Biochimica et Biophysica Acta, 1151 (1993) 28–34

BBAMEM 76053

Influence of hydroxylation and conjugation in cross-inhibition of bile acid transport across the human trophoblast basal membrane

Maria Angeles Serrano [b], Pilar Bravo [a], Mohamad Yehia A. El-Mir [a] and Jose Juan G. Marin [a]

[a] *Departamento Fisiologia y Farmacologia, Facultad de Farmacia, Universidad de Salamanca, Salamanca (Spain) and*
[b] *Departamento Bioquimica y Biologia Molecular, Facultad de Farmacia, Universidad de Salamanca, Salamanca (Spain)*

(Received 10 March 1993)

Key words: Bile acid; Placenta; Transport; Basal membrane; (Human)

Taurocholate (TC) transport across the basal plasma membrane of the human trophoblast is a carrier-mediated process, whose specificity is probably not restricted to TC. The aim of this work was to gain further insight into the role of hydroxylation and conjugation in the behavior of the carrier system vs. bile acid (BA) species. Radiolabeled TC transport by basal plasma membrane (BPM) vesicles obtained from human term placenta was measured by a rapid filtration technique. Glycocholate (GC), taurochenodeoxycholate (TCDC) and taurodeoxycholate (TDC) inhibited TC binding to BPM. These bile acids compete with TC for the binding sites. Symmetry properties for GC- and TCDC-induced inhibition of TC transport was found in experiments where GC or TCDC were at the *cis*-side of the membrane (uptake and efflux experiments). GC and TCDC-induced inhibition seems to be of mixed type. By contrast, TDC was observed to affect TC transport differently, depending on whether the experiments addressed uptake or efflux. At the intracellular side of the membrane (uptake), TDC induced a marked increase in both V_{max} and K_t. However, at the fetal side (efflux) a significant reduction in both V_{max} and K_t was found. In spite of these peculiarities, the values for K_i were very close for GC, TCDC and TDC at the intracellular side but not at the fetal side, where the decreasing order for K_i was GC > TCDC > TDC. TC uptake by BPM vesicles was not modified in the presence of a wide range of estrone sulfate concentrations (0.002–1.0 mM). In summary, these results indicate that a particular bile acid molecular structure is necessary for steroid-related compounds to interact with the bile acid carrier located in BPM. They also suggest that changes in the number and position of hydroxy groups, as well as in the amino-acid moiety in amidated bile acids modify the behavior of the carrier, which may play an important role in the net vectorial transfer of bile acids across the placenta.

Introduction

Bile acids enter the human trophoblast from the fetal blood as the first step in the placental transfer of these compounds towards the maternal blood. This is performed via a carrier-mediated process [1]. In previous studies we have reported that taurocholate transport across this membrane is activated by an inversely-directed bicarbonate gradient [2] and that the specificity of this transporter is probably not restricted to taurocholate; rather it would include another bile acid species, as well as certain non-bile-acid cholephilic organic anions [3]. The particular interactions of the carrier with bile acids of different molecular structure may be important in the control of the overall transfer process. In this respect, it should be noted that there are differences in both the maternal and fetal bile acid pool compositions [4]. This is partially accounted for by differences in the synthesis and biotransformation of bile acids by the mother and the fetus [5].

The aim of the present work was to gain further insight into the role of hydroxylation and conjugation in the behavior of the carrier system vs. bile acid species, a requisite for understanding the relevance of placental bile acid transfer in fetal-maternal physiology and physiopathology. Because the role of simple diffusion across the trophoblast seems to be minor, due to the low permeability of lipid bilayers for anionic bile acid molecules [6], impairments in carrier-mediated transfer may be the origin of fetal and/or maternal diseases. Additionally, in pathological situations inducing hypercholanemia, different carrier specificities may determine a differential net accumulation of certain types of bile acids in fetal blood. This may be very important for the fetus because the toxic properties of bile acids, such as teratogenicity [7], differ widely. The

Correspondence to: J.J.G. Marin, Departamento de Fisiologia y Farmacologia, Facultad de Farmacia, 37007 Salamanca, Spain.

biological effects of bile acids depend on their physicochemical characteristics and these are mainly determined by the number and position of their hydroxy groups and the presence of polar polyatomic groups, such as sulfation, glucuronation and, most important, amidation of the side chain with glycine or taurine [8].

In order to perform this work, taurocholate (3α,7α,12α-trihydroxy-5β-cholanoyltaurinate) binding and transport (efflux and uptake) by basal plasma membrane (BPM) vesicles obtained from human term trophoblast were investigated in the presence of three model bile acid species, i.e., glycocholate (3α,7α,12α-trihydroxy-5β-cholanoylglycinate), a primary trihydroxy bile acid resembling taurocholate but conjugated with glycine instead of taurine; taurochenodeoxycholate (3α,7α-dihydroxy-5β-cholanoyltaurinate), another primary bile acid similar to taurocholate, except for the lack of the 12-hydroxy group, and taurodeoxycholate (3α,12α-dihydroxy-5β-cholanoyltaurinate), a secondary bile acid which differs from taurocholate in the lack of the 7-hydroxy group. The effect of one of the major circulating steroids in human, i.e., estrone sulfate – a non-bile acid molecule that like bile acids has a steroid ring – was also investigated.

Part of this work was presented at the 26th meeting of the European Association for the Study of the Liver, September 11–14, 1991, Palma de Mallorca, Spain and it has appeared in part in abstract form in the Journal of Hepatology 13, S13, 1991.

Materials and Methods

Materials. Labeled taurocholate, L-alanine and dihydroalprenolol (DHAP) were obtained from New England Nuclear (Itisa, Madrid, Spain). Unlabeled bile acids and estrone sulfate were purchased from Sigma (St. Louis, MO, USA). *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) was purchased from Boehringer-Mannheim (Mannheim, Germany). All other reagents were from Merck (Darmstadt, Germany) or were of similar analytical grade. The purity of labeled and unlabeled bile acids was higher than 95% as indicated by the manufacturers by TLC and constated by us by HPLC.

Preparation of plasma membrane vesicles. Basal plasma membrane (BPM) vesicles were purified from normal term human placentas kindly supplied by the Gynaecology and Obstetrics Department of the Virgen de la Vega Hospital (Salamanca, Spain). The BPM vesicles were prepared by an adaptation of the method of Kelley et al. [9], as described elsewhere [1]. The purity, integrity and orientation of the BPM vesicles were assayed, as indicated previously [1] by dihydroalprenolol binding [10], Na^+/K^+-ATPase activity latency (EC 3.6.1.37) [11], ouabain binding latency [12], alkaline phosphatase activity (EC 3.1.3.1) [13], L(+)-tartrate-sensitive acid phosphatase (EC 3.1.3.2) [14], glucose-6-phosphatase (EC 3.1.3.9) [15] and succinic dehydrogenase (EC 1.3.99.1) [16]. These measurements gave results (Table I) similar to those reported previously [1]. Protein was determined by the method of Lowry et al., as modified by Markwell [17], with bovine serum albumin as standard.

BPM vesicles were stored in buffer A (250 mM sucrose, 100 mM KNO_3, 10 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 mM Hepes-Tris (pH 7.40)) at −80 °C until used. No significant difference in taurocholate uptake or efflux between fresh and frozen/thawed vesicles was found up to four weeks (data not shown). Before carrying out the experiments, the vesicles were first quickly thawed at 37°C and diluted with buffer A, except for loading purposes (10 mg protein/ml), to approx. 5 mg protein/ml. They were then vesiculated by 6 passages through a 25-gauge needle.

TABLE I

Purity, integrity and orientation of BPM vesicles

Results are means ± S.E.. Number in parentheses represents the number of determinations in different preparations tested. The integrity of the BPM vesicles was calculated from the Na^+/K^+-ATPase latency. The proportion of sealed vesicles was 56.3 ± 0.6%. The orientation of the vesicles was calculated by considering the ouabain binding latency as due to the inside-out sealed vesicles. Most (70.4%) of the sealed vesicles were found to be inside-out oriented.

Fraction	Homogenate	BPM vesicles	BPM vesicles + SDS	Enrichment [a]
DHAP binding (pmol DHAP/mg protein)	0.013 ± 0.002 (n = 5)	0.33 ± 0.09 (n = 4)	–	25.15
Alkaline phosphatase (μmol/h per mg protein)	6.9 ± 1.5 (n = 4)	34.6 ± 9.2 (n = 5)	–	5.01
Na^+/K^+-ATPase (μmol/h per mg protein)	–	0.48 ± 0.07 (n = 7)	1.09 ± 0.14 (n = 7)	–
Ouabain binding (arbitrary units/mg protein)	–	1489 ± 224 (n = 5)	2466 ± 129 (n = 7)	–

[a] Enrichment is the ratio of marker enzyme activity in the BPM preparation to the activity in the homogenate.

Binding and transport studies. To perform binding studies and thereafter to carry out efflux experiments, the vesicles were preloaded with bile acids as follows: a double-concentrated suspension of purified BPM preparation was diluted 1:1 with buffer A containing $2 \times C$ mM unlabeled bile acid plus approx. 5000 dpm/μl [^{14}C]taurocholate (spec. act. 46.7 mCi/mmol), where C was the desired final concentration of bile acid. The BPM vesicles were incubated with the buffer containing labeled and unlabeled bile acids at 25 °C for 2 h before use.

To carry out transport studies, bile acid retention by the BPM vesicles was measured by a rapid filtration technique [18]. Experiments were initiated by adding 80 μl of incubation buffer to 20 μl of BPM suspension (approx. 5 μg vesicle protein/μl). The composition and conditions of the different incubation and loading buffers are indicated in the legend of the tables and figures. The incubations were ended by the addition of 4 ml of ice-cold stop solution (250 mM KCl, 25 mM $MgSO_4$, 10 mM Hepes-Tris (pH 7.40)) and immediate filtration through 0.65 μm Millipore cellulose-nitrate filters (Millipore, Madrid, Spain). The incubation test-tubes and subsequently the filters were rinsed once again with the same stop solution and then three additional times with similar stop solution containing 0.1 mM unlabeled bile acid. This procedure, selected on the base of preliminary studies (Fig. 1), reduces the retention of radioactivity by the filters (blank). Typically, $5 \cdot 10^4$ dpm applied to the filters gave an average blank of less than 50 dpm. In efflux experiments, the total loaded radioactivity after 2 h loading (Q_o) was measured by rapid filtration after adding 20 μl of the vesicle suspension and 80 μl of the incubation medium directly to 4 ml of stop solution. Net efflux was calculated by subtracting the actual value of radioactivity found at the incubation time considered (Q_t) from Q_o, determined for each specific loaded vesicle preparation. Radioactivity on the filters was measured in a liquid scintillation counter (LS-1800-Beckman: Beckman Instruments, Madrid, Spain) using Ready Safe Scintillation Cocktail, also from Beckman, as a scintillant.

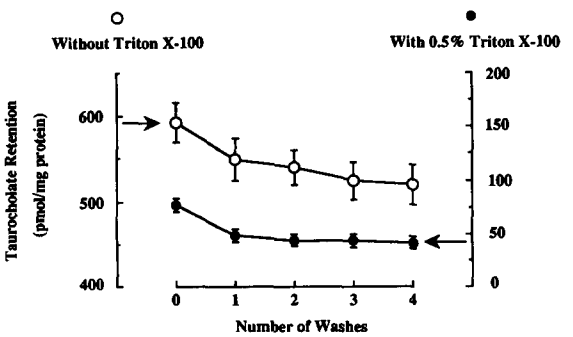

Fig. 1. Effect of washing 0–4-times with 4 ml of ice-cold stop solution (250 mM KCl, 25 mM $MgSO_4$, 10 mM Hepes-Tris (pH 7.40)) containing 0.1 mM unlabeled taurocholate (TC). Before washing and filtering, the vesicles were incubated for 60 min with 0.1 mM unlabeled taurocholate solution containing approx. 50000 dpm of [^{14}C]taurocholate (○). Some vesicles were also incubated in the presence of 0.5% Triton X-100 (•). Values are mean ± S.E. from duplicate values obtained in three different BPM preparations.

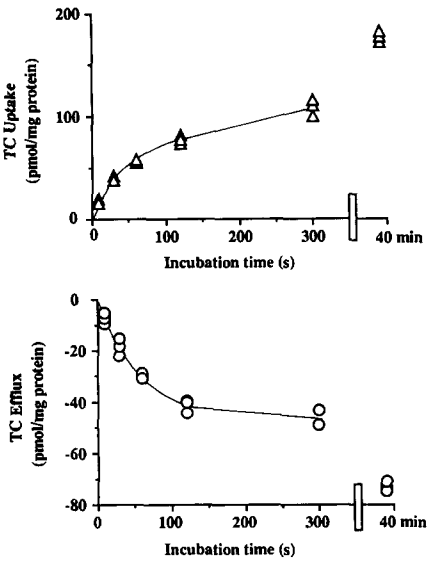

Fig. 2. Typical time course of taurocholate (TC) uptake (upper panel) and efflux (lower panel) by BPM vesicles. In uptake experiments, BPM vesicles (20 μl) were incubated with 80 μl buffer A (250 mM sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 100 mM KNO_3, 10 mM Hepes-Tris (pH 7.40)) containing 0.1 mM of TC. In efflux experiments, 20 μl of 0.1 mM TC-loaded BPM vesicles were incubated with 80 μl bile acid-free buffer A. As shown in the figure, values are usually obtained in triplicate in the same BPM preparation.

Data analysis. As indicated in the legend of the tables and figures, all incubations were performed in duplicate or triplicate and all observations confirmed in three or more separate BPM preparations. Values are given as means ± S.E. Significance was determined by the paired t-test or the Bonferroni method of multiple range testing, as appropriate. Kinetic studies were carried out at 37°C under initial velocity conditions; i.e., at 30 s incubation time (Fig. 2). For kinetic analysis, the values for taurocholate uptake or efflux rate were fitted to an equation comprising the sum of saturable and linear components. The estimations were made by nonlinear regression analysis using the software described by Yamaoka et al. [19]. The inhibition constant K_i for BA was determined using Lineweaver-Burk plots. Regression lines were calculated by the least-squares method. Statistical analysis was done on a Macintosh SE Computer (Apple Computer, Cupertino, CA, USA).

Results

Bile acid binding to BPM vesicles

To understand the mechanism of a selective transport system it is important to analyze each kinetic process independently. Thus, the first step in the car-

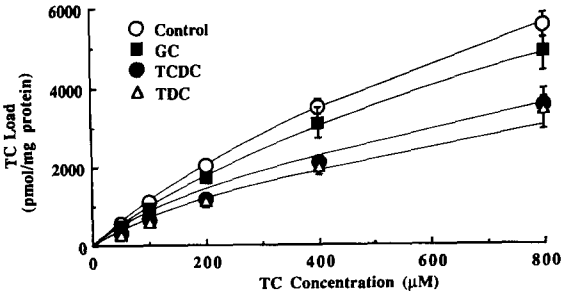

Fig. 3. Effect of 0.3 mM glycocholate (GC), taurochenodeoxycholate (TCDC) or taurodeoxycholate (TDC) on taurocholate (TC) load by BPM vesicles measured after 2 h incubation at 25°C in the presence of the indicated TC concentrations in the media. Double-concentrated BPM suspensions were loaded by incubation (1:1 (v/v)) with a similar buffer containing 0.1, 0.2, 0.4, 0.8 or 1.6 mM TC and 0.6 mM GC, TCDC or TDC. Values are means ± S.E. obtained from experiments (Control, $n = 13$; GC, $n = 6$; TCDC, $n = 6$; TDC, $n = 6$) carried out in triplicate on five different vesicle preparations.

rier-mediated transport process, i.e., the binding of the ligand (TC) to the carrier, was investigated in the presence of one of the bile acid species chosen. In experiments where TC associated with BPM was measured after incubation for 2 h at 25°C together with GC, TCDC or TDC (Fig. 3), there were two major findings: (i) The plot of TC load (Q_o) vs. TC concentration in the loading media was not linear. (ii) The extent of Q_o was reduced in the presence of GC, TCDC or TDC.

These findings suggest that a saturable component is involved in the process of bile acid loading by BPM vesicles. Such saturability is unlikely to be due to the fraction of TC in solution within the vesicle because the concentration of this compound in the loading media was lower than the so-called critical micellar concentration (CMC), which is in the range of 3–12 mM for most endogenous bile acids [20]. Therefore, aggregation due to micelle formation or loss of vesicular content due to detergent effects on the plasma membrane can be reasonably ruled out and a binding of bile acid to BPM vesicles should be postulated to account for the saturable component of Q_o. To confirm this point, four BPM preparations were incubated with 0.1 mM L-[^{14}C]alanine for 2 h in the presence or absence of 1.0 mM TC. The amount of radioactivity found in BPM incubated with both L-[^{14}C]alanine and TC was 103.9% (±4.7, S.E.) of that found in BPM incubated without TC. This suggests the absence of vesicle disruption in the presence of TC, at least, up to 1.0 mM. These experiments were also used to obtain an approximate value for intravesicular volume (I_v). To do so, it was assumed that at equilibrium, equal L-[^{14}C]alanine concentrations within and outside the vesicles was reached and that L-[^{14}C]alanine binding to BPM was negligible [21]. The calculated I_v value was 0.776 μl/mg protein. Using this value we fitted the experimental Q_o values to the following equation:

$$Q_o = ((C \cdot B_{max})/(K_b + C)) + (U_b \cdot C) + (I_v \cdot C) \quad (1)$$

The saturable term of the equation, $((C \cdot B_{max})/(K_b + C))$, presumably accounts for TC binding to specific sites on BPM. In this term, B_{max} is the maximal saturable binding and K_b is the half-maximal concentration for saturable binding. Two additional linear terms are also included in the model. The first, $(U_b \cdot C)$, is due to nonsaturable binding and, therefore, U_b is the affinity constant. The second linear term, $(I_v \cdot C)$, stands for the amount of TC assumed to be dissolved within the vesicle, and hence I_v is the intravesicular volume. The values for these constants were calculated by nonlinear regression and the results are shown in Table II. GC, TCDC and TDC were found to significantly reduce the efficiency of the saturable TC binding to BPM vesicles, expressed as B_{max}/K_b. The effect was mainly due to an increase in the K_b value. The inhibitory potency for these bile acids, was TDC > TCDC > GC. Contrariwise, Table II also shows that the nonsaturable term was not significantly affected by these bile acids.

Bile acid transport by BPM vesicles

The study of TC transport in the presence of GC, TCDC or TDC at the *cis*-side of the membrane, i.e., both these bile acids and TC outside the vesicle in

TABLE II

Fitting parameters of TC load by BPM vesicles

Double-concentrated BPM suspensions were loaded after 2 h incubation at 25°C (1:1 (v/v)) with similar buffer containing 0.1, 0.2, 0.4, 0.8 or 1.6 mM TC and 0.6 mM GC, TCDC or TDC. Results are means ± S.E. from data obtained in duplicate or triplicate measurements carried out in six BPM preparations. Best fitting by nonlinear regression analysis of loading data points was that obtained using the following equation: $Q_o = ((C \cdot B_{max})/(C + K_b)) + (U_b \cdot C) + (I_v \cdot C)$, where C is the value of taurocholate concentration inside and outside the vesicles, B_{max} is maximal saturable binding, K_b is the half-maximal concentration for saturable binding, U_b is the affinity constant for nonsaturable binding and I_V is the intravesicular volume, assumed to be 0.776 μl/mg protein from experimental measurements. The results were compared to control experiments by the Bonferroni method of multiple range testing.

	B_{max} (nmol/mg protein)	K_b (mM)	U_b (nl/mg protein)	B_{max}/K_b (% of control)
Control	10.55 ± 1.02	0.95 ± 0.07	105 ± 9	100
GC	9.85 ± 1.44	1.02 ± 0.06	112 ± 6	86.71 ± 2.20 *
TCDC	8.14 ± 1.80	1.38 ± 0.17	120 ± 7	53.21 ± 2.30 *
TDC	9.55 ± 1.44	1.71 ± 0.16 *	117 ± 11	50.43 ± 5.70 *

* $P < 0.05$.

uptake experiments or both inside the vesicle in efflux experiments (Fig. 4), clearly indicates the existence of GC-, TCDC- and TDC-induced inhibition of TC transport. The inhibition due to GC shows certain symmetry characteristics. The efficiency of TC transport (V_{max}/K_t) was similarly reduced both in uptake (Fig. 5) and efflux experiments (Fig. 6). Analysis of TCDC-induced inhibition revealed that this was stronger in the efflux (Fig. 6) than in the uptake (Fig. 5) experiments. The mechanism of GC and TCDC-induced inhibition seems to be similar at both sides of the membrane and hence a mixed inhibition with a very important effect on V_{max} is suggested. TDC showed a very peculiar effect; for this bile acid different results were obtained depending on whether the experiments addressed uptake or efflux. TDC was found to inhibit TC uptake by a mechanism involving an increase in both V_{max} and K_t (Fig. 5). However, a reduction in both V_{max} and K_t was found in efflux experiments (Fig. 6). In both cases the efficiency of TC transport was significantly decreased.

The values for K_i were very close for GC, TCDC and TDC on the intracellular side of the membrane (uptake experiments) (Fig. 7). By contrast, from efflux

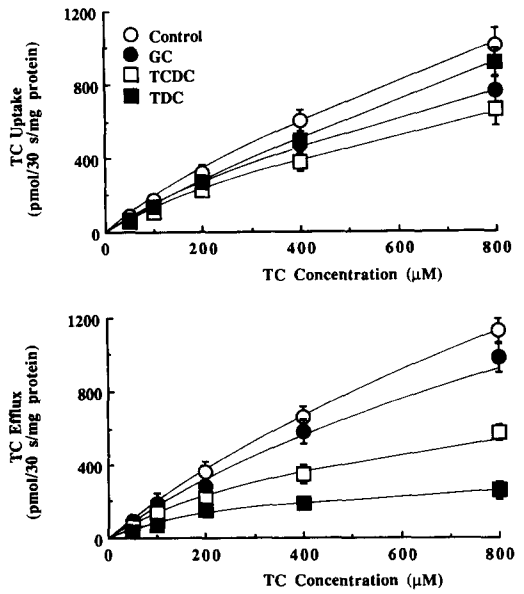

Fig. 4. Effect of 0.3 mM glycocholate (GC), taurochenodeoxycholate (TCDC) or taurodeoxycholate (TDC) on the initial rate of taurocholate (TC) uptake (upper panel) and efflux (lower panel) by BPM vesicles. In uptake experiments, aliquots (20 μl) of the vesicle suspension were incubated with 80 μl buffer A (250 mM sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 100 mM KNO_3, 10 mM Hepes-Tris (pH 7.40)) containing varying concentrations of TC and 0.3 mM GC, TCDC or TDC. The initial rate of taurocholate efflux by BPM vesicles was studied using aliquots (20 μl) of the vesicle suspension preloaded with varying concentrations of taurocholate and 0.3 mM GC, TCDC or TDC that were incubated with 80 μl bile acid-free buffer A. Values are means ± S.E. obtained from experiments (Uptake: Control, $n = 9$; GC, $n = 6$; TCDC, $n = 8$; TDC, $n = 6$. Efflux: Control, $n = 13$; GC, $n = 6$; TCDC, $n = 6$; TDC, $n = 6$) carried out in triplicate on five different vesicle preparations.

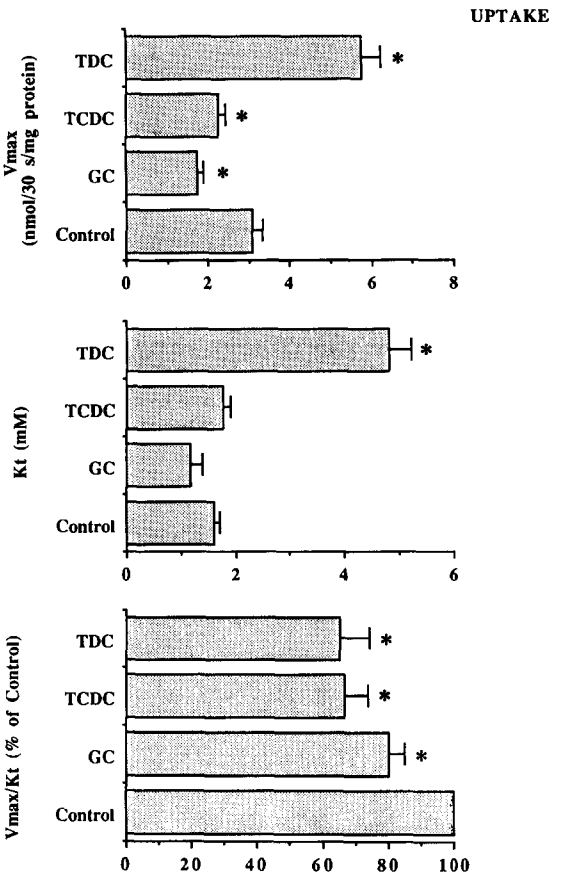

Fig. 5. Effect of 0.3 mM glycocholate (GC), taurochenodeoxycholate (TCDC) or taurodeoxycholate (TDC) on apparent maximal velocity (V_{max}), apparent dissociation constant (K_t) and apparent efficiency (V_{max}/K_t) of taurocholate uptake by BPM vesicles. The values were obtained by fitting the data from experiments shown in Fig. 4 (upper panel) to a Michaelis-Menten equation plus a linear (diffusional) term as described in Materials and Methods. Values are means ± S.E.
* $P < 0.05$ as compared with control by the paired t-test.

experiments a different inhibitory constant at the fetal side was calculated. In this case, the decreasing order for K_i was GC > TCDC > TDC (Fig. 7).

To assess the importance of the steroid ring in the interaction of bile acids with the carrier, the ability of estrone sulfate to inhibit TC transport was investigated. Two facts justify this choice. Firstly, the human placenta takes up relatively large amounts of steryl sulfates of both fetal and maternal origin. In part due to the high steryl-sulfatase activity in this organ, these steroids are biotransformed to estriol which is then released into the maternal compartment [22]. Secondly, the uptake of estrone sulfate by rat hepatocytes is a carrier-mediated process and a mutual competition between TC and estrone sulfate for hepatic uptake has been reported [23]. This, however, does not appear to be the case as far as the trophoblast is concerned. The results shown in Table III indicate that no effect on TC uptake by BPM vesicles occurred in the presence of a broad range of estrone sulfate concentrations (2–1000

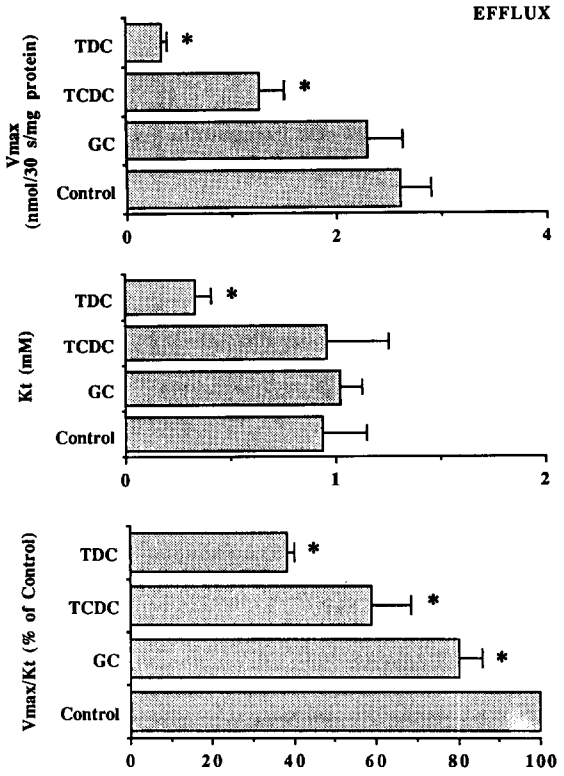

Fig. 6. Effect of 0.3 mM glycocholate (GC), taurochenodeoxycholate (TCDC) or taurodeoxycholate (TDC) on apparent maximal velocity (V_{max}), apparent dissociation constant (K_t) and apparent efficiency (V_{max}/K_t) of taurocholate efflux by BPM vesicles. The values were obtained by fitting the data from experiments shown in Fig. 4 (lower panel) to a Michaelis-Menten equation plus a linear (diffusional) term as described in Materials and Methods. Values are means ± S.E. * $P < 0.05$ as compared with control by the paired t-test.

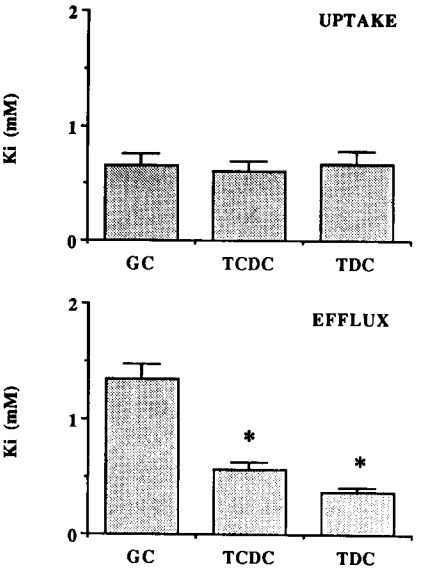

Fig. 7. Comparison of the inhibition constant (K_i) for glycocholate (GC), taurochenodeoxycholate (TCDC) and taurodeoxycholate (TDC) obtained in experiments of taurocholate uptake (upper panel) or efflux (lower panel) by BPM vesicles. The values for K_i were obtained by Lineweaver-Burk transformation of the experimental data shown in Fig. 4. Values are means ± S.E. * $P < 0.05$ as compared with that located on the left by the Bonferroni method of multiple-range testing.

μM), suggesting that a particular bile acid molecular structure is necessary for steroid-related compounds to interact with this carrier.

Discussion

The findings reported in this paper indicate that different bile acids reduce both TC binding and transport by BPM vesicles. Interactions more complex than pure competition occur and will be discussed below. From the results obtained in binding, uptake and efflux experiments it can be suggested that GC and TCDC may interact mainly (but not completely) with the carrier at a site other than the one used by TC. The interaction clearly decreases the translocation-dissociation rate, regardless of the direction of the transport process (uptake or efflux) and it slightly modifies the affinity of the carrier for TC. The study of GC-, TCDC- and TDC-induced inhibition of TC transport revealed that this inhibition is stronger at the fetal side of the membrane except for GC which was similarly effective at both sides. To interpret this finding, it should be remembered that most BPM vesicles are oriented in-side-out (Table I); i.e., the fetal-facing side is located inside the vesicle while the outside surface of the vesicle corresponds to the intracellular face of the trophoblast plasma membrane. Maximal effect was observed for TDC in efflux experiments where TC transport efficiency was reduced to less than 40%. Moreover, as has been described in Results the mechanism

TABLE III

Effect of estrone sulfate on taurocholate uptake by BPMV

Aliquots of BPM vesicles (20 μl) were incubated for 30 s at 37°C with 80 μl buffer A (250 mM sucrose, 0.2 mM $CaCl_2$, 10 mM $MgCl_2$, 100 mM KNO_3, 10 mM Hepes/Tris (pH 7.40)) containing 15 μM taurocholate and varying concentrations of estrone sulfate. Results are means ± S.E. ($n = 3$). The results were compared by the paired t-test. No significant difference was found.

Estrone sulfate concentration (μM)	Taurocholate uptake (pmol/mg protein per 30 s)
0	26.8 ± 1.2
2	29.5 ± 1.7
5	29.8 ± 1.6
10	29.7 ± 0.4
25	31.2 ± 3.1
50	26.9 ± 0.7
100	25.3 ± 1.4
250	26.4 ± 0.7
500	24.5 ± 2.4
1000	24.7 ± 1.5

of TDC-induced inhibition was both different from that found for GC and TCDC and different inside and outside the vesicle. TDC interactions occurring on the intracellular side (uptake experiments) induce a marked increase in both the apparent K_t and V_{max}. This suggests a double interaction, i.e., both competing for the TC-binding site and increasing the translocation-dissociation rate for the putatively TDC-loaded carrier. The mechanism of inhibition on the fetal side of the membrane (efflux experiments) appears to be entirely different; i.e., an increase in the affinity of the carrier for TC (decrease in apparent K_t) and a decrease in the translocation-dissociation rate were observed.

These results indicate that the bile acid carrier located in the basal plasma membrane of the human term trophoblast is sensitive to interaction with different bile acid species. This occurs in a complex way depending on the particular molecular structure and the side of the membrane. Changes in the amino-acid moiety in amidated bile acids and in the number and position of the hydroxyl groups do modify the behavior of the transporter, which is probably able to carry out rectifying and cooperative processes among different bile acids. This may play an important role in the control of net placental transfer by favouring the fetal-maternal excretion of bile acids synthesized by the fetus, as well as by reducing the maternal-fetal transfer of some bile acids present at higher concentrations in maternal blood. If a selective barrier for certain bile acid types does exist, this may partially protect the fetus from the deleterious properties of some of these substances, such as secondary-bile-acid-induced teratogenicity [7].

Acknowledgements

The authors would like to thank Dr. C.A.R. Boyd for his helpful discussion during the preparation of the manuscript. Drs. Antonio Lopez Salva and Tomas Rodriguez Bravo and the rest of the Gynaecology and Obstetrics Department of the Virgen de la Vega Hospital, Salamanca, Spain, for providing the human placentas. Thanks are also given to Nicholas Skinner for reviewing the manuscript. The expert secretarial work of Alicia Escobar and M. Isabel Hernandez and the excellent technical assistance of M. Carmen Gonzalez Mesonero are gratefully acknowledged. This study was supported in part by the Fondo de Investigaciones Sanitarias de la Seguridad Social (93/0419) and by the Junta de Castilla y Leon, Spain. P.B. is a Research Fellow of the Ministerio de Educacion y Ciencia, Spain. M.Y.A.E.-M. is a Research Fellow of the Ministerio de Asuntos Exteriores, Spain.

References

1 Marin, J.J.G., Serrano, M.A., El-Mir, M.Y., Eleno, N. and Boyd, C.A.R. (1990) Gastroenterology 99, 1431–1438.
2 El-Mir, M.Y.A., Eleno, N., Serrano, M.A., Bravo, P. and Marin, J.J.G. (1991) Am. J. Physiol. 260, G887–G894.
3 Bravo P, El-Mir, M.Y.A., Serrano, M.A. and Marin. J.J.G. (1992) J. Physiol. 446, 34P.
4 Colombo, C., Roda, P., Roda, E., Buscaglia, M., Dell'Agnola, C.A., Filippetti, P., Ronchi, M. and Sereni. F. (1985) Pediatr. Res. 19, 227–231.
5 Watkins, J.B. (1983) J. Pediatr. Gastroenterol. Nutr. 2, 365–373.
6 Cabral, D.J., Small, D.M., Lilly, H.S. and Hamilton, J.A. (1987) Biochemistry 26, 1801–1804.
7 Zimmerli, B.,Valantinas, J. and Meier, P.J. (1989) J. Pharmacol. Exp. Ther. 250, 301–308.
8 Hofmann, A.F. and Roda, A. (1984) J. Lipid Res. 25, 1477–1489.
9 Kelley, L.K., Smith, C.H. and King, B.F. (1983) Biochim. Biophys. Acta 734, 91–98.
10 Williams, L.T., Jarett, L. and Lefkowitz, R.J. (1976) J. Biol. Chem. 251, 3096–3104.
11 Scharschmidt, B.F., Keeffe, E.B., Blankenship, N.M. and Ockner, R.K. (1979) J. Lab. Clin. Med. 93, 790–799.
12 Bers, D.M., Philipson, K.D. and Nishimoto, A.Y. (1980) Biochim. Biophys. Acta 601, 358–371.
13 Bretaudiere, J.P. and Spilmann, T. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 75–82, Verlag Chemie, Weinheim.
14 Moss, D.W. (1984) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 92–101, Verlag Chemie, Weinheim.
15 Baginski, E.S., Foa, P.P. and Zak, B. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), pp. 876–880, Verlag Chemie, Weinheim.
16 Pennington, R.J. (1961) Biochem. J. 80, 649–654.
17 Markwell, M.A.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–210.
18 Hopfer, U., Nelson, K., Perroto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32.
19 Yamakoa, K., Tanigawara, Y., Nakagawa, T. and Uno, T.A. (1981) J. Pharmacol. Dyn. 4, 879–885.
20 Roda, A., Hofmann, A.F. and Mysels, K.J. (1983) J. Biol. Chem. 258, 6362–6370.
21 Alonso de la Torre, S.R., Serrano, M.A., Medina, J.M. and Alvarado, F. (1992) Biochem. J. 288, 47–53.
22 Dibbelt, L. and Kuss, E. (1991) Biol. Chem. Hoppe-Seyler 372, 173–185.
23 Schwenk, M. and Lopez del Pino, V. (1980) J. Steroid Biochem. 13, 669–673.