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INVESTIGATIONS ON THE SODIUM DEPENDENCE OF BILE ACID FLUXES IN THE ISOLATED PERFUSED RAT LIVER

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SUMMARY

At $[Na^+]_o = 118$ mM the concentrative transfer of cholic and taurocholic acid from the perfusate into the isolated rat liver displays saturation kinetics (taurocholate: $V = 299$ nmol · min^{-1} · g^{-1}, $K_m = 61$ μM; cholate: $V = 327$ nmol · min^{-1} · g^{-1}, $K_m = 436$ μM). Perfusion with an isotonic sodium-free medium did not change the feature of a carrier-mediated transport but did markedly reduce V without affecting K_m (taurocholate: $V = 65$ nmol · min^{-1} · g^{-1}, $K_m = 78$ μM; cholate: $V = 104$ nmol · min^{-1} · g^{-1}, $K_m = 354$ μM).

It was experimentally assured that the observed reduction of bile salt uptake was not a consequence of regurgitation of bile salts or due to an excessive intracellular accumulation during cholestasis in the sodium-free state.

The rate of taurocholate efflux is very low when compared with the rapid rate of the uptake. A stimulatory action of extracellular sodium on this pathway was also observed.

Inhibition of the $(Na^+ + K^+)$-ATPase by 1 mM ouabain resulted in a decrease of bile salt uptake. Activation of the enzyme by potassium readmission to a K^+-deprived liver enhanced bile salt uptake. The immediate response to alteration of the enzyme activity suggests a close association of a fraction of bile acid active transport with the sodium pump.

INTRODUCTION

Sodium-dependent transport processes [1, 2] have been implicated in the stimulatory action of choleretic drugs on bile flow [3–5]: Co-transport of sodium and ouabain across the sinusoidal pole of the hepatocyte is believed to increase sodium flux in the sinusoid-to-canaliculus direction [3]. This leads to stimulation of basal bile flow which depends on sodium net transport in the same direction [6, 7]. In addition to their well known osmotic effect, bile acids might influence hepatic fluid secretion by a similar mechanism [4, 5]. Simultaneous increase of sodium influx during accumulation of bile acids in the isolated liver [4, 5], and dependence of uptake kinetics on extracellular sodium have been reported for cholate [8] and taurocholate [3, 9].

Because of the possible relevance of a sodium-linked transport to the choleretic effect of bile acids, the sodium dependence of hepatic bile acid fluxes was investigated further. The interaction of the $(Na^+ + K^+)$-ATPase with bile acid transport was also studied because a role of this enzyme has been inferred in the sodium-dependent uptake of organic solutes in various epithelia [1, 2, 10, 11] as well as in the stimulation of basal bile flow [2].

MATERIALS AND METHODS

Animals. Male Wistar rats (Mus Rattus AG, Brunnthal, Germany) weighing from 180 to 250 g were used as liver donors. The animals were fed a rat chow and had free access to water.

Liver perfusions. Surgical preparation and perfusion of the isolated liver were done as described previously [3]. Non-recirculating perfusion systems were used in all experiments. Four groups of experiments were performed: (a) Na^+ dependence of bile acid uptake by the isolated liver, (b) Na^+ dependence of bile acid efflux from the liver, (c) inhibition of the $(Na^+ + K^+)$-ATPase and bile acid uptake, (d) activation of the $(Na^+ + K^+)$-ATPase and bile acid uptake.

(a) Two perfusion systems were arranged in a temperature constant (37 °C) box so that an immediate shift from one system to the other was possible. The perfusate consisted of bovine erythrocytes washed and suspended in either solution A or B. Solution A ("normal sodium"): 118.4 mM NaCl, 4.75 mM KCl, 2.57 mM $CaCl_2$, 1.19 mM KH_2PO_4, 1.18 mM $MgSO_4$, 25.0 mM Tris, 5.05 mM glucose; 10 mg/l Microcillin (Bayer, Germany). pH 7.4 was adjusted by addition of 1 M HCl. Solution B (sodium free): NaCl was replaced isotonically by choline chloride.

(b) The same experimental design was used as described under (a). Livers were first loaded with ^{14}C-labelled taurocholic acid (261–407 μM in perfusate A) for 12 min and then perfused alternatively with two taurocholate-free "chase solutions" (perfusate A and B). Taurocholate efflux from the liver was calculated from release of radioactivity into the perfusate.

(c) Four experiments were performed with an erythrocyte-containing medium (perfusate A) as described before, six experiments were done using the hemoglobin-free perfusion system of Scholz [12] with Krebs-Henseleit bicarbonate buffer as perfusion medium. The first perfusion system contained the bile salts only, the second one bile salts and 1.0 mM ouabain.

(d) The effect of $(Na^+ + K^+)$-ATPase activation on bile acid uptake was studied by readmission of K^+ to K^+-deprived preparations in a hemoglobin-free perfusion system [12]. The sum of $(Na^+ + K^+)$ in the perfusion medium was kept constant [13]. After 70 min of K^+-free perfusion 12–25 mM K^+ were readmitted. Bile salts were infused into the portal cannula at a constant rate to achieve a concentration of 8 μM cholate or 30 μM taurocholate in the inflowing perfusate.

Reagents and analytical methods. Sodium cholate and taurocholate were obtained from Fluka AG (Buchs, Switzerland) and from Serva (Heidelberg, Germany). [*Carboxy*-^{14}C]Cholic acid (sodium salt, 59.5 Ci/mol) and [carbonyl-^{14}C]-taurocholic acid (51.2 Ci/mol, sodium salt) were from the Radiochemical Centre (Amersham, England).

Radioactivity in perfusate samples or in single bile drops was counted in a

2,5-diphenyloxazole/1,4-bis-(5-phenyloxazolyl-2)-benzene/toluene cocktail by liquid scintillation methods.

For thin-layer chromatography of bile acids ethanolic extracts were prepared from evaporated samples of perfusate (3 ml) and from liver specimens ground under liquid N_2. The adsorbent was silica gel G (Merck, Germany). The plates were developed first for 6 cm with *n*-butanol/acetic acid/water (100 : 10 : 10, v/v), air dried and then developed with toluene/acetic acid/water (50 : 50 : 5, v/v) for 15 cm. Radioactivity was determined either directly on the plate with a gas flow thin-layer scanner (Berthold, Germany) or by liquid scintillation counting after extraction of the adsorbent with ethanol.

Calculations. Bile acid fluxes (v) were calculated from concentrations in the inflowing (c_{in}) and the outflowing perfusate (c_{out}) and from perfusate flow rate, and are expressed as μmol/g wet weight per min. The average sinusoidal concentration (c_{av}) was calculated using the formula $c_{av} = (c_{in} - c_{out})/(\ln c_{in} - \ln c_{out})$.

RESULTS

Sodium dependence of bile acid uptake

When isolated livers were perfused with a constant concentration of cholate or taurocholate, respectively, in a non-recirculating perfusion system, the concentration of bile acids in the effluent perfusate attained a constant level after few minutes (Fig. 1). Switching to a sodium-free medium immediately increased the effluent concentration indicating a reduction of bile acid uptake. Comparable results were obtained when sodium was replaced by potassium instead of choline (not shown).

As previously shown, substitution of sodium by choline (and other cations) reduced bile secretion by the isolated liver [7]. Total omission of sodium caused a complete but reversible cholestasis (Fig. 1c). The possibility that the reduced bile acid uptake during the sodium-free period is due to an intracellular bile acid overload caused by the cholestasis was considered. However, calculation of the intracellular bile salt content showed that there was no correlation between the rate of uptake and the amount of bile salt already present in the cells: It is seen from Figs. 1a and 1b that, although the intracellular taurocholate content is continuously increasing, the uptake velocity of taurocholic acid at normal extracellular sodium is not affected, whereas sodium-free perfusion results in an immediate reduction of taurocholate transport, even when the intracellular content is low. Therefore, it is concluded that bile acid uptake is directly influenced by the extracellular sodium concentration, and the decreased bile acid transport is not the consequence of intracellular accumulation of bile salts.

Regurgitation of conjugated bile acids during cholestasis should be considered as another reason for elevated bile acid levels during sodium-free perfusion. If this is the case, appreciable amounts of taurocholic acid should be detected in the effluent during perfusion with cholic acid, as it occurs after bile duct ligation [14]. As determined by thin-layer chromatography, only small amounts of taurocholate were detected in the effluent during sodium-free perfusion (Table I) which certainly cannot explain the reduced uptake of cholic acid under these conditions.

Sodium dependence of bile acid uptake was studied at perfusate concentrations (c_{in}) ranging from 17.8 μM to 1.76 mM cholate, and from 27.3 μM to 0.69 mM

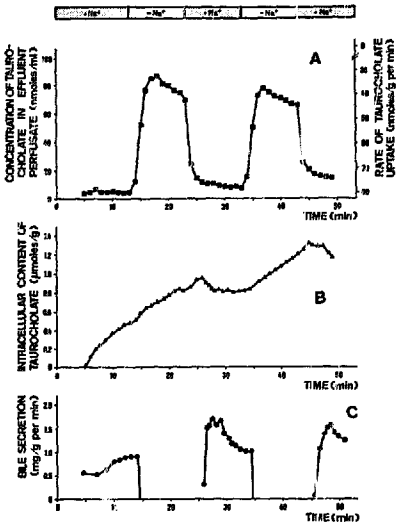

Fig. 1. Bile salt uptake, intracellular concentration, and bile secretion by the isolated perfused rat liver during perfusion with "normal sodium" (118 mM) and sodium-free perfusion media. Taurocholate concentration of the inflowing perfusate (c_{in}) in this experiment was 139 μM. Similar patterns were obtained for different c_{in} of taurocholate (four experiments) and cholate (six experiments).

TABLE I

APPEARANCE OF TAUROCHOLATE (PERCENT OF TOTAL BILE ACID CONCENTRATION) IN THE EFFLUENT PERFUSATE DURING CHOLATE UPTAKE OF THE ISOLATED LIVER IN THE SODIUM-FREE STATE

c_{in} (μM)	c_{out} (μM)		%
Cholate	Cholate	Taurocholate	
35.5	21.1	1.6	7.0
88.8	49.9	9.5	16.0
177	112	4.2	3.6
1776	1523	31.1	2.0

TABLE II

LIVER/PERFUSATE CONCENTRATION RATIOS OF BILE SALTS DURING PERFUSION OF THE ISOLATED LIVER WITH "NORMAL SODIUM" (118 mM) AND SODIUM-FREE PERFUSATES

At the end of the perfusion experiment (cf. Fig. 1) the intracellular concentration (c_{li}) was calculated from the difference between total uptake and biliary excretion of radioactivity. In case of cholate the obtained figure was corrected for the amount of conjugated bile salts which was determined by thin-layer chromatography.

Bile salt	c_{in} (nmol/ml)	c_{av} (nmol/ml)	c_{li} (nmol/g)	c_{li}/c_{av}
Taurocholate				
$[Na^+] = 118$ mM	27.3	5.0	95.3	19.1
	139	46.8	1297	27.7
	693	484	2199	4.5
$[Na^+] = 0$ mM	27.3	10.9	201	18.4
	139	105	1325	12.6
	694	652	2783	4.3
Cholate				
$[Na^+] = 118$ mM	17.8	8.5	52.8	6.2
	355	176	1393	7.9
	1776	1417	2708	1.9
$[Na^+] = 0$ mM	17.8	12.0	109	9.1
	355	299	1408	4.7
	1776	1662	3478	2.1

taurocholate. Both substances were accumulated in the isolated liver. Liver/perfusate ratios greater than 1 were observed at various concentrations in the inflowing perfusate (Table II). Not unexpected from these results, plotting of uptake rates at different average sinusoidal concentrations revealed saturability of the hepatic uptake of both taurocholic and cholic acid (Fig. 2).

Substitution of perfusate sodium by choline markedly reduced the rate of cholate and taurocholate uptake but did not abolish their concentrative transfer into the isolated liver. Also at zero $[Na^+]_o$ the bile salt content of the liver is still higher than the perfusate concentrations (Table II) and a dependence of the uptake rates on the sinusoidal concentration characteristic for a carrier-mediated process is still preserved (Fig. 2).

The influence of extracellular sodium on the transport kinetics of bile acids was found exclusively on the maximal transport velocity (V). V of taurocholate uptake was reduced in the sodium-free state from 299 to 65 nmol/g per min, and of cholate uptake from 327 to 104 nmol/g per min. The apparent Michaelis-Menten constants remained virtually unchanged. For taurocholate uptake K_m is 61 μM at normal extracellular sodium and 78 μM at zero perfusate sodium. The corresponding figures for cholate are 436 and 354 μM, respectively.

Sodium dependence of bile acid efflux from the liver

Because cholate undergoes metabolic changes in the liver it is not a suitable

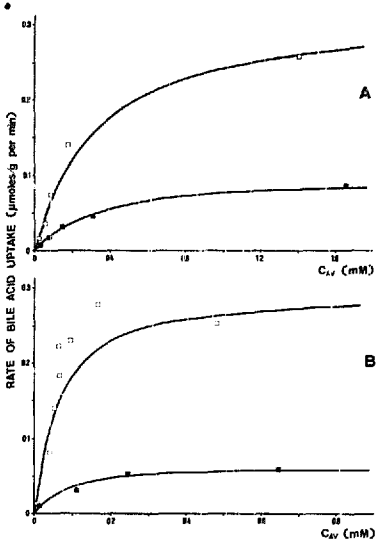

Fig. 2. Rate of cholate (A) and taurocholate (B) uptake by the isolated perfused liver at different average sinusoidal concentrations (c_{av}).

candidate for studying pathways of bile acid efflux from the liver. Therefore, only taurocholate could be used in this type of experiments.

Taurocholate efflux was first followed in the sodium-free state (Fig. 3). Restoring the normal sodium concentration by switching to perfusate A increased taurocholate efflux from the isolated organ. Bile secretion which was completely arrested in the sodium-free phase, was immediately resumed. The biliary excretion of taurocholate contributed to the steady decline of the intracellular concentration (Fig. 3). Therefore, the fractional efflux (percent of intracellular content per min) was chosen for representation of the outward movement of taurocholate. Table III shows that the efflux of taurocholate increases with rising intracellular concentration and is stimulated further by rendering the extracellular sodium to normal. Thereby the fractional efflux is enhanced 2–4-fold.

The efflux in both the sodium-free and the "normal sodium" state is low when compared to the corresponding uptake rates (Fig. 2). At an intracellular concentration of 1 μmol/g taurocholate efflux is 7.6 nmol/g per min (Table III). At the same perfusate concentration the uptake of taurocholic acid would have reached the saturation

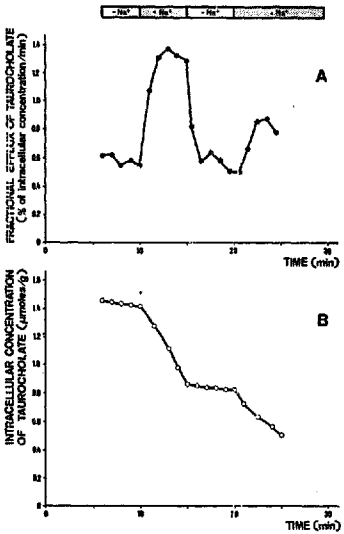

Fig. 3. Efflux of taurocholate into the perfusate during perfusion of the isolated liver with "normal sodium" (+Na^+) and sodium-free (−Na^+) perfusion media (one out of three experiments).

TABLE III

STIMULATION OF TAUROCHOLATE EFFLUX FROM THE ISOLATED LIVER BY EXTRACELLULAR SODIUM

c_{in}* (μM)	c_{il}** (μmol/g)	Average taurocholate efflux (nmol/g per min)		Fractional taurocholate efflux (percent c_{il}/min)	
		−Na^+	+Na^+	−Na^+	+Na^+
261	1.01	4.0	7.6	0.40	0.76
284	1.45	7.7	13.3	0.59	1.38
407	2.50	14.0	51.7	0.56	2.40

* Concentration in the inflowing perfusion medium during loading of the isolated liver with taurocholate.

** Intracellular taurocholate content at the end of the loading period.

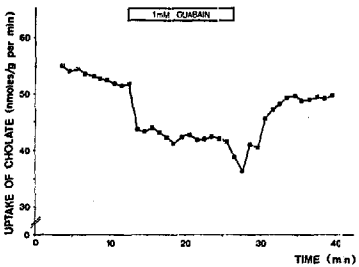

Fig. 4. Influence of ouabain on bile salt uptake, exemplified by perfusion of the liver with 23.2 μM cholate. Additional experiments are listed in Table IV.

level by far (Fig. 2) and a rate close to $V = 299$ nmol/g per min can be assumed. The corresponding figures for the sodium-free state are 4 and 65 nmol/g per min, respectively. It is obvious that the efflux contributes only minimally to taurocholate net transport. Determination of the uptake velocity, therefore, gives a reasonable estimate of bile acid influx.

Inhibition of the $(Na^+ + K^+)$-ATPase and bile acid uptake

Inhibitors of the $(Na^+ + K^+)$-ATPase also inhibit sodium-dependent transport processes [1, 10, 11]. Relatively high concentrations have to be used to inhibit the rat liver enzyme. 1 mM ouabain brings about a 100 % inhibition in vitro [15]. In the

TABLE IV

INFLUENCE OF OUABAIN ON BILE SALT UPTAKE BY THE ISOLATED RAT LIVER

Bile salt	c_{in} (μM)	Rate of cholate uptake (nmol/g per min) with addition of ouabain		Reduction by ouabain (%)
		None	1.0 mM	
Cholate	35.5	21.1	17.7	16
	35.5	14.7	13.2	10
	35.5	17.0	16.6	2
	35.5	12.9	12.4	4
	23.2*	51.8	41.8	19
	23.2*	67.1	43.9	35
Taurocholate				
	55.8*	172.4	129.8	25
	55.8*	165.4	127.9	23
	55.8*	165.0	63.5	61
	55.8*	152.0	79.9	47

* In these experiments the liver was perfused with hemoglobin-free perfusate (for details see Materials and Methods).

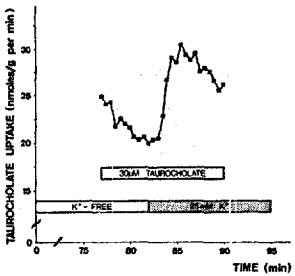

Fig. 5. Effect of potassium readmission on bile salt uptake by the isolated perfused rat liver (for experimental details see Materials and Methods). Results of additional experiments with cholate and taurocholate are given in Table V.

isolated perfused rat liver this concentration reduces bile acid uptake (Fig. 4). An average decrease of the uptake of cholic acid by 14 % and of taurocholic acid by 39 % was observed (Table IV). The inhibitory effect on bile acid transport is completely reversible. Omission of ouabain from the perfusion medium restores the former rate of bile acid uptake immediately.

Activation of the $(Na^+ + K^+)$-ATPase and bile acid uptake

Readmission of K^+ to K^+-deprived isolated liver preparations activates the $(Na^+ + K^+)$-ATPase [13]. When 12–25 mM K^+ were added to the perfusion medium after 70 min of K^+-free perfusion a transitory increase of bile acid uptake was observed (Fig. 5). The enhancement over K^+-free values was 16 % in the case of cholic acid and 51 % in that of taurocholic acid (Table V).

TABLE V

EFFECT OF K^+ READMISSION TO A K^+-FREE PERFUSATE ON BILE SALT UPTAKE

Bile salt	Perfusate K^+ after readmission (mM)	Uptake rate (nmol/g per min)		Increase (%)
		$-K^+$	$+K^+$	
Cholate	25.4	17.8	20.2	13.5
	23.1	7.8	9.7	24.4
	12.6	6.4	7.1	10.9
Taurocholate				
	25.0	34.2	66.5	94.4
	25.0	19.0	28.5	50.0
	12.5	81.3	87.6	7.7

DISCUSSION

The liver/perfusate concentration ratios, which cannot be explained by a diffusion-controlled entry of bile acids, together with the saturability of the uptake kinetics suggest an active transport of bile acids in rat liver at $[Na^+]_o = 118$ mM as well as at zero perfusate $[Na^+]$. Stimulation of the maximal transport velocity by extracellular Na^+ without affecting K_m provides a basis for the speculation, that Na^+ might facilitate the movement of a substrate-carrier complex across the plasma membrane rather than affects the binding of a transferred bile acid to its carrier. Thereby, a fraction of bile acid active transport might become sodium dependent. Consequently, the necessary energy for the active uptake of bile acids might be derived not only from metabolic sources but also from the action of the $(Na^+ + K^+)$-ATPase [1, 2, 10, 11]. According to the "sodium gradient" hypothesis [16] this enzyme provides energy for sodium-dependent transport processes by maintenance of the existing sodium gradient. The observed parallel changes of bile acid uptake with activation or inhibition of the $(Na^+ + K^+)$-ATPase suggest a close relation of hepatic bile acid uptake with the function of the enzyme and thereby substantiate also the sodium dependence of a fraction of bile acid transport.

From the symmetrical model of sodium-dependent substrate transport [16] it would be expected that active influx and efflux of bile acids likewise are driven by an electrochemical sodium gradient. Surprisingly, taurocholate efflux is enhanced when the outward sodium gradient, which exists during sodium-free perfusion, is reversed by 118 mM $[Na^+]_o$. Although this effect of extracellular Na^+ on the efflux is difficult to explain, it probably precludes a symmetrical mode of operation of sodium-dependent bile acid transport. In addition, the lack of a saturable component suggests that the slow outward movement of taurocholate is by diffusion alone and casts some doubt on the existence of an efficient extrusion of bile acids at the sinusoidal pole of the hepatocyte.

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