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Competition of bile acids on the sulfobromophthalein uptake in basolateral rat liver plasma membrane vesicles

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Abstract—The effect of different bile acids (BA) on the hepatic uptake of sulfobromophthalein (BSP) was investigated in liver plasma membrane vesicles enriched in basolateral fraction. BSP uptake was measured either in the absence (electroneutral component) or in the presence of a membrane potential (electrogenic component) induced by the addition of valinomycin in the presence of an inwardly-directed potassium gradient. BSP uptake was also measured in the presence of different BA [cholate (C), taurocholate (TC), ursodeoxycholate (UDC) and tauroursodeoxycholate (TUDC)]. Electrogenic BSP uptake was not affected by BA. Conversely, the electroneutral portion of the BSP uptake was inhibited with an inhibition constant (K_i, μM) of 230 ± 40 for C, 103 ± 33 for TC, 99 ± 34 for UDC and 120 ± 39 for TUDC, respectively (means ± SD, N = 4). The Dixon and Cornish-Bowden plot of the data revealed an uncompetitive type of inhibition for each BA. These data indicate that the electroneutral, but not the electrogenic, BSP transport system is modulated by BA.

Key words: organic anions; bile acids; membrane carrier proteins; hepatic uptake; competition

The hepatic uptake of OA* occurs via a carrier-mediated process. At least three putative basolateral plasma membrane proteins have been reported for non-bile acid cholephilic organic anions (BTL, BBBP and OABP) [1–9]. The uptake is sodium-independent [3, 6]. The hepatic uptake of BSP, a prototype of non-bile acid-OA, occurs via both BTL and BBBP, the former mechanism being electrogenic and the latter electroneutral [10]. Both proteins have been isolated and characterized. OAPB was also characterized and its function reported to be chloride-sensitive in the presence of albumin [9]. In addition, a fourth oatp, described to be involved in the sodium-independent transport of OA, has recently been cloned from rat liver [11].

On the contrary, BA, in particular taurocholate, are transported into the hepatocyte mainly by a sodium-dependent system [12], and a carrier protein was characterized to be involved in this function [13]. Based on competitive studies, it has been suggested that an additional system, the so-called basolateral multispecific organic anion transporter (bMOAT), takes part in both OA and sodium-independent bile acid transport. The affinity of bMOAT appears to be low and its specificity broad [14]. The oatp also appears to be involved in the transport of both conjugated and unconjugated bile acids.

The substrate specificity of the sodium-independent transport systems is poorly defined and some overlapping in the descriptions has occurred. Taurocholate does not show inhibition of BSP uptake in isolated hepatocytes [15] while competition of BSP on taurocholate uptake has been reported [16].

The aim of this study was to investigate the effect of different bile acids on both the electrogenic and electroneutral component of BSP uptake.

* Abbreviations: OA, organic anions; BTL, bilitranslocase; BSP, sulfobromophthalein; BBBP, BSP/bilirubin binding protein; OABP, organic anion binding protein; bMOAT, basolateral multispecific organic anion transporter; LPMV, liver plasma membrane vesicles; BA, bile acids; oatp, organic anion transporting polypeptide; C, cholate; TC, taurocholate; UDC, ursodeoxycholate; TUDC, tauroursodeoxycholate; RSA, relative specific activities.

Materials and Methods

Female Wistar rats (60–90 days old) weighing 200–250 g were used throughout the study. Animals were allowed free access to standard laboratory chow (Nissan, Milan, Italy) and tap water, and were housed in a constant temperature–humidity environment.

LPMV were prepared as described previously [17] in a medium containing 10 mM HEPES, 250 mM sucrose, pH 7.4, and stored in liquid nitrogen until use (within 4 weeks). The degree of purity was determined by measuring the activity of Na^+,K^+-ATPase [18], 5′nucleotidase [19], glucose-6-phosphatase and succinate-cytochrome *c* reductase [20]. The release of inorganic phosphate was measured according to Widnell [21]. Protein concentration was assessed by the bicinchoninic acid protein assay according to Smith *et al.* [22]. The RSA of Na^+,K^+-ATPase and 5′ nucleotidase was enriched 17 and 3 times over the starting homogenate, respectively, while contamination with either endoplasmic reticulum (RSA of glucose-6-phosphatase 0.25) or mitochondria (RSA of cytochrome *c* reductase 0.16) was low.

[^{35}S]BSP was prepared according to Kurisu *et al.* [23] with a specific activity of 37 Ci/mmol. [^{35}S]BSP uptake by LPMV was measured by the rapid filtration technique as already described [10]. Electroneutral uptake was measured in the incubation medium, whereas electrogenic uptake was measured in the incubation of the above composition but with the isoosmotic substitution of 100 mM KCl for sucrose. Valinomycin dissolved in methanol (50 μg/mg protein) was added at time 0 to the electrogenic incubation medium. Radioactivity of the filters was measured in a βcounter (Betamatic V, Kontron) after addition of 10 mL of scintillation liquid. Nonspecific binding to LPMV was determined in each experiment by the addition of 25 μL LPMV to ice-cold incubation medium. This value was subtracted from that obtained at 37° and the difference referred to as specific binding. All determinations were performed in triplicate and all observations confirmed with two or more separate membrane preparations.

The effect of C, TC, UDC and TUDC was assayed by measuring BSP initial uptake rate at a BSP concentration of 5 and 20 μM (electrogenic and electroneutral, respectively) after the addition of each bile acid (100 μM) to the incubation medium. The type of inhibition was determined

Table 1. Effect of the addition of C, TC, UDC and TUDC (100 μM) on electroneutral and electrogenical BSP uptake measured at 20 and 5 μM, respectively

BA (100 μM)	Electroneutral uptake	Electrogenic uptake
Control	100 ± 7	100 ± 10
C	87 ± 3*	91 ± 9
TC	67 ± 5†	93 ± 15
UDC	72 ± 8†	97 ± 15
TUDC	65 ± 9†	98 ± 12

* $P < 0.05$; † $P < 0.01$.

Results are expressed as percentage of control values. Control values (nmol BSP/mg protein/15 sec) were 0.513 ± 0.040 for the electroneutral and 0.519 ± 0.050 for the electrogenic component, respectively.

by measuring the inhibitory effect of increasing bile acid concentrations (5, 50, 100, 150 and 250 μM) on BSP uptake. Initial uptake rates were measured and plotted according to Dixon [24] and Cornish-Bowden [25].

Chemicals were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and were analytical grade purity. Bile acids were more than 98% pure.

All results are expressed as means ± SD. Significance of difference was assessed with the Student's *t*-test for paired data.

Results and Discussion

The effect of BA on electroneutral and electrogenic BSP uptake in sinusoidal liver plasma membrane vesicles was measured at a BSP concentration of 20 and 5 μM, respectively, as this concentration was shown to be close to the apparent K_m of each transport system [10]. As shown in Table 1 the addition of BA significantly reduced ($P < 0.05$) the electroneutral BSP uptake component, while the electrogenic portion was not affected. The effect was much greater for TC, UDC and TUDC (30% inhibition) than for C (10% inhibition).

In order to assess the type of inhibition exerted by each BA, the inhibitory effect was also measured in the presence of increasing concentrations (5–250 μM) of each BA, and the data analysed according to Dixon plot (Fig. 1). The electroneutral uptake of BSP was assessed at two BSP concentrations (10 and 20 μM, respectively). Two parallel lines were obtained for each BA, indicating that each one exerted an uncompetitive inhibition of the electroneutral BSP uptake. When data were plotted according to Cornish-Bowden (Fig. 2), two non-parallel lines were obtained which intersected above the *x* axis, confirming what was observed in Fig. 1, i.e. an uncompetitive type of inhibition [25]. The inhibition constants (K_i, μM) obtained from the Cornish-Bowden plot are reported in Table 2.

BA did not modify electrogenic BSP uptake, but did inhibit the electroneutral portion of the process. At a BA concentration of 100 μM, the extent of inhibition differed, ranging from 10% for cholate to 30% for the other BA tested. This finding fits with that derived from the inhibition constants (Table 2). The K_i differed significantly between cholate and the three other BA, the value being somehow twice as high for the former than that for the latter. This difference cannot be accounted for by a conjugation with taurine, as comparable inhibition constants were found for the unconjugated and conjugated moiety of ursodeoxycholic acid (UDCA and TUDCA). As the length and charge of the lateral chain, the distortion of the steroid backbone, and the number of OH substitutes influence the affinity of the transport system [26], the discrepancy may reside in the different molecular arrangement of the different BA tested.

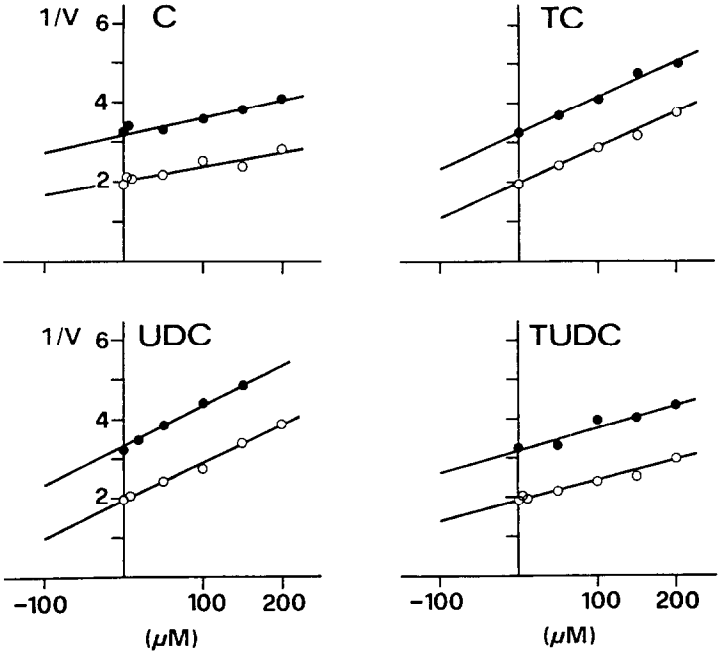

Fig. 1. Dixon plot of the inhibitory effect of BA on electroneutral hepatic BSP uptake. 1/V = 1/uptake rate (nmoles BSP/mg protein/15 sec)$^{-1}$. (●) = BSP uptake at 10 μM BSP; (○) = BSP uptake at 20 μM BSP. Each point represents the mean of four different experiments.

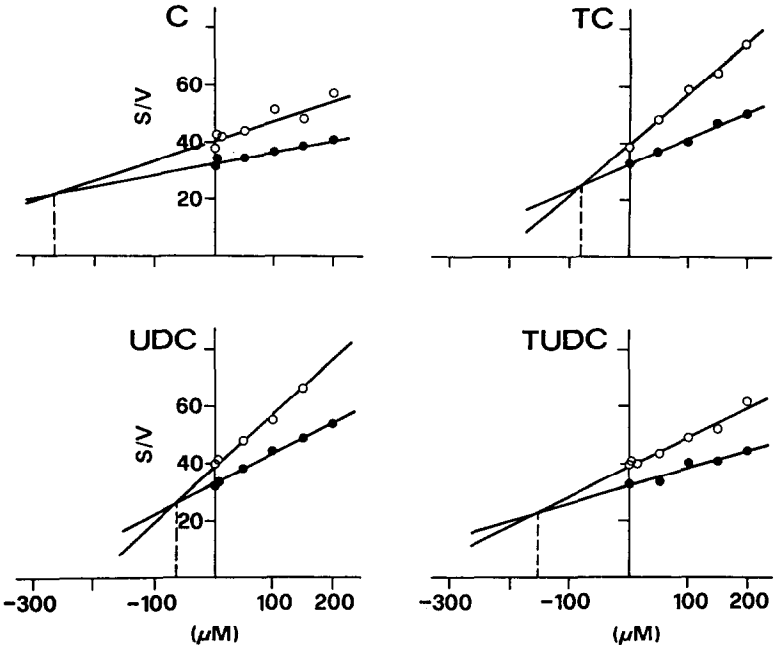

Fig. 2. Cornish-Bowden plot of the inhibitory effect of BA on electroneutral hepatic BSP uptake. S/V [S = BSP concentration (μM); V = uptake rate (nmol BSP/mg protein/15 sec)]. (●) = BSP uptake at 10 μM; (○) = BSP uptake at 20 μM BSP. Each point represents the mean of four different experiments.

Table 2. Inhibition constant values (μM) of individual bile acids on electroneutral BSP uptake as derived from the Cornish-Bowden plot of the data (see Fig. 2)

	K_i (μM)
C	230 ± 40
TC	103 ± 33
UDC	99 ± 34
TUDC	120 ± 39

Data are expressed as means ± SD.

These data indicate that bile acids, in the absence of sodium, are not transported across the basolateral membrane by the electrogenic, bilitranslocase-mediated BSP uptake. They also confirm and expand previous evidence obtained in isolated hepatocytes which indicated that TC did not show any inhibition on membrane potential-sensitive BSP transport [15]. On the contrary, when BSP transport was measured in the absence of membrane potential, BA inhibited BSP uptake. The interaction of BA with BBBP may be ruled out based on previous evidence indicating that antibodies raised against BBBP did not show any inhibition on the transport of either C or TC in isolated hepatocytes [6]. The function of OABP is sensitive to chloride in the presence of albumin [8, 9]. As our experimental set up included neither albumin nor chloride, the possible effect of BA on this carrier molecule appears unlikely. Both bMOAT and oatp, on the contrary, seem to be the most likely candidates to account for the observed interaction of BA on the electroneutral component of BSP transport.

The uncompetitive inhibition observed suggests that BA do not compete with the electroneutral BSP carrier system(s) at the level of the binding site, but, rather bind to the membrane thus perturbing the function of the carrier molecule(s).

Collectively, data reported show that the electroneutral but not the electrogenic portion of BSP hepatic uptake is modulated by BA. The interaction seems to occur at the level of the so-called, still undefined bMOAT or of the recently described oatp.

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Centro Studi Fegato and Dipartimento BBCM
University of Trieste
Trieste
Italy

ADRIANA M. TORRES*
ANA ISABEL GALAN†
CLAUDIO TIRIBELLI‡

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* Permanent address: Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Argentina.

† Permanent address: Departamento de Fisiología, Universidad de Salamanca, Spain.

‡ Corresponding author: Claudio Tiribelli, Centro Studi Fegato, Dipartimento BBCM, Via Giorgieri 1, 34127 Trieste, Italy. Tel. (39) 40-399 4927; FAX (39) 40-912881.

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