

## Molecular Cloning, Chromosomal Localization, and Functional Characterization of a Human Liver Na<sup>+</sup>/Bile Acid Cotransporter

Bruno Hagenbuch and Peter J. Meier

Division of Clinical Pharmacology and Toxicology, Department of Medicine, University Hospital, CH-8091 Zürich, Switzerland

### Abstract

We have used a cDNA probe from a cloned rat liver Na<sup>+</sup>/taurocholate cotransporting polypeptide (Ntcp) to screen a human liver cDNA library. A 1,599-bp cDNA clone that encodes a human Na<sup>+</sup>/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<sup>+</sup>-dependent taurocholate uptake. Saturation kinetics indicated that the human NTCP has a higher affinity for taurocholate (apparent  $K_m = 6 \mu\text{M}$ ) than the previously cloned rat protein (apparent  $K_m = 25 \mu\text{M}$ ). NTCP-mediated taurocholate uptake into oocytes was inhibited by all major bile acid derivatives (100  $\mu\text{M}$ ), bumetanide (500  $\mu\text{M}$ ), and bromosulphophthalein (100  $\mu\text{M}$ ). 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. Phenomenologically, 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* oo-

cytes, a rat liver Na<sup>+</sup>-taurocholate cotransporting polypeptide (Ntcp)<sup>1</sup> 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<sup>+</sup>-dependent taurocholate cotransporter and its chromosomal localization.

### Methods

**Cloning of a human Na<sup>+</sup>/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)<sup>+</sup> 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  $2 \times 10^5$  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 [ $\alpha$ -<sup>32</sup>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  $\mu\text{g}/\text{ml}$  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 ( $2 \times 10^6$  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<sup>+</sup>-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<sup>+</sup>/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-

Address correspondence to Bruno Hagenbuch, Ph.D., Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital, CH-8091 Zürich, Switzerland.

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

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/94/03/1326/06 \$2.00

Volume 93, March 1994, 1326–1331

1. Abbreviations used in this paper: BSP, bromosulphophthalein; Ntcp, rat liver Na<sup>+</sup>/taurocholate cotransporting polypeptide; NTCP, human liver Na<sup>+</sup>/taurocholate cotransporting polypeptide.

terminated 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 [ $G-^3H$ ]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<sup>6</sup> cpm/ml of labeled probe (a PfuI/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.

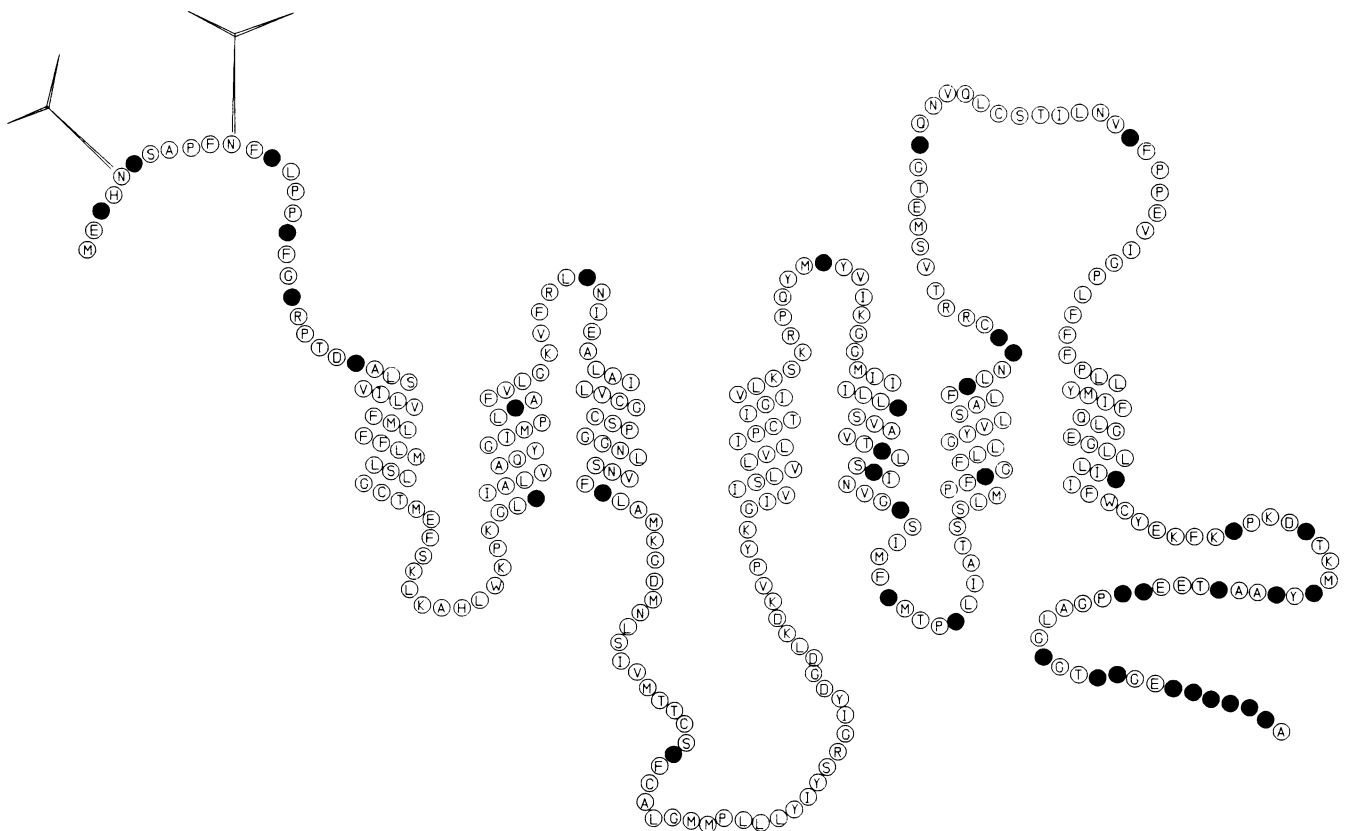
	1	A	AAG	AAG	GCA	TCC	AGC	AAG	AAC	TGC	ACA	AGA	AAC	GGA	GTC	AGC	CGG	AGA	ACA	AGG	AGT	GGT	CTT	CCA	CTG	CCT	CAC	AGG	AGG	82	
Rat		V		V						S				G		H		A		K											
Human	1	M	E	A	H	N	A	S	A	P	F	N	F	T	L	P	P	N	F	G	K	R	P	T	D	L	A	L	S	28	
	83	ATG	GAG	GCC	CAC	AAC	GCG	TCT	GCC	CCA	TTC	AAC	TTC	ACC	CTG	CCA	CCC	AAC	TTT	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	

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)<sup>+</sup> RNA using as a probe a <sup>32</sup>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<sup>+</sup>-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<sup>+</sup>/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



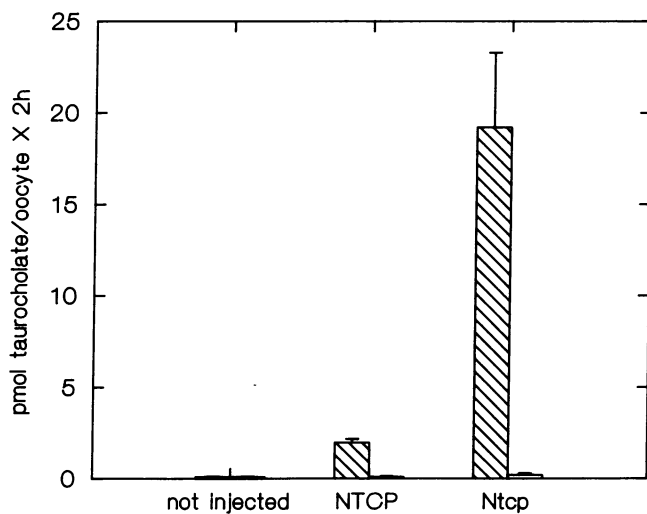
**Figure 2.** Proposed secondary structure model of the human NTCP. The protein is depicted with seven transmembrane segments (see text) of 21 residues each. Glycosylation at Asn5 and Asn11 is indicated. Closed circles, nonconservative amino acid replacements between the human and the rat transporters.

and location of the transmembrane domains. The differences in the amino acid sequence between the human and the rat  $\text{Na}^+$ /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).

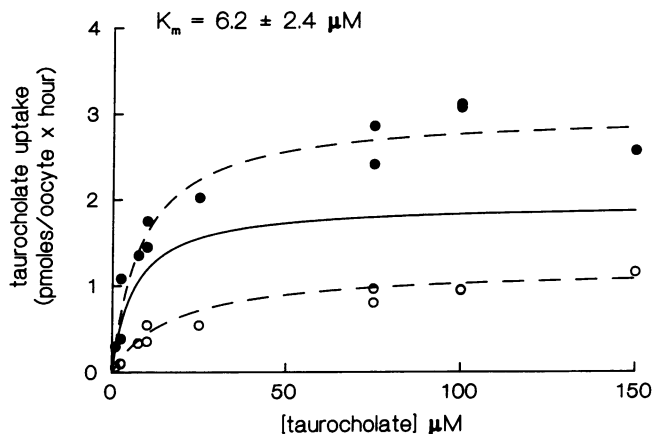
**Functional characterization of NTCP.** As demonstrated in Fig. 3, injection of 5 ng of NTCP-cRNA resulted in  $\text{Na}^+$ -dependent taurocholate uptake that was  $\sim 20$ -fold above the background of noninjected oocytes. Interestingly, injection of the same amount of rat Ntcp-cRNA resulted in  $\sim 10$ -fold higher expression of  $\text{Na}^+$ -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  $\text{Na}^+$ -dependent glucose cotransporters (12, 13) and the human and canine cardiac  $\text{Na}^+/\text{Ca}^{2+}$  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  $\text{Na}^+$ /bile acid cotransport system showed clear saturability with an apparent  $K_m$  for taurocholate of  $6.3 \pm 2.4 \mu\text{M}$ . This value is significantly lower than the previously reported  $25 \mu\text{M}$  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  $\text{Na}^+$ -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 ( $17 \mu\text{M}$ ) 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  $\pm$  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 ( $2.5$ – $150 \mu\text{M}$ ) was determined. Values represent means of 12–15 determination from two out of three experiments performed with different batches of oocytes.  $\text{Na}^+$ -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  $\text{Na}^+$ -dependent Taurocholate Uptake into NTCP-cRNA-Injected Oocytes

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

Oocytes were injected with 5 ng of NTCP-cRNA and then cultured for 3 d. 1-h uptake of  $10 \mu\text{M}$  taurocholate was measured in the presence of  $100 \mu\text{M}$  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

Hybrid	NTCP	Human chromosome																								
		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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
324	-	-	-	-	Dq	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
423	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
507	+	-	+	-	+	-	-	-	-	-	-	+	-	65	-	-	-	-	-	-	-	40	-	25	-	+
683	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+	-	+	-	-	-
734	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
750	+	-	-	-	D	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
756	+	-	-	-	D	+	-	-	-	-	-	+	65	45	-	-	-	-	-	+	+	+	-	-	+	+
803	-	-	-	+	-	-	-	+	-	-	15	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
811	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	65	65	-	-	-	-	-	-	-
852	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
854	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
860	-	-	-	-	+	-	-	-	-	15	-	-	-	-	-	-	-	-	-	35	-	-	-	-	-	-
862	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
867	+	60	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-
904	-	-	-	-	D	+	-	-	-	-	-	+	-	-	-	5	-	-	-	-	-	+	-	-	+	+
909	+	-	-	-	D	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
937	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-
940	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
967	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
968	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
983	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	-
1006	-	-	-	55	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
1049	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1079	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1099	-	65	-	-	D	-	-	-	-	-	-	-	+	-	-	10	+	-	-	40	-	+	-	-	-	-
Discordant no. of hybrids	7	7	8	8	16	6	6	9	9	8	8	4	6	1	7	9	9	8	8	5	5	9	6	7	6	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  $\text{Na}^+$ -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  $\text{Na}^+$ /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.

### References

1. Van Dyke, R. W., J. E. Stephens, and B. F. Scharschmidt. 1982. Bile acid transport in cultured rat hepatocytes. *Am. J. Physiol.* 243:G484-G492.
2. Frimmer, M., and K. Ziegler. 1988. The transport of bile acids in liver cells. *Biochim. Biophys. Acta.* 947:75-99.
3. Novak, D. A., F. C. Ryckman, and F. J. Suchy. 1989. Taurocholate transport by basolateral plasma membrane vesicles isolated from human liver. *Hepatology.* 10:447-453.
4. Zimmerli, B., J. Valantinas, and P. J. Meier. 1989. Multispecificity of  $\text{Na}^+$ -dependent taurocholate uptake in basolateral (sinusoidal) rat liver plasma membrane vesicles. *J. Pharmacol. Exp. Ther.* 250:301-308.
5. Hagenbuch, B., H. Lübbert, B. Stieger, and P. J. Meier. 1990. Expression of the hepatocyte  $\text{Na}^+$ /bile acid cotransporter in *Xenopus laevis* oocytes. *J. Biol. Chem.* 265:5357-5360.
6. Hagenbuch, B., B. Stieger, M. Foguet, H. Lübbert, and P. J. Meier. 1991. Functional expression cloning and characterization of the hepatocyte  $\text{Na}^+$ /bile acid cotransport system. *Proc. Natl. Acad. Sci. USA.* 88:10629-10633.
7. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156-159.
8. Jacobson, A. 1987. Purification and fractionation of poly(A)<sup>+</sup> RNA. *Methods Enzymol.* 152:254-261.
9. Klein, P., M. Kanehisa, and C. DeLisi. 1985. The detection and classification of membrane-spanning proteins. *Biochim. Biophys. Acta.* 815:468-476.
10. Devereux, J., P. Haeberli, and O. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12:387-395.
11. Boyer, J. L., B. Hagenbuch, M. Ananthanarayanan, F. Suchy, B. Stieger, and P. J. Meier. 1993. Phylogenetic and ontogenic expression of hepatocellular bile acid transport. *Proc. Natl. Acad. Sci. USA.* 90:435-438.
12. Hediger, M. A., M. J. Coady, T. S. Ikeda, and E. M. Wright. 1987. Expression cloning and cDNA sequencing of the  $\text{Na}^+$ /glucose co-transporter. *Nature (Lond.).* 330:379-381.
13. Hediger, M. A., E. Turk, and E. M. Wright. 1989. Homology of the human intestinal  $\text{Na}^+$ /glucose and *Escherichia coli*  $\text{Na}^+$ /proline cotransporters. *Proc. Natl. Acad. Sci. USA.* 86:5748-5752.
14. Nicoll, D. A., S. Longoni, and K. D. Phillipson. 1990. Molecular cloning and functional expression of the cardiac sarcolemmal  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchanger. *Science (Wash. DC).* 250:562-565.
15. Komuro, I., K. E. Wenninger, K. D. Phillipson, and S. Izumo. 1992. Molecular cloning and characterization of the human cardiac  $\text{Na}^+$ / $\text{Ca}^{2+}$  exchanger cDNA. *Proc. Natl. Acad. Sci. USA.* 89:4769-4773.
16. Carey, M. C., and M. J. Cahalane. 1988. Enterohepatic circulation. In *The Liver: Biology and Pathobiology*. I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press Ltd., New York. pp. 573-616.
17. Hardison, W. G. M., P. J. Lowe, and E. Gosink. 1988. Nature of taurodehydrocholic acid uptake in rat hepatocytes. *Am. J. Physiol.* 254:G269-G274.
18. Jacquemin, E., B. Hagenbuch, B. Stieger, A. W. Wolkoff, and P. J. Meier. 1991. Expression of the hepatocellular chloride-dependent sulfobromophthalein uptake system in *Xenopus laevis* oocytes. *J. Clin. Invest.* 88:2146-2149.