Molecular Cloning, Chromosomal Localization, and Functional Characterization of a Human Liver Na^+/Bile Acid Cotransporter

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Abstract

We have used a cDNA probe from a cloned rat liver Na^+/taurocholate cotransporting polypeptide (Ntcp) to screen a human liver cDNA library. A 1,599-bp cDNA clone that encodes a human Na^+/taurocholate cotransporting polypeptide (NTCP) was isolated. The human NTCP consists of 349 amino acids (calculated molecular mass of 38 kD) and exhibits 77% amino acid homology with the rat Ntcp. In vitro translation experiments indicate that the protein is glycosylated and has a molecular weight similar to the rat Ntcp. Injection of in vitro transcribed cRNA into *Xenopus laevis* oocytes resulted in the expression of Na^+-dependent taurocholate uptake. Saturation kinetics indicated that the human NTCP has a higher affinity for taurocholate (apparent K_m = 6 μM) than the previously cloned rat protein (apparent K_m = 25 μM). NTCP-mediated taurocholate uptake into oocytes was inhibited by all major bile acid derivatives (100 μM), bumetanide (500 μM), and bromosulphophthalein (100 μM). Southern blot analysis of genomic DNA from a panel of human/hamster somatic cell hybrids mapped the human *NTCP* gene to chromosome 14. (*J. Clin. Invest.* 1994. 93:1326–1331.) Key words: bile salts • hepatocytes • organic anion transport • sinusoidal • taurocholate

Introduction

Bile formation is an important function of hepatocytes and involves transport of bile acids and other organic anions from portal blood into bile. Conjugated bile acids such as taurocholate or glycocholate enter hepatocytes predominantly via a sodium-dependent cotransport system. Phenomenologicaly, this secondary active bile acid uptake system has been well characterized in a variety of experimental systems including the perfused rat liver, isolated rat hepatocytes, isolated rat, and human sinusoidal membrane vesicles and *Xenopus laevis* oocytes (1–5). Using functional expression cloning in *Xenopus laevis* oocytes, a rat liver Na^+-taurocholate cotransporting polypeptide (Ntcp)[1] has been cloned (6). In this study, we have used an Ntcp-derived cDNA probe to screen a human liver cDNA library. We report the isolation and functional characterization of the human hepatocellular Na^+-dependent taurocholate cotransporter and its chromosomal localization.

Methods

Cloning of a human Na^+/bile acid cotransporter cDNA. Total human liver RNA was prepared from pieces of frozen liver (obtained from kidney donors and kindly provided by U. A. Meyer, Biozentrum Basel, Switzerland) using a single-step acid guanidinium thiocyanate-phenol-chloroform extraction method (7). mRNA was isolated using oligo(dT)-cellulose chromatography (8). A cDNA library was constructed from total poly(A)$^+$ RNA (Superscript kit; Life Technologies, Inc., Gaithersburg, MD). The cDNA was unidirectionally ligated into NotI/SalI cut pSPORT1, and recombinant plasmids were introduced into *Escherichia coli* WM1100 by electroporation (Gene Pulser; Bio-Rad Laboratories, Hercules, CA). Replica filters containing 2×10^5 recombinants of the cDNA library were screened using an EcoRI fragment (nucleotides 261–1,187 of the rat Ntcp cDNA) (6) that was labeled with 3,000 Ci/mmol [α-^{32}P]dCTP (Amersham International, Buckinghamshire, United Kingdom) using the Random Primed DNA Labeling Kit (Boehringer Mannheim GmbH, Mannheim, Germany). After 2 h of prehybridization at 42°C in 50% formamide, 0.75 M NaCl/0.075 M sodium citrate at pH 7.0 (5× SSC), 5× Denhardt's solution, 0.5% SDS, and 200 μg/ml of denatured salmon sperm DNA, the filters were hybridized for 16 h at 42°C in the same solution containing, in addition, the labeled probe (2×10^6 cpm/ml). After hybridization, the filters were washed twice for 15 min at room temperature in 2× SSC, 0.1% SDS, followed by one wash for 1 h at 42°C in 1× SSC, 0.1% SDS, and one final wash for 15 min at 50°C in 0.1× SSC, 0.1% SDS. Washed filters were exposed to x-ray film at −70°C overnight. After two rounds of screening, single positive colonies, which were functionally tested using *Xenopus laevis* oocytes, were obtained. 5 ng of in vitro–transcribed cRNA were injected into *Xenopus laevis* oocytes and Na^+-dependent taurocholate uptake was measured as described (5). Sequence analysis was performed using double-stranded cDNA as template and the T7 Sequencing kit (Pharmacia Biotech Inc., Piscataway, NJ). Human liver Na^+/taurocholate cotransporting polypeptide (NTCP) cDNA was sequenced in both directions using either unidirectionally deleted clones (Erase-a-base; Promega Corp., Madison, WI) or especially synthesized oligonucleotide primers. Nucleotide and amino acid sequence analyses were performed with the DNA and protein sequence analysis program DNASIS/PROSIS (Pharmacia Biotech Inc., Piscataway, NJ). Putative membrane spanning domains were de-

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Received for publication 27 July 1993 and in revised form 22 October 1993.

J. Clin. Invest.

0021-9738/94/03/1326/06 $2.00
Volume 93, March 1994, 1326–1331

1. *Abbreviations used in this paper:* BSP, bromosulfophthalein; Ntcp, rat liver Na^+/taurocholate cotransporting polypeptide; NTCP, human liver Na^+/taurocholate cotransporting polypeptide.

termined according to Klein et al. (9). The GCG software package (Genetics Computer Group, Inc., Madison, WI) (10) was used to perform sequence comparison and multiple alignments.

Transport assays in oocytes. Oocytes were maintained in culture and uptake of 2.1 Ci/mmol [G-^{3}H]taurocholic acid (Du Pont-New England Nuclear, Boston, MA) was determined as described (5).

Chromosomal localization of NTCP. Southern blots containing genomic DNA samples from 26 individual somatic cell hybrids digested with PstI were obtained from BIOS Corp. (New Haven, CT). After 1.5 h of prehybridization at 42°C in 50% formamide, 5× SSC, 5× Denhardt's solution, 0.5% SDS, and 200 μg/ml of denatured salmon sperm DNA the filters were hybridized for 16 h at 42°C in the same solution containing, in addition, 2×10^6 cpm/ml of labeled probe (a PflMI/EcoRI fragment corresponding to nucleotides −8 to 987 encompassing most of the coding region of NTCP). After hybridization, the filters were washed twice for 5 min at room temperature in 2× SSC, 0.1% SDS, followed by one wash for 15 min at 63°C in 0.1× SSC, 0.1% SDS. Filters were exposed to x-ray film at −70°C.

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          1  A AAG AAG GCA TCC AGC AAG AAC TGC ACA AGA AAC GGA GTC AGC CGG AGA ACA AGG AGT GGT CTT CCA CTG CCT CAC AGG AGG   82
Rat              V           V                           S               G           H       A           K
Human     1  M   E   A   H   N   A   S   A   P   F   N   F   T   L   P   P   N   F   G   K   R   P   T   D   L   A   L   S     28
         83  ATG GAG GCC CAC AAC GCG TCT GCC CCA TTC AAC TTC ACC CTG CCA CCC AAC TTT GGC AAG CGC CCC ACA GAC CTG GCA CTG AGC  166
Rat          I               L           L   L
Human    29  V   I   L   V   F   M   L   F   F   I   M   L   S   L   G   C   T   M   E   F   S   K   I   K   A   H   L   W     56
        167  GTC ATC CTG GTG TTC ATG TTG TTC TTC ATC ATG CTC TCG CTG GGC TGC ACC ATG GAG TTC AGC AAG ATC AAG GCT CAC TTA TGG  250
Rat                          V   I   V                       F                       A           L               I       H
Human    57  K   P   K   G   L   A   I   A   L   V   A   Q   Y   G   I   M   P   L   T   A   F   V   L   G   K   V   F   R     84
        251  AAG CCT AAA GGG CTG GCC ATC GCC CTG GTG GCA CAG TAT GGC ATC ATG CCC CTC ACG GCC TTT GTG CTG GGC AAG GTC TTC CGG  334
Rat              S                                   I                                               L       T
Human    85  L   K   N   I   E   A   L   A   I   L   V   C   G   C   S   P   G   G   N   L   S   N   V   F   S   L   A   M    112
        335  CTG AAG AAC ATT GAG GCA CTG GCC ATC TTG GTC TGT GGC TGC TCA CCT GGA GGG AAC CTG TCC AAT GTC TTC AGT CTG GCC ATG  418
Rat                                                          S       S                                           V
Human   113  K   G   D   M   N   L   S   I   V   M   T   T   C   S   T   F   C   A   L   G   M   M   P   L   L   L   Y   I    140
        419  AAG GGG GAC ATG AAC CTC AGC ATT GTG ATG ACC ACC TGC TCC ACC TTC TGT GCC CTT GGC ATG ATG CCT CTC CTC CTG TAC ATC  502
Rat                  K                                                                   M                   I
Human   141  Y   S   R   G   I   Y   D   G   D   L   K   D   K   V   P   Y   K   G   I   V   I   S   L   V   L   V   L   I    168
        503  TAC TCC AGG GGG ATC TAT GAT GGG GAC CTG AAG GAC AAG GTG CCC TAT AAA GGC ATC GTG ATA TCA CTG GTC CTG GTT CTC ATT  586
Rat                                                      H       V   P       I   L                           T   F
Human   169  P   C   T   I   G   I   V   L   K   S   K   R   P   Q   Y   M   R   Y   V   I   K   G   G   M   I   I   I   L    196
        587  CCT TGC ACC ATA GGG ATC GTC CTC AAA TCC AAA CGG CCA CAA TAC ATG CGC TAT GTC ATC AAG GGA GGG ATG ATC ATC ATT CTC  670
Rat              L                       A           V                   N                   V               H       L
Human   197  L   C   S   V   A   V   T   V   L   S   A   I   N   V   G   K   S   I   M   F   A   M   T   P   L   L   I   A    224
        671  TTG TGC AGT GTG GCC GTC ACA GTT CTC TCT GCC ATC AAT GTG GGG AAG AGC ATC ATG TTT GCC ATG ACA CCA CTC TTG ATT GCC  754
Rat                                  S               M           I                       Q           P   S
Human   225  T   S   S   L   M   P   F   I   G   F   L   L   G   Y   V   L   S   A   L   F   C   L   N   G   R   C   R   R    252
        755  ACC TCC TCC CTG ATG CCT TTT ATT GGC TTT CTG CTG GGT TAT GTT CTC TCT GCT CTC TTC TGC CTC AAT GGA CGG TGC AGA CGC  838
Rat              I                       F           I                                       T
Human   253  T   V   S   M   E   T   G   C   Q   N   V   Q   L   C   S   T   I   L   N   V   A   F   P   P   E   V   I   G    280
        839  ACT GTC AGC ATG GAG ACT GGA TGC CAA AAT GTC CAA CTC TGT TCC ACC ATC CTC AAT GTG GCC TTT CCA CCT GAA GTC ATT GGA  922
Rat                                                              A                   I       I           R
Human   281  P   L   F   F   F   P   L   L   Y   M   I   F   Q   L   G   E   G   L   L   L   I   A   I   F   W   C   Y   E    308
        923  CCA CTT TTC TTC TTT CCC CTC CTC TAC ATG ATT TTC CAG CTT GGA GAA GGG CTT CTC CTC ATT GCC ATA TTT TGG TGC TAT GAG 1006
Rat              I       P               Q           I   T       K           A           D   A   T       A       E   K
Human   309  K   F   K   T   P   K   D   K   T   K   M   I   Y   T   A   A   T   T   E   E   T   I   P   G   A   L   G   N    336
       1007  AAA TTC AAG ACT CCC AAG GAT AAA ACA AAA ATG ATC TAC ACA GCT GCC ACA ACT GAA GAA ACA ATT CCA GGA GCT CTG GGA AAT 1090
Rat                  H   N       N   I   P   P   L   Q   P   G   P   S   P   N   G   L   N   S   G   Q   M   A   N
Human   337  G   T   Y   K   G   E   D   C   S   P   C   T   A                                                                350
       1091  GGC ACC TAC AAA GGG GAG GAC TGC TCC CCT TGC ACA GCC TAG CCC TTC CCC TGG TGG CCT GGA TTC TGG TCC CAA AGC AAT TCT 1174
       1175  GAA AGC CAG TGT GGT AAA CTA GAG AGA GCA GCA AAA ACA CCA GTC TTG CCT GAG TCT TTC TCC AGC ATT TCC AGT ACA TCT ATC 1258
       1259  AGA ATC ATC AAG TCT TGG CCG GGA ACA CAG ACA GGG TGT CTA CCC AAG AAG CCT CAC CTA TCC CCA ACT TAG AAT TTG CTA CTT 1342
       1343  ATT TTA AAG ACT TGT TCA GTG ACT GTA AAC TCT ATG AAA CCA GAA ACC GAA TCT GCC TCT TGC TGG GAT CTC TAA AAG TGT CTG 1426
       1427  ATA AGC ATC TTA AAG TCA CTC AAT TCC TGA ACT AAT CAA TAT ATA TGT TTA ACC CAT TAC TCA AAT ACC CAA ATC CCA TTC CAA 1510
       1511  GTT TTG TGA CCC AAA AGA GAA ATA AAT GCT CAC AAG TGC TGT AGA ATT AAA CTT CAG AAG TTC TAA CCT TAA AAA AAA AAA AAA 1594
       1595  AAA AA                                                                                                          1599
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Figure 1. Nucleotide and deduced amino acid sequences of NTCP cDNA. Amino acid residues that differ between the human and the rat protein are shown above the amino acid sequence. (The sequence reported in this paper has been submitted to the GenBank Data Bank with the accession number L21893.)

Results and Discussion

Nucleotide and amino acid sequences of NTCP. To clone the NTCP, we screened ~ 200,000 colonies of a cDNA library prepared from human liver poly(A)$^+$ RNA using as a probe a ^{32}P-labeled EcoRI fragment complementary to the major part of the coding region of the rat Ntcp cDNA (6). After two rounds of screening, five single clones were identified. cDNA sequence analysis revealed that four of them were identical. mRNA was synthesized in vitro from these four clones and injected into Xenopus laevis oocytes. Indeed, all four cRNAs led to the expression of Na^+-dependent taurocholate uptake. One of these functionally active clones was sequenced and the cDNA and deduced amino acid sequences are shown in Fig. 1. The total cDNA insert of the human NTCP consists of 1,599 nucleotides. Starting with the initiation site at nucleotide 83, an open reading frame extends over the next 1,047 nucleotides coding for a protein of 349 amino acids with a calculated molecular mass of ~ 38 kD. Although the presence of a polyadenylation signal ~ 40 bases upstream of the poly(A) tail together with hybridization of the cDNA to a 1.6-kb human liver mRNA on a Northern blot (data not shown) indicate the isolation of a full-length clone, additional primer extension experiments shall reveal the location of the cap site and thus establish the real length of the 5′ untranslated sequence. The amino acid sequence of the human and the rat Na^+/bile acid cotransporters is compared in Figs. 1 and 2. There is 88% similarity between the human and the rat amino acid sequences, 77% of the residues being identical and another 11% being conservative replacements. Whether the clustering of nonconservative amino acid replacements at the COOH-terminal end of the protein has any functional significance is not yet known. At the DNA level, the lowest identities were found within the 5′-noncoding region (70%) and the highest identities within the coding and the 3′-noncoding regions (83%). The overall identity of only 78% with the rat cDNA explains the weak signal previously obtained on Northern blots (6, 11). Based on hydrophobicity analysis (9) and in analogy to the rat Ntcp, we propose the secondary structure model with seven transmembrane domains as depicted in Fig. 2. In vitro translation experiments performed as described (6) resulted in the synthesis of an unglycosylated 34-kD polypeptide on SDS-PAGE in the absence of a glycosylated 40-kD polypeptide in the presence of dog pancreatic microsomes (data not shown). The 6-kD difference is compatible with two sites being glycosylated. Experiments using site-directed mutagenesis to determine the natural glycosylation sites of the rat Ntcp revealed that Asn5 and Asn11 of the rat protein are glycosylated (Hagenbuch, B., and P. J. Meier, manuscript in preparation). In addition, immunostaining of primary rat hepatocytes with a polyclonal antibody against the COOH-terminal end of the rat Ntcp was only achieved in the presence of detergents, suggesting an intracellular localization of the COOH-terminal domain (unpublished observation). These data support the predicted model shown in Fig. 2, but additional experiments are required to definitively determine the exact location of all cytoplasmic loops and of the number

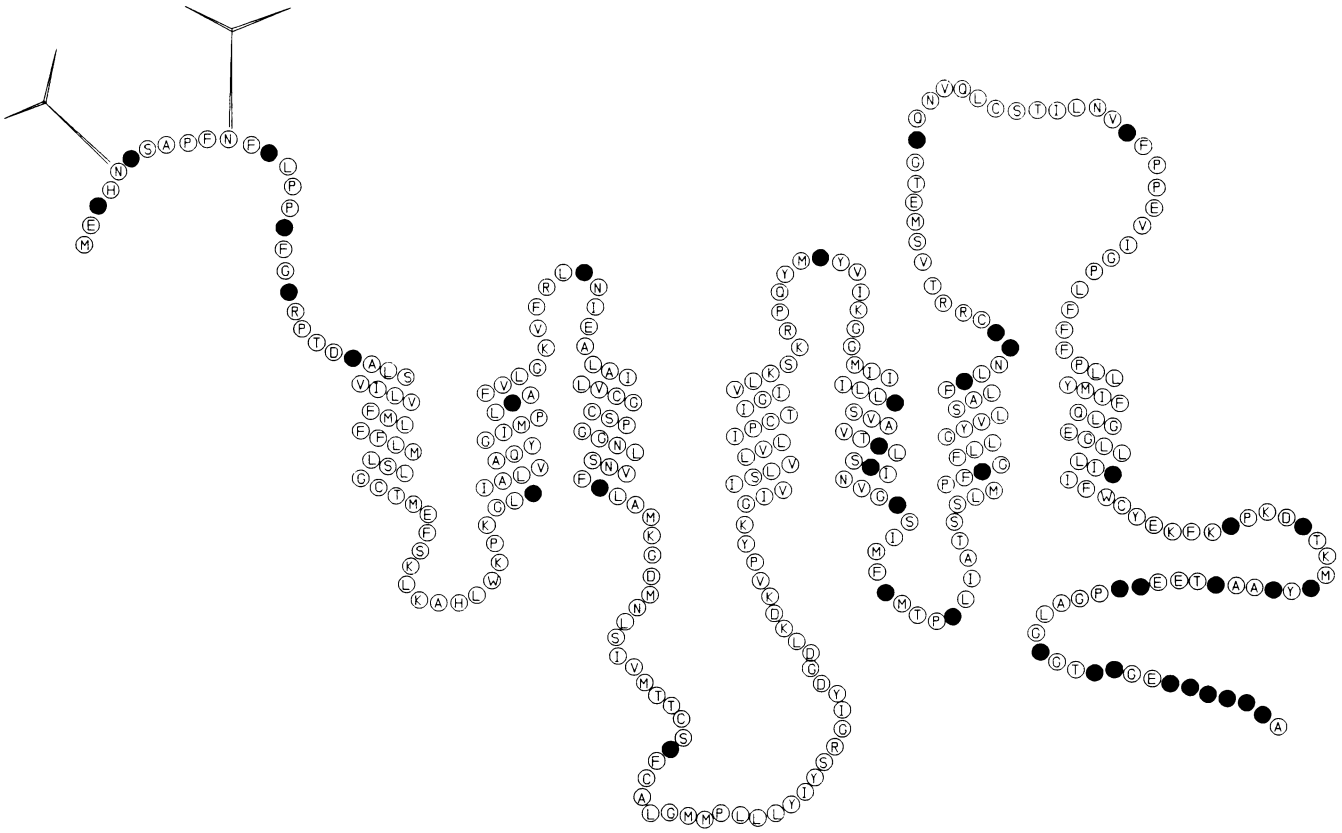

Figure 2. Proposed secondary structure model of the human NTCP. The protein is depicted with seven transmembrane segments (see text) of 21 residues each. Glycosylation at Asn5 and Asn11 is indicated. *Closed circles,* nonconservative amino acid replacements between the human and the rat transporters.

and location of the transmembrane domains. The differences in the amino acid sequence between the human and the rat Na^+/bile acid cotransporting polypeptides together with their different affinities for taurocholate (see below) might be a good starting point for mutational analysis to define functionally important amino acids (Fig. 2, *closed circles*).

Functional characterization of NTCP. As demonstrated in Fig. 3, injection of 5 ng of NTCP-cRNA resulted in Na^+-dependent taurocholate uptake that was ~ 20-fold above the background of noninjected oocytes. Interestingly, injection of the same amount of rat Ntcp-cRNA resulted in ~ 10-fold higher expression of Na^+-dependent taurocholate uptake, suggesting that the human transporter might exhibit a much lower bile acid transport capacity as compared to the rat system. Alternatively, the expression of the human NTCP-cRNA may be less efficient in oocytes as compared to the rat Ntcp-cRNA, as it was also the case for the human and rabbit intestinal Na^+-dependent glucose cotransporters (12, 13) and the human and canine cardiac Na^+/Ca^{2+} exchangers (14, 15).

To further characterize the human NTCP, we next investigated its transport kinetics and its substrate specificity. As demonstrated in Fig. 4, the cloned human Na^+/bile acid cotransport system showed clear saturability with an apparent K_m for taurocholate of 6.3±2.4 μM. This value is significantly lower than the previously reported 25 μM for the rat transporter (6). This higher affinity of the human NTCP would allow more efficient extraction of bile acids at low plasma concentrations and might help to keep the human systemic bile acid concentrations at the known physiological low levels (16).

To compare the substrate inhibition pattern of NTCP with the rat Ntcp, as well as with reported studies performed with human basolateral membrane vesicles (3), we measured Na^+-dependent taurocholate uptake into oocytes in the presence of the major bile acid derivatives and some other organic anions. Table I summarizes these inhibition studies and demonstrates

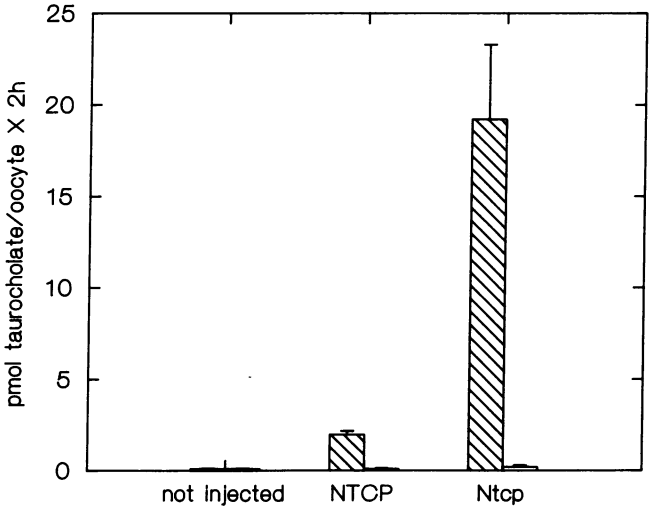

Figure 3. Functional expression of NTCP cRNA in oocytes. *Xenopus laevis* oocytes were either not injected or were injected with 5 ng of in vitro synthesized human (*NTCP*) or rat (*Ntcp*) cRNA. Oocytes were cultured for 2 d and 2-h taurocholate (17 μM) uptake values were determined in the presence of either 100 mM NaCl (*hatched bars*) or choline chloride (*open bars*) as described (5). Values represent mean±SE of 12–15 determinations in one of four oocyte preparations.

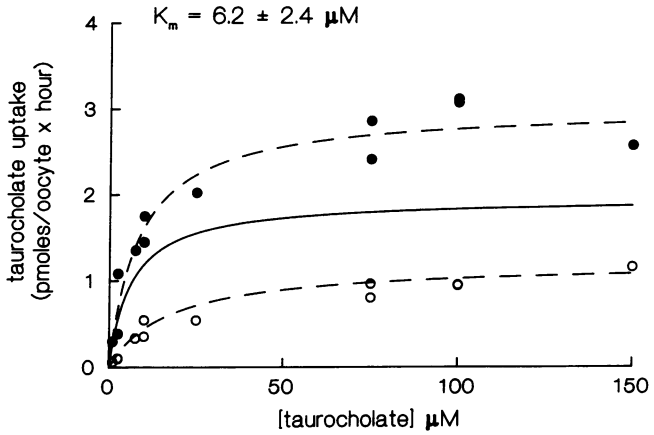

Figure 4. Kinetics of taurocholate uptake by NTCP cRNA-injected oocytes. *Xenopus laevis* oocytes were injected with 5 ng of cRNA. After 2 d in culture, 1-h uptake of taurocholate (2.5–150 μM) was determined. Values represent means of 12–15 determination from two out of three experiments performed with different batches of oocytes. Na^+-dependent taurocholate transport was calculated as the difference between the uptake in the presence of 100 mM NaCl (*closed circles*) and uptake in the presence of 100 mM choline chloride (*open circles*). The curves were fitted by nonlinear regression analysis assuming Michaelis-Menten kinetics.

that similar to previous observations for the rat Ntcp (6) and experiments in intact hepatocytes (17) the synthetic keto-bile acid taurodehydrocholate had no inhibitory effects. All major physiological bile acids on the other hand inhibited the cloned human NTCP by 40–90%. Unconjugated bile acids exhibited a less pronounced inhibition as compared to the taurine or glycine conjugates with the exception of chenodeoxycholate

Table I. Effect of Various Organic Anions on Na^+-dependent Taurocholate Uptake into NTCP-cRNA-Injected Oocytes

Inhibitor	Taurocholate uptake (mean±SE)	Percent of control
	pmol/oocyte per h	
None	1.175±0.114	100±10
Taurodehydrocholate	1.066±0.080	91±7
Cholate	0.675±0.089	57±8
Taurocholate	0.351±0.036	30±3
Glycocholate	0.522±0.046	44±4
Chenodeoxycholate	0.156±0.022	13±2
Taurochenodeoxycholate	0.223±0.035	19±2
Glycochenodeoxycholate	0.161±0.020	14±2
Ursodeoxycholate	0.531±0.067	45±6
Tauroursodeoxycholate	0.090±0.010	8±1
Lithocholate	0.737±0.090	63±8
Taurolithocholate	0.389±0.073	33±6
Glycolithocholate	0.279±0.051	24±4
Taurodeoxycholate	0.116±0.009	10±1
Probenecid (1 mM)	1.194±0.152	101±13
Bumetanide (0.5 mM)	0.831±0.087	71±7
BSP (0.1 mM)	0.483±0.052	41±4

Oocytes were injected with 5 ng of NTCP-cRNA and then cultured for 3 d. 1-h uptake of 10 μM taurocholate was measured in the presence of 100 μM bile acids or the indicated concentrations of other organic anions.

Table II. Segregation of the NTCP Gene with Human Chromosome 14 in Human–Hamster Cell Hybrids

		Human chromosome																							
Hybrid	*NTCP*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
212	−	−	−	−	−	Dq	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
324	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
423	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
507	+	−	−	+	−	+	−	−	−	−	−	−	+	−	65	−	−	−	−	−	40	−	25	−	+
683	+	−	−	−	−	+	−	−	−	−	−	−	+	−	+	−	−	−	−	+	−	+	+	−	−
734	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
750	+	−	−	−	−	D	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−
756	+	−	−	−	−	D	+	+	−	−	−	−	+	65	45	−	−	−	−	+	+	+	−	−	+
803	−	−	−	−	+	+	−	−	+	−	−	15	−	−	−	−	−	−	−	−	−	−	+	+	−
811	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	65	65	−	−	−	−	−	−
852	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
854	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
860	−	−	−	30	−	+	+	−	−	−	15	−	−	−	−	−	−	−	−	35	−	+	−	−	−
862	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
867	+	60	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	+	+	−	−	−	−	−
904	−	−	−	−	−	D	+	−	−	−	−	−	+	−	−	−	5	−	−	−	−	+	−	−	+
909	+	−	−	−	−	D	+	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−
937	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+	−	−	−	+	−	−	−
940	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
967	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
968	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	65	−
983	−	−	−	−	−	+	−	−	−	−	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1006	−	−	−	−	55	+	−	+	+	−	−	−	−	+	−	+	−	−	−	+	−	+	−	−	−
1049	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−
1079	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	10	+	−	−	−	−	−	−	−	−
1099	−	65	−	−	−	D	−	−	−	−	−	−	−	+	−	−	−	−	−	40	−	+	+	−	−
Discordant no. of hybrids		7	7	8	8	16	6	6	9	9	8	8	4	6	1	7	9	8	8	5	5	9	6	7	6

D, a deletion at 5p15.1-5p15.2; Dq, multiple deletions in 5q; numbers represent the percentage of the cell population containing the noted chromosome; +, >75%.

which was as potent an inhibitor as its taurine and glycine conjugates. Probenecid and bumetanide did not or only slightly inhibited NTCP mediated Na^+-dependent taurocholate uptake, while bromosulfophthalein (BSP) exerted a stronger inhibitory effect. This *cis*-inhibition pattern of NTCP is similar to the one previously observed with rat Ntcp (6), which is also inhibited by BSP, but does not transport this nonbile acid organic anion (18). Whether or not the inhibitory conjugated bile acids are in fact transported by NTCP is currently under further investigation.

Chromosomal localization of NTCP. To determine the chromosomal location of the human *NTCP* gene, we used Southern blot analysis of a DNA panel of human–hamster somatic cell hybrids. PstI-digested DNA samples of 26 human–hamster cell hybrids were analyzed by hybridization with a PflMI/EcoRI fragment of the cloned human NTCP. The results of this hybridization analysis are summarized in Table II. Correlation of the presence of a human specific hybridization signal with the human chromosome content of the hybrid cell lines showed that the *NTCP* gene is located on chromosome 14. The single discordance with hybrid 937 could be explained by a possible small deletion of chromosome 14 in this hybrid, which is not detected in karyotype analysis.

In conclusion, we have cloned a human Na^+/bile acid cotransporter that has a significantly higher affinity for taurocholate than the rat Ntcp. The protein showed a 77% amino acid homology to rat Ntcp and the gene could be localized to chromosome 14. These studies provide the basis for future investigation and characterization of the human NTCP gene in various physiological and pathophysiological situations.

Acknowledgments

We thank Prof. U. A. Meyer, Biozentrum Basel, Switzerland for supplying us with frozen human liver tissue, Ani Ohannessian for excellent technical assistance, and Prof. B. F. Scharschmidt for critical reading of the manuscript.

This study was supported by the Swiss National Science Foundation Grant 32-29878.90. Dr. Hagenbuch is a recipient of a Cloetta Foundation fellowship.

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