Characteristics of bile salt uptake into skate hepatocytes

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The substrate specificity for the transporter that mediates the hepatic uptake of organic anions in freshly isolated hepatocytes of the elasmobranch little skate (*Raja erinacea*) was determined for bile salts and bile alcohols. The Na⁺-independent transport system exhibits a substrate specificity, which is different from the specificity of Na⁺-dependent bile salt transport in mammals. Unconjugated and conjugated di- and tri-hydroxylated bile salts inhibit uptake of cholytaurine and cholate competitively. Inhibition is significantly greater with unconjugated as opposed to glycine- or taurine-conjugated bile salts. However, the number of hydroxyl groups in the steroid moiety of the bile salts has only minor influences on the inhibition by the unconjugated bile salts. Since the transport system seems to represent an archaic organic-

anion transport system, other anions, such as dicarboxylates, amino acids and sulphate, were also tested, but had no inhibitory effect on bile salt uptake. To clarify whether bile alcohols, the physiological solutes in skate bile, share this transport system, cholytaurine transport was studied after addition of 5 β -cholestane-3 β ,5 α ,6 β -triol, 5 α -cholestan-3 β -ol and 5 β -cholestane-3 α ,7 α ,12 α -triol. These bile alcohols inhibit cholytaurine uptake non-competitively. In contrast, uptake of 5 β -cholestane-3 α ,7 α ,12 α -triol, which is Na⁺-independent, is not inhibited by cholytaurine. The findings further characterize a Na⁺-independent organic-anion transport system in skate liver cells, which is not shared by bile alcohols and has preference for unconjugated lipophilic bile salts.

INTRODUCTION

Bile salts are the major solutes in mammalian bile and are cleared from portal blood by the liver. To maintain vectorial transport of bile salts from the blood sinusoids to the bile canaliculus, hepatocytes possess highly efficient transport systems in their sinusoidal and canalicular plasma membranes (Anwer and Hegner, 1978; Meier et al., 1984; Ruetz et al., 1987; Frimmer and Ziegler, 1988). Kinetic studies and photoaffinity-labelling studies suggest that in rat liver at least two transport systems with overlapping substrate specificity are involved in hepatocellular bile salt uptake (von Dippe and Levy, 1983; Fricker et al., 1987a; Buscher et al., 1987; Frimmer and Ziegler, 1988; Zimmerli et al., 1989). Recent experiments (Schramm et al., 1993) show that both cellular transport systems are active in the presence of an inwardly directed Na⁺ gradient as well as under Na⁺-depleted conditions.

In marine vertebrates, such as the elasmobranch little skate (*Raja erinacea*), sulphated bile alcohols rather than bile acids are found in bile (Tammar, 1974; Karlaganis et al., 1989). Nevertheless, bile salts are selectively taken up into skate liver from plasma by a Na⁺-independent organic-anion transport system (Boyer et al., 1976; Smith et al., 1987a; Fricker et al., 1987b) and are secreted into bile. Photoaffinity-labelling studies and mutual competition experiments using bile salts and photolabile bile salt derivatives suggest that a single polypeptide with an apparent molecular mass of 54000 Da might be involved in this transport process (Fricker et al., 1987b). To clarify the structural requirements for recognition by this transport system, which is different from the mammalian Na⁺-dependent bile salt transport system(s) (Boyer et al., 1993), we determined cholytaurine uptake in the absence and in the presence of other bile salts and organic anions. In addition, mutual interactions between cholytaurine and bile alcohol transport were analysed to gain information about whether bile alcohols share a common transport system with bile salts in hepatocytes of this marine species.

MATERIALS AND METHODS

All experiments were performed at the Mount Desert Island Biological Laboratory in Salsbury Cove, ME, U.S.A.

Animals

Male skates (*Raja erinacea*; 1.0 ± 0.3 kg), were netted off Southwest Harbor, ME. The animals were maintained in aerated tanks supplied with seawater at 15 °C and were used within 4 days of capture. All fishes were anaesthetized before study with pentobarbital sodium (2.5 mg/kg) injected via a caudal vein.

Chemicals

[6-³H]Cholytaurine (6.6 Ci/mmol) and [2,4-³H]cholic acid (10 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). [¹⁴C]Carboxy-inulin (5.0 Ci/mmol) was obtained from Amersham International (Amersham, Bucks., U.K.). 5 β -Cholestane-3 β ,5 α ,6 β -triol and 5 α -cholestan-3 β -ol were purchased from Sigma (St. Louis, MO, U.S.A.). [7 β -³H]5 β -Cholestane-3 α ,7 α ,12 α -triol was kindly provided by Professor Dr. G. Kurz, Institute of Organic Chemistry and Biochemistry D-7800 Freiburg, Germany. It was synthesized as described by Gengenbacher et al. (1990). The bile acids and bile alcohols were shown by high-performance t.l.c. to have purity > 98 %. All other reagents were obtained from commercial sources in the highest purity available.

Hepatocyte Isolation

Skate hepatocytes were isolated by a collagenase perfusion technique described in detail elsewhere (Smith et al., 1987b). Briefly, the liver was removed from the abdominal cavity of the anaesthetized fish and was perfused via the portal vein at 15 °C with Ca²⁺/Mg²⁺-free elasmobranch Ringer solution (270 mM NaCl, 4 mM KCl, 3 mM MgCl₂, 2.5 mM CaCl₂, 0.5 mM

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Na_2SO_4 , 1 mM KH_2PO_4 , 8 mM NaHCO_3 and 350 mM urea) before perfusion with collagenase (0.07–0.1 % in elasmobranch Ringer solution). Then the liver was placed in 80 ml of Ringer solution containing 100 units/ml DNAase, and the cells were suspended after removing the connective-tissue capsule with a forceps. The cells were resuspended in elasmobranch Ringer solution to yield a final concentration of $\sim (5\text{--}6) \times 10^6$ cells/ml. Most of the hepatocytes were isolated in clusters of 3–5 cells surrounding a single bile canaliculus, indicating the maintenance of their structural polarity even in the isolated state (Reed et al., 1982; Smith et al., 1987b). The viability of the cells was assessed by Trypan Blue exclusion (normally > 97 % of the cells excluded Trypan Blue). All kinetic experiments were performed within 2.5 h after cell isolation.

Uptake studies with isolated hepatocytes

Freshly isolated hepatocytes [$(2.5\text{--}3) \times 10^6$ cells] were incubated at 15 °C in 2.5 ml of elasmobranch Ringer solution containing various concentrations of radiolabelled and unlabelled bile salts or bile alcohol. When other organic anions were used in inhibition experiments, the cell medium was supplemented with the respective compound in concentrations as indicated in Figure legends and Tables. The flasks were agitated by hand to minimize unstirred water-layer effects and to keep the cells in suspension. After the indicated time intervals, 500 μl portions of cell suspension were removed and centrifuged in 1.5 ml polyethylene tubes in a Beckman microfuge at 12000 g for 10 s. The supernatant was removed with a Pasteur pipette and the surface of the remaining cell pellet was washed with ice-cold elasmobranch Ringer solution and carefully blotted with filter tips to remove residual fluid on the surface of the cell pellet. Substrate trapped in extracellular fluid was determined by incubation of cells with [^3H]inulin and calculation of extracellular space in the pellet after centrifugation. The tubes were cut with a razor blade, and the tips containing the cell pellets were transferred to a scintillation vial. The vials were vigorously shaken and the dispersed cells were solubilized with 250 μl of 4 % sulphosalicylic acid. After 3 h, 5 ml of scintillation liquid (Optifluor; Packard Instruments, Downers Grove, IL, U.S.A.) was added, and the radioactivity was determined in a Packard Tri-Carb scintillation counter, by using the external-standard-ratio method to correct for quenching. Initial uptake rates were calculated from the cell-incorporated radioactivity, and curve analyses were performed with the data-analysis program Enzfitter (Elsevier Publishers, Amsterdam, The Netherlands).

RESULTS

Transport of conjugated and unconjugated bile salts

Previous studies from this laboratory indicated that the uptake of the conjugated bile salt cholytaurine into freshly isolated skate hepatocytes is a Na^+ -independent process, which is mediated by at least one transport system (Fricker et al., 1987b; Smith et al., 1987a). In the present study, at a given initial concentration, the accumulation of the unconjugated cholate and the conjugated cholytaurine in skate hepatocytes was linear for at least 90 s. Therefore the initial rates of transport (0–60 s at 15 °C) were plotted as a function of increasing bile salt concentrations. The uptake rates into isolated skate hepatocytes of cholytaurine and cholate as function of increasing concentrations exhibited curvilinearity. However, no clear saturability of total uptake could be observed for both bile salts. Analysis of the kinetic data gave

evidence that the uptake of both bile salts occurs by more than one process. But it did not allow a clear distinction whether, in addition to the saturable transport process, which was easily accessible to kinetic analysis at low concentrations, a second transport system is active with a K_m far outside the concentration range used, becoming better recognizable at much higher concentrations, or whether the bile salts under observation are also taken up into isolated skate hepatocytes by passive diffusion, which becomes the predominant transport component at very high bile salt concentrations. Under the assumption that the second transport component represents passive diffusion, we subtracted it from total uptake to obtain an estimate for the kinetic constants of the transport system that predominantly contributes to total uptake of cholytaurine at low bile salt concentrations. It exhibited a K_m of $45 \pm 12 \mu\text{M}$ and a $V_{\max}$ of $100 \pm 15 \text{ pmol/min per mg}$ of protein for cholytaurine. The uptake of cholate exhibited a saturable component with a K_m of $25 \pm 12 \mu\text{M}$ and a $V_{\max}$ of $52 \pm 18 \text{ pmol/min per mg}$ of protein (Figures 1a and 1b). For both bile salts, no significant difference in uptake rates could be observed, when sodium salts in the medium were replaced by the respective choline salts (Figure 1).

Figure 1 Initial rates of bile salt uptake into skate hepatocytes: dependence on bile salt concentration

Isolated skate hepatocytes were incubated with various concentrations (0–500 μM) of [^3H]cholate (a) or [^3H]choleytaurine (b). At each substrate concentration, uptake was determined at 15, 30, 45 and 60 s, and the initial uptake rates were calculated from the slope of linear regression lines. The data represent means $\pm$ S.E.M. of triplicate determinations in 8 separate cell preparations. Black symbols, total uptake: ●, ▲, uptake in the presence of Na^+ ; ■, ◆, Na^+ replaced by choline $^+$. Broken lines: transport component assumed to be diffusion. White symbols: total uptake minus diffusional component.

Figure 2 Competition between different bile salts for uptake into isolated skate hepatocytes

The uptake of cholytaurine into skate liver cells was determined in the presence of 100 μ M concentrations of different bile salts. Initial rates of uptake were determined as described in Figure 1, and the data were plotted in an Eadie-Scatchard diagram to analyse the type of inhibition. The data represent means $\pm$ S.E.M. of triplicate determinations in 3 separate cell preparations: $\bullet$, control; $\square$, in presence of chenodeoxycholytaurine; $\blacktriangle$, in presence of ursodeoxycholate; $\circ$, in presence of chenodeoxycholate; $\blacklozenge$, in presence of cholate. Units: v , pmol/min per mg of protein; s , μ M.

Table 1 Uptake of cholytaurine (10 μ M) into isolated skate hepatocytes in the presence of other unconjugated, taurine- and glycine-conjugated bile salts

The data represent means $\pm$ S.E.M. from triplicate determinations on at least three separate hepatocyte preparations.

Bile salt	Uptake	
	(pmol/min per mg of protein)	(%)
Control	20.0 $\pm$ 5.0	100
3 α ,7 α ,12 α -OH	4.8 $\pm$ 0.8	24 $\pm$ 4
Glyco-3 α ,7 α ,12 α -OH	8.8 $\pm$ 0.8	44 $\pm$ 4
Tauro-3 α ,7 α ,12 α -OH	11.0 $\pm$ 1.8	55 $\pm$ 9
3 α ,7 β -OH	12.0 $\pm$ 0.4	60 $\pm$ 2
3 α ,7 α -OH	8.0 $\pm$ 0.8	40 $\pm$ 4
Glyco-3 α ,7 α -OH	8.6 $\pm$ 1.0	43 $\pm$ 5
Tauro-3 α ,7 α -OH	13.6 $\pm$ 0.4	68 $\pm$ 2
3 α ,12 α -OH	3.0 $\pm$ 0.8	15 $\pm$ 4
Glyco-3 α ,12 α -OH	7.0 $\pm$ 0.8	35 $\pm$ 4
Tauro-3 α ,12 α -OH	15.0 $\pm$ 2.8	75 $\pm$ 14
3 α ,6 β -OH	10.0 $\pm$ 1.2	50 $\pm$ 6
Tauro-3 α ,6 β -OH	6.0 $\pm$ 1.0	30 $\pm$ 5
3 α -OH	9.8 $\pm$ 2.0	49 $\pm$ 10
3,7,12-oxo	9.6 $\pm$ 0.6	48 $\pm$ 3
Tauro-3,7,12-oxo	14.0 $\pm$ 3.0	70 $\pm$ 15

Inhibition studies with bile salts

To determine whether different bile salts share the same system for hepatocellular uptake in skate liver, the transport of cholytaurine into isolated skate hepatocytes was measured in the presence of various other bile salts (100 μ M). Only cholytaurine concentrations below 50 μ M were used for these measurements, in order to assign more clearly the effect of each inhibitor to the identified transport system. Cholate, ursodeoxycholate, chenodeoxycholate and chenodeoxycholytaurine significantly in-

hibited cholytaurine uptake. As illustrated in an Eadie-Scatchard diagram (Figure 2), cholytaurine uptake was competitively inhibited by these other bile salts. Inhibition was strongest in the presence of cholate (apparent K_i 20 $\pm$ 5 μ M). Chenodeoxycholate, ursodeoxycholate and chenodeoxycholytaurine inhibited cholytaurine uptake with an apparent K_i of 97 $\pm$ 9 μ M, 151 $\pm$ 11 μ M and 526 $\pm$ 56 μ M respectively. Competitive inhibition was also observed with those bile salts, when cholate was used as the radioactively labelled substrate. K_i values were in the same range as with cholytaurine as substrate (cholytaurine 47 $\pm$ 8 μ M, chenodeoxycholate 86 $\pm$ 4 μ M, ursodeoxycholate 143 $\pm$ 6 μ M, chenodeoxycholytaurine 490 $\pm$ 40 μ M). As expected, the K_i values for cholate and cholytaurine were in the same range as the respective K_m values of both bile acids.

To determine whether the number and/or the position of the hydroxyl groups of C-24 bile salts influenced the extent of inhibition of this Na⁺-independent transport system, cholytaurine (initial concn. 10 μ M) uptake was measured in the presence of various unconjugated, glycine- and taurine-conjugated mono- di- and tri-hydroxylated bile salts (initial concn. 100 μ M) as well as in the presence of bile salts, in which the hydroxyl groups were replaced by keto residues. As illustrated in Table 1, all bile salts were able to inhibit cholytaurine uptake. However, the unconjugated bile salts had a greater inhibitory capacity than their respective glycine or taurine conjugates (unconjugated > glycine conjugate > taurine conjugate). The 3 α ,6 β -hydroxy bile salts were the one exception, where the taurine conjugate inhibited cholytaurine uptake more effectively. Inhibition by ursodeoxycholate (3 α ,7 β -hydroxy) was significantly less than that by its 3 α ,7 α -enantiomer, chenodeoxycholate (40 $\pm$ 2% versus 60 $\pm$ 4% inhibition of cholytaurine uptake). When all hydroxyl groups of the steroid nucleus were replaced by keto residues (3,7,12-oxocholanoate, dehydrocholate), the inhibition was similar to that by the hydroxylated bile salts. Again, the taurine conjugate was significantly less effective than the unconjugated bile salt. The extent of inhibition with lithocholate (3 α -hydroxy) was questionable, since this bile salt was difficult to solubilize in elasmobranch Ringer solution. Since cholytaurine uptake is taken up by an organic-anion transport system, whose substrate specificity has not yet been fully determined, we examined whether certain amino acids, dicarboxylates or sulphate interfered with bile salt transport. These anions have been described to be taken up into hepatocytes by transport systems with overlapping substrate specificity in mammalian hepatocytes (Hugentobler and Meier, 1986). Using cholytaurine at an initial concentration of 10 μ M as substrate, we added the amino acids glutamine, glutamate and taurine (1 mM), the dicarboxylic acids oxalate and succinate (1 mM) and the anion sulphate (10 mM) as inhibitors to the incubation medium. However, cholytaurine uptake was not inhibited by any of the added compounds suggesting separate uptake systems for these anions in skate hepatocytes (Table 2).

Inhibition studies with bile alcohols

Elasmobranch bile contains bile alcohols rather than bile salts. These might be recognized by the same organic-anion transport system, due to their structural similarity. Thus 5 β -cholestane-3 α ,7 α ,12 α -triol, 5 β -cholestane-3 β ,5 α ,6 β -triol and 5 α -cholestan-3 β -ol were tested as inhibitors. Because of their low solubility in elasmobranch Ringer solution, the bile alcohols were dissolved in 2% DMSO to a final concentration of 25 μ M, whereas the concentration of cholytaurine was 1 μ M in these experiments. DMSO was also added to the incubation solution of control experiments. As illustrated in Figure 3, each bile alcohol inhibited

Table 2 Uptake of cholytaurine (10 μ M) into isolated skate hepatocytes in the presence of other organic anions

The data represent means $\pm$ S.E.M. of triplicate determinations from three different hepatocyte preparations.

	Uptake (%)
Control	100
Glutamine (1 mM)	95 $\pm$ 9
Glutamate (1 mM)	94 $\pm$ 8
Oxalate (1 mM)	109 $\pm$ 5
Succinate (1 mM)	85 $\pm$ 14
Sulphate (10 mM)	89 $\pm$ 8

Figure 3 Non-competitive inhibition of cholytaurine uptake by bile alcohols

Uptake of various concentrations of cholytaurine into skate liver cells in the absence and in the presence of bile alcohols (25 μ M). Data are plotted in an Eadie-Scatchard diagram to demonstrate the non-competitive type of inhibition. The data represent means $\pm$ S.E.M. of triplicate determinations in 3 separate cell preparations: ●, control; ■, 25 μ M 5 α -cholestan-3 β -ol; ○, 25 μ M 5 β -cholestan-3 β ,5 α ,6 β -triol; ▲, 25 μ M 5 β -cholestan-3 α ,7 α ,12 α -triol. Units as in Figure 2.

Table 3 Initial rates of uptake of [7 β - 3 H]5 β -cholestan-3 α ,7 α ,12 α -triol in the absence and in the presence of Na $^+$ and cholytaurine

The data represent the means $\pm$ S.E.M. of triplicate determinations in three separate cell preparations.

Concn.	Uptake (pmol/min per mg of protein)			
	Control	Na $^+$ -depleted medium	100 μ M cholytaurine	200 μ M cholytaurine
5	47.6 $\pm$ 5.9	43.2 $\pm$ 4.8	51.8 $\pm$ 8.3	50.0 $\pm$ 5.6
6.6	67.6 $\pm$ 8.7	68.3 $\pm$ 5.3	64.5 $\pm$ 7.4	60.5 $\pm$ 4.5
10	107.5 $\pm$ 26.5	102.0 $\pm$ 11.4	83.3 $\pm$ 11.9	96.4 $\pm$ 8.8
20	142.5 $\pm$ 29.3	135.0 $\pm$ 12.2	147.5 $\pm$ 29.4	140.5 $\pm$ 4.3

cholytaurine uptake, but none of them exhibited competitive inhibition, suggesting that they do not share the same transport system with bile salts. When the uptake of [7 β - 3 H]5 β -cholestan-3 α ,7 α ,12 α -triol into isolated skate hepatocytes was measured in the presence of 100 μ M or 200 μ M cholytaurine, no competition for transport was seen (Table 3). Initial studies suggest that the uptake rates of [7 β - 3 H]5 β -cholestan-3 α ,7 α ,12 α -triol were also

not changed when Na $^+$ in the incubation medium was replaced by choline (Table 3). Furthermore, in preliminary studies bile alcohols inhibited each other's uptake, supporting the hypothesis that the mechanism for bile alcohol uptake into skate hepatocytes is different from that of bile salt uptake.

DISCUSSION

The clearance of bile salts and other organic anions from the portal blood by the liver requires a variety of highly versatile transport systems for their efficient hepatocellular uptake and canalicular secretion. However, significant differences exist among species with respect to their bile composition. In contrast with mammalian bile, where bile salts are the major biliary solutes, sulphated bile alcohols are predominant in the bile of marine elasmobranchs such as the little skate (*Raja erinacea*) or sharks. Nevertheless cholytaurine is transported into skate hepatocytes by an organic-anion membrane carrier system (Fricker et al., 1987b; Smith et al., 1987a). The present study demonstrates that conjugated and unconjugated bile salts are taken up into skate hepatocytes by a saturable and Na $^+$ -independent process, since initial rates of uptake were similar when Na $^+$ in the incubation medium was replaced by choline. This phenomenon is different from observations in mammalian liver (e.g. rat and human), where conjugated bile salts in physiological concentrations are transported into hepatocytes by two Na $^+$ -dependent processes (Schramm et al., 1993). Recent evidence from expression cloning experiments (Boyer et al., 1993) suggests that, in contrast with the system in higher vertebrates, the Na $^+$ -independent bile salt transport of cholytaurine in the little skate also depends on the presence of extracellular chloride.

In contrast with studies with isolated mammalian hepatocytes (Buscher et al., 1987; Schramm et al., 1993), where total uptake of cholytaurine was saturable in the presence of Na $^+$ and under Na $^+$ -depleted conditions, total cholytaurine and cholate uptake into skate hepatocytes was not saturable at higher bile salt concentrations. Kinetic analysis of the uptake process could not resolve whether the transport component, which is predominant at the higher bile salt concentrations, represents a second saturable uptake system with a very high K_m or reflects passive diffusion. Nevertheless, this transport component accounts for less than 10% of total uptake at cholate or cholytaurine concentrations lower than 25 μ M, i.e. the physiological concentration range, in which cholytaurine has been used as substrate for the inhibition experiments. Passive diffusion is rather unlikely for a conjugated bile salt such as cholytaurine with its highly polar and negatively charged taurine residue. In contrast, non-ionic diffusion through lipid membranes has been proposed as a possible uptake process for cholate, which does not have such a polar residue, in addition to carrier-mediated saturable transport (Cafilisch et al., 1990). We also cannot exclude the possibility that the linear uptake component, which contributes significantly to the measured uptake values at high bile salt concentrations, represents unspecific binding to the lipophilic surface of the hepatocytes. Therefore, all inhibition studies were performed with low substrate concentrations (10 μ M), where carrier-mediated transport is presumed to occur.

Photoaffinity labelling and mutual inhibition studies suggest that the Na $^+$ -dependent bile salt transport in rat liver is multi-specific (Hardison et al., 1984, 1991; Buscher et al., 1986; Zimmerli et al., 1989; Cafilisch et al., 1990). However, in contrast with the Na $^+$ -dependent transporter, no information is available concerning the substrate requirements of the Na $^+$ -independent organic-anion transport system in skate liver. Therefore we

Figure 4 Inhibition of cholytaurine uptake into skate hepatocytes by bile salts as a function of the lipophilicity of the various inhibitors

The lipophilicity of the inhibitors was estimated from their octanol/water partition coefficients (P). Abbreviations: TDCA, deoxycholytaurine; GCA, cholyglycine; TCA, cholytaurine; GCDCA, chenodeoxycholyglycine; THDCA, hydoxycholytaurine; GDCA, deoxycholyglycine; CA, cholate; CDCA, chenodeoxycholate; UDCA, ursodeoxycholate; DCA, deoxycholate.

measured the uptake of cholytaurine in the presence of other bile salts, in order to assess the structural criteria for recognition of this putative transporter. However, these inhibition studies do not exclude the possibility that these bile salts could also be transported by other systems, nor do they define the affinity between the transport system and substrate, since only one concentration of cholytaurine and one inhibitor concentration were used. The main findings indicate that the hepatic uptake of both cholytaurine and cholate is competitively inhibited by other bile salts. Thus the same Na^+ -independent transport system is shared by conjugated and unconjugated bile salts. The number of hydroxyl groups at the steroid nucleus had less influence on the ability of the bile salts to inhibit cholytaurine transport than has been reported for the Na^+ -dependent bile salt carrier system in rat hepatocyte basolateral plasma membranes (Zimmerli et al., 1989) or for isolated rat hepatocytes (Hardison et al., 1984). Moreover, for a given core structure, glycine- and taurine-conjugated bile salts were less potent inhibitors than the unconjugated bile salts. This is also different from findings with a Na^+ -dependent sinusoidal bile salt carrier in rat hepatocytes (Hardison et al., 1984). Interestingly, there is a rough correlation between the extent of inhibition of cholytaurine transport by other bile salts and their lipophilicity (Figure 4), as estimated by their respective octanol/water partition coefficients (Heuman, 1989; Roda et al., 1990). Two dihydroxy bile salts, chenodeoxycholate and ursodeoxycholate, are the only ones that do not fit this correlation.

It could not be determined with certainty how the orientation of the hydroxyl groups, i.e. α or β , at the steroid nucleus influences the inhibition of cholytaurine transport, since only two bile salts with a β -orientated hydroxyl group were tested ($3\alpha,7\beta$ - and $3\alpha,6\beta$ -hydroxyl). However, ursodeoxycholate ($3\alpha,7\beta$ -hydroxyl) inhibited cholytaurine uptake to a lesser extent than its 7α -enantiomer chenodeoxycholate (40% versus 60% inhibition). In contrast with our results, significant differences in inhibition of the Na^+ -dependent cholytaurine uptake by chenodeoxycholate and ursodeoxycholate have not been observed in isolated rat hepatocytes (Hardison et al., 1984).

When the amino acids glutamine, glutamate, taurine or the dicarboxylates succinate and oxalate, which are potential substrates for organic-anion carrier systems, were used as inhibitors, no inhibition of cholytaurine uptake could be detected, indicating that they do not interact with this organic-anion transporter in hepatocytes of the little skate. Recent experiments show that glutamine shares a common Na^+ -dependent transport system with alanine, which is different from the taurine uptake system. Taurine is also taken up into skate hepatocytes by a Na^+ -dependent transport process (Ballatori and Boyer, 1988, 1992), which is not inhibited by cholytaurine. Thus our studies indicate the presence of separate systems for these amino acids. Sulphate also had no influence on the initial uptake rates of cholytaurine. Previous studies with skate hepatocyte isolated basolateral plasma-membrane vesicles demonstrated inhibition of pH-gradient-stimulated sulphate uptake by cholate, but not by taurocholate (Hugentobler et al., 1987). In that study the suggestion was made that cholate inhibits sulphate transport not by direct competition, but by dissipation of the transmembrane pH gradient, which might then diminish the driving force for sulphate transport.

Bile salts in elasmobranchs appear to be largely sulphated bile alcohols, and sulphated scymnol [5β -cholestane- $3\alpha,7\alpha,12\alpha,24\xi$, $26,27$ -hexol 26(or 27)-sulphate] has been identified as the predominant bile alcohol in the bile of the little skate by means of fast-atom-bombardment mass spectrometry (Haslewood, 1968; Karlaganis et al., 1989). Because of their structural similarity, bile alcohols might potentially use the same system for hepatic uptake as determined for bile acids. In isolated rat hepatocytes, a precursor of cholic acid, 5β -cholestane- $3\alpha,7\alpha,12\alpha$ -triol, has been demonstrated to compete with bile acids for uptake, and photoaffinity labelling studies revealed that this bile alcohol and bile acids may interact with the same proteins in rat hepatocyte membranes (Buscher et al., 1986). Yet in skate hepatocytes a non-competitive competition of Na^+ -independent cholytaurine uptake by 5β -cholestane- $3\alpha,7\alpha,12\alpha$ -triol and also other bile alcohols was seen. Furthermore, since cholytaurine does not inhibit the uptake of 5β -cholestane- $3\alpha,7\alpha,12\alpha$ -triol into isolated skate hepatocytes, which is also Na^+ -independent, bile alcohols do not appear to share the same transport system as bile acids for hepatocellular uptake in skate liver. Rather, preliminary experiments in isolated perfused skate livers suggest that there are separate mechanisms for efficient concentrative transport of bile alcohol (5β -cholestane- $3\alpha,7\alpha,12\alpha$ -triol) into bile of the little skate. Analysis of skate bile by t.l.c. suggests that the bile alcohol is metabolized to a more polar metabolite, indicating a transcellular rather than a paracellular hepatic pathway. Characterization of uptake, metabolism and hepatocellular excretion of bile alcohols in skate is the subject of future studies.

In summary, the previously identified Na^+ -independent organic-anion transport system in skate hepatocytes exhibits a specificity for unconjugated and conjugated bile salts which is significantly different from that of the Na^+ -dependent bile salt transport systems in rat liver. Unconjugated and more lipophilic bile salts are preferred rather than conjugated bile salts. Furthermore, bile alcohols, which are more natural components of skate bile, do not appear to share the same transport system as bile salts for uptake into skate hepatocytes.

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