Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter

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Craddock, Ann L., Martha W. Love, Rebecca W. Daniel, Lyndon C. Kirby, Holly C. Walters, Melissa H. Wong, and Paul A. Dawson. Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G157–G169, 1998.—The enterohepatic circulation of bile acids is maintained by Na+-dependent transport mechanisms. To better understand these processes, a full-length human ileal Na+-bile acid cotransporter cDNA was identified using rapid amplification of cDNA ends and genomic cloning techniques. Using Northern blot analysis to determine its tissue expression, we readily detected the ileal Na+-bile acid cotransporter mRNA in terminal ileum and kidney. Direct cloning and mapping of the transcriptional start sites confirmed that the kidney cDNA was identical to the ileal Na+-bile acid cotransporter. In transiently transfected COS cells, ileal Na+-bile acid cotransporter-mediated taurocholate uptake was strictly Na+-dependent and chloride independent. Analysis of the substrate specificity in transfected COS or CHO cells showed that both conjugated and unconjugated bile acids are efficiently transported. When the inhibition constants for other potential substrates such as estrone-3-sulfate were determined, the ileal Na+-bile acid cotransporter exhibited a narrower substrate specificity than the related liver Na+-bile acid cotransporter. Whereas the multispecific liver Na+-bile acid cotransporter may participate in hepatic clearance of organic anion metabolites and xenobiotics, the ileal and renal Na+-bile acid cotransporter retains a narrow specificity for reclamation of bile acids.

Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine, where they facilitate the absorption of fat-soluble vitamins and cholesterol (6). The majority of bile acids are efficiently reabsorbed from the intestine and returned to the liver via the portal venous circulation. At the liver, bile acids are extracted and resecreted into bile (7). A fraction (10–50% depending on the bile acid species) of the absorbed bile acids escapes hepatic uptake from the portal blood and spills over into the systemic circulation. The binding of bile acids to plasma proteins prevents their glomerular filtration and minimizes urinary excretion. In addition, bile acids in the glomerular filtrate are actively reabsorbed from the renal tubules and returned to the liver for uptake (27, 31). Thus the amount of bile acid filtered through the glomerulus exceeds urinary excretion, and this process may contribute to the increased concentration of bile acids found in peripheral blood during obstructive liver disease (18).

Active uptake of bile acids from both the ileum and kidney is mediated by an Na+-gradient driven transporter located on the epithelial apical membrane (6, 31). As in the ileum, bile acid transport in the renal proximal tubules is thought to act as a salvage mechanism to conserve bile acids. The relationship between the hepatic, ileal, and renal Na+-bile acid cotransporter systems has only recently been resolved with the cloning of the bile acid carriers from those tissues (4). The liver and ileal Na+-bile acid cotransporters are related gene products that share 35% sequence identity and are predicted to be structurally similar (13, 32). In contrast, the ileal and renal carriers appeared to be products of the same gene based on Northern blotting studies in the hamster (32) and rat (22). This finding was subsequently confirmed in a study of the ontogeny of ileal and renal bile acid transport that demonstrated the expression of both ileal Na+-bile acid cotransporter mRNA and protein in rat kidney (3).

We have previously cloned a partial cDNA encompassing the entire coding region for the human ileal Na+-bile acid cotransporter (33) and analyzed inherited mutations associated with primary bile acid malabsorption (14). Whereas the ileal Na+-bile acid cotransporter has been cloned from a number of different species (4), a full-length clone has not been described. This is due in part to the large size of the message, ~4.0 kb in the hamster (32) and 5.0 kb in the rat (22), which far exceeds the 1,047-nucleotide coding region. In addition, although previous studies in rodents have shown ileal Na+-bile acid cotransporter mRNA expression in the ileum, kidney, cecum, and colon (22, 32) and liver Na+-bile acid cotransporter mRNA expression in liver and kidney (13), there is no information on the tissue expression of these carriers in humans. In this study, we describe the cloning of the full-length human ileal Na+-bile acid cotransporter cDNA and compare its human tissue expression with the related liver Na+-bile acid cotransporter NTCP (13).

The transport kinetics and specificity of Na+-bile acid cotransport have been examined in everted gut sacs, isolated ileal enterocytes, and ileal brush-border membranes from a variety of species (6, 11, 30). However, there is a paucity of information on the human ileal Na+-bile acid cotransporter (1, 10). The identification of the Na+-bile acid cotransporters from human liver (13) and ileum (33) makes it possible to determine their transport properties in the absence of other potential bile acid carriers by expression in transfected cells. In this study, the ion dependence and substrate specificity of the ileal and renal Na+-bile acid cotransporter were analyzed in transiently transfected COS and stably
transfected CHO cells. In contrast to the multispecific liver Na\textsuperscript{+}-bile acid cotransporter (13), these studies indicate that the ileal and renal Na\textsuperscript{+}-bile acid cotransporter retains a narrow substrate specificity for the reclamation of bile acids.

**MATERIALS AND METHODS**

Materials. Human ileum (obtained within 10 cm of the ileocecal valve), cecum, and kidney tissue samples were obtained from surgical specimens excised as a result of colon carcinoma, inflammatory bowel disease, or kidney carcinoma. Only normal tissues, as identified by the attending pathologist, were used for RNA isolation. Human liver (obtained from liver donors) was kindly provided by Dr. Benjamin Shenider (Department of Pediatrics, Mount Sinai School of Medicine). Tissues were frozen in liquid N\textsubscript{2} and stored at −70°C until use.

**General methods**. Total cellular RNA was isolated by the guanidinium isothiocyanate-CsCl centrifugation procedure. Poly(A) RNA was isolated using oligo(dT)-cellulose spin columns from Pharmacia-LKB Biotechnology (Piscataway, NJ). For Northern blot analysis, poly(A) RNA was fractionated on guanidinium isothiocyanate-CsCl centrifugation procedure.

A human placental genomic DNA library in λEMBL3 (catalog no. HL1067; Clontech; Palo Alto, CA) was screened as described (33). A single positive clone, λHG8, was identified after screening 2 × 10\textsuperscript{8} bacteriophages. After restriction enzyme digestion and Southern blot analysis, fragments corresponding to exon and flanking sequences were subcloned into pBluescript for DNA sequencing. These results showed that λHG8 encompassed only the 3' half of the human ileal Na\textsuperscript{+}-bile acid cotransporter gene (14, 33). To identify the 3' end of the human ileal Na\textsuperscript{+}-bile acid cotransporter cDNA, we employed the rapid amplification of cDNA ends (RACE) procedure. Reverse transcription was performed with 3 μg of human ileal poly(A) RNA and an oligo(dT) adapter primer, 5'-AAGGATCCCTGTCGACATC(CT)\textsuperscript{3} (nucleotides 3036 to 3059) for the subsequent amplification reactions. For the polymerase chain reaction (PCR) amplification (50 μl; 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 2 min), the reactions contained 1 μl of cDNA, 0.5 μM primers (HIBAT 52 and Universal Adapter primer, 5'-AAGGATCCCTGTCGACATC-3', 0.2 mM dNTPs, 1.5 mM MgCl\textsubscript{2}, and 0.5 U of Taq polymerase. Following PCR amplification, the 3' RACE products were isolated from a 1.2% (wt/vol) agarose gel, treated with T4 DNA polymerase, phosphorylated with T4 polynucleotide kinase, and ligated into Smal-digested pBluescript II KS. Individual clones were identified by colony hybridization using a \[32\text{P}\] labeled Pst I-Xba I fragment from the λHG8 clone that extended from nucleotides 2963 to 3839 downstream of the transcription start site. Fourteen clones that were positive by colony hybridization were selected for sequencing by the dideoxynucleotide method.

To identify the 5' end of the human ileal Na\textsuperscript{+}-bile acid cotransporter cDNA, a fragment of genomic DNA encompassing the putative transcription start site was isolated from a P1 library (14). Oligonucleotides for primer extension analysis were designed using this sequence. For primer extension analysis, HIBAT 34 (5'-TCACGTGCTCATAGAGTCTAATTTTC-3'; nucleotides 3036 to 3059) for the 5' RACE product was used to amplify the 3' end of the human ileal Na\textsuperscript{+}-bile acid cotransporter cDNA. A pair of oligonucleotide primers 5' GAGCCACGTTAATTGCTGGAATCC-3' (nucleotides 511 to 530) or HIBAT 67 (5'-GGGCTGAACTTATGTAATGC-3'; nucleotides 251 to 271) were labeled at the 5' end using \[32\text{P}\] ATP and T4 polynucleotide kinase, annealed to human ileal or renal poly(A) RNA, and extended with a modified Moloney murine leukemia virus reverse transcriptase (SuperScript II RNase H\textsuperscript{−}; Life Technologies). The primer extension products were resolved on a 6% acrylamide gel containing 7 M urea. The ends of the products were localized by simultaneous electrophoresis of a dideoxynucleotide sequencing reaction using HIBAT 34.

cDNA cloning from human kidney RNA. First-strand cDNA was synthesized from human kidney RNA using a cDNA synthesis kit (SuperScript kit; Life Technologies). A pair of oligonucleotide primers 5' GCTTCTGGACATCAGGCTC-3' (nucleotides 560 to 578) and 5' CTTTACTGAGAATCTCTGGCCTC-3' (nucleotides 1663 to 1682) were labeled at the 5' end using \[32\text{P}\] ATP and T4 polynucleotide kinase, annealed to human ileal or renal poly(A) RNA, and extended with a modified Moloney murine leukemia virus reverse transcriptase (SuperScript II RNase H\textsuperscript{−}; Life Technologies). The primer extension products were resolved on a 6% acrylamide gel containing 7 M urea. The ends of the products were localized by simultaneous electrophoresis of a dideoxynucleotide sequencing reaction using HIBAT 34.

The insert was subcloned into a pT7Blue T vector (Novagen). The insert was sequenced by the dideoxynucleotide method. The sequences were determined with an Applied Biosystems model 373A automated sequencer.
was sequenced by the dideoxynucleotide method using human ileal Na\(^+\)-bile acid cotransporter sequence-specific or pT7Blue T vector-specific primers.

Construction of pCMV-human liver bile acid transporter (NTCP) plasmid. A human liver Na\(^+\)-bile acid cotransporter (the Na\(^+\)-taurocholate co-transporting polypeptide, NTCP; Ref. 13) expression plasmid was constructed as follows. First-strand cDNA was synthesized from human liver poly(A\(^+\)) RNA using a cDNA synthesis kit (SuperScript kit; Life Technologies). A pair of oligonucleotide primers, 5\('\) AGGAG-GATGGAGGCCCCAACAGCGTCT 3\('\) and 5\('\) GCTAGGTGTTG-GCAAGGGGAGGATGCTCT 3\('\), corresponding to human liver in monolayer culture at 37°C in an atmosphere of 5% CO\(_2\) in on both strands by the dideoxynucleotide method.

Individual clones were screened by COS cell transfection. Clones that expressed taurocholate uptake activity were sequenced vidual clones were screened by COS cell transfection. Clones containing 700 µg/ml of G-418. CHO-K1 cells were seeded at 1.5 x 10\(^4\) cells/well on 48-well plates (700 µg/ml streptomycin; Life Technologies). A pair of oligonucleotide primers, 5\('\) AGGAG-GATGGAGGCCCCAACAGCGTCT 3\('\) and 5\('\) GCTAGGTGTTG-GCAAGGGGAGGATGCTCT 3\('\), corresponding to human liver Na\(^+\)-bile acid cotransporter nucleotides 77 to 103 and 1133 to 1107 (13) was used for PCR with human liver cDNA at an annealing temperature of 72°C. Following PCR amplification, an appropriate size product (1.056 base pairs) was isolated from a 0.8% (wt/vol) agarose gel. The fragment was treated with T4 DNA polymerase, phosphorylated with T4 polynucleotide kinase, and ligated into Sma I-digested pCMV5. Individual clones were screened by COS cell transfection. Clones that expressed taurocholate uptake activity were sequenced on both strands by the dideoxynucleotide method.

Cell culture and transfection. COS-1 cells were maintained in monolayer culture at 37°C in an atmosphere of 5% CO\(_2\) in medium A [Dubecco's modified Eagle's medium (DMEM) containing 4,500 mg/l D-glucose, 10% (vol/vol) fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin; Life Technologies]. CHO-K1 cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in medium B, which consisted of a 1:1 (vol/vol) mixture of DMEM containing 4,500 mg/l D-glucose and Ham's F-12 medium, 10% (vol/vol) fetal calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Life Technologies). For bile acid uptake assays, COS or CHO cells were incubated in medium C, which consisted of a Hanks' balanced salt solution containing 137 mM NaCl (33) or the indicated concentrations of cations and anions.

Stable overexpression of the human ileal Na\(^+\)-bile acid cotransporter was achieved by cotransfecting CHO-K1 cells with pCMV5-human ileal Na\(^+\)-bile acid cotransporter and pSV\(_{-}\)Neo using the calcium phosphate precipitation procedure (16) and selecting for resistant colonies. In parallel assays, the cell extracts were brought to 3% sodium dodecyl sulfate (SDS), 5% glycerol, 30 µM Tris-HCl, pH 7.4, 10 mM EDTA, and 100 mM dithiothreitol; the samples were boiled for 5 min and then alkylated by incubation with 330 mM iodoacetamide at 37°C for 30 min. The samples were resolved by SDS-polyacrylamide gel electrophoresis on 10% acrylamide gels and subjected to immunoblotting as previously described (33) using rabbit anti-ileal Na\(^+\)-bile acid cotransporter peptide antibody. The rabbit antibody was visualized using a horseradish peroxidase-conjugated goat anti-rabbit antibody and an enhanced chemiluminescence detection system (ECL; Amersham International, Buckinghamshire, UK).

Bile acid uptake assays. For transfection, the plasmids were isolated using the Wizard Maxiprep DNA purification procedure (Promega, Madison, WI). On day 0, 1.5 x 10\(^4\) COS cells per 100-mm dish were plated in medium A. On day 1, COS cells were transfected with 5 µg of either pCMV5-human ileal Na\(^+\)-bile acid cotransporter, pCMV5-human liver Na\(^+\)-bile acid cotransporter, or pCMV2-β-galactosidase DNA by the DEAE-dextran method (33). On day 2, the transfected cells were trypsinized, pooled, and replated in 24-well culture plates at 7 x 10\(^6\) cells/well in medium A. On day 4, the cells were incubated at 37°C for 10 min in the indicated media containing radiolabeled bile acid in the presence or absence of competitor. After incubation, the medium was removed, and each cell monolayer was washed three times with ice-cold PBS plus 0.2% (wt/vol) bovine serum albumin and 1 mM taurocholate and once with ice-cold PBS alone. The cell monolayer was dissolved in 0.1 N NaOH, and aliquots were taken to determine cell-associated protein and radioactivity. CHO cells were plated on day 0 at 3.8 x 10\(^5\) cells per 35-mm dish in medium B. On day 1, the cells were refed medium B containing 10 mM sodium butyrate. After 20 h, the dishes were washed and incubated in duplicate with Hanks' balanced salt solution containing 137 mM NaCl and the indicated concentration of [\(^{3}H\)]-labeled solute for 10 s at 37°C. The cell monolayers were processed as described for the COS cells. Uptake values were corrected for the background at each concentration of solute by subtracting the uptake values from parallel assays performed in the absence of Na\(^+\) or uptake values from parallel dishes of parental CHO-K1 cells. Kinetic parameters for taurocholate uptake were derived using a computer-based least-squares fit of individual data points. Substrate saturation curves were analyzed using Hanes-Woolf plots (21). Inhibition of Na\(^+\)-dependent taurocholate uptake by various substrates was evaluated kinetically by Dixon and Cornish-Bowden plot analysis.

**RESULTS**

Analysis of the human ileal Na\(^+\)-bile acid cotransporter cDNA. The full-length sequence for the human ileal Na\(^+\)-bile acid cotransporter cDNA was derived from cDNA and genomic DNA cloning and is shown schematically in Fig. 1A. The polyadenylation signal and poly(A) tail were identified using 3' RACE; the translation termination codon is followed by a long 3'-untranslated region of 2,134 nucleotides. The assignment of the 3' end was confirmed by Northern blot analysis. No transcript was detected in ileal or kidney RNA after hybridization of a \(^{32}\)P-labeled Xba I-Sac I
fragment that extended from nucleotides 3840 to 4357 downstream of the transcription start site; however, a 4.0-kb transcript was readily detected using a 32P-labeled Pst I-Xba I fragment that extended from nucleotides 2963 to 3839 downstream of the transcription start site (data not shown).

The transcriptional start sites were identified by primer extension analysis. Figure 2A (lane 4) shows the results of a primer extension analysis performed using a 20-nucleotide primer located 68 nucleotides upstream of the initiator methionine. Two prominent primer products (194 and 529 nucleotides in length) that extended from HIBAT 34 to positions 261 and 598 nucleotides upstream of the initiator methionine were obtained. To confirm the presence of the upstream start site, the primer extension was repeated using a 24-nucleotide primer located 325 nucleotides upstream of the initiator methionine (Fig. 2A, lane 2). The major product was a closely spaced doublet (267 and 273 nucleotides in length) that extended from HIBAT 67 to positions 598 and 592 nucleotides upstream of the initiator methionine (Fig. 2A, lane 2). The transcription start sites at position +1 and +338 are separated by two highly conserved head-to-tail repeats of 127 and 129 nucleotides, respectively (Fig. 1B).

Tissue expression of the human ileal Na⁺-bile acid cotransporter. Northern blot analysis of the ileal Na⁺-bile acid cotransporter mRNA in hamster (32) and rat (22) revealed single major transcripts of ~4.0 and 5.0 kb, respectively. To determine the size of the human ileal Na⁺-bile acid cotransporter message, Northern blot analysis was performed with human ileal poly(A) RNA. As shown in Fig. 3A, a 4.0-kb transcript was detected in human ileum. This transcript size is in close agreement with the composite size of the human ileal Na⁺-bile acid cotransporter cDNA as determined using a combination of RACE, cDNA, and genomic DNA cloning (3, 779 nucleotides without the poly(A) tail). The different transcripts arising from the two transcriptional start sites could not be readily distinguished under these Northern blotting conditions because of the large size of the full-length transcript.

Na⁺-dependent bile acid uptake has also been demonstrated in brush-border membranes of the kidney (31) and the sinusoidal membranes of the liver (13). To examine the tissue-specific expression of the human ileal Na⁺-bile acid transporter, Northern blot analysis was performed. A 4.0-kb transcript was readily detected in human kidney, but absent from liver, heart, brain, placenta, lung, skeletal muscle, and pancreas (Fig. 3B). In addition, no hybridization was detected after Northern blot analysis of poly(A) RNA from human spleen, thymus, prostate, testis, ovar, and peripheral blood leukocytes (data not shown). A very faint hybridization signal for the ileal Na⁺-bile acid cotransporter was detectable in the cecum upon longer
Strong hybridization of the ileal but not the liver Na⁺-bile acid cotransporter cDNA to a 4.0-kb transcript in ileum and kidney as well as the previous localization of a single gene for the ileal Na⁺-bile acid cotransporter to human chromosome 13q33 (14) suggest that the ileal Na⁺-bile acid cotransporter and the previously reported renal Na⁺-bile acid cotransporter activity (27, 31) are encoded by the same gene. To directly address this question, an Na⁺-bile acid cotransporter cDNA clone was isolated from human kidney total cDNA using a reverse transcriptase-PCR cloning strategy. The sequence of the kidney cDNA clone was identical to the human ileal Na⁺-bile acid cotransporter (data not shown). To determine whether the kidney Na⁺-bile acid cotransporter mRNA used the same transcriptional start sites as the human ileal message, primer extension analysis was performed with human kidney RNA. As shown in Fig. 2B, two prominent primer extended products were obtained that comigrated with those obtained from human ileum. These results indicate that the ileal Na⁺-bile acid cotransporter gene also encodes a renal Na⁺-bile acid cotransporter and confirm the results from a previous characterization of the properties and ontogeny of the rat renal Na⁺-bile acid cotransporter (3).

Ion dependence of taurocholate transport. A critical property of the ileal bile acid transporter is the dependence on an external Na⁺ gradient (30). To extend our understanding of the Na⁺-bile acid cotransport process, we examined the cation and anion dependence of the transporter. pCMV5-human ileal Na⁺-bile acid cotransporter-transfected COS cells were incubated in a modified Hanks’ buffer containing 5 μM [³H]taurocholate for 10 min at 37°C. Preliminary studies showed that uptake of [³H]taurocholate by ileal Na⁺-bile acid cotransporter-transfected COS cells was linear for up to 15 min at 37°C (data not shown). When 137 mM Na⁺ was replaced by K⁺, Li⁺, Rb⁺, Cs⁺, choline, or tetraethylammonium, taurocholate uptake was reduced at least

Fig. 2. Primer extension analysis of transcription initiation sites of human ileal Na⁺-bile acid cotransporter gene. A: positions of start sites, oligonucleotide primers, and repeat sequences are shown schematically at top. Transcription start sites are located at positions 1 and 338; the initiator methionine is located at position 599. Large arrows indicate repeat sequences. Small arrows (67 and 34) indicate oligonucleotides used for primer extension analysis. ATG, codon for the initiator methionine; open bar, coding region. 

B: 32P-labeled oligonucleotides HIBAT 67 (lanes 1 and 2) or HIBAT 34 (lanes 3 and 4) were annealed to 5 μg of tRNA (lanes 1 and 3) or human ileal poly(A) RNA (lanes 2 and 4) and extended using a modified Moloney murine leukemia virus reverse transcriptase. Primer extension products were resolved on a 6% acrylamide gel containing 7 M urea. Dried gel was exposed to Amersham Hyperfilm for 20 h at −70°C with an intensifying screen. Ends of the products were localized by simultaneous electrophoresis of a dideoxynucleotide sequencing reaction using HIBAT 34. *Primer extension products terminating at position +1 that were detected with HIBAT 34 and HIBAT 67. B: 32P-labeled oligonucleotide HIBAT 34 was annealed to 5 μg of tRNA (lane 1), 5 μg of liver poly(A) RNA (lane 2), 15 μg of kidney poly(A) RNA (lane 3), or 5 μg of ileal poly(A) RNA (lane 4) and extended using a modified Moloney murine leukemia virus reverse transcriptase. Primer extension products were resolved on a 6% acrylamide gel containing 7 M urea. Dried gel was exposed to Amersham Hyperfilm for 20 h at −70°C with an intensifying screen. M, molecular weight markers.
In contrast to the strict Na\textsuperscript{+} requirement, the ileal bile acid transporter does not exhibit a specific anion dependence. In addition to chloride ion, bicarbonate, bromide, and sulfate were able to support taurocholate uptake (Table 2). Figure 4 shows the uptake of taurocholate by human ileal Na\textsuperscript{+}-bile acid cotransporter-transfected COS cells as a function of Na\textsuperscript{+} concentration. Uptake increased as a sigmoidal function of Na\textsuperscript{+} concentration from 10 to 137 mM. Maximal taurocholate uptake was observed above 30 mM Na\textsuperscript{+} and was half-maximally stimulated at an Na\textsuperscript{+} concentration of 20 mM. This is similar to the value of 23 mM Na\textsuperscript{+} previously measured in human ileal brush-border membrane vesicles (1). When the data were analyzed by plotting taurocholate uptake vs. taurocholate uptake/[Na\textsuperscript{n}], where n is equal to the activator:substrate stoichiometry (26), the correlation coefficients for this analysis were as follows: n = 1, 0.487; n = 2, 0.937; n = 3, 0.848; and n = 4, 0.791. The lack of linearity of the plot for n = 1 suggests the involvement of more than one Na\textsuperscript{+} per taurocholate transport event. The inset in Fig. 4 shows a plot of taurocholate uptake vs. taurocholate uptake/[Na\textsuperscript{2}].

30-fold with no significant difference between various cations (Table 1). In contrast to the strict Na\textsuperscript{+} requirement, the ileal bile acid transporter does not exhibit a specific anion dependence. In addition to chloride ion, bicarbonate, bromide, and sulfate were able to support taurocholate uptake (Table 2).

Figure 4 shows the uptake of taurocholate by human ileal Na\textsuperscript{+}-bile acid cotransporter-transfected COS cells as a function of Na\textsuperscript{+} concentration. Uptake increased as a sigmoidal function of Na\textsuperscript{+} concentration from 10 to 137 mM. Maximal taurocholate uptake was observed above 30 mM Na\textsuperscript{+} and was half-maximally stimulated at an Na\textsuperscript{+} concentration of 20 mM. This is similar to the value of 23 mM Na\textsuperscript{+} previously measured in human ileal brush-border membrane vesicles (1). When the data were analyzed by plotting taurocholate uptake vs. taurocholate uptake/[Na\textsuperscript{n}], where n is equal to the activator:substrate stoichiometry (26), the correlation coefficients for this analysis were as follows: n = 1, 0.487; n = 2, 0.937; n = 3, 0.848; and n = 4, 0.791. The lack of linearity of the plot for n = 1 suggests the involvement of more than one Na\textsuperscript{+} per taurocholate uptake event. The inset in Fig. 4 shows a plot of taurocholate uptake vs. taurocholate uptake/[Na\textsuperscript{2}].

Specificity of uptake. To examine the substrate specificity of the human ileal Na\textsuperscript{+}-bile acid cotransporter, direct uptake experiments were performed using radio-labeled bile acids. As shown previously for taurocholate (33), the human ileal Na\textsuperscript{+}-bile acid cotransporter mediated the uptake of the unconjugated bile acid cholate, as well as glycine conjugates of deoxycholate, chenodeoxycholate, and ursodeoxycholate (Fig. 5). The apparent Michaelis constant (K\textsubscript{m}) values for uptake in transfected COS cells are listed in Table 3.
Table 1. Cation dependence of taurocholate uptake by human ileal Na\(^+\)-bile acid cotransporter-transfected COS cells

<table>
<thead>
<tr>
<th>Cation</th>
<th>Taurocholate Uptake, pmol·min(^{-1})·mg protein(^{-1})</th>
<th>% of Control</th>
</tr>
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<tbody>
<tr>
<td>Sodium</td>
<td>9.2 ± 0.41</td>
<td>100</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.3 ± 0.17</td>
<td>3</td>
</tr>
<tr>
<td>Lithium</td>
<td>0.3 ± 0.04</td>
<td>3</td>
</tr>
<tr>
<td>Rubidium</td>
<td>0.3 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Cesium</td>
<td>0.2 ± 0.01</td>
<td>2</td>
</tr>
<tr>
<td>Choline</td>
<td>0.0 ± 0.06</td>
<td>0</td>
</tr>
<tr>
<td>Tetraethylammonium</td>
<td>0.1 ± 0.02</td>
<td>1</td>
</tr>
</tbody>
</table>

Values for taurocholate uptake are means ± SD of triplicate measurements and are corrected for uptake into pCMV2-β-galactosidase-transfected cells. Data are also shown as percentage of control (uptake in the presence of Na\(^+\)). COS cells were transfected with pCMV5-human ileal Na\(^+\)-bile acid cotransporter or pCMV2-β-galactosidase DNA (5 µg) and assayed for uptake of 5 µM \(^{3}\)H]taurocholate (0.76 Ci/mmol) in presence of 137 mM Na\(^+\) or an equal concentration of potassium, lithium, rubidium, cesium, choline, or tetraethylammonium. After 10 min at 37°C, the medium was removed, and each cell monolayer was washed and processed to determine protein and cell-associated radioactivity.

Table 2. Anion dependence of taurocholate uptake by human ileal Na\(^+\)-bile acid cotransporter-transfected COS cells

<table>
<thead>
<tr>
<th>Anion</th>
<th>Taurocholate Uptake, pmol·min(^{-1})·mg protein(^{-1})</th>
<th>% of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>7.5 ± 0.7</td>
<td>100</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>8.1 ± 1.0</td>
<td>108</td>
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<tr>
<td>Bromide</td>
<td>6.2 ± 0.4</td>
<td>83</td>
</tr>
<tr>
<td>Acetate</td>
<td>2.1 ± 0.2</td>
<td>28</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.3 ± 0.3</td>
<td>31</td>
</tr>
<tr>
<td>Sulfate</td>
<td>5.2 ± 0.8</td>
<td>69</td>
</tr>
</tbody>
</table>

Values for taurocholate uptake are means ± SD of triplicate measurements and are corrected for uptake into pCMV2-β-galactosidase-transfected cells. Data are also shown as percentage of control (uptake in the presence of sodium chloride). COS cells were transfected with pCMV5-human ileal Na\(^+\)-bile acid cotransporter or pCMV2-β-galactosidase DNA (5 µg) and assayed for uptake of 5 µM \(^{3}\)H]taurocholate (0.52 Ci/mmol) in presence of 137 mM sodium chloride or an equal concentration of sodium acetate, bicarbonate, bromide, phosphate, or sulfate. After 10 min at 37°C, medium was removed, and each cell monolayer was washed and processed to determine protein and cell-associated radioactivity.

To extend our understanding of the substrate specificity of the human ileal and renal Na\(^+\)-bile acid cotransporter, cis-inhibition studies were also performed with a variety of other organic anions. The apparent \(K_i\) was determined by assaying \(^{3}\)H]taurocholate uptake at various concentrations of substrate and inhibitor; the kinetic types of inhibition were evaluated by complementary Dixon/Cornish-Bowden plot analysis and Line-weaver-Burk plot analysis. A comparison of the inhibition of taurocholate uptake in transfected COS cells at a 20-fold excess of competitor is shown in Fig. 7. Cyclosporin A was a potent inhibitor (inhibition type: noncompetitive) of taurocholate uptake, whereas bromosulfophthalein (inhibition type, noncompetitive) and chenodeoxycholate-3-sulfate (inhibition type, competitive) were relatively poor inhibitors. In contrast, a number of other organic anions and amphipathic molecules showed little inhibition. These included olsalazine, 17β-estradiol-3-sulfate, estrone-3-sulfate, taurodeoxycholate, and bilirubin ditaurate conjugate. In general, the human ileal Na\(^+\)-bile acid cotransporter was inhibited by fewer organic anions than previously determined for the liver Na\(^+\)-bile acid cotransporter (34). For example, bromosulfophthalein and 17β-estradiol-3-sulfate are potent inhibitors of the rat liver Na\(^+\)-bile acid cotransporter (33). In contrast, bromosulfophthalein was a weak inhibitor (apparent \(K_i\) = 144 µM) and 17β-estradiol-3-sulfate and estrone-3-sulfate were extremely poor inhibitors of the ileal Na\(^+\)-bile acid cotransporter.

To directly examine these substrate specificity differences, \(^{3}\)H]estrone-3-sulfate transport was analyzed in human liver or ileal Na\(^+\)-bile acid cotransporter-transfected COS cells. As shown in Fig. 8, both the human ileal and liver Na\(^+\)-bile acid cotransporters expressed saturable taurocholate uptake activity with apparent \(K_m\) and \(V_{max}\) values of (ileal) 18 µM and 48 pmol·min\(^{-1}\)·mg protein\(^{-1}\) and (liver) 10 µM and 333 pmol·min\(^{-1}\)·mg protein\(^{-1}\), respectively. The differences in the apparent \(V_{max}\) for taurocholate transport between the liver and ileal Na\(^+\)-bile acid cotransporter expression plasmids may reflect transfection efficiency or possibly differences in the substrate turnover number. 

<table>
<thead>
<tr>
<th>[NaCl] (mM)</th>
<th>[H] Taurocholate Uptake (pmol min(^{-1}) mg protein(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>25</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>100</td>
<td>0.63 ± 0.04</td>
</tr>
<tr>
<td>150</td>
<td>0.67 ± 0.05</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of Na\(^+\) concentration on taurocholate uptake by human ileal Na\(^+\)-bile acid cotransporter-transfected COS cells. On day 0, COS cells were seeded (1.5 × 10\(^4\) cells/100-mm dish) in DMEM and transfected the following day with pCMV5-human ileal Na\(^+\)-bile acid cotransporter or pCMV2-β-galactosidase DNA (5 µg) by the DEAE-dextran method. On day 2, each group of transfected cells was treated with trypsin, pooled, and replated in 24-well culture plates at 7 × 10\(^3\) cells/well. On day 5, cells were incubated at 37°C for 15 min in Hanks’ balanced salt solution containing 50 µM \(^{3}\)H]taurocholate (0.52 Ci/mmol) and the indicated concentration of NaCl (chloride chloride was included as osmotic replacement for NaCl). Medium was then removed, and each cell monolayer was washed and processed to determine protein and cell-associated radioactivity. Values for taurocholate uptake are means ± SD of triplicate measurements. Inset: plot of V/[Na\(^+\)] vs. V, where V = \(^{3}\)H]taurocholate uptake in pmol·min\(^{-1}\)·mg protein\(^{-1}\).
ber of the two transporters. In the same transfected COS cells, the transport of [3H]estrone-3-sulfate by the human liver Na\(^{+}\)-bile acid cotransporter-transfected cells was also saturable, with an apparent \(K_m\) of 60 µM and \(V_{max}\) of 111 pmol·min\(^{-1}\)·mg protein\(^{-1}\). Thus in COS cells from the same pooled transfection, the human liver Na\(^{+}\)-bile acid cotransporter exhibited a sixfold decreased affinity and a threefold lower maximal transport rate for estrone-3-sulfate compared with taurocholate. In contrast, uptake of estrone-3-sulfate by human ileal Na\(^{+}\)-bile acid cotransporter-transfected COS cells was indistinguishable from the mock-transfected cell background.

The inability to measure uptake of low-affinity potential substrates such as estrone-3-sulfate may represent detection problems as a result of low uptake activities in the transiently transfected COS cells. To overcome this problem, stably transfected CHO cell lines expressing the human ileal Na\(^{+}\)-bile acid cotransporter were generated. As shown in Fig. 9A, the untreated and sodium butyrate-induced stably transfected CHO cells express higher levels of human ileal Na\(^{+}\)-bile acid cotransporter protein and activity. Prior incubation for 20 h with 10 mM sodium butyrate increased the taurocholate uptake activity, and taurocholate uptake was linear at least up to 1 min in these cells. Eadie-Hofstee analysis of taurocholate uptake by the stably transfected CHO cells (Fig. 9B) revealed a \(K_m\) value of 18 µM for taurocholate, similar to values determined in transiently transfected COS cells. The overall taurocholate uptake activity was substantially increased in the stably transfected CHO cells, with an apparent \(V_{max}\) value of \(\sim 2,200\) pmol·min\(^{-1}\)·mg cell

### Table 3. Bile acid uptake in human ileal Na\(^{+}\)-bile acid cotransporter-transfected COS cells

<table>
<thead>
<tr>
<th>Bile Acid</th>
<th>Apparent (K_m), µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate</td>
<td>13.3 ± 5.5</td>
</tr>
<tr>
<td>Cholate</td>
<td>33.3 ± 12.8</td>
</tr>
<tr>
<td>Glycodeoxycholate</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>Glycochenodeoxycholate</td>
<td>5.7 ± 1.6</td>
</tr>
<tr>
<td>Glycoursodeoxycholate</td>
<td>4.1 ± 0.7</td>
</tr>
</tbody>
</table>

Data are means ± SE. \(K_m\), Michaelis constant. COS cells were transfected with pCMV5-human ileal Na\(^{+}\)-bile acid cotransporter or pCMV2-β-galactosidase DNA (5 µg) and assayed for uptake of radiolabeled bile acid as described in the legend to Fig. 5.

Fig. 5. Bile acid uptake activity of human ileal Na\(^{+}\)-bile acid cotransporter in transfected COS cells. COS cells were transfected with pCMV5-human ileal Na\(^{+}\)-bile acid cotransporter or pCMV2-β-galactosidase DNA (5 µg) and assayed for uptake in presence of the indicated concentration of [3H]cholate (1.0 Ci/mm mol; A), [14C]glycodeoxycholate ([14C]GDCA, 43.3 mCi/mm mol; B), [14C]glycochenodeoxycholate ([14C]GCDCA, 48.6 mCi/mm mol; C), or [14C]glycoursoxycholate ([14C]GUDCA, 40.2 mCi/mm mol; D). After 10 min at 37°C, medium was removed, and each cell monolayer was washed and processed to determine protein and cell-associated radioactivity. Uptake values were corrected for nonspecific uptake by mock (pCMV2-β-galactosidase) transfected cells and are means of duplicate measurements.Insets: Eadie-Hofstee analysis of uptake data.
protein⁻¹. However, as shown in the transiently transfected COS cells (Fig. 9B), uptake of estrone-3-sulfate by the human ileal Na⁺-bile acid cotransporter expressing CHO cells was statistically indistinguishable from the parental CHO-K1 cells (P > 0.05).

**DISCUSSION**

Analysis of the human ileal Na⁺-bile acid cotransporter cDNA. In this study, we report the cloning of the full-length human ileal Na⁺-bile acid cotransporter cDNA and mapping of its 5’ transcriptional start sites and 3’ end. Whereas a partial cDNA sequence (33) and gene structure (14) for the human ileal Na⁺-bile acid cotransporter have been identified, identification of the transcription start sites and 3’ end has not been described. The entire human ileal Na⁺-bile acid cotransporter cDNA sequence was elucidated using a combination of cDNA and genomic DNA cloning, 3’ RACE, and primer extension analysis. Analysis of the 5’ end of the human ileal Na⁺-bile acid cotransporter message revealed two major transcriptional start sites located ~337 nucleotides apart. At this time it is not known whether this unusual heterogeneity results from the use of multiple promoters. The two start sites are separated by an intervening head-to-tail dimer of highly conserved repeat sequences. The two sequences, 127 and 129 nucleotides in length, respectively, are 91% identical but do not show identity to any other sequences in the current versions of several DNA sequence data bases. This repeat sequence is a recent event in evolution and is not present in the mouse ileal Na⁺-bile acid cotransporter gene (Dawson, unpublished data). The effect of these sequences on ileal Na⁺-bile acid cotransporter gene transcription is not known. However, instability of this repeat sequence could affect ileal Na⁺-bile acid cotransporter expression and lead to a phenotype such as primary bile acid malabsorption (14). In addition, there are obvious translational consequences associated with such widely separated transcription start sites. The upstream start site generates a transcript with a 5’-untranslated region of 598 nucleotides that encodes 14 upstream AUG codons. In contrast, the downstream start site generates a 5’-untranslated region of 261 nucleotides with only 3 upstream AUG codons. Since the translation scanning hypothesis (9) predicts that an abundance of upstream AUG codons would inhibit translation from the downstream authentic initiator methionine, the shorter transcript would be preferentially translated. Conditions that selectively increase transcription initiation at the downstream start site may lead to increased ileal Na⁺-bile acid cotransporter protein expression.

The human ileal Na⁺-bile acid cotransporter message contains a long 3’-untranslated region of 2,134 nucleotides and utilizes a single polyadenylation site.

Table 4. Inhibition of taurocholate uptake in human ileal Na⁺-bile acid cotransporter-transfected COS

<table>
<thead>
<tr>
<th>BileAdd</th>
<th>Apparent Kᵢ, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate</td>
<td>24.8±10.0</td>
</tr>
<tr>
<td>Cholate</td>
<td>41.5±9.3</td>
</tr>
<tr>
<td>Taurodeoxycholate</td>
<td>17.2±3.1</td>
</tr>
<tr>
<td>Deoxycholate</td>
<td>6.3±2.5</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>6.1±4.6</td>
</tr>
<tr>
<td>Chenodeoxycholate</td>
<td>3.3±1.6</td>
</tr>
<tr>
<td>Tauroursodeoxycholate</td>
<td>28.0±6.1</td>
</tr>
<tr>
<td>Ursodeoxycholate</td>
<td>75.0±13.5</td>
</tr>
</tbody>
</table>

Data are means ± SD of 3 independent COS cell transfections. Kᵢ, inhibition constant. COS cells were transfected with pCMV5-human ileal Na⁺/bile acid cotransporter or pCMV2-ß-galactosidase DNA (5 µg) and assayed for uptake at 5, 10, and 25 µM [³H]taurocholate (0.76 Ci/mmol) in absence or presence of 10, 50, or 100 µM bile acid competitor. After 10 min at 37°C, medium was removed, and each cell monolayer was washed and processed to determine protein and cell-associated radioactivity. Inhibition of taurocholate uptake was evaluated by Dixon and Cornish-Bowden plot analysis.
analysis of human liver Na\(^+\)-bile acid cotransporter expression. The lack of liver Na\(^+\)-bile acid cotransporter mRNA expression in kidney agrees with previous studies in the hamster (5) but differs from studies in the rat where a faint signal was observed (13). This may be due to species differences or the liver Na\(^+\)-bile acid cotransporter signal may be below our level of detection. Interestingly, the liver Na\(^+\)-bile acid cotransporter cDNA also hybridized to a smaller 1.1-kb transcript in placenta. Screening of a human placental cDNA library identified a partial liver Na\(^+\)-bile acid cotransporter cDNA consistent with the placental transcript arising from the same gene (Walters and Dawson, unpublished results).

In humans, only 1–2 µmol of bile acids are excreted in the urine per day, implying a highly efficient tubular reabsorption (23). Even in patients with liver disease where plasma bile acid concentrations are elevated, the 24 h urinary excretion of nonsulfated bile acids remains significantly less than the quantity that undergoes glomerular filtration (15, 18, 23, 24). This diminished urinary excretion was explained in subsequent studies that identified an Na\(^+\)-dependent active bile acid reabsorption process in the proximal renal tubules (27, 31). More recently, a careful study of the ontogeny of rat ileal Na\(^+\)-bile acid cotransporter activity, protein, mRNA, and gene transcription indicated that this gene is also responsible for renal bile acid transport (3). In the present study, we have confirmed and extended those results. Using a reverse transcriptase-PCR cloning and sequencing strategy, we have shown the coding region of the cross-hybridizing human kidney cDNA to be identical to the ileal Na\(^+\)-bile acid cotransporter. Analysis of the 5′ end of the kidney transcript revealed that the same transcriptional start sites are utilized in adult ileum and kidney. These results indicate that both the ileal and renal Na\(^+\)-bile acid cotransporters are encoded by identical transcripts derived from the SLC10A2 gene. In addition to the physiological implications, these results also have therapeutic consequences. Recently, potent inhibitors of the ileal Na\(^+\)-bile acid cotransporter have been developed as potential therapies for hypercholesterolemia (12, 29). Since the same transporter is also expressed in the kidney, these inhibitors should also block renal reclamation of nonsulfated bile acids and increase urinary bile acid output.

Ion dependence of taurocholate uptake. Taurocholate uptake by human ileal Na\(^+\)-bile acid cotransporter-transfected COS cells exhibited a strict requirement for external Na\(^+\) (Table 1). This distinguishes the ileal Na\(^+\)-bile acid cotransporter from other Na\(^+\)-solute transporters such as the glucose or succinate transporters, where Li\(^+\) will weakly support solute uptake. The Na\(^+\)-coupled transport of some amino acids and neurotransmitters has been shown to require an accompanying Cl\(^-\) anion (8). In contrast, the human ileal Na\(^+\)-bile acid cotransporter does not exhibit a strict Cl\(^-\) requirement for taurocholate uptake in transfected COS cells. These findings extend the results of earlier studies that examined the electrogenic nature of taurocholate uptake by ileal brush-border membrane vesicles (1, 30).

As noted previously for the rat ileal Na\(^+\)-bile acid cotransporter cDNA, the human ileal Na\(^+\)-bile acid cotransporter 3′-untranslated region contains a number of di- and trinucleotide repeats as well as multiple copies of an ATTTA motif that is associated with mRNA instability. These elements may be important in explaining previously noted differences between ileal Na\(^+\)-bile acid cotransporter steady-state mRNA levels and gene transcription rates in developing ileum (3, 22).

Analysis of the human ileal Na\(^+\)-bile acid cotransporter mRNA expression. By Northern blot analysis, the ileal Na\(^+\)-bile acid cotransporter mRNA has been found in hamster ileum, distal jejunum, and kidney (32) and in rat ileum, kidney, cecum, and proximal colon (22). In this study of mRNA expression in human tissues, a 4.0-kb transcript was also found in ileum and kidney, whereas no hybridization was detected in pancreas, brain, placenta, lung, skeletal muscle, and a variety of other human tissues. A very weak signal was also detected in human cecum and verified by reverse transcriptase-PCR analysis. Under similar high-stringency Northern blotting conditions, human liver Na\(^+\)-bile acid cotransporter mRNA was detected in liver, but not in kidney. This is the first report of a Northern blot
those studies, taurocholate uptake was still observed after Cl⁻ was replaced with SCN⁻, SO₄²⁻, or isethionate. These results argue against a role for a cotransported anion in taurocholate transport and rule out a specific requirement for the Cl⁻ anion. Analysis of taurocholate transport as a function of Na⁺ concentration revealed a sigmoidal relationship suggesting an Na⁺:taurocholate stoichiometry greater than 1:1. However, electrogenic Na⁺:taurocholate cotransport has not been a universal finding in earlier studies (30). Ultimately, the ability to overexpress the Na⁺:bile acid cotransporters in heterologous systems such as Xenopus oocytes and CHO cells will permit the use of sensitive voltage-clamp techniques to unambiguously resolve this question (28).

Specificity of bile acid transport. Taurocholate uptake by the expressed human ileal Na⁺:bile acid cotransporter was saturable with an apparent Kₘ of ~13 µM. This apparent Kₘ was similar to values reported using a human ileal mucosa technique and human isolated brush-border membrane vesicles (1). In the studies of transport specificity, the human ileal Na⁺:bile acid cotransporter had a higher affinity for the dihydroxy bile acids chenodeoxycholate and deoxycholate than ursodeoxycholate or the trihydroxy bile acid, cholate. Although there is a paucity of information on the specificity of the human ileal bile acid transport system (10), these data generally agree with earlier studies in the rat and guinea pig (29). The ileal Na⁺:bile acid cotransporter had a similar or slightly lower affinity for taurosodeoxycholate than taurocholate. In contrast, the liver Na⁺:bile acid cotransporter exhibited a much higher affinity (almost 3-fold) for taurosodeoxycholate than taurocholate (13). The relative affinities of the other bile acids tested and the effect of conjugation on the apparent Kₘ were similar between the human ileal and liver Na⁺:bile acid cotransporters. For the dihydroxy bile acids, chenodeoxycholate and deoxycholate, taurine conjugation had little effect or slightly increased the apparent Kₘ. In contrast, taurine conjugation lowered the apparent Kₘ for the more hydrophilic bile acids cholate and ursodeoxycholate.

Sulfation is an important pathway in bile acid metabolism (7) and is thought to promote the normal fecal excretion of lithocholate. In cholestasis, hepatic or renal addition of a sulfate moiety to the 3α and 7α positions of bile acids increases renal excretion and represents a major route for bile acid elimination (16, 24). In this study, chenodeoxycholate-3-sulfate was shown to be a weak inhibitor of taurocholate uptake by the human ileal and renal Na⁺:bile acid cotransporter. However, in direct uptake studies with Na⁺:bile acid cotransporter-transfected COS cells, [¹⁴C]chenodeoxycholate-3-sulfate was not transported by the ileal bile acid transporter and was only weakly transported by the liver bile acid transporter (data not shown). This result agrees with earlier studies in guinea pig ileum (11) and rat liver that suggested there was little or no Na⁺:dependent transport of chenodeoxycholate-3-sulfate. Thus at the molecular level, sulfation increases fecal and urinary bile acid excretion by decreasing its binding and blocking its transport by the ileal and renal Na⁺:bile acid cotransporter. This concept has been applied to increase the delivery of ursodeoxycholic acid to the colon by administering ursodeoxycholic acid as the sulfate conjugates (17).

Cyclosporin A has been shown to inhibit the rat ileal bile acid transporter (19). However, those studies did not determine whether the affected step was uptake across the apical brush-border membrane or transcellu-
lary transport and efflux across the basolateral membrane of the ileal enterocyte. In the present study, cyclosporin A was shown to be a potent noncompetitive inhibitor of the ileal Na\(^+\)-bile acid cotransporter. It is not clear why a significantly higher dose of cyclosporin A was required to inhibit glycocholate transport in everted gut sacs (50% transport inhibition at 2.69 mM cyclosporin A) than in the transfected COS cells (apparent \(K_i = 25 \mu M\) for inhibition of taurocholate uptake). This apparent \(K_i\) value (25 \(\mu M\)) is similar to that determined for the liver Na\(^+\)-bile acid cotransporter in isolated rat hepatocytes and sinusoidal rat liver plasma membrane vesicles (34).

Another potential inhibitor that was examined is olsalazine. Olsalazine is a therapeutic agent for inflammatory bowel disease that is composed of two 5-aminosalicylic acid molecules joined by an azo bond. At millimolar concentrations, this agent has been shown to be a noncompetitive inhibitor of Na\(^+\)-dependent bile acid transport in rat ileum (2). In this study, olsalazine had little inhibitory effect on taurocholate uptake by the human ileal Na\(^+\)-bile acid cotransporter at concentrations below 1 mM and acted as only a weak noncompetitive inhibitor at concentrations between 1 and 5 mM (the highest concentration used; data not shown). Thus, although it is possible that inhibition of ileal bile acid absorption by olsalazine contributes to the diarrhea associated with this agent, the very high apparent \(K_i\) argues that mechanisms other than direct inhibition of bile acid uptake are also involved.

Estrone-3-sulfate transport. A comparison of the pattern of cis-inhibition revealed a limited overlap of substrate specificity between the liver and ileal Na\(^+\)-bile acid cotransporters. A striking example is estrone-3-sulfate and 17\(\beta\)-estradiol-3-sulfate. Preliminary reports (20) and this study indicate that estrone-3-sulfate is also transported by the human liver Na\(^+\)-bile acid cotransporter. In contrast, estrone-3-sulfate showed little ability to inhibit taurocholate transport by human ileal Na\(^+\)-bile acid cotransporter and was not a substrate in direct uptake experiments.

In conclusion, these studies indicate that the ileal and renal Na\(^+\)-bile acid cotransporter has a more limited substrate specificity compared with the multiphasic liver Na\(^+\)-bile acid cotransporter. Whereas the liver Na\(^+\)-bile acid cotransporter may have evolved to aid in the hepatic clearance of steroid sulfates, organic anion metabolites, and xenobiotics (13), the ileal and renal Na\(^+\)-bile acid cotransporter retained a narrow specificity for the reclamation of bile acids. This narrow substrate specificity agrees with the physiological location of the transporter in the enterohepatic circulation.
(6, 7). In the lumen of the ileum or the renal proximal tubules, bile acids are efficiently recovered, whereas many non-bile acid metabolites and xenobiotics are destined for elimination in the feces or urine.

The nucleotide sequence reported in this study has been submitted to the GenBank/EMBL Data Bank with accession no. U10417.

This work was supported by the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant DK-47587 and by an American Gastroenterology Association/Janssen Pharmaceutical Research Scholar Award to P. A. Dawson. P. A. Dawson is an American Heart Association Established Investigator. R. W. Daniel was supported by National Heart, Lung, and Blood Institute (NHLBI) Cardiovascular Pathology National Service Training Award HL-07115. M. W. Love was supported by NHLBI Cardiovascular Pathology National Service Training Award HL-07115. M. H. Wong is the recipient of NIDDK Predoctoral Fellowship DK-08718.

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Received 7 August 1997; accepted in final form 9 October 1997.

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