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themes

Bile Acid Regulation of Hepatic Physiology
I. Hepatocyte transport of bile acids

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Wolkoff, Allan W., and David E. Cohen. Bile Acid Regulation of Hepatic Physiology. I. Hepatocyte transport of bile acids. *Am J Physiol Gastrointest Liver Physiol* 284: G175–G179, 2003; 10.1152/ajpgi.00409.2002.—Bile acids are cholesterol derivatives that serve as detergents in bile and the small intestine. Approximately 95% of bile acids secreted by hepatocytes into bile are absorbed from the distal ileum into the portal venous system. Extraction from the portal circulation by the hepatocyte followed by reexcretion into the bile canaliculus completes the enterohepatic circulation of these compounds. Over the past few years, candidate bile acid transport proteins of the sinusoidal and canalicular plasma membranes of the hepatocyte have been identified. The physiology of hepatocyte bile acid transport and its relationship to these transport proteins is the subject of this Themes article.

organic anion transporting protein; Na^+-taurocholate cotransporting polypeptide; bile salt export pump

BILE ACIDS ARE A FAMILY OF cholesterol derivatives that serve important roles as detergents in bile and the small intestine. In humans, bile acid secretion by the hepatocyte approximates 1 g/h, 95% of which is recovered following absorption from the distal ileum into the portal venous system. Extraction from the portal circulation by the hepatocyte followed by reexcretion into the bile canaliculus completes the enterohepatic circulation of bile acids. This Themes article examines mechanisms for hepatocyte uptake and excretion of bile acids.

PHYSIOLOGY OF HEPATOCYTE BILE ACID UPTAKE

Bile acids circulate in plasma bound with relatively high affinity to albumin (26). There is also significant binding of bile acids to lipoproteins, especially HDL (28). Despite their low free concentrations, bile acids are extracted from the circulation quickly by the liver with single-pass extraction rates as high as 80% (25). Initial studies in isolated rat hepatocytes performed by Schwarz et al. (29) showed saturable uptake of taurocholate was decreased by 75% following replacement of extracellular Na^+ by K^+ or by sucrose. Similar Na^+ dependence of taurocholate uptake was subsequently demonstrated in the isolated perfused rat liver (25). Although limited availability of radiolabeled compounds restricted studies of hepatocyte transport, several groups were able to demonstrate efficient, Na^+-dependent uptake of a variety of conjugated bile acids (31). The mechanistic dependence of bile acid transport on Na^+ has been the subject of a number of studies in hepatocytes and basolateral plasma membrane vesicles. These studies (17) revealed Na^+-bile acid cotransport with a Na^+/bile acid stoichiometry of $>$1:1.

IDENTIFICATION AND CHARACTERIZATION OF HEPATOCYTE BASOLATERAL PLASMA MEMBRANE BILE ACID TRANSPORTERS

The characteristics of bile acid transport by hepatocytes strongly suggest carrier mediation (Fig. 1). Considerable effort has been focused on defining a basolateral plasma membrane bile acid transporter(s). Initial studies utilized a photoaffinity approach to covalently attach radiolabeled bile acids to transporters contained in liver cell plasma membrane preparations as well as hepatocytes. Candidate transport proteins with apparent molecular mass of 48,000 and 54,000 Da were identified (33, 36). Interestingly, radiation inactivation analysis indicated that the minimal functional molecular mass of the Na^+-dependent taurocholate transporter in rat liver basolateral plasma membrane vesicles was 170,000 Da (3).

The advent of expression cloning techniques resulted in the identification of a Na^+-dependent bile acid transporter that has been termed the Na^+-taurocholate-cotransporting polypeptide (ntcp) (18). This cloned cDNA encodes a protein with a predicted molecular mass of 39,000 Da that migrates at 49,000 Da on SDS-PAGE due to glycosylation. Expression of ntcp is restricted to the hepatocyte, where it resides on the basolateral (sinusoidal) plasma membrane.

The possibility that ntcp represents "the" Na^+-dependent bile acid transporter of the hepatocyte has been asserted (18) but not proven. Evidence supportive

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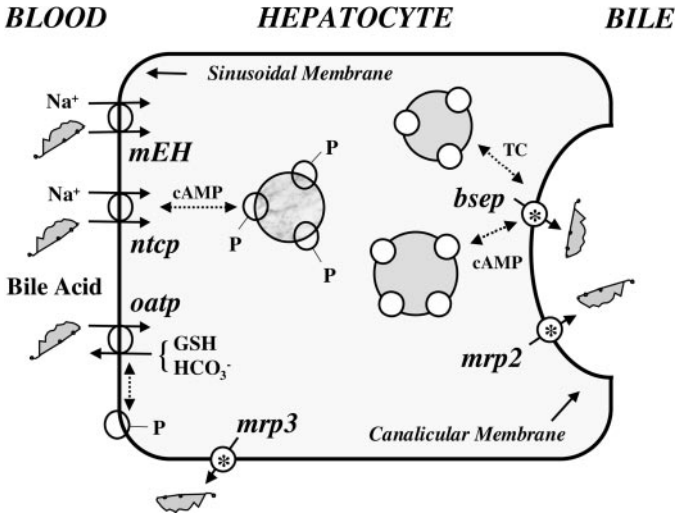

Fig. 1. Activities and short-term regulation of major known hepatocellular bile acid transport proteins. Solid arrows indicate transport of the indicated substrate. Dotted arrows denote regulatory pathways of vesicular transport and phosphorylation. * indicates ATP-dependent transport. bsep, Bile salt export pump; GSH, glutathione; mEH, microsomal epoxide hydrolase; mrp2/mrp3, multidrug resistance-associated protein 2/3; ntcp, Na^+-taurocholate cotransporting polypeptide; oatp, organic anion transporting protein; TC, taurocholate; P, phosphorylated transporter.

of a major role for ntcp in bile acid transport includes similar K_m values for bile acid transport by hepatocytes compared with ntcp-transfected cells as well as parallel decreases of Na^+-dependent taurocholate transport and ntcp expression in cultured rat hepatocytes and during rat liver regeneration (18). It is important to point out, however, that these correlations do not establish cause and effect. The function of other potential transporters could also be altered when hepatocytes are cultured or during regrowth of the liver. The strongest functional evidence that has been used to imply a major transport role for ntcp is a 95% reduction of Na^+-dependent taurocholate uptake following inhibition of ntcp expression in rat liver cRNA-injected *Xenopus laevis* oocytes by antisense nucleotides (8, 18). Whereas this experimental strategy offers a direct test of ntcp function, current database searching reveals that the antisense oligonucleotide that was used to inhibit ntcp translation (TAACCCATCAGAAAGCCAGA) (8) is not actually specific for ntcp. It is also antisense for a number of other rat liver proteins, including αII-spectrin and α-fodrin, as well as *X. laevis* proteins including MAPK kinase 7 and Ca^{2+}/calmodulin-dependent kinase. Thus it is possible that expression or trafficking of other rat liver mRNA-encoded transport proteins could have been altered in these studies. Conclusive evidence of the role of ntcp may have to await preparation of a knockout mouse model. As of this time, spontaneously occurring disorders in experimental animals or humans in which altered bile acid uptake is attributable to mutations in ntcp have yet to be described.

Microsomal epoxide hydrolase (mEH) has been proposed as another candidate Na^+-dependent bile acid transporter (32). This 49,000-Da protein was first implicated as a transporter based on its ability to reconstitute Na^+-dependent taurocholate uptake in proteoliposomes. Subsequent analysis revealed that this protein was identical to mEH. To explain its presence on the plasma membrane, evidence was presented that insertion into the endoplasmic reticulum membrane occurs in two distinct topological orientations, one of which is targeted to the plasma membrane (37). Although expression of mEH in *X. laevis* oocytes and in a Syrian hamster kidney fibroblast cell line was not associated with development of Na^+-dependent taurocholate uptake (12), expression of this protein in Madin-Darby canine kidney cells was sufficient to produce Na^+-dependent taurocholate transport (32). Targeted disruption of the mEH gene in the mouse has been reported (20). Because serum bile acid levels and hepatocyte transport were not among those parameters included in the phenotypic characterization of this mouse, a potential physiological role of mEH in bile acid transport remains to be elucidated.

Although ~75% of bile acid uptake by the hepatocyte appears to be Na^+-dependent, a substantial amount of uptake occurs by a Na^+-independent mechanism(s). The organic anion transporting proteins (oatps) are candidate Na^+-independent bile acid transporters (18). The oatp family of proteins has greatly expanded over the past few years as additional members have been described in humans, mice, rats, skates, and cows. Identity among these proteins ranges from 40 to 95%. In general, the oatps have wide and overlapping substrate specificities. Several are present in the liver, where they reside on the basolateral plasma membrane of the hepatocyte. When transfected into cultured cells, these proteins mediate Na^+-independent transport of various bile acids with affinities similar to that of ntcp-mediated transport. Moreover, oatps are the targets of nuclear hormone receptors, which regulate bile acid metabolism and transport (30). Notwithstanding these suggestive data, the physiological roles of oatps in bile acid transport remain to be established.

PHYSIOLOGY OF HEPATOCYTE BILE ACID EXCRETION

The concentration gradient of bile acids between the hepatocyte and the bile canaliculus is large. Studies performed in canalicular membrane vesicle preparations revealed ATP-dependent transport of taurocholate (22, 23) that could provide the energy necessary for this concentrative process. On the basis of reconstitution of transport activity in proteoliposomes, a 110,000-Da glycoprotein was designated as the ATP-dependent canalicular transporter (22, 27). However, more recent studies have shown that the 160,000-Da protein initially named the sister of P-glycoprotein (spgp) was able to mediate ATP-dependent taurocholate transport in cRNA-injected *X. laevis* oocytes and in vesicles isolated from spgp-transfected Sf9 cells (6). This protein has been renamed bile salt export pump (bsep), and the gene has been formally designated as *Abcb11*. Most convincingly, it was found that

the human counterpart, *ABCB11*, is mutated in the group of patients with progressive familial intrahepatic cholestasis type 2 (PFIC2), a disorder associated with markedly elevated serum bile acid levels and low content of bile acids in bile (15). Interestingly, targeted inactivation of *Abcb11* in mice is associated with a less-severe phenotype. These mice have mild intrahepatic cholestasis with biliary bile acid excretion ~30% of that in wild-type mice (34). There was little excretion of hydrophobic bile acids in bile of these knockout mice. In contrast, biliary excretion of the more hydrophilic derivatives of muricholic acid was intact, suggesting that there may be an alternative canalicular transporter that can mediate excretion of hydrophilic bile acids (34).

Multidrug resistance-associated protein 2 (mrp2; ABCC2), a member of the ATP-binding cassette (ABC) family of proteins, serves as a canalicular exporter of bilirubin glucuronides and is mutated in the Dubin-Johnson syndrome (24), an inheritable disorder characterized by chronic conjugated hyperbilirubinemia. It is also a candidate to be an alternative canalicular bile acid transporter. Mrp2 can mediate excretion into bile of 3-sulfate and 3-glucuronide dianionic bile salts as well as ester sulfates of lithocholic acid (1). Although mrp2 does not transport taurocholic acid, mrp3, a closely related protein that resides on the basolateral plasma membrane of the hepatocyte, does transport this common bile acid (11). Upregulation of mrp3 in mrp2-deficient rat liver correlates with higher rates of taurocholic acid efflux (2), suggesting a compensatory mechanism for bile acid elimination from the hepatocyte. Also of note is the observation that substitution of the positively charged amino acid arginine with the neutral amino acid leucine at position 586 or position 1096 of mrp2 is sufficient to enable this protein to transport taurocholic acid (13). This intriguing finding should stimulate more-detailed studies regarding structure-function relationships of all of these transport proteins.

REGULATION OF TRANSPORTER FUNCTION

Sinusoidal and canalicular bile acid transport are under both long-term and short-term regulation. The mechanism for long-term regulation, such as the downregulation of transport that accompanies cholestasis and liver regeneration, is regulation of protein expression (5, 14). These changes are largely mediated by interactions of bile acids with specific nuclear receptors and transcription factors that will be the subject of another Themes article in this series. In contrast, short-term regulation occurs via changes in protein targeting between plasma membrane and intracellular locations, as well as via posttranslational modifications that result in altered transport activity (Fig. 1). Under normal physiological circumstances, e.g., between fasted and fed states, the hepatocyte is exposed to rapidly fluctuating levels of circulating bile salts. The liver responds in the short term by increasing hepatocellular bile salt transport over a time frame that is too rapid to be controlled by transcriptional mechanisms. Several mechanisms that can be utilized for this response have been described for the bile acid transporters that have been described in the preceding sections.

Studies in rat hepatocytes showed that cAMP pretreatment increased the maximal Na^+-dependent taurocholic acid transport rate by ~50% and that this was associated with translocation of ntcp from an intracellular location to the plasma membrane (21). Subsequent studies showed that, within the hepatocyte, ntcp exists in a phosphorylated form and that it undergoes dephosphorylation in response to cAMP and subsequent translocation to the plasma membrane (21). Evidence indicates that cAMP activates protein phosphatase 2B (PP2B) and that PP2B-mediated dephosphorylation of ntcp is required for its trafficking to the cell surface (35). Although the mechanism mediating ntcp trafficking has not been fully elucidated, it appears to be mediated by a phosphatidylinositol-3-kinase-dependent pathway that also may require intact actin filaments (35).

Regulation of Na^+-independent transport activity has been examined using sulfobromophthalein (BSP). In rat hepatocytes, maximal uptake of BSP is reduced by 75% following a 10-min exposure to extracellular ATP but not to ADP (7). This effect is rapidly reversible on removal of ATP and has characteristics that suggest a regulatory role for a purinergic receptor. Downregulation of BSP transport is also seen after exposure of hepatocytes to the phosphatase inhibitors okadaic acid or calyculin A (7) and is accompanied by serine phosphorylation (7). In contrast to results with ntcp, studies using a cell-impermeant biotinylation protocol reveal that oatp1 phosphorylation does not result in its removal from the plasma membrane (7). Rather, this transporter remains on the cell surface, apparently in an inactive conformation. Together with studies on ntcp, these findings indicate that the phosphorylation state must be considered when assessing functional expression of transporters in pathophysiological states.

Similar to other canalicular ABC transporters, Abcb11 (bsep) resides in an intracellular compartment from which it can be delivered rapidly to the canalicular membrane under situations of increased physiological functional demand (16). In isolated perfused rat liver, bile secretion is increased by intravenous infusion of cAMP or taurocholic acid (9, 10). Subsequent studies showed that these agents recruit ABC proteins, including bsep, to the canalicular plasma membrane from intracellular stores. This recruitment is inhibited by the microtubule disruptor colchicine (4) and by the phosphatidylinositol 3-kinase inhibitor wortmannin (19). These studies also suggested that the effects of cAMP and taurocholate on recruitment of bsep to the canalicular membrane were independent, suggesting the existence of two intracellular pools of Abcb11 (16). Recent evidence also indicates that Abcb11 transport activity is stimulated by lipid products of phosphatidylinositol 3-kinase (16).

FUTURE PERSPECTIVES

Whereas considerable progress has been made toward understanding uptake and secretion of bile acids by hepatocytes, important questions remain unanswered. Definition of the relative contributions in vivo of ntcp, oatps, and mEH to clearance of bile acids from portal blood will likely require the creation of mice with targeted disruption of genes encoding one or more of these transporters. Additional structure-function analyses will be essential to establish the molecular mechanisms of transport for both sinusoidal and canalicular transporters. Whether these proteins act in isolation or as multimers that homo- or heteroassociate is similarly unknown. Finally, continued efforts to elucidate both the transcriptional control and trafficking of bile acid transporters will no doubt lead to fundamental insights into important aspects of the cellular biology of the hepatocyte.

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