# ATP-DEPENDENT BILE SALT TRANSPORT IN SKATE (*RAJA ERINACEA*) HEPATOCYTES IS MEDIATED BY A FUNCTIONAL ANALOGUE OF bsep, THE BILE SALT EXPORT PUMP

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Hepatic bile secretion is initiated at the level of the bile canaliculus by continuous vectorial secretion of bile salts and other solutes across this apical membrane domain. In mammals, bile salts are the major solutes secreted into bile, whereas sulfated bile alcohols predominate in many species of fish, including sharks and rays (Karlaganis et al., *J. Lipid Res.* 30:317-322, 1989).

The molecular mechanism by which bile salts are transported across the canalicular membrane into bile has remained elusive, until recently (Gerloff et al., *J. Biol. Chem.* 273:10046-10050, 1998). Earlier studies in rat and human liver canalicular membrane vesicles had demonstrated that transport of bile salts is mediated by an ATP-dependent system that appears relatively selective for bile salts and structurally related compounds, but the molecular identity was unknown. Gerloff and coworkers (*ibid.*) recently demonstrated that the 'sister of P-glycoprotein', spgp, functions as a canalicular bile salt export pump in mammalian liver. The full length sequence of spgp was thus renamed bsep, the bile salt export pump. Bsep is a member of the ABC (ATP binding cassette) superfamily of membrane transporters, and is expressed predominantly in the liver. Moreover, bsep is selectively localized to the canalicular membrane of hepatocytes, indicating an important role in biliary secretion. When bsep was expressed in *Xenopus laevis* oocytes and Sf9 cells, it was found to transport bile salts with high affinity, in an ATP-dependent manner. The substrate specificity of bsep is relatively narrow when compared to the broad specificity of some ABC transporters, and appears restricted to primary bile salts, in agreement with studies in rat canalicular membrane vesicles. A yeast ABC protein that also transports bile salts, Bat1p, has recently been described (Ortiz et al., *J. Biol. Chem.* 272:15358-15365, 1997). This yeast bile salt transporter displays a comparable substrate specificity, although its sequence homology with bsep is relatively low (23% amino acid identity).

The present study examined whether a similar ATP-dependent bile salt transport activity exists in the liver of the evolutionarily primitive marine fish *Raja erinacea*, the little skate, an animal that synthesizes mainly sulfated bile alcohols rather than bile salts. As in mammals, the skate actively secretes these bile alcohols across the canalicular membrane into bile (Boyer et al., *Am. J. Physiol.* 230:974-981, 1976), but the mechanism is unknown. We tested for the presence of a bsep analogue in skate liver using a polyclonal antibody to the rat liver protein. ATP-Dependent bile salt transport activity was measured in skate liver plasma membrane vesicles using [$^3$H]taurocholate as the substrate. Vesicles were isolated by a modification of the method of Song and coworkers (*J. Cell Biol.* 41:124-132, 1969), as previously described from our laboratory (Sellinger et al., *Toxicol. Appl. Pharmacol.* 107:369-376, 1991).

Western blot analysis of skate liver membranes indicated a predominant band that displayed an apparent molecular mass of ~210 kDa. This predicted molecular mass is somewhat larger than that seen for bsep in rat liver canalicular membranes, ~160 kDa. Immunofluorescence analysis of isolated, polarized skate hepatocyte clusters demonstrated positive staining in bile canaliculi, consistent with its selective apical localization in mammalian liver.

[$^3$H]Taurocholate uptake into skate liver plasma membrane vesicles was markedly stimulated by the inclusion of ATP in the incubation medium. In this mixed population of skate liver membrane vesicles (sinusoidal and canalicular), the ATP-sensitive component represented about 75% of the total taurocholate uptake. Uptake was not stimulated by a nonhydrolyzable analogue of ATP (AMP-PNP) and was inhibited by vanadate, indicating that ATP hydrolysis is required. Gramicidin D had no effect, demonstrating that the ATP effect is not secondary to an induced electrical or proton gradient. The ATP-dependent component of uptake was found to be saturable both in terms of taurocholate (apparent $K_m$ of 40±7 μM) and ATP (apparent $K_m$ of 0.6±0.1 mM).

Scymnol sulfate, the major bile alcohol of the skate, was an excellent inhibitor of ATP-dependent taurocholate uptake in the vesicles. The inhibition was competitive in nature, with a Ki of 23 μM, suggesting that this endogenous bile alcohol is a high affinity substrate of this transport system. ATP-Dependent [$^3$H]taurocholate uptake was also inhibited by other primary bile salts and bile salt derivatives: cholate and glycocholate, 0.1 mM, decreased uptake to ~65% of control, whereas NBD-taurocholate, taurodeoxycholate, and glycodeoxycholate decreased uptake to ~40% of control. Interestingly, lithocholate was a powerful *cis*-inhibitor, whereas taurolithocholate and glycolithocholate had only small effects. In contrast to the bile salts, ATP-dependent [$^3$H]taurocholate uptake was not inhibited by selective inhibitors of mrp2 or mdr1, canalicular membrane proteins that function to secrete other organic solutes into bile. The glutathione *S*-conjugates DNP-SG (0.1 mM), $LTC_4$ (0.7 μM), and GSSG (0.1 mM) had no effect. GSH itself (10 mM), verapamil (0.01 mM), a P-glycoprotein substrate, and *p*-aminohippurate (0.1 mM) also had no effect. In contrast, vincristine (0.1 mM), another P-glycoprotein substrate, and two organic anions, BSP (0.1 mM) and DIDS (0.5 mM), were effective inhibitors. The latter observations are consistent with previous studies in rat liver canalicular membrane vesicles that have also reported inhibition of ATP-dependent taurocholate transport by BSP and DIDS.

Overall, our findings provide functional and structural evidence for a bsep-like protein in the canalicular membrane of the skate liver. This transporter is expressed early in vertebrate evolution and transports both bile salts and bile alcohols. (Supported by ES03828, ES01247, DK34989, DK25636, DK48823, and by NSF BIR9531348, ESI9452682 and DBI9531348).