TRANSPORT OF BILE ALCOHOLS IN THE LIVER OF THE ELASMOBRANCH LITTLE SKATE (*RAJA ERINACEA*)

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Bile acids are the major bile salts in mammalian species. In contrast, the sulfated bile alcohol scymnol sulfate, 3α,7α,12α,24ξ,26,27-hexahydroxy-5ß-cholestane-26(27)-sulfate is the major bile salt in the bile of the elasmobranch little skate. Nevertheless, skate liver is also able to transport bile acids. But their uptake occurs by a Na^+-independent mechanism that is different from Na^+-dependent mammalian bile acid transport (Smith et al., Am. J. Physiol:252,G479-484,1987; Fricker et al., Biochem. J. 299:665-670,1994). Thus, the animal represents an important comparative model to study Na^+-independent bile acid transport and the overlapping specificity with bile alcohol transport. In the present study we used [^{3}H]-5ß-cholestane-3α,7α,12α-triol (CT), [^{3}H]-5ß-cholestane-3α,7α, 12α,24ξ26,27-hexol-26-sulfate (scymnol sulfate) and [^{3}H]-taurocholate as model compounds to investigate bile acid and bile acohol transport in isolated skate hepatocytes and perfused liver preparations.

[^{3}H]-CT was from Prof. G. Kurz, University Freiburg, FRG. [^{3}H]-labelled scymnol sulfate was sampled over a time period of 3 days from the bile of a free swimming skate which had received an i.v. injection of [^{3}H]-CT. Isolated hepatocytes were prepared by collagenase perfusion as described previously (Smith et al., Am.J.Physiol 252:G479-484, 1987) and then maintained at 12 °C in elasmobranch Ringer´s solution. The initial rates of uptake of bile salts were determined by the rapid centrifugation technique. Isolated perfused skate livers were perfused at a flow rate of 30 ml/min and a hydrostatic perfusion pressure of 5-6 cm with 100 ml elasmobranch Ringer´s solution in a recirculating system. Scymnol sulfate was injected into the portal vein of the perfused livers to give an initial concentration in the perfusate of 100 µM.

Analysis of the uptake of the bile salts into isolated hepatocytes exhibited a saturable and a nonsaturable transport component for all three compounds. The transport was temperature sensitive and was not altered when Na^+ in the medium was replaced by $choline^+$ The uptake of CT was not inhibited by taurocholate, whereas taurocholate uptake into the cells was inhibited in a noncompetitive manner by CT, indicating that uncharged bile alcohols are transported by a system separate from the taurocholate transporting system. The uptake of taurocholate was competitively inhibited by scymnol sulfate and vice versa, suggesting that taurocholate shares the physiological system for scymnol sulfate.

To examine the biliary secretion of bile alcohols, scymnol sulfate was injected into the portal vein of isolated perfused skate livers. The secreted bile was collected over 7-9 hours, because of the very slow bile flow of only 40-50 µl/h. Scymnol sulfate exhibited a significant

choleretic potential as shown by a stimulation of biliary excretion (Figure 1). Bile flow increased about 2-fold after addition of the sulfated bile alcohol within 15-30 min after addition into the perfusate, indicating a rapid hepatic uptake and secretion of the compound. The result supplements our earlier observation of a choleretic potential of CT, which is a metabolic precursor of scymnol sulfate. It is secreted only as the sulfated metabolite. Approximately 25 % [^{3}H]-scymnol sulfate was recovered in the secreted bile within the sampling period of 7 hours.

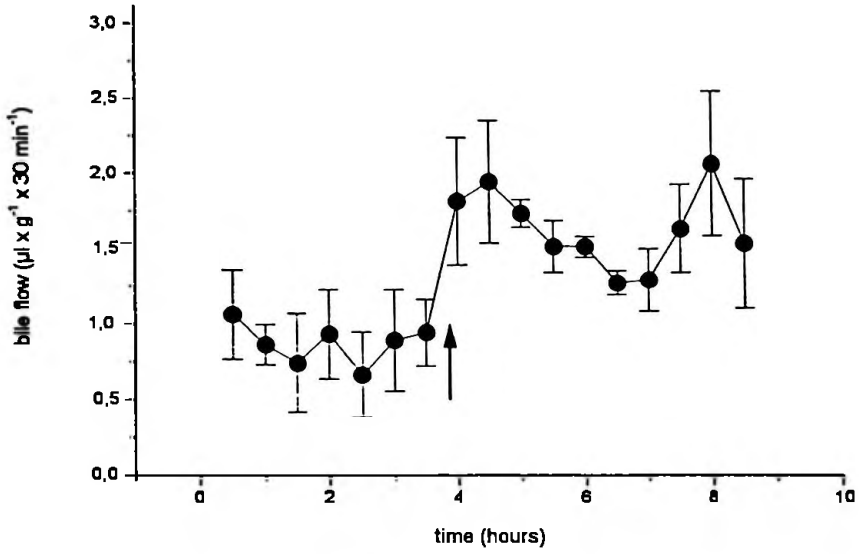

Figure 1: Bile flow of isolated perfused skate livers (n=4); the arrow indicates the addition of scymnol sulfate to the perfusate (final concentration 100 µM). The addition of the bile alcohol has a significant choleretic effect.

In summary, the experiments demonstrate that uncharged bile alcohols are transported into skate liver by a separate system from taurocholate, whereas taurocholate is transported by the hepatocellular uptake system for scymnol sulfate. The secretion of scymnol sulfate appears to be a major driving force for bile formation in elasmobranch liver similar to the secretion of taurocholate in mammalian liver.

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