BILE ALCOHOL TRANSPORT IN SKATE (RAJA ERINACEA) LIVER

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Recent studies from this laboratory have demonstrated that bile salts are taken up into skate hepatocytes by a saturable and Na^+-independent system (Smith et al., J. Exp. Zool. 241, 291-296, 1987; Fricker et al., Am. J. Physiol. 253, G816-G8221,1987), which is sensitive towards SH-group reacting agents (Blumrich et al., MDIBL-Bull. 30, 43-45,1990). However, bile salts are only minor constituents of skate bile, whereas sulfated bile alcohols are found as the major solutes (Karlaganis et al., J. Lipid. Res. 30, 317-322,1989). Therefore, we studied the mechanisms underlying the hepatocellular uptake and secretion of bile alcohols and their contribution to bile formation in isolated skate hepatocytes and isolated perfused skate liver. For our investigations we used two bile alcohols, which were recently synthesized in our laboratory, namely 3α,7α,12α-trihydroxy-5β-cholestane and its derivative 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol, which is metabolically inert at positions 26 and 27. Both bile alcohols were available as unlabeled and radiolabeled (^3H) compound.

We have employed freshly isolated skate hepatocytes and isolated perfused skate liver as models for the investigation of hepatocellular uptake and biliary secretion of both compounds. Initial rates of uptake of bile alcohols into the liver cells were measured by a rapid centrifugation technique (Fricker et al., Am. J. Physiol. 253, G816-G821,1987). The initial rates of uptake were measured from 0 to 2 min in 20 sec intervals. Both bile alcohols were taken up by a saturable transport component as well as by simple diffusion. The overall transport process was temperature sensitive and the saturable component showed no Na^+-dependency. This result is in accordance with our previous observations on bile salts in isolated skate hepatocytes. Both bile alcohols exhibited mutual competition. No decrease in uptake rates was seen in the presence of cholyltaurine, whereas cholyltaurine uptake was inhibited by both bile alcohols (Fig. 1). Kinetic analysis of the intial rates of uptake as a function of concentration indicated a noncompetitive type of inhibition. These findings suggest, that the anionic bile acid and the neutral bile alcohols do not share common pathways for hepatocellular uptake into skate liver.

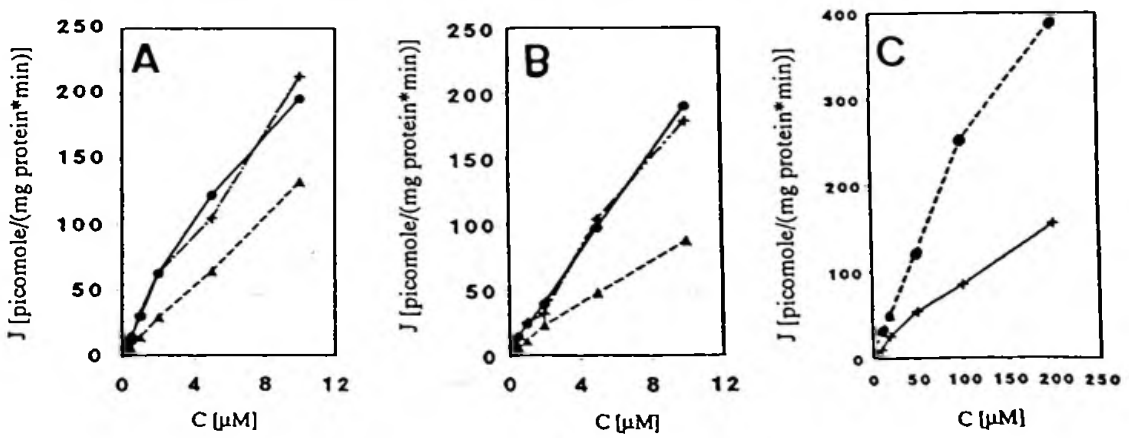

Fig. 1: Initial rates of uptake of bile alcohols and cholyltaurine into skate hepatocytes as function of substrate concentration

A: 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol: • alone, + in the presence of 50 μM cholyltaurine and ▲ in the presence of 10 μM 3α,7α,12α-trihydroxy-5β-cholestane

B: 3α,7α,12α-trihydroxy-5β-cholestane: • alone, + in the presence of 50 μM cholyltaurine and ▲ in the presence of 10 μM 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol

C: cholyltaurine: • alone,+ in the presence of 10 μM 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol

When isolated skate livers were perfused with elasmobranch Ringer solution containing 1 μM of the respective isotope diluted substrate, both bile alcohols exhibited a choleretic effect, which was more distinct for the fluorinated compound. Whereas infusion of 3α,7α,12α-trihydroxy-5β-cholestane resulted in a slightly increased bile flow of about 1.5 fold, infusion of 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol stimulated bile flow 3- to 5-fold. (Fig. 2). This result is in contrast to previous bile salt infusion, which had only an insignificant choleretic effect in skate liver. The distribution of the radiolabeled bile alcohol was also determined. 85 to 95% of the applied fluorinated alcohol remained in the livers after 5 hours of perfusion. Analysis of the biliary secreted radioactivity indicated, that the bile alcohol was almost completely metabolized to a more polar metabolite. Identification of the chemical structure of the metabolite(s) and its contribution to bile formation in skate liver is the subject of ongoing studies in our laboratory.

The results suggest, that bile alcohols play an essential role in modulating bile formation and bile flow in this species, similar to bile acids in mammals.

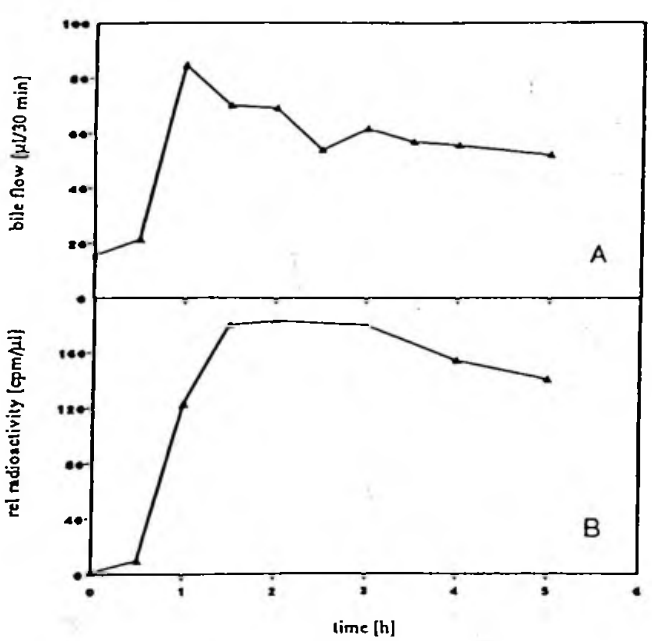

Fig. 2: Bile flow (A) and appearance of radioactivity in the bile (B) of isolated perfused skate livers. Values are corrected for biliary dead space (Reed et al., Am. J. Physiol. 242, G313-318, 1982).

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