ENTEROHEPATIC CIRCULATION IN THE LITTLE SKATE (RAJA ERINACEA)

Ralph Wössner[1], Gert Fricker[2], Gerhart Kurz[1], and James L. Boyer[3]
[1]Institut für Organische Chemie und Biochemie, D-78104 Freiburg, Germany,
[2]Sandoz Pharma AG, CH-4002 Basel, Switzerland,
[3]Yale Medical School, New Haven, CT 06510

Bile acids and bile alcohols are taken up by the liver of the little skate (Raja erinacea). Bile acids are taken up by diffusion and a saturable, Na^+-independent transport system (Fricker et al., Am. J. Physiol. 253, G816-G821,1987; Smith et al., J. Exp. Zool. 241,291-296, 1987; Fricker et al., Biochem. J. 1994, in press), which is sensitive towards SH-group reacting reagents (Blumrich et al., MDIBL-Bull. 30, 43-45,1990). The bile alcohols 5β-cholestane-3α,7α,12α-triol and 26,26,26,-27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol are taken up predominantly by simple diffusion and only in small part by a saturable Na^+-independent transport mechanism (Fricker et al., MDIBL-Bull. 32, 59-60, 1993). Recent studies on biliary secretion of these bile alcohols showed that the alcohols themselves cannot be secreted into bile without being metabolized to a more polar compound. The structure of the metabolites has not yet been defined, but both metabolites show a chromatographic behaviour in reversed-phase HPLC similar to bile acid and bile alcohol conjugates and sulfates. 5β-cholestane-3α,7α,12α-triol acts likely as a natural precursor of scymnol sulfate (5β-cholestane-3α,7α,12α,24,26,27-hexol-26-sulfate), which is the major constituent of skate bile. In contrast, 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol cannot be metabolized at position C-26 and C-27, and its metabolite has a polar group located elsewhere in the molecule.

The aim of this study was to investigate the biliary secretion of bile alcohol metabolites in isolated perfused skate liver and to demonstrate the existence of an enterohepatic circulation in this marine species.

[7ß-^{3}H]5β-cholestane-3α,7α,12α-triol, 26,26,26,27,27,27-hexafluoro-[7ß-^{3}H]5β-cholestane-3α,7α,12α-triol and the unlabeled alcohols were synthesized in our home laboratory. Isolated skate livers were perfused at a flow rate of 30 ml/min and a hydrostatic perfusion pressure of 5-6 cm with elasmobranch Ringer´s solution. Bile alcohols (2mM in 500 µl DMSO) were injected at a rate of 5 µl/sec just in front of the liver. Bolus injections of bile alcohol metabolites were done with various amounts of radioactivity obtained from liver perfusions with the corresponding bile alcohols and bile collection. To study intestinal reabsorption of bile alcohol metabolites they were injected into the small intestine of bile duct ligated fish. All compounds were diluted in 1 ml bile of control animals before injection. Bile was collected by means of balloons connected with cannulas inserted into the common bile ducts of the free swimming fish. The bile and liver samples were analyzed for radioactivity to assess the existence of an enterohepatic circulation in these fishes.

Perfusions of skate livers with the two bile alcohols 5β-cholestane-3α,7α,12α-triol (THC) and 26,26,26,27,27,27-hexafluoro-5β-cholestane-3α,7α,12α-triol (HFC) resulted in biliary secretion of the corresponding polar metabolites. No parent bile alcohols were secreted, as was checked by thin layer chromatography of the bile samples. In the case of THC-perfusions the secreted compound was concentrated up to 24-fold in bile as compared to the concentration of the original compound in the perfusate. The metabolite of HFC was concentrated up to 13-fold in the bile when HFC was

administered to the perfusate, respectively. The bile containing the radioactive labelled metabolites was collected and was administered by injection into the small intestine of free swimming fish 1 cm below the entrance of the common bile duct. The bile alcohols themselves were also administered into the intestine. Bile was collected up to for 4 days in one-day-fractions. Table 1 shows the recovery rates of the different injected compounds.

substance	intestine	liver	bile	total
HFC	16.6 %	5.6 %	14.2 %	36.4 %
metabolite of HFC	10.9 %	42.2 %	34.3 %	87.4 %
THC	-	0.1 %	94.8 %	94.9 %
metabolite of THC	-	-	97.2 %	97.2 %

Table 1:Recovery of radioactivity in the bile after administration into the intestine.Values represent means of two experiments. For abbreviations see text.

When isolated skate livers were perfused with the bile alcohol metabolites, an effective secretion of both compounds into bile could be observed (Fig. 1), indicating that the metabolites themselves can also be taken up by the liver.

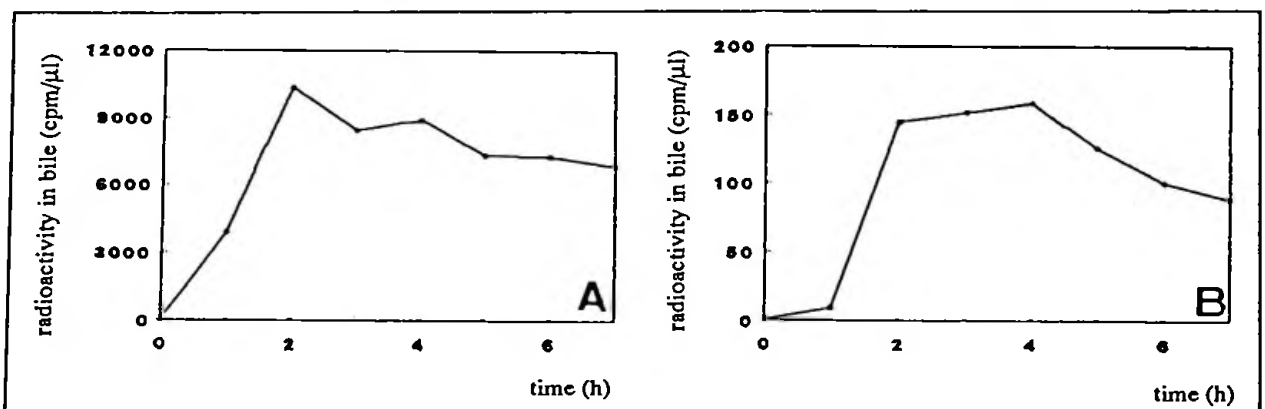

Fig.1: Biliary secretion of radioactivity in liver perfusion experiments after bolus injection of the metabolite of 5β-cholestane-3α,7α,12α-triol (A; 12.9 x 10^6 cpm) and 26,26,26,27,27,27- hexafluoro-5β-cholestane-3α, 7α, 12α - triol (B; 0.6 x 10^6 cpm)

The results demonstrate the presence of an enterohepatic circulation in the skate. Only the natural precursor and its metabolite are recovered in high yields in bile, but not exogenous, metabolically stable bile alcohol nor its metabolite. This indicates that at least one of the transport systems involved in the enterohepatic cirulation is specific for physiological substances. From the observation made in liver perfusion experiments, that both metabolites are readily taken up by the liver and are secreted again into bile, we conclude that the observed specificity for the enterohepatic circulation can be attributed to a specific transport system for reabsorption of bile alcohols and/or their sulfate esters in the intestinal tract.

Acknowledgements: This study was supported by grants from the CMTS to G.F. and the Deutsche Forschungsgemeinschaft SFB 154, F1 to R.W.