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The ABC of Solute Carriers

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The sodium bile salt cotransport family SLC10

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Abstract The SLC10 family of sodium/bile salt cotransporters contains over 50 members in animal, plant and bacterial species. In man, two well-characterized members and three orphan transporters are known. The Na^+/taurocholate cotransporting polypeptide (NTCP; *SLC10A1*) and the apical sodium-dependent bile salt transporter (ASBT; *SLC10A2*) are critical components of the enterohepatic circulation of bile salts. NTCP and ASBT are cotransporters that mediate sodium-dependent, electrogenic uptake of mainly bile salts into hepatocytes (NTCP), biliary epithelial cells, ileal enterocytes and renal proximal tubular cells (ASBT).

Keywords Bile salt · Enterohepatic circulation · NTCP · ASBT · Liver · Intestine · Proximal tubule · Cotransport

Introduction

Bile formation is essential for normal intestinal lipid digestion and absorption, cholesterol homeostasis, and for the hepatic excretion of lipid-soluble xenobiotics, drugs, and heavy metals. Bile secreted by the liver is composed mainly of bile salts (~67%), phospholipids, cholesterol and proteins. After their secretion, bile salts travel down the biliary tree and are stored in the gallbladder. In response to a meal the gallbladder empties its contents into the duodenum where the bile salts play an essential role in the efficient digestion and absorption of lipids and lipid soluble vitamins (i.e. vitamins A, D, E and K). In the terminal ileum bile salts are absorbed almost quantitatively and return to the liver with the portal circulation. The small amount of fecal bile salt loss (~5% of the total) is balanced by hepatic conversion of cholesterol to bile salts, a process representing an important route for elimination of cholesterol from the body. In the liver, bile salts are removed actively from portal blood and re-secreted into bile (Fig. 1). Because this continuous flow of bile salts is restricted to the hepatocytes, the biliary tree, the intestine, and the portal blood, the system is called the enterohepatic circulation. This enterohepatic circulation is dependent on several transport systems, among which the two sodium-dependent bile salt cotransporters of the SLC10 family play a crucial role. The Na^+/taurocholate cotransporting polypeptide (NTCP; *SLC10A1*) located at the sinusoidal plasma membrane of hepatocytes (Fig. 1) extracts bile salts efficiently from portal blood for re-secretion across the canalicular membrane into the bile. In parallel, the apical sodium-dependent bile salt transporter (ASBT; *SLC10A2*), expressed at the brush border membrane of ileocytes (Fig. 1), actively removes bile salts from the intestinal lumen and allows their return to the liver via the portal circulation. Most of the bile salts secreted by hepatocytes are not newly synthesized and have undergone enterohepatic recycling. As such, disturbances in bile salt synthesis, biliary secretion, and enterohepatic cycling all have profound effects on hepatic and gastrointestinal physiology.

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Brief history

The rat Ntcp (*Slc10a1*) was isolated by expression cloning using the *Xenopus laevis* oocyte system [1]. This cDNA was used subsequently to identify a human NTCP ortholog [2]. Two splice variants have been isolated subsequently from mouse liver [3]. Expression cloning was also employed to isolate the ASBT from a hamster ileal cDNA library [4]. This resulted in the subsequent identification of the rat [5], human [6], rabbit [7], and mouse [8] ASBT orthologs (Table 1).

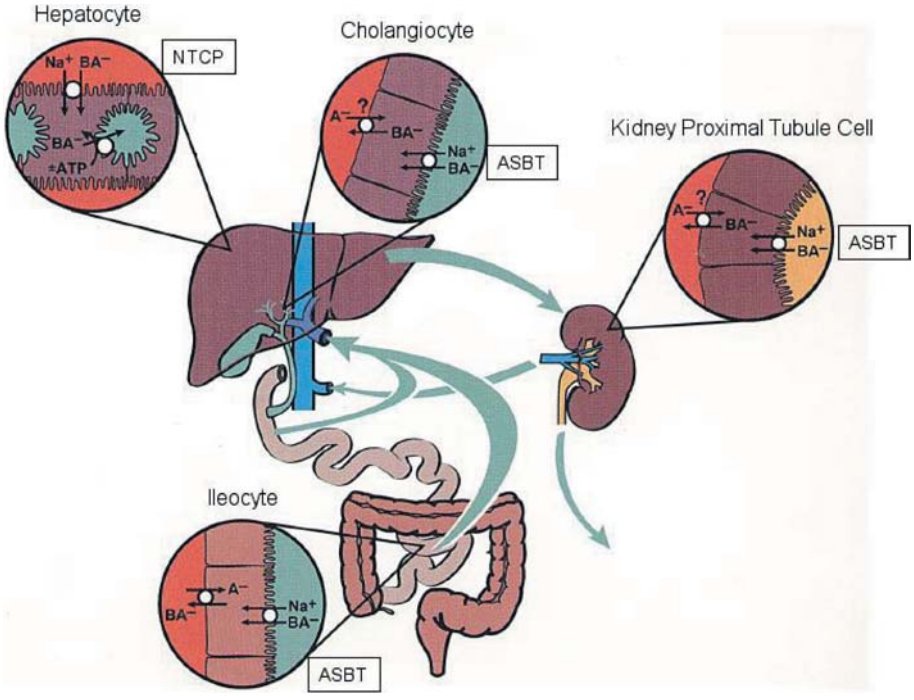

Fig. 1 Cellular localization of the SLC10 sodium bile salt cotransporters Na^+/taurocholate cotransporting polypeptide (*NTCP*) and the apical sodium-dependent bile salt transporter (*ASBT*). While expression of NTCP is restricted to the sinusoidal membrane of *hepatocytes*, ASBT has been detected in the apical membranes of ileal enterocytes (*ileocytes*), of biliary endothelial cells (*cholangiocytes*) and of renal *proximal tubular cells*. Secretion of bile salts across the canalicular membrane of hepatocytes is accomplished by the ATP-dependent bile salt export pump (BSEP), while the proposed exchanger in the basolateral membrane of ileocytes has not yet been identified

Table 1 SLC10—the sodium bile salt cotransporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transporter type/ coupling ions*	Tissue distribution and cellular/ subcellular expression	Link to disease (OMIM)	Human gene locus	Sequence accession ID	Splice variants and their specific features
SLC10A1	NTCP	LBAT	Bile salts	C/Na^+	Liver, pancreas		14q24.1	NM_003049	Ntcp1/Ntcp2 (mouse)
SLC10A2	ASBT	IBAT, ISBT	Bile salts	C/Na^+	Ileum, kidney, biliary tract	PBAM (601295)	13q33	NM_000452	tASBT (rat)
SLC10A3	P3			O	Ubiquitously (based on EST data)		Xq28	NM_019848	
SLC10A4	Hypothetical protein MGC29802			O	Brain neuroblastoma (based on EST data)		4p11	NM_152679	
SLC10A5	Similar to P3 protein			O	Fetal brain (based on EST data)		8q21	XM_294493	

**C* Cotransporter, *O* orphan, *EST* expressed sequence tag

Functional characteristics

Both NTCP and the ASBT have been expressed in different recombinant systems including *Xenopus laevis* oocytes [1], COS-7 [9], CHO [10], HPCT-1E3 [11], MDCK [12, 13], HEK 293 [14], and rat hepatoma cells [15]. Both transport systems mediate Na^+-dependent uptake of conjugated and unconjugated bile salts and the only non-bile salt substrate identified so far for NTCP is estrone-3-sulfate [10]. The driving force for NTCP- and ASBT-mediated bile salt uptake is provided by the inwardly directed Na^+ gradient maintained by the basolateral Na^+, K^+-ATPase as well as the negative intracellular potential. Detailed analysis has revealed that transport is electrogenic with a sodium:taurocholate stoichiometry of at least 2:1 [16, 17]. Very recent studies of ASBT chimeras have shown that the benzothiazepine-based inhibitors interact with specific residues in the carboxyl-terminal transmembrane domain [18]. These data, as well as previous chemical modification experiments, indicate strongly that the two C-terminal putative transmembrane domains constitute an important part of the binding pocket for bile salts [14]. The importance of the C-terminal part of NTCP and ASBT is supported by the recent demonstration that the glutamic acid at position 257 as well as the cysteine at position 266 in rat Ntcp (both completely conserved among eight members of the

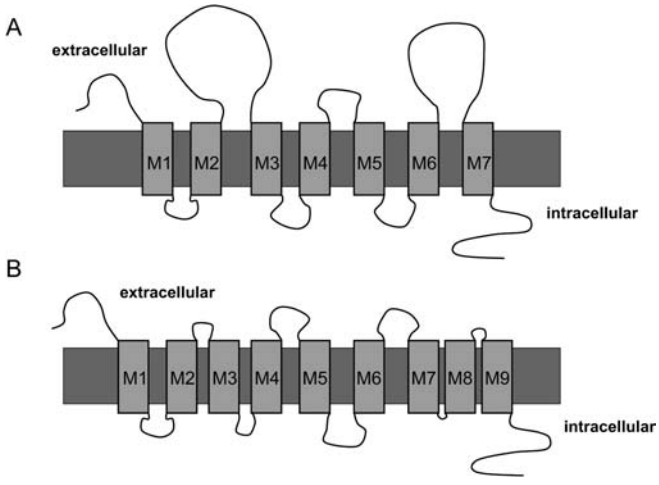

Fig. 2A, B Topological models of the SLC10 sodium bile salt cotransporters. Computer modeling suggests seven transmembrane (*M*) domains (**A**) while experimental evidence is compatible with a nine-transmembrane domain model (**B**)

SLC10 transporters) are essential for taurocholate transport [19].

Computer programs have favored a seven-transmembrane domain topology for both NTCP and ASBT [2, 20]. However, recent experimental evidence based on translation/insertion scanning, alanine insertion and glycosylation site mutagenesis suggest rather a nine-transmembrane domain topology (Fig. 2) [21, 22]. Ultimately, an approach such as X-ray crystallography will be required to determine the topology.

Individual members

SLC10A1

The human NTCP is a 349-amino acid polytopic membrane glycoprotein with an apparent molecular mass of approximately 56 kDa [2]. NTCP's expression at the basolateral (sinusoidal) membrane of human hepatocytes and high affinity for conjugated bile salts (taurocholate apparent K_m ~6 μM, taurochenodeoxycholate ~2 μM) promotes efficient extraction of bile salts from portal blood to keep plasma concentrations at a minimum. In addition to the liver, Ntcp is also expressed at the luminal (apical) membrane of pancreatic acinar cells where it may function to clear any bile salts that leak into the terminal acini [23]. NTCP orthologs have been identified in the rat [1] (362 amino acids, 77% amino acid identity to human NTCP), and mouse [3]. In contrast to humans and rat, the mouse *Ntcp* gene encodes two splice variants, Ntcp1 with 362 amino acids and the less-abundant Ntcp2 with 317 amino acids and a shorter C-terminal end [3].

NTCP's properties satisfy all the functional criteria for hepatocytic Na^+-coupled bile salt uptake including: (a) preferential high affinity transport of conjugated bile salts, (b) kinetics for taurocholate transport similar to isolated hepatocytes, (c) electrogenic Na^+-taurocholate uptake, (d) appropriate tissue-specific expression in the liver, and (e) similar ontogeny for Na^+-dependent bile salt uptake and NTCP expression in development. The overwhelming evidence indicates that NTCP accounts for most, if not all, hepatic Na^+-dependent bile salt transport [24, 25]. NTCP mRNA and protein expression is decreased in various animal models of cholestasis and liver disease [26, 27]. In addition, recent studies have also shown similar decreased NTCP expression in percutaneous liver biopsies of patients with cholestatic liver disease [28]. An inherited defect in NTCP has not yet been reported. It is possible that an isolated NTCP gene defect is asymptomatic since the liver also expresses Na^+-independent bile salt transporters. However, a rare disorder characterized by a relatively isolated hypercholanemia has been described recently, and an NTCP defect remains a candidate for at least a subset of those patients [29].

SLC10A2

The human ASBT is a 348-amino acid polytopic membrane glycoprotein with an apparent molecular mass of approximately 50 kDa [20]. ASBT transports all major species of bile salts, but favors trihydroxy (cholic acid, CA) over dihydroxy bile salts, and conjugated over unconjugated species. ASBT is expressed on the apical brush border membrane at high levels in terminal ileum and at lower levels in renal proximal tubules [30] and biliary epithelium [31]. In the ileum and kidney, ASBT functions as a salvage mechanism for efficient conservation of bile salts. In the bile ducts, ASBT's function may be to permit cholangiocytes to sample biliary contents to activate cellular signaling pathways rather than to transport significant quantities of bile salts [31]. ASBT shares approximately 35% amino acid identity with NTCP, and ASBT orthologs have been identified in the rat [5], mouse [7], hamster [4] and rabbit [7]. Alternative splicing gives rise to a novel truncated, 19-kDa form of ASBT in the rat. A significant proportion of the ASBT transcript undergoes exon-2 skipping, resulting in a frameshift at codon 126 and truncation of the ASBT protein from 348 to 154 amino acids. Preliminary evidence suggests that this truncated protein (tASBT) functions on the basolateral membrane as a bile salt efflux pump [32]. Notably, this is the first example of a solute carrier gene encoding both the solute uptake and efflux mechanisms.

The properties of ASBT satisfied all the functional criteria for ileal active bile salt uptake including: (a) a strict sodium dependence for bile salt transport, (b) narrow substrate specificity encompassing only conjugated and unconjugated bile salts with negligible uptake of other organic anions, (c) specific intestinal expression in the terminal ileum, and (d) similar ontogeny for ileal sodium-dependent taurocholate uptake and ASBT expression [33]. Finally, inherited mutations in the human ASBT gene cause primary bile salt malabsorption, an

idiopathic intestinal disorder associated with interruption of the enterohepatic circulation of bile salts and fat malabsorption [34]. This last finding demonstrates clearly that most intestinal bile salt absorption in humans is mediated by ASBT.

SLC10A3

P3 cDNA was originally cloned from placenta and encodes a protein of 477 amino acids [35]. The function of the P3 protein is currently unknown. However, on the basis of its wide tissue expression and its conservation in distant animal species, P3 protein is thought to encode a protein with housekeeping functions. Since P3 shares approximately 27% amino acid identity with the ASBT and NTCP, it has been assigned to the "sodium/bile acid symporter" family. However, to date there is no experimental data indicating that the P3 protein functions in solute transport and its function remains a mystery.

SLC10A4

This hypothetical protein MGC29802 of 437 amino acids shares ~37% identity with human NTCP. The function of MGC29802 is currently unknown. Based on expressed sequence tag (EST) database searches, MGC29802 appears to be expressed predominantly in neuroblastomas. However, its definitive tissue distribution as well as its function will have to be determined experimentally.

SLC10A5

This is the third predicted orphan transporter and consists of 297 amino acids and, based on EST data, is expressed in the fetal brain.

SLC10 transporters as therapeutic targets

Inhibitors for treatment of hypercholesterolemia and cholestasis

Inhibition of intestinal reabsorption of bile salts using binding resins was an important early therapeutic strategy for increasing hepatic cholesterol demand in order to elevate low-density lipoprotein (LDL) receptor levels and decrease plasma LDL. An alternative to luminal sequestration of bile salts by binding resins is to inhibit the intestinal absorption of bile salts directly. As a result of its intestinal expression and role in the enterohepatic circulation, ASBT is a potential target for cholesterol-lowering therapy and a variety of high-affinity inhibitors have been developed. These inhibitors fall into two general classes, bile salt-derivatives including bile salt dimers [36], and non-bile salt compounds including benzothiazepine [37] and benzothiepine analogs [38]. Two ileal bile salt transporter inhibitors have entered phase-2 clinical trials with an associated reduction in LDL cholesterol of 10% reported in one trial [39].

SLC10 transporters as portals for drug delivery

With its restricted expression to hepatocytes NTCP is ideally suited to target drugs to the liver. Thus, any kind of liver-specific disease is a potential target for treatment with drugs that are conjugated to bile salts, but which, in an unconjugated form, do not home efficiently to the liver. Possible examples are e.g. anti-viral drugs or pro-drugs to treat hepatitis, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors to lower cholesterol levels or cytostatic drugs to treat hepatocellular carcinomas. The feasibility of such an approach has been shown for several compounds such as e.g. antisense oligonucleotides, amino acids and peptides [40] as well as for cytostatic drugs which keep their potency even after conjugation to bile salts [41]. Similarly, oligopeptides coupled to bile salts are readily absorbed in the small intestine [42], which could be a useful way to improve the bioavailability of orally applied peptide drugs.

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