Biochimica et Biophysica Acta, 1111 (1992) 139–141

BBAMEM 70715

Rapid report

Substrate-specific differences in the rate of bile acid carrier reorientation: studies on human placental basal vesicles

N. Eleno [a,b], C.A.R. Boyd [a] and J.J.G. Marin [b]

[a] *Department of Human Anatomy, University of Oxford, Oxford (UK) and* [b] *Department of Physiology, Faculty of Pharmacy, University of Salamanca, Salamanca (Spain)*

(Received 11 August 1992)

Key words: Transport kinetics; Placenta; Membrane vesicle

The initial rate of transport of the bile acid glycocholic acid (GCA) has been measured in influx and efflux across placental basal membrane vesicles, and the mechanism of inhibition of its transport by the analogue taurochenodeoxycholic acid (TCDCA) analysed kinetically. This analogue, although trans-stimulating GCA efflux, inhibits influx in a way which does not depend upon substrate concentration; moreover, its potency as an inhibitor is markedly influenced by whether it is placed on one or on both sides of the vesicles membrane. These findings can be accounted for by postulating that both GCA and TCDCA are translocated through the carrier, but that the rate of loaded carrier reorientation is higher than that of the free carrier only when loaded with TCDCA and not with GCA.

Previous studies have shown that in the trophoblast [1,2] there are carriers for bile acids providing a route for the excretion of these compounds from the fetus. We have used membranes isolated from the basal surface of this epithelium as a model to investigate some features that determine the interaction of this carrier with differing substrates using the labelled trihydroxy bile acid glycocholic acid (GCA) as a substrate and the dihydroxy bile acid taurochenodeoxycholic acid (TCDCA) as an inhibitor.

In other transport systems the kinetics of inhibition have been shown to be influenced markedly by the side on which the inhibitor is placed with respect to substrate [3,4]. We have therefore investigated the influence of localisation of both inhibitor and of substrate on the pattern of inhibition.

Preparation of basal membrane vesicles. Basal plasma membrane vesicles (BPMV) were prepared from fresh human full-term placentae obtained by either caesarian section or vaginal delivery according to a modified preparation of Kelley et al. [5] as described [6]. Membrane vesicles were finally suspended in 10 mM Hepes-Tris (pH 7.4) buffer containing 250 mM sucrose, 100 mM KNO_3, 10 mM $MgCl_2$ and 0.20 mM $CaCl_2$ to give a final protein concentration of 6–8 mg/ml. The purity of this membrane preparation is similar to the values found in the original report.

The vesicles were frozen at −70°C before use. Transport activity was observed to be intact after the membrane suspension had been stored for 8 weeks under these conditions. To carry out each experiment, frozen membranes were thawed and then vesiculated by ten passages through a 25-gauge needle.

Transport measurements. Experiments were performed to measure the influx of [^{14}C]glycocholate (GCA) in the presence or absence of taurochenodeoxycholate (TCDCA), which has been shown to be an inhibitor of GCA transport. Transport was measured by rapid filtration.

For influx, 20 μl of BPMV (120–160 μg protein) were incubated in 80 μl of a medium containing 10 mM Hepes-Tris (pH 7.4), 250 mM sucrose, 100 mM KNO_3, 10 mM $MgCl_2$ and 0.20 mM $CaCl_2$ with the addition of [^{14}C]GCA plus unlabelled GCA. For efflux BPMV were preincubated for 2 h at room temperature in the presence of [^{14}C]GCA plus unlabelled GCA; at the start of the efflux experiment 20 μl of this vesicle suspension was added to 80 μl of the medium described above.

All experiments were performed at 37°C, and the time of incubation as well as the concentrations of GCA or TCDCA used are indicated in the figure

Correspondence to: N. Eleno, Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

legends. The concentrations of bile acids used did not alter the pH of the media, and did not have a disrupting effect on the vesicles.

The assays were terminated by diluting the sample with a 40-fold excess of an ice-cold (4°C) buffer composed of 10 mM Hepes-Tris (pH 7.40), 250 mM KCl and 25 mM $MgSO_4$. The diluted sample was immediately filtered through a Millipore cellulose filter (0.65 μm) and washed once with the same solution. Three additional washes were performed to reduce the non-specific binding of the isotope to the filter as described previously [2]. The filters were dissolved in aqueous scintillant (ACSII, Amersham) and counted in a liquid scintillation counter.

Protein estimation. Protein concentration of the vesicle preparation was determined by the method of Lowry using bovine serum albumin as standard.

Chemicals. [^{14}C]Glycocholate was obtained from New England Nuclear. Unlabelled GCA and TCDCA (Na salts) were obtained from Sigma. All other chemicals were of the highest purity commercially available.

Results. Fig. 1a, b shows the results of an experiment in which the inhibitory effect of TCDCA on the initial rate of labelled GCA influx was examined firstly under zero-trans entry, with inhibitor outside the vesicles, at differing substrate concentrations; and secondly under identical conditions but with TCDCA present at the same concentration both inside and outside. Very strikingly the pattern of inhibition differs in the two experiments. Two important conclusions emerge; firstly in both experiments the inhibition is not dependent on substrate concentration; secondly the localisation of the inhibitor strongly influences the concentration of TCDCA giving half-maximal inhibition. The potency of TCDCA as an inhibitor of GCA influx (given by the slope of the plot) is approximately four times lower when TCDCA is present on both sides (K_i = 2.4 mM) than when it is confined to the outside (K_i = 0.6 mM).

Fig. 2 shows the results of an experiment in which the efflux of GCA (50 μM) from preloaded vesicles has been studied either in the presence or in the absence of TCDCA (300 μM) confined to the external compartment. There is marked trans-stimulation of GCA efflux by the TCDCA, the rate constant being increased more than 4-fold from a value of 0.58 to 2.54 min^{-1}.

To determine the effect of substrate binding on the rate of carrier movement we have also looked at the efflux of GCA under zero-trans and equilibrium exchange conditions at two substrate concentrations (25 and 200 μM). The results are shown in Table I and indicate that the movement of the free carrier across

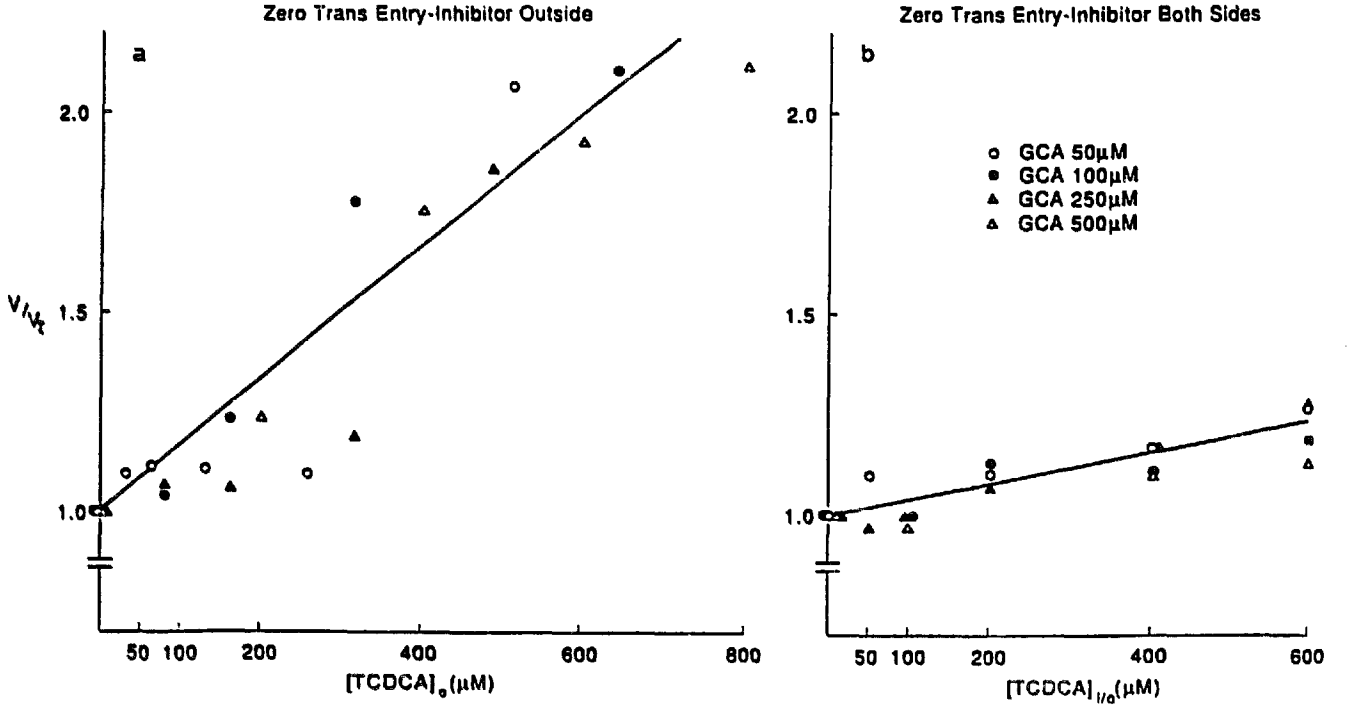

Fig. 1. Effect of the sidedness on the inhibition of GCA influx by different concentrations of TCDCA. The inhibitory effect of varying concentrations of TCDCA (0–800 μM) was measured on the influx of each of the following concentrations of GCA: 50 (○), 100 (●), 250 (▲) and 500 μM (△). The assay was performed under two conditions: (a) with TCDCA outside the vesicles (zero-trans entry with the inhibitor outside). BPMV were incubated for 10 s in an incubation medium containing both GCA and TCDCA; (b) with TCDCA at the same concentration on both sides of the membrane (zero-trans entry with the inhibitor on both sides). BPMV were preloaded for 2 h at room temperature with the indicated concentrations of TCDCA, and then incubated for 10 s in the presence of both GCA and TCDCA. These graphs represent the relative velocity (ratio between the velocity in the absence and in the presence of the inhibitor) plotted against the different concentrations of inhibitor under initial rate conditions.

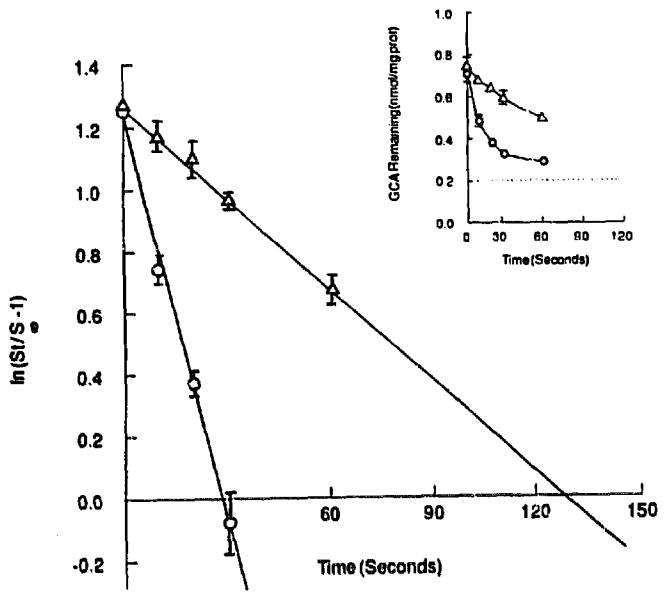

Fig. 2. Trans-stimulation of GCA efflux by TCDCA. GCA (50 μM) remaining in the vesicles was measured in the absence ($\triangle$) (mean ± S.E., $n = 9$) or in the presence ($\circ$) of 300 μM TCDCA outside the vesicles at different times of incubation. After preincubation total radioactivity loaded was ascertained at time zero by adding 20 μl of BPMV suspension to 80 μl of incubation medium previously diluted with 4 ml of ice-cold stop solution. The equation of the line is ln $(S_t/S_x - 1) = \ln B - kt$. The Y-axis represents ln $(S_t/S_x - 1)$; S_t is the quantity of isotope remaining in the vesicles at time t, and S_x is the quantity of isotope remaining after infinite time (dotted line indicated on inset). The rate constant k is obtained from the slope of the plot.

the membrane is not different from that of the substrate-loaded carrier at either concentration.

Conclusions. Since GCA efflux is not changed by the presence of unlabelled substrate on the trans-side of the membrane we can conclude that the velocity of reorientation of the free carrier and of the carrier bound to GCA are the same. However, TCDCA placed in the trans-compartment clearly does trans-stimulate. This means that TCDCA is translocated through the carrier; furthermore, the rate of translocation of the carrier loaded with TCDCA across the membrane must be greater than that both of the free carrier and of the carrier loaded with GCA. But how do we explain the findings shown in Fig. 1 in which we see that the inhibition is much less strong when TCDCA is present on both sides (rather than only outside) of the membrane? This is the predicted result if TCDCA is itself translocated and if the carrier loaded with TCDCA reorientates more rapidly than does the free carrier. With TCDCA present only on the outside the carrier will be found predominantely in an inward-facing conformation and GCA influx will be strongly inhibited; when TCDCA is also present inside there will also be accelerated return of the carrier loaded with this bile salt to the outside and thus less carrier depletion at the external face.

The other important observation shown in Fig. 1 is that the extent of inhibition produced by external TCDCA is not dependent on the concentration of GCA. This finding is also readily explained if TCDCA but not GCA binding to the carrier depletes the available pool of outward facing carrier molecules.

All of the experimental observations we have made can be explained by a carrier model postulating accelerated reorientation of the TCDCA-loaded but not of the GCA-loaded carrier. The pattern of inhibition observed will then follow due to the fact that substrates in the external compartment cannot complete for carrier sequestered to the internal face of the membrane. That such a small change in substrate structure (between a dihydroxy and a trihydroxy bile acid) can produce a detectable change in the membrane protein transport cycle may have more general implications for substrate interactions with other carriers.

TABLE I

Efflux of GCA across placental basal plasma membrane vesicles

Values (means ± S.E., $n = 3$) of GCA efflux in two conditions: (a) zero-trans exit (with GCA only inside the vesicles) and (b) equilibrium exchange (with GCA at the same concentration on both sides of the membrane). GCA was used at two concentrations, 25 and 200 μM.

	GCA efflux (pmol/mg protein per 30 s)	
	zero-trans exit	equilibrium exchange
GCA 25 μM	59.3 ± 0.8	61.5 ± 1.6
GCA 200 μM	430.9 ± 18.9	467.0 ± 26.4

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 Iioka, H. (1985) Jap. J. Obstet. 50, 100–110.
3 Devés, R. and Krupka, R.M. (1989) Methods Enzymol. 171, 113–132.
4 Devés, R. (1991) in New Methods to Study Membrane Transport (Yudilevich, D.L., Devés, R., Peran, S. and Cabantchik, Z.I., eds.), pp. 3–19, Plenum Press, New York.
5 Kelley, L.K., Smith, C.H. and King, B.F. (1983) Biochim. Biophys. Acta 734, 91–98.
6 Kudo, Y. and Boyd, C.A.R. (1990) J. Physiol. 426, 381–395.