Passage of S(+) and R(–) γ -vinyl-GABA across the human isolated perfused placenta

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- 1 The maternal to foetal transfers of S(+)- and R(–)- γ -vinyl-GABA (VGB) across the human isolated perfused placenta were low and comparable with those of acidic α -amino acids.
- 2 The placental uptake of the active S(+)-isomer from the maternal circulation exceeded that of the R(–)-isomer and this was reflected by a corresponding difference in placental tissue concentrations.
- 3 During perfusion with recirculation of the foetal medium, the two enantiomers were present at a similar concentration and did not concentrate in foetal perfusate, indicating that the excess amount of S(+)-VGB cleared from the maternal circulation was not accessible to the foetal perfusate. Furthermore, stable concentrations of both isomers in the foetal perfusate suggested a lack of placental metabolism.
- 4 Possible explanations of these findings include the operation of a stereoselective sodium-dependent-GABA placental uptake system on the maternal side, similar to that observed in neuronal tissue, or stereoselective binding to a placental GABA transaminase.

Keywords human placenta transport vigabatrin enantiomers

Introduction

γ -aminobutyric acid (GABA) is a major inhibitory neuromediator in the central nervous system. An efficient uptake of GABA mediated by a sodium-dependent process has been demonstrated in synaptosomes (Martin, 1973). GABA is a decarboxylated derivative of glutamate. In contrast to its precursor glutamate, GABA has apparently no other function except neurotransmission. Glutamate uptake in the human placenta (Schneider & Dancis, 1974) has been related to the metabolic functions of the foetus. The uptake of GABA has not been studied in the human placenta, although GABA decarboxylase and GABA receptors (Erdo *et al.*, 1985) have been described in this organ.

By altering GABA metabolism, γ -vinyl-GABA (VGB) increases the concentration of GABA in the central nervous system (Grove *et al.*, 1981). It has a potential therapeutic role in the treatment of a number of neurological disorders. VGB enters the central nervous system where it inhibits the activated GABA degrading enzyme GABA transaminase. VGB is a racemic mixture of S(+)- and R(–)-isomers. After oral administration of VGB, lower peak plasma concentrations of the S(+)-

enantiomer were observed (Haeghele & Strechter, 1986). This may indicate a greater absorption of the R(–)-isomer, faster elimination of the S(+)-isomer or more extensive tissue distribution of the S(+)-isomer.

We have used the perfused human placenta, which displays a stereoselectivity in many transport systems (amino acids and glucose) and lacks innervation, as a model to investigate a possible role of non-neuronal selective transport systems in the disposition of VGB.

Methods

Perfusion

Placentas were collected from normal pregnancies terminated by Caesarean section. A foetal artery and a foetal vein were cannulated and small cannulae were inserted in the intervillous space, as described by Schneider *et al.* (1972). Plasma from patients with malaria (1 l per experiment) was used to prepare the perfusion

medium. This plasma was rejected for transfusion by our blood bank. It was dialysed against 0.9% (w/v) sodium chloride and defibrinated with excess calcium chloride (Guerre-Millo *et al.*, 1982). The final medium was diluted (1:1) in Earle's solution containing 1 g l⁻¹ of (+)-glucose. Both foetal and maternal media were gassed with a mixture of 95% O₂ and 5% CO₂ throughout the experiments. The pH and temperature of the media were 7.4 and 37° C, respectively. The flow rates were between 3.5 and 6.7 ml min⁻¹ on the foetal side and between 11.0 and 13.1 ml min⁻¹ on the maternal side of the placenta. Arterial pressures remained below 40 mmHg in both circuits. The wet weight of the perfused placental lobule varied from 8 to 46 g.

Four compounds were added to the perfusion medium: *N*-methyl-[¹⁴C]-antipyrine (N.E.N., 50 mCi mmol⁻¹), (-)-(4-5-[³H])-leucine (Amersham, 147 Ci mmol⁻¹) and a racemic mixture of S(+)- and R(-)-γ-vinyl-GABA (Vigabatrin[®], Merrell Dow). The radioactive compounds were dissolved in the medium to give activities of 6 μCi l⁻¹ and 12.5 μCi l⁻¹, respectively. VGB was added to the medium to give a final concentration of 50 μg ml⁻¹ of each enantiomer.

The perfusions were continued for 150 min. In the first 60 min period, used to assess clearance and uptake, the foetal and maternal media were not recirculated (5 experiments). The compounds were present only in maternal medium. In the period from 60 to 150 min, used to examine maternal to foetal active transport, the foetal medium was recirculated whilst the maternal circulation was single-pass (4 experiments). During this period, foetal and maternal media contained equimolar concentrations of the compounds.

Tissue extraction

Frozen tissues from four experiments (0.3 to 0.5 g) were homogenized in 2 to 4 ml of chilled 0.9% (w/v) sodium chloride using Potter-Elvehjem homogenizer with a Teflon[®] pestle and centrifuged for 15 min at 10,000 *g*. The supernatants were assayed for antipyrine, (-)-leucine, S(+)-VGB and R(-)-VGB.

Radioactivity and drug analysis

Venous effluent samples (3 ml) from the foetal and maternal circulations were collected every 5 min and maintained at 4° C until the end of the experiment. Aliquots (0.5 ml) were counted in Aqualuma[®] (4 ml) in a Beckman spectrometer LS 1801. Further aliquots (0.5 ml to 2 ml) were frozen and assayed subsequently for S(+)-VGB and R(-)-VGB as described by Rey *et al.* (1990). This method involved formation of *N*-trifluoroacetyl-*O*-propyl ester derivatives of S(+)-VGB and R(-)-VGB, separation and measurement by gas chromatography-mass spectrometry using a Chirasil-L-val capillary column (Chrompack). The lower limit of the assay was 2 mg l⁻¹ (both isomers) and coefficients of variation were 4.1% and 5.3% for 5 mg l⁻¹ S(+)-VGB and R(-)-VGB, respectively.

Data analysis

Maternal-foetal (CL_{MF}) and maternal-placental (CL_{MP}) clearances in the first phase and the foetal-maternal concentration ratio (R_{FM}) in the second phase were calculated as follows:

$$-CL_{MF} = C_{Fv} * Q_F / C_{Ma}$$

This indicates the volume of maternal perfusate from which compound was removed to the foetal circulation per unit time.

$$-CL_{MP} = (C_{Ma} - C_{Mv}) * Q_M / C_{Ma}$$

This indicates the volume of maternal perfusate from which compound was taken up into the placenta per unit time.

$$-R_{FM} = C_{Fv} / C_{Ma}$$

Where *C* = concentration or radioactivity (μg ml⁻¹ or d min⁻¹ ml⁻¹); F = foetal; M = maternal; Q_F = foetal flow rate; Q_M = maternal flow rate; a = artery and v = vein.

The data were expressed as means ± s.e. mean. Statistical significance was determined using Student's *t*-test for paired values (tissue content), two-way ANOVA with replication (open circuit and recirculation periods) and linear regression (recirculation period) with a test for parallelism according to Tallarida & Murray (1986).

Results

Open circuit perfusion

The maternal-foetal clearances (CL_{MF}) of antipyrine and (-)-leucine increased steadily up to 35 min of perfusion and then were stable up to 60 min (Figure 1). The mean CL_{MF} values from 25 to 45 min were 2.09 ± 0.19 and 1.73 ± 0.16 ml min⁻¹, respectively (*F*(1,40) = 5.2; *P* < 0.05). The CL_{MF} values of S(+)-VGB and R(-)-VGB increased in parallel over 60 min and their

Figure 1 Maternal-foetal clearances of antipyrine (○), (-)-leucine (●), S(+)-VGB (△) and R(-)-VGB (▲) in the open circuit period (0 to 60 min). Each point represents mean ± s.e. mean data from five perfusions.

mean values between 25 and 45 min (0.53 ± 0.04 and 0.55 ± 0.04 ml min⁻¹, respectively) were not significantly different ($F(1,40) = 0.04$).

The maternal-placental clearances (CL_{MP}) of antipyrine and (-)-leucine decreased steadily over 35 min (Figure 2). From 30 to 45 min, the mean values were 3.83 ± 0.39 ml min⁻¹ for (-)-leucine and 2.94 ± 0.26 ml min⁻¹ for antipyrine ($F(1,30) = 18.5$; $P < 0.01$). During the same period, the CL_{MP} of S(+)-VGB was significantly higher than that of R(-)-VGB ($F(1,30) = 17.0$; $P < 0.01$). Mean values were 1.80 ± 0.24 and 0.69 ± 0.18 ml min⁻¹, respectively. The CL_{MP} of the S(+)-enantiomer was also significantly greater ($F(1,30) = 33.3$, $P < 0.01$) than its CL_{MF} value (0.56 ± 0.04 ml min⁻¹).

Recirculation of foetal medium

After an initial 10 min period of equilibration, the R_{FM} of antipyrine remained constant whereas that of (-)-leucine increased steadily (Figure 3). The R_{FM} of antipyrine averaged 0.96 ± 0.005 from 70 to 140 min. During this period, the slope of the regression line of (-)-leucine R_{FM} against time ($y = 3.7 \times 10^{-3}x + 0.7$; $r = 0.63$; $n = 60$) was higher and significantly different ($t = 4.7$; $P < 0.01$; $n = 120$) from that obtained with antipyrine ($y = 6.6 \times 10^{-4}x + 0.9$; $r = 0.35$; $n = 60$). The mean R_{FM} value observed at 150 min for (-)-leucine was 1.32 ± 0.14 .

In contrast to (-)-leucine, there was no foetal to maternal concentration gradient for either of the isomers of VGB (Figure 4). The mean R_{FM} values between 95 to 140 min ($n = 40$) for S(+)-VGB and R(-)-VGB were 0.91 ± 0.01 and 0.89 ± 0.01 , respectively and did not differ significantly ($F(1,72) = 0.98$).

Tissue content of radioactivity and drug

The contents of tritium and ¹⁴C radioactivities derived from (-)-leucine and antipyrine and the concentrations of S(+)-VGB and R(-)-VGB in the placental tissue extracts are shown in Table 1. The tissue ³H/¹⁴C radioactivity ratios were two to ten times greater than those of the maternal arterial medium indicating a relative

accumulation of tritiated material in the tissue. The tissue S/R VGB concentration ratio averaged 1.12 and was significantly higher than the mean ratio of 0.98 ± 0.031 observed in the arterial maternal medium ($t = 4.4$, $P < 0.05$, $n = 4$).

Discussion

The CL_{MF} values of S(+)-VGB and R(-)-VGB observed in the period of open circuit perfusion (0.53 ± 0.04 and 0.55 ± 0.04 ml min⁻¹, respectively) were low for a compound of small molecular weight (MW: 129) and in comparison to the CL_{MF} value of (-)-leucine (1.73 ± 0.16 ml min⁻¹; MW: 131). The clearance index of VGB (0.26), which relates the CL_{MF} of the drug to that of antipyrine, was comparable with the value of 0.27 and greater than the value of 0.19 reported for α -aminoisobutyric and glutamic acids, respectively (Schneider *et al.*, 1979a). Thus, these γ -amino acid derivatives cross the human placenta at a similar rate to acidic α -amino acids.

The CL_{MF} values of S(+)-VGB and R(-)-VGB and the CL_{MP} value of R(-)-VGB (0.53 ± 0.04 , 0.55 ± 0.04 ,

Figure 3 Steady state ratios (R_{FM}) of (-)-leucine (●) and antipyrine (○) in the recirculation period (60 to 150 min). Each point represents mean $\pm$ s.e. mean data from four determinations obtained in four perfusions.

Figure 2 Maternal-placental clearances of (-)-leucine (●), antipyrine (○), S(+)-VGB (△) and R(-)-VGB (▲) in the open circuit period (0 to 60 min). Each point represents mean $\pm$ s.e. mean data from five measurements obtained in five perfusions.

Figure 4 Steady state ratios (R_{FM}) of S(+)-VGB (○) and R(-)-VGB (●) in the recirculation period (60 to 150 min). Each point represents mean $\pm$ s.e. mean data from four measurements obtained in four perfusions.

Table 1 Placental tissue content of drugs and radioactivity

Experiment	Tissue extract			Medium	Tissue extract			Medium
	[³ H]-Leu (d min ⁻¹ ml ⁻¹)	[¹⁴ C]-Ant (d min ⁻¹ ml ⁻¹)	³ H/ ¹⁴ C ratio	³ H/ ¹⁴ C ratio	S(+) (μg ml ⁻¹)	R(-) (μg ml ⁻¹)	S/R ratio	S/R ratio
1	51294	9214	5.52	1.85	11.5	10.3	1.117	1.057
2	49388	15709	3.14	1.78	41.6	37.3	1.115	0.912
3	51774	3340	15.32	1.61	54.7	49.0	1.116	1.002
4	11972	4920	2.43	1.66	8.5	7.6	1.118	0.956

Leu: (-)-leucine; Ant: antipyrine; S(+): S(+)-γ-vinyl-GABA; R(-): R(-)-γ-vinyl-GABA.

0.69 ± 0.18 ml min⁻¹, respectively) were similar, but the CL_{MP} value of S(+)-VGB (1.80 ± 0.24 ml min⁻¹) was about three times higher. Similarly, the CL_{MP} of (-)-leucine (3.83 ± 0.39 ml min⁻¹) exceeded that of antipyrine (2.94 ± 0.26 ml min⁻¹) confirming the high uptake of this amino acid by the placenta from the maternal side (Schneider *et al.* 1987) associated with significant placental metabolism (Roberson *et al.*, 1976; Scislawski *et al.*, 1983). Because the high maternal uptake of the S(+)-VGB compared with R(-)-VGB is not reflected by its placental release to the foetal medium, it is likely that the maternal-foetal concentration gradient, which was similar for the two isomers, constitutes the main force driving the transplacental passage. Nevertheless, because these compounds were transferred in low amount, their passage might not be distinguished at foetal to maternal flow ratios between 0.3 and 0.6.

The consistent foetal-maternal concentration gradient observed for (-)-leucine in the period of recirculation (R_{FM}: 1.32 ± 0.14) implies an active placental transport of this α-amino acid or its metabolic products. Under the same conditions, the VGB enantiomers did not accumulate in the foetal circulation (R_{FM}: 0.91 ± 0.01 and 0.89 ± 0.01) and the mean S(+)/R(-) ratio was close to unity (1.02 ± 0.02, *n* = 40). From the high CL_{MP} of S(+)-VGB observed in the period of open circuit perfusion, the R_{FM} of S(+)-VGB was expected to be higher than that of R(-)-VGB in foetal perfusate over the recirculation period. If the two enantiomers were discharged with the same facility to the foetal perfusate in the recirculation period, the concentration of S(+)-VGB would be higher than that of R(-)-VGB. Since the two enantiomers were detected at similar concentrations and did not concentrate in the foetal perfusate, the excess of S(+)-VGB taken up from the maternal side was not available for transfer from placenta to foetal perfusate.

Placental uptake without foetal accumulation could be explained by placental drug metabolism. Thus, glutamic and aspartic acids accumulate in placenta tissue slices (Schneider & Dancis, 1974) but not in foetal

recirculation medium (Schneider *et al.*, 1979b). The concentrations of these two amino acids were shown by Schneider *et al.* (1979b) to decrease in the foetal medium with time. In contrast, in the present study the R_{FM} values of both enantiomers of VGB remained constant thereby excluding any placental metabolism.

S(+)-VGB was selectively accumulated by the placental tissue (S(+)/R(-) ratio of 1.12 in tissue and 0.98 ± 0.031 in medium). The radioactivity associated with (-)-leucine was also concentrated by the placental tissue (Table 1). However, in this case, this also reflects radiolabelled metabolites of leucine (Schneider *et al.*, 1979a; 1987). The high uptake of S(+)-VGB from perfusion medium compared to R(-)-VGB in the open perfusion period and the selective tissue uptake of S(+)-VGB suggest a stereospecific binding to a protein carrier at the maternal-placental interface.

A selective uptake of S(+)-VGB has also been reported in neurons and astrocytes (Schousboe *et al.*, 1986). Although S(+)-VGB exhibits a high affinity uptake in neurons, the high IC₅₀ necessary to inhibit the uptake of GABA (Schousboe *et al.*, 1986) indicates that this uptake does not involve transport by the sodium-dependent-GABA system demonstrated in brain. GABA as well as GABA_A receptors are present in the placenta (Erdo *et al.*, 1985). This neuromediator can be imported from blood or locally synthesized from glutamic acid by placental GABA decarboxylase (Erdo *et al.*, 1985). It is not known whether the placenta contains a sodium-dependent-GABA transport system. S(+)-VGB uptake mediated by a carrier process may operate independently of GABA transport as in neurons, but our study does not allow any conclusions regarding relationships between S(+)-VGB and GABA transporters.

An alternative explanation of the high placental S(+)-VGB uptake and its stereospecific uptake from the maternal circulation could be a selective binding to GABA transaminase, imposing a gradient for the diffusion of the active S(+)-VGB from the maternal circulation to the tissue. However, it is not known whether this enzyme is present in the human placenta.

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