magnification for G–I. CV, central vein; PV, portal vein.

**Fig. 4.** Immunolocalization of recombinant OATP8 in transfected HEK293 (A and C) and MDCKII (B and D) cells. Cells were grown on Transwell membranes and fixed either with methanol/acetone (HEK293 cells) or with paraformaldehyde (MDCKII cells). OATP8 was stained with the SKT antibody (dilution 1:50). A and B show the optical sections in the XY-plane. C and D show the optical sections in the XZ plane as indicated by the yellow lines in A and B. In the HEK293 cells OATP8 is localized in the whole plasma membrane, whereas it is localized to the lateral membrane in the polarized MDCKII cells. The bar corresponds to 10 μm, same magnification in A–D.

**Immunolocalization of Recombinant OATP8 in Transfectants—**OATP8 showed a similar localization as OATP2 in transfected cells. OATP8 was sorted to the lateral membrane in the polarized MDCKII cells (Fig. 4, B and D). In the nonpolarized HEK293 cells, OATP8 was localized over the entire plasma membrane (Fig. 4, A and C).

**Immunoblot Analysis of Endogenous and Recombinant OATP8—**The specificity of the polyclonal antibodies SKT (Fig. 1) and ESL (5) was first established in immunoblot analyses using crude membranes from HEK293 cells transfected either with OATP2 (HEK-OATP2) or with OATP8 (HEK-OATP8). As shown in Fig. 5, antibody SKT was specific for OATP8 and antibody ESL was specific for OATP2. The recombinant OATP8 in HEK293 cells has an apparent molecular mass of about 90 kDa. When the cells were treated with tunicamycin to inhibit
N-glycosylation, the molecular mass of the recombinant OATP8 was reduced to about 65 kDa (Fig. 5). In the basolateral membrane from human liver, however, the polyclonal antibody SKT detected a signal with an apparent molecular mass of about 120 kDa. Deglycosylation with peptide N-glycosidase F reduced the molecular mass of OATP8 to about 60 kDa. Apparently, OATP8 is heavily glycosylated in the hepatocytes. Interestingly, when OATP8 was expressed in the hepatocyte-derived cell line HepG2, it showed a similar molecular mass of about 90 kDa as in transfected HEK293 or MDCKII cells (not shown). Thus, the glycosylation of OATP8 in the liver differs from the glycosylation in the transfected cultured cells.

Transport Activity of Recombinant OATP8—Several organic anions were tested as substrates for uptake by OATP8-transfected HEK293 cells. Uptake of BSP by OATP8 was time-dependent (Fig. 6). Transport activity of OATP8 was not sodium-dependent because replacement of sodium chloride by choline chloride did not significantly affect the transport activity of OATP8. The apparent \( K_m \) values for BSP and \( E_{17\beta G} \) were 3.3 and 5.4 \( \mu M \), respectively. Leukotriene \( C_4 \) and dehydroepiandrosterone sulfate (Table II) were also transported by recombinant human OATP8. The bile salts cholate, cholyl taurine, and cholyl glycine tested under the same experimental conditions at a substrate concentration of 5 \( \mu M \) were not taken up into OATP8-transfected cells at an enhanced rate.

Reduced glutathione, an important endogenous antioxidant, was tested both in cis-inhibition studies and in direct uptake experiments. At a concentration of 5 mM, reduced glutathione did not cis-inhibit OATP8-mediated uptake of 1 \( \mu M \) BSP. Moreover, no significant difference in uptake rates of \(^{3}^{3}^{3}H\)-reduced glutathione, at a concentration of 100 \( \mu M \), was detected between HEK293 cells expressing OATP8 and HEK293 cells transfected with control vector alone, suggesting that reduced glutathione is not a substrate for OATP8.

**Fig. 5.** Immunoblot analyses. Membrane proteins were separated by SDS-polyacrylamide gel electrophoresis. OATP2 was detected by the polyclonal antibody ESL (5), and OATP8 was detected by the polyclonal antibody SKT (Fig. 1). A, ESL detected only OATP2 and SKT detected only OATP8. B, glycosylation of OATP8 in human liver. OATP8 has an apparent molecular mass of about 120 kDa in comparison to 90 kDa of OATP2. The high molecular mass of OATP8 in hepatocytes is a result of different glycosylation as deglycosylation with N-peptidyl glycosidase F reduced the molecular mass of OATP8 to about 60 kDa. C, glycosylation of OATP8 in transfected HEK293 cells. Recombinant OATP8 in the transfected HEK293 cells is glycosylated differently as compared with liver. Both OATP2 and OATP8 have an apparent molecular mass of about 90 kDa. After treatment of the cells with tunicamycin (10 \( \mu g/\text{ml} \)), the molecular mass of OATP8 was reduced to about 65 kDa.

**Fig. 6.** Transport activity of recombinant OATP8. A, uptake of BSP was measured at 200 nM BSP using HEK293 cells transfected with OATP8 cDNA (HEK-OATP8) or control vector (HEK-Co). B and C, determination of \( K_m \) values for BSP and \( E_{17\beta G} \). Transport rate for OATP8 was calculated by subtracting values determined with HEK-Co cells from those with HEK-OATP8 cells. Data points represent the means \( \pm \) S.D. (n = 3).
of the differences between OATP8-transfected and control vector-transfected HEK293 cells was determined by unpaired Student’s t test.

| Table II: Substrate specificity of OATP8 |
|-----------------------------------------|
| Uptake of BSP, E,17βG, and dehydroepiandrosterone sulfate (DHEAS) at 37 °C was measured at a substrate concentration of 5 μM. Uptake of leucotriene C4 was determined at 500 nm. Uptake experiments were performed at 37 °C. Data are the means±S.D. (n = 3). The significance of the differences between OATP8-transfected and control vector-transfected HEK293 cells was determined by unpaired Student’s t test. |
| Substrate | HEK-OATP8 | HEK-Co | Uptake ratio (OATP8/Co) |
|-----------|-----------|--------|------------------------|
| BSP (5 μM) | 7.2 ± 1.3 | 38.5 ± 0.8 | 2.3* |
| E,17βG (5 μM) | 0.92 ± 0.30 | 0.12 ± 0.07 | 7.4* |
| DHEAS (5 μM) | 0.75 ± 0.26 | 0.15 ± 0.11 | 5.2* |
| Leucotriene C4 (500 nm) | 0.21 ± 0.02 | 0.09 ± 0.03 | 2.3* |

*p < 0.05.

DISCUSSION

We have cloned and characterized a new member of the human OATP family belonging to the solute carrier superfamily. The newly identified transporter is the eighth member of the SLC21A family, and the gene symbol accordingly is SLC21A8. By following the numbering of the gene nomenclature, the translation product of this new gene was termed OATP8. All known OATPs within the SLC family exhibit common features. They all are glycosylated and have a similar secondary structure with 12 predicted transmembrane helices (1, 5). Within the OATP family, OATP8 shows the highest degree of identity, both on nucleotide and amino acid level, to human OATP2 (4, 5, 24). Both are preferentially, if not exclusively, expressed in human liver (Fig. 2) (4, 5, 24), whereas other OATPs are expressed in a variety of tissues as shown for human OATP1 with its expression in brain, kidney, liver, and testis (2).

The genomic organization of human OATP1 (SLC21A3), OATP2 (SLC21A6), and OATP8 is very similar. All three genes contain 14 exons with an average exon size of about 150 bp. All 13 introns share identical phases with a high proportion of class 1 phases, where the codon is interrupted between the first and the second nucleotide (Fig. 1). The genomic organization of human OATP2 (SLC21A6) and OATP8 (SLC21A8) is closely related with 13 exons identical in length. The genomic organization of OATP8 compared with OATP1 revealed 9 exons identical in length. Figure 1 demonstrates that the 5 exons differing in length exhibit gaps in the amino acid alignment leading to the different number of base pairs. OATP1 has been mapped to chromosome 12p12 (13), and for OATP8 the genomic clone localization was restricted to the lateral membrane, whereas in the nonpolarized HEK293 cells the recombinant protein was localized to the whole plasma membrane (Fig. 4). Using the HEK293 cells stably expressing the recombinant protein, several typical organic anions including BSP, E,17βG, and dehydroepiandrosterone sulfate were transported by OATP8 (Table II and Fig. 6). The apparent Km values for BSP and E,17βG were 3.3 and 5.4 μM, respectively. These Km values were in the same range as the ones for transport by recombinant rat Oatp1 (25). The Km value for E,17βG was comparable with the one determined with human OATP2 (5). A major difference between the hepatic basolateral uptake transporters OATP2 and OATP8 concerns their ability to transport certain bile salts. In contrast to human OATP2, which transports cholyli taurine and whose 17β-glucuronosyl estradiol transport was inhibited by cholate (5), OATP8 did neither transport cholyli taurine nor cholate nor cholyli glycine. Both transporters thus have overlapping substrate specificities as well as clear differences that require further detailed characterization. An additional marked difference between OATP2 and OATP8 became apparent in the deglycosylation experiments indicating that OATP8 in human liver is much more glycosylated than OATP2 (Fig. 5). Deglycosylation yields a similar molecular mass for both proteins that corresponds to the mass predicted on the basis of the number of amino acids. It will be of interest to examine in more detail a role of the different oligosaccharide moieties of OATP8 and OATP2 in human hepatocytes.

In summary, human OATP8 represents the third member of the human OATP family of transporters that was cloned, localized, and functionally examined. OATP8 is the second uptake transporter, in addition to OATP2 (3–5), which is predominantly, if not exclusively, expressed in human liver and mediates the sodium-independent, high affinity uptake of important physiological amphiphilic organic anions into hepatocytes. It will be of interest to identify additional mutually exclusive endogenous substrates for human OATP2 and OATP8, in addition to cholate and cholyli taurine.

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