SH-GROUPS ARE ESSENTIAL FOR UPTAKE OF BILE ACIDS IN HEPATOCYTES OF THE LITTLE SKATE (RAJA ERINACEA)

M. Blumrich[1], E. Petzinger[1] and J. L. Boyer[2]
[1]Institut fuer Pharmkologie und Toxikologie, Justus Liebig Universitat Giessen, 6300 Giessen, Germany
[2]Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, CT 06510

A single sinusoidal bile salt transport system has been characterized for the uptake of taurocholate in isolated hepatocytes from the elasmobranch Raja erinacea (Fricker et al., Am. J. Physiol. 253: G816, 1987). In this species conjugated bile acids are taken up by Na^+-independent transport (Smith et al., Am. J. Physiol. 252: G479, 1987). The elasmobranch bile acid transporter is thought to be an archaic transport system which is also found in mammalian species together with an additional Na^+-dependent sinusoidal transport system for conjugated bile acids. We have previously distinguished these transport systems in rat hepatocytes by examining the effects of several sulfhydryl-reagents on the uptake of taurocholate and cholate (Blumrich and Petzinger, BBA 1029: 1, 1990). In these studies, the organic mercurial parachloromercuribenzenesulfonate (PCMBS) strongly inhibited (^{3}H)-taurocholate uptake, whereas N-ethylmaleimide (NEM) preferentially blocked the uptake of (^{14}C)-cholate, supporting the view that rat hepatocytes maintain two distinct transport systems for the uptake of conjugated vs. unconjugated bile acids. It was therefore of interest to assess the effects of sulfhydryl reagents on the hepatic transport system(s) for bile acids in Raja erinacea.

Hepatocytes were isolated from male skates by a collagenase perfusion technique (Smith et al., J. Exp. Zool. 241: 291,1987) and resuspended in elasmobranch Ringers. (^{14}C)-cholate and (^{3}H)-taurocholate uptake was measured by a rapid centrifugation method at 15 ^{0}C (Ballatori and Boyer, Am. J. Physiol. 254: R801, 1988) as described in detail (Blumrich and Petzinger, BBA 1029: 1, 1990).

Table 1 illustrates the initial uptake rates for (^{3}H)-taurocholate and (^{14}C)-cholate for the various conditions used in these experiments. Uptake was partially inhibited at 4 ^{0}C by 100 μM cholate or taurocholate confirming previous findings (Smith et al. Am. J. Physiol. 252:G479, 1987). However digitoxin, a strong inhibitor of the unconjugated bile acid uptake transporter in rat hepatocytes, reduced initial uptake rates (V_i) for both bile acids by 90 %, supporting the hypothesis that these bile acids share a common transport system in this marine species. We assumed that the bile acid transporter of the skate hepatocyte is functionally comparable to the cholate transport system of mammalian hepatocytes.

This assumption was confirmed with sulfhydryl reagents. PCMBS (100 μM) blocked the uptake rates of taurocholate by 27 % and the uptake for cholate by 31 %, whereas NEM (200 μM), a covalent-binding SH-reagent, inhibited 48 % of the V_i of cholate transport. By contrast, another SH-blocking agent dithiobis-nitropyridine (DTNP), 200 μM, reduced V_i by 55 % for both bile acids. Since the tested SH-group blockers reduced the transport of taurocholate as well as

Table 1 shows the effects of sulfhydryl reagents on the initial uptake rates (V_i) of (^{3}H)-taurocholate and (^{14}C)-cholate in isolated skate hepatocytes 3 * 10^6 hepatocytes/ml suspension were preincubated at 15 ^{0}C or 4 ^{0}C for at least 30 minutes. V_i rates of the uptake of 11 nM (^{3}H)-taurocholate/10 μM taurocholate or 1.25 μM (^{14}C)-cholate/5 μM cholate were determined by linear regression from the 15, 45, 75 and 105 seconds values under various conditions.

n = 4, X ± SD

	TAUROCHOLATE (pmol/mg protein)		**CHOLATE** (pmol/mg protein)	
	Vi ± SD	% Inh.	Vi ± SD	% Inh.
15 ^{0}C +Na$^+$				
Contr.	20.9 ± 5.2		28.5 ± 7.4	
100 μM TC	9.4 ± 2.7	55	15.7 ± 3.8	44
100 μM C	12.4 ± 3.4	40	12.9 ± 3.1	54
100 μM Digitoxin	3.5 ± 1.2	88	3.2 ± 1.4	89
200 μM NEM	17.9 ± 4.5	11	15.1 ± 3.8	48
200 μM DTNP	8.7 ± 2.1	58	13.4 ± 2.3	53
100 μM PCMBS	14.7 ± 3.7	27	19.4 ± 3.6	31
15 ^{0}C -Na$^+$				
Contr.	19.4 ± 4.7		25.4 ± 5.4	
200 μM NEM	17.4 ± 3.7	10	14.5 ± 4.1	42
100 μM PCMBS	14.8 ± 4.5	24	18.3 ± 3.8	27
4 ^{0}C +Na$^+$				
Contr.	8.7 ± 2.9		17.4 ± 4.7	
100 μM TC	5.4 ± 1.4	38	8.7 ± 3.8	51
100 μM C	6.7 ± 1.9	23	10.6 ± 2.7	40

of cholate identically, a single transport system for the uptake of conjugated and unconjugated bile acids is suggested in the elasmobranch hepatocyte. In contrast, in isolated hepatocytes from the rat, taurocholate transport is specifically inhibited by PCMBS. Residual uptake of taurocholate in the presence of 100 μM PCMBS is less than 10 % but 70 % of cholate in the rat. However, in the mammalian hepatocytes identical inhibition of both bile acids by 100 μM PCMBS is seen in sodium-free choline buffer. Therefore uptake of taurocholate and cholate into skate hepatocytes was investigated in sodium-free choline elasmobranch Ringer solution. Identical inhibition by PCMBS and NEM occured. Inhibition by PCMBS of the uptake of both bile acids was nearly completely antagonized by addition of 500 μM dithiothreitol (DTT). Within 15 minutes, DTT normalized the transport rates.

In contrast to previous findings in rat hepatocytes, neither 100 μM $CdCl_2$ nor 100 μM $HgCl_2$ inhibited significantly V_i for both bile acids in uptake experiments in skate hepatocytes.

In summary, these results indicate that SH-groups are present in the organic anion transport system for bile acids in hepatocytes of the small skate, Raja erinacea and are essential for the function of the transporter. The pattern of sulfhydryl inhibition is consistent with the hypothesis that conjugated and unconjugated bile acids share one common carrier system in this marine species. In mammals (at least) two transport systems, one for conjugated bile acids and one for unconjugated bile acids are present which could be distinquished by SH-group blockers.

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