EFFECT OF ALBUMIN ON BILE ACID UPTAKE BY ISOLATED RAT HEPATOCYTES. IS THERE A COMMON BILE ACID CARRIER?

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SUMMARY

Cholate and taurocholate uptakes were studied in presence of albumin using isolated rat hepatocytes. Albumin decreased nonspecific binding of both bile acids and inhibited cholate uptake noncompetitively and taurocholate uptake competitively. Although different bile acids except dehydrocholate inhibited both cholate and taurocholate uptake, their relative inhibitory potency was not the same for both bile acids. Uptake of both bile acids was characterized by a saturable as well as an unsaturable process both in presence and in absence of albumin. The results suggest that both bile acids may be transported by more than one carrier and taurocholate is transported more efficiently than cholate by hepatocytes.

INTRODUCTION

Burke et al. (1) have shown that human albumin binds both cholate and taurocholate strongly. Cholate (90-95%) is more avidly bound than its taurine conjugate (45-50%). Recently both cholate (2) and taurocholate (3) were postulated to be transported by carrier-mediated process into isolated rat hepatocytes. In addition, isolated hepatocytes were found to bind both bile acids nonspecifically. The nonspecific binding of cholate was further supported by studies using isolated rat liver plasma membranes (4). These studies were done in absence of albumin. Albumin binding could influence uptake and nonspecific binding of bile acids seen in isolated hepatocytes. Moreover uptake parameters obtained in presence of albumin would be more representative of in-vivo situation. For these reasons, we have studied nonspecific binding and uptake of cholate and taurocholate by isolated rat

hepatocytes to determine the uptake parameters in presence of albumin and to investigate the effect of albumin on those parameters.

MATERIALS AND METHODS

Tritiated inulin and [24-^{14}C]cholic acid were purchsed from Amersham Buchler, Braunschweig and [24-^{14}C]taurocholic acid was obtained from New England Nuclear, Dreieichenhain, Germany. Solubilizer Soluene-350 and scintillation fluid Instagel were obtained from Packard Instruments, Frankfurt. Unlabeled bile acids (Calbiochem, San Diago, California) were of A grade. Crystalline bovine serum albumin (contained 1-3% globulin) was obtained from Sigma Chemical Co., Munich and silicon oil from Wacker Chemie, Munich, Germany.

Male Wistar rats (250-300 g) fed standard laboratory diet were used as liver donor. Hepatocytes were prepared as described before (2, 5). The cells were finally resuspended in modified Hanks buffer (pH 7.4) without bicarbonate (bicarbonate was replaced by 10 mM phosphate) for transport studies. For studies in presence of albumin, the cells were resuspended in modified Hanks buffer containing specified amount of albumin. The cell viablity (85-95% by 0.2% trypan blue) and metabolic states were as described before (5). All solutions were adjusted to pH 7.4.

Bile acid uptake by isolated rat hepatocytes was studied at 37^{o}C as described earlier (2, 3). Briefly, the cells (1.0 to 1.5 mg cell protein) were incubated with radioactive bile acid (cholate or taurocholate) of different concentrations in presence or absence of albumin (or other bile acids) in microtubes for 15, 30 and 45 sec in atleast duplicates. The uptake was stopped and the cells were separated from the media by centrifugal filtration through silicon oil layer (AR 200: AR 20 = 3:1). The cell pellet from the bottom of the microtube was cut out, dissolved in solubilizer Soluene-350 and counted for radioactivity after adding Instagel. The radioactivity in the cell pellet was corrected for adherent fluid radioactivity determined from tritiated inulin content in the pellet. The radioactivity of the cell free media and cell pellets was determined using external standard ratio to correct for quenching of ^{14}C and tritium simultaneously. The cellular protein content was determined by a modified biuret method (6). The cellular uptake of bile acid was calculated from corrected pellet radioactivity and was expressed as nmol of bile acid per mg cell protein.

RESULTS AND DISCUSSION

The effect of albumin on bile acid uptake and nonspecific binding at a single concentration of cholate and taurocholate is shown in Fig. 1. The uptake is linear for 45 sec. The zero-time intercept (Fig. 1) is considered to represent nonspecific binding (2, 3). In both cases, albumin decreased nonspecific binding of bile acids to hepatocytes. At a particular albumin concentration, the percent decrease of this binding remained relatively

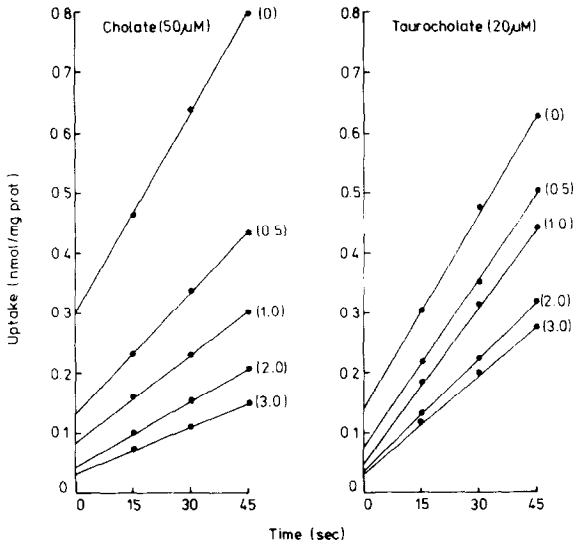

Fig. 1. Time dependent uptake of cholate and taurocholate at 37°C in presence of albumin at concentrations in percent shown in parenthesis. Each value represents an average of three determinations and each solid line the regression line. Nonspecific binding is estimated from zero-time intercept and uptake rate from the slope.

Table I. Effect of albumin on nonspecific binding of bile acids

Albumin conc. (%)	Nonspecific binding (% control)	
	Cholate	Taurocholate
0	100	100
0.5	44 ± 2	55 ± 2
1.0	32 ± 2	41 ± 2
2.0	22 ± 1	33 ± 3
3.0	14 ± 1	27 ± 2

Values are mean ± SEM of four different concentrations of cholate (20, 50, 100 and 200 μM) and taurocholate (10, 20, 50 and 100 μM) from three different cell preparations.

constant for different bile acid concentrations. This would be expected since percent of both cholate and taurocholate bound to a particular albumin concentration is relatively constant for a wide range of bile acid concentrations (1). Thus the binding values for different bile acid concentrations were combined together and summarized in table I. Albumin decreased nonspecific binding of cholate more than that of taurocholate to hepatocytes. Higher binding affinity of albumin for cholate than taurocholate (1) could explain this difference.

Bile acid uptake rates were calculated from the slopes of the uptake curves (Fig. 1) and expressed as nmol x min^{-1} x mg cell $protein^{-1}$. The standard error of uptake rate represents the standard error of the slope. Albumin inhibited uptake rates of both bile acids. However, Lineweaver-Burk plot of uptake rates of both bile acids at different albumin and bile acid concentrations clearly show (Fig. 2) that cholate uptake is noncompetitively and taurocholate uptake is competitively inhibited by albumin. The effect of albumin is unexpected since bile acids are suggested to be transported by

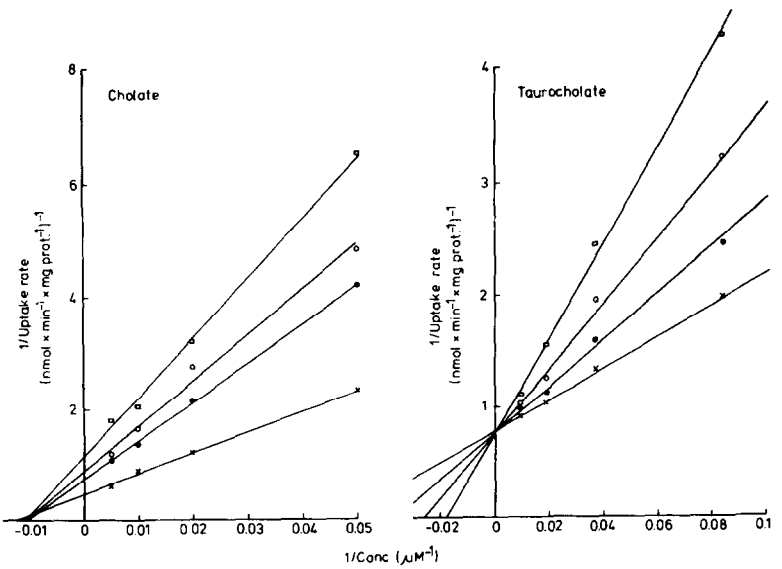

Fig. 2. Lineweaver-Burk plot of cholate and taurocholate uptake showing inhibition by 0(X), 1(O), 2(O) and 3( ) percent albumin. Uptake rates are estimated as in Fig. 1. Solid lines represent the regression lines.

a common carrier (7). Binding of bile acids to albumin alike would not explain this difference. There could be two likely possiblities: 1) There could be different carrier- one for cholate and another for taurocholate. Either albumin or albumin bound taurocholate (or both) has affinity for taurocholate carrier but not for cholate carrier. There are some indirect evidences to support different carrier hypothesis. The activation energy of taurocholate uptake (3) is about twofold higher than that of cholate uptake (2). Ouabain inhibited taurocholate uptake (3) but not cholate uptake (2). Dehydrocholate failed to affect initial plasma disappearence of taurocholate in rat (8). 2) Bile acids are transported by a common carrier. However, albumin binds more polar taurocholate differently than cholate such that albumin bound taurocholate and not cholate has affinity for the carrier. A common carrier hypothesis is supported by the following evidences. Taurocholate uptake is competitively inhibited by taurochenodeoxycholate in isolated rat hepatocytes (3) and in dog (7). Chenodeoxycholate and taurocholate induced countertransport of cholate in isolated rat hepatocytes (2). Cholate and taurocholate inhibited each other competitively in isolated perfused rat liver (9).

If bile acids are transported by a common carrier into hepatocytes and different bile acids have different affinities for the carrier then the relative ability of different bile acids to inhibit both cholate and taurocholate uptake rate would be the same. In order to investigate this possiblity, effects of seven different bile acids on cholate and taurocholate uptake are studied in absence of albumin. All bile acids except dehydrocholate inhibited both cholate and taurocholate uptake rate (Table II). In general dihydroxy bile acids inhibited both cholate and taurocholate more than their corresponding trihydroxy bile acids. However the relative inhibitory potency of different bile acids are not the same for both cholate and taurocholate uptake. Although conjugated bile acids with the exception of glycocholate inhibited taurocholate uptake more than the unconjugated bile acids, this does not seem to be case for cholate uptake. Taurochenodeoxycholate inhibited taurocholate uptake more than chenodeoxycholate but cholate uptake less than chenodeoxycholate. Glycochenodeoxycholate inhibited cholate uptake more than taurochenodeoxycholate, while both bile acids inhibited tauro-

Table II. Effect of bile acids on cholate and taurocholate uptake.

Bile acids[a] (100 μM)	Cholate (20 μM)		Taurocholate (20 μM)	
	Uptake rate	Percent inhibition	Uptake rate	Percent inhibition
Control	298 ± 15	0	517 ± 44	0
C	166 ± 11	44	345 ± 26	36
TC	136 ± 14	54	237 ± 25	54
GC	169 ± 17	43	321 ± 30	38
CDC	105 ± 12	65	295 ± 26	43
TCDC	135 ± 13	55	136 ± 14	74
GCDC	61 ± 10	80	117 ± 16	77
DHC	274 ± 17	8	548 ± 39	-6

	Inhibitory potency[b]
Cholate uptake	GCDC > CDC > TCDC = TC > GC = C
Taurocholate uptake	GCDC = TCDC > TC > CDC = GC = C

The uptake rates (pmol x min^{-1} x mg $prot.^{-1}$) are slopes of uptake curves as in Fig. 1 ± standard error for six determinations.

[a] Bile acids are C = cholate, TC = taurocholate, GC = glycocholate, CDC = chenodeoxycholate, TCDC = taurochenodeoxycholate, GCDC = glycochenodeoxycholate and DHC = dehydrocholate

[b] The symbol (>) indicates significant difference.

cholate uptake to the same extent. The different inhibitory potency and the different effect of albumin on cholate and taurocholate uptake would suggest the possiblity that hepatic uptake of bile acids is mediated by more than one carrier and a particular carrier has affinity for more than one bile acid. This would explain the competition between different bile acids for uptake. The different effect of albumin would then probably be due to different affinity of albumin or albumin bound taurocholate for the carriers. It is interesting that dehydrocholate, a synthetic bile acid with no hydroxyl

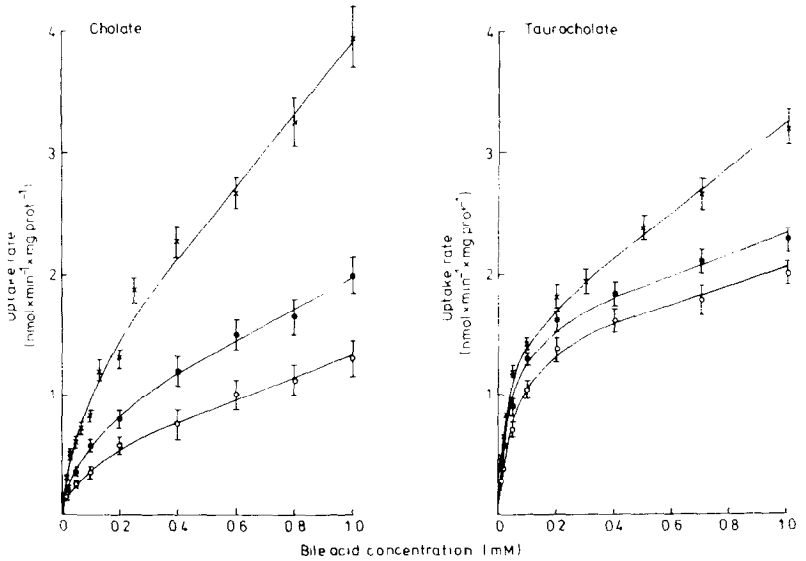

Fig. 3. Relationship between incubation concentration and uptake rate of cholate and taurocholate in presence of 0(X), 1.5(O) and 3.0(O) percent albumin. Each value represents uptake rate and its standard error from six determinations. The solid lines represent uptake rates calculated from estimated uptake parameters in Table III. The calculated curves account for more than 95% of the total variation in experimental values.

group, did not inhibit cholate or taurocholate uptake. This may indicate that hydroxyl group or groups may be necessary for transport by cholate and taurocholate carriers. More studies would surely be required to establish the multiple carrier hypothesis for hepatic bile acid uptake.

The uptake studies using wide range of cholate and taurocholate concentrations were then carried out to determine uptake parameters in presence of albumin. The result show that the uptake rates for both bile acids are biphasic and increase linearly with increasing bile acid concentrations above 400 μM (Fig. 3). This linear component has been suggested to represent an unsaturable process and believed to represent passive diffusion (10). However, in view of the above discussion, it is also possible that the unsaturable process represents uptake by other carrier which has relatively low affinity. The contribution of unsaturable process was calculated from the slope of the linear part of the curve and subtracted from total uptake rates to obtain uptake rates due to saturable process as described

Table III. Effect of albumin on bile acid uptake parameters.

Bile acids	Albumin conc. (%)	Uptake parameters[a] Unsaturable component (pmol x min^{-1} x mg prot.$^{-1}$ x μM^{-1})	Km (μM)	Vmax (nmol x min^{-1} x mg prot.$^{-1}$)
Cholate	0	2.79 ± 0.232	74 ± 7.8	1.17 ± 0.115
	1.5	1.25 ± 0.137	76 ± 7.3	0.78 ± 0.028
	3.0	0.89 ± 0.095	73 ± 9.1	0.48 ± 0.022
Taurocholate	0	1.78 ± 0.031	26 ± 2.2	1.53 ± 0.048
	1.5	0.80 ± 0.064	38 ± 4.1	1.63 ± 0.065
	3.0	0.64 ± 0.032	55 ± 5.4	1.52 ± 0.062

[a]The uptake parameters are calculated from data in Fig. 3 as described in the text. The values are parameters ± standard error of the parameters.

by others (10). The apparent Michaelis constant (Km) and maximum uptake rate (Vmax) were then calculated from the resulting uptake rates using Eadie and Hofstee plot (Table III). The unsaturable component for cholate uptake was shown before in absence of albumin (2). Schwarz et al. (3) reported no such unsaturable component for taurocholate uptake in absence of albumin. The reason may be that they used taurocholate concentrations only upto 200 μM. The contribution of unsaturable component would be relatively insignificant under normal portal blood bile acid concentration (56 μM in rats). However, this component may be quantitatively important in diseases where portal blood concentration of bile acids is increased for example in stagnant loop syndrome. Albumin decreased Vmax for cholate and increased Km for taurocholate indicating further that albumin inhibited uptake of cholate non-competitively while that of taurocholate competitively. The kinetic parameters for saturable process suggest that hepatocytes transport taurocholate more efficiently than cholate in presence as well as in absence of albumin.

This may be responsible in part for higher plasma unconjugated bile acids in stagnant loop syndrome.

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