Foetal glutamate as a possible precursor of placental glutamine in the guinea pig

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The role of foetal glutamate as a source of placental glutamine was investigated in the near-term pregnant guinea-pig placenta perfused *in situ* through the umbilical vessels. With normal foetal amino acid concentrations there was a significant two-way exchange of glutamate between the placenta and foetal perfusate, but a net release of the amino acid from the placenta. Radioactively labelled glutamate carbon entering the placenta by this exchange was freely incorporated into intracellular glutamine, but only 1.5% of it was found in glutamine transported out into the foetal circulation. In the guinea pig, therefore, foetal glutamate does not appear to be a precursor of glutamine released from the placenta on the foetal side.

Glutamine is important for foetal growth as a nitrogen donor for synthetic pathways, including the synthesis of purines for the adenine and guanine nucleotides, NAD, RNA and DNA (e.g. Meister, 1965). The sources of glutamine reaching the foetus are therefore of considerable interest.

Glutamine itself has been shown to flow relatively rapidly, compared with other amino acids, from the placenta to the foetus in several species, including the sheep, human and rat (Lemons *et al.*, 1976; Hayashi *et al.*, 1978; Yamamoto *et al.*, 1974). However, the precursor of glutamine, glutamate, is not transferred across the placenta to the foetus in the pregnant rhesus monkey (Stegink *et al.*, 1975) or rat (Dierks-Ventling *et al.*, 1971). Furthermore, no net uterine uptake of glutamate was found by Holzman *et al.* (1979) in the conscious pregnant ewe near term, but a significant negative umbilical venous–arterial difference indicated that in the sheep glutamate flows, in steady-state conditions, from the foetus to the placenta (Lemons *et al.*, 1976; Holzman *et al.*, 1979). Measurements in umbilical-cord blood during delivery suggest that the same is true in the human (Rooth & Nilsson, 1964; Prenton & Young, 1969; Hayashi *et al.*, 1978). These results imply that in the sheep and the human near term there is a net foetal production of glutamate, the excess entering the placenta.

Consistent with these findings, Schneider *et al.*

(1979), perfusing the human placenta cotyledon closed circuit, found an increase in glutamine plus asparagine concentration in the foetal perfusate, but a fall in glutamate. They interpreted these results as indicating that, *in vivo*, glutamate produced by the foetus is taken up into the placenta and converted there into glutamine, which is then transported back to the foetus. This could be a mechanism for salvaging excess foetal glutamate carbon, produced, for example, as a by-product of glutamine deamidation in foetal purine synthesis.

However, earlier work of Hill & Young (1973) had not revealed an uptake of glutamate on the foetal side of the perfused guinea-pig placenta. Since there was otherwise nothing known about the disposition of glutamate and glutamine across the guinea-pig placenta, we set out to investigate this further, and to find whether in this species foetal glutamate is a quantitatively important precursor of glutamine released from the placenta.

Materials and methods

Animals

Experiments were carried out with pregnant albino guinea pigs (Duncan–Hartley strain) weighing 1.28 ± 0.03 kg (S.E.M., 14 animals), kept on a 12 h-light (07:00–19:00 h)/12 h-dark cycle. They were used between days 58 and 65 of gestation (mean 62; term = 67 days) estimated from the foetal weights [72 ± 2.9 (61) g], referred to the gestational-age-versus-weight curves of Draper (1920) and Bell (1941). Crown-to-rump lengths of the foetuses were 109 ± 1.5 mm ($n = 61$).

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Tissue sampling

For all experiments the animals were anaesthetized with 25 mg of sodium pentobarbitone B.P. (Macarthy's Surgical, Dagenham, Essex, U.K.)/kg (10 mg/ml) injected into the brachial vein after producing local analgesia with 1% lignocaine hydrochloride (Antigen, Rosecrea, Ireland). Maternal blood samples were taken from a carotid-artery catheter, and foetal blood samples for measurements *in vivo* were taken from the umbilical vein after Caesarean delivery of the foetus. Placentas were rapidly removed, frozen between clamps cooled in liquid nitrogen, weighed and stored for assay in liquid nitrogen.

Placental perfusions

Guinea-pig placentas were perfused as described by Reynolds & Young (1971) and Young (1980). The method exploits the unique advantage of the placenta in having two circulations; the organ is left *in situ* in the uterus, supplied with nutrients and oxygen by uterine arterial blood as it is *in vivo*. The foetal circulation of the placenta is perfused open circuit through the umbilical vessels after cannulation of the two umbilical arteries and the vein, and removal of the foetus. The flow rate of 2 ml/min and pressure of 35–45 mmHg (4.7–6.0 kPa) approximate to those found *in vivo* (Young, 1980). The perfusion medium was Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964) equilibrated with the gas mixture O₂/CO₂/N₂ (4:5:91) to give partial pressures of approx. 30 mmHg (4 kPa) for O₂ and 36 mmHg (4.8 kPa) for CO₂, equivalent to those in the umbilical-vein blood *in utero*.

The medium contained 3 g of bovine albumin (fraction V, reagent grade; Miles Laboratories, Stoke Poges, Bucks., U.K.)/100 ml, 5 mM-glucose and physiological concentrations of the L-amino acids and glycine. L-Glutamine and L-glutamate were added at concentrations of 840 and 48 μ M respectively, determined from initial experiments (Table 1), and the remaining amino acids at concentrations found previously for foetal guinea pig blood plasma (Hill & Young, 1973). L-Cysteine, glutamate and glutamine were made up freshly for each experiment, and added immediately before the start. As a result of spontaneous glutamine deamidation there was a slow increase in perfusate arterial glutamate concentration during perfusions. Values of glutamine and glutamate were, respectively, 806 ± 12 and $69 \pm 8 \mu$ M at 5–10 min and 804 ± 10 and $74 \pm 12 \mu$ M at 15–20 min. The 25–30 min values are given in Table 2.

Placentas were initially perfused for 30 min to reach steady state, then at designated zero time perfusion proper was started with the same medium except that the glutamate was labelled with L-

[U-¹⁴C]glutamate (The Radiochemical Centre, Amersham, Bucks., U.K.) to give a specific radioactivity of 8.3 μ Ci/ μ mol. The venous effluent was collected for 5 min periods, together with corresponding reservoir (arterial) samples, placed immediately into ice and stored in liquid nitrogen. The 5–10, 15–20 and 25–30 min samples were assayed. At the end of perfusion (30 min) the placentas, which weighed 5.56 ± 0.39 (8) g, were freeze-clamped (see above) and stored in liquid nitrogen for assay. Maternal blood was sampled at 0 and 30 min of perfusion (see above).

Glutamate and glutamine determinations

Frozen placenta samples were deproteinized for assay by homogenization in 2 vol. of ice-cold 1.5 M-HClO₄; blood and perfusion media were deproteinized by mixing with 1 vol. of cold 1.5 M-HClO₄. The supernatants after centrifugation (1500 g) for 10 min were neutralized with cold 6 M-KOH/0.6 M-K₂HPO₄ mixture to pH 6.8–7.2. Glutamate was measured in neutralized extracts by the enzymic method of Beutler & Michal (1974), by using glutamate dehydrogenase (bovine liver, in glycerol), diaphorase (Boehringer Corp., Lewes, Sussex, U.K.) and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-tetrazolium chloride (BDH Chemicals, Poole, Dorset, U.K.). Glutamine was determined in the same way after treatment of samples (Lund, 1974) with L-glutaminase (grade V; Sigma Chemical Co., Poole, Dorset, U.K.).

Radioisotope measurements

The L-¹⁴C radioactivities of glutamate and glutamine were measured as described by Squires & Brosnan (1978), the glutamate radioactivity by treating samples with L-glutamate decarboxylase (type V, Sigma) and counting the radioactivity of the ¹⁴CO₂ released with an Intertechnique model SL30 liquid-scintillation counter (with Packard Instagel scintillator), and the glutamine radioactivity in the same way after addition of L-glutaminase (grade V, Sigma).

Placental blood-space measurements

Measurements of metabolite concentrations and radioactivities in placentas were all corrected for values in both the foetal and maternal blood spaces. To estimate these spaces 1 mg of inulin (BDH)/ml was added to the perfusion medium, and 100 μ Ci (10 mg) of [G-³H]inulin (New England Nuclear, Southampton, Hants., U.K.) was injected into the maternal circulation through the jugular vein before the start of perfusions. The inulin concentrations in maternal blood, placenta and perfusate were determined enzymically by the method of Renschler (1963), except that the concentrations of NADP, ATP and Mg²⁺ in the reaction mixture were

doubled. From these values and [^3H]inulin radioactivities in maternal blood and placenta, the inulin spaces were found to be: foetal, 0.277 ± 0.014 (8) ml/g placenta wt.; maternal, 0.447 ± 0.011 (8) ml/g.

Results

Glutamine and glutamate concentrations in maternal, placental and foetal compartments

The steady-state concentrations of glutamine and glutamate in maternal blood, placenta and foetal blood in near-term pregnant guinea pigs are shown in Table 1. The glutamine concentration in foetal plasma was high, 7.9 times the maternal value, but considerably higher still in placental intracellular water: 29.7 times the maternal concentration and 3.8 times the foetal.

Glutamate, however, had a very low foetal plasma concentration and small maternal-to-foetal gradient, but an extremely high placental concentration, $22\,400\ \mu\text{M}$, i.e. 595 times the maternal concentration and 470 times the foetal.

Table 1. *Glutamine and glutamate concentrations in maternal blood plasma, placental intracellular water and foetal plasma of the pregnant guinea pig*

The glutamine and glutamate concentrations were measured enzymically as described in the Materials and methods section. The placenta values are corrected for the concentrations in both maternal and foetal blood spaces, determined as outlined in the Materials and methods section. Values are means $\pm$ S.E.M. for the numbers of animals given in parentheses. Significance of difference from maternal concentration: * $P < 0.001$; † $P < 0.02$; significance of difference from placental concentration, ‡ $P < 0.001$.

	Concentration (μM)		
	Maternal plasma (8)	Placental intracellular water (8)	Foetal plasma (10)
Glutamine	106 ± 5	3154 ± 552	$840 \pm 53^{*†}$
Glutamate	38 ± 2	$22\,434 \pm 1551$	$48 \pm 3^{†‡}$

Glutamine and glutamate fluxes

Table 2 shows the glutamine and glutamate fluxes across the foetal side of the perfused guinea-pig placenta, after 25–30 min of perfusion. The umbilical venous–arterial difference of glutamine concentration was positive, indicating a net release into the medium, but this was not statistically significant, probably because of the difficulty of measuring differences as small as 3%. At 5–10 and 15–20 min the average rates of release were 5.5 ± 5.3 and 10.6 ± 7.7 nmol/min per g.

However, there was a significant net release of glutamate from the placenta, 6.4 ± 1.5 (8) nmol/min per g, made up of an influx of 4.1 ± 0.9 and an efflux of 10.5 ± 1.1 nmol/min per g (Table 2). At 5–10 min of perfusion the rate of net outflow was 6.0 ± 1.1 nmol/min per g (influx, 3.6 ± 0.7 ; efflux, 9.6 ± 0.8 nmol/min per g) and at 15–20 min net outflow was 6.3 ± 1.1 (influx, 3.7 ± 0.7 ; efflux, 10.0 ± 0.8 nmol/min per g), all these values being statistically different from zero ($P < 0.002$ or better).

Conversion of glutamate into glutamine

When ^{14}C -labelled glutamate was added to the perfusion medium, labelled glutamine emerged from the placenta on the foetal side, indicating that glutamate was converted into glutamine in the placenta. The $1\text{-}^{14}\text{C}$ specific radioactivity of glutamine in the venous perfusion medium was 0.19 ± 0.06 d.p.m./nmol at 5–10 min, 0.36 ± 0.11 d.p.m./nmol at 15–20 min and 0.38 ± 0.08 d.p.m./nmol at 25–30 min, showing that the incorporation of label into glutamine reached steady state by about 30 min.

The specific $1\text{-}^{14}\text{C}$ radioactivity of placental intracellular glutamine after 30 min of perfusion was 6.6 ± 1.4 (8) d.p.m./nmol, remarkably close to that of glutamate, 14.1 ± 3.3 (8) d.p.m./nmol. Thus, within the trophoblast, glutamate carbon was readily incorporated into glutamine carbon (see the Discussion section).

On the other hand, at 25–30 min, the rate of release of glutamine $1\text{-}^{14}\text{C}$ radioactivity from the

Table 2. *Glutamine and glutamate fluxes across the foetal side of the perfused guinea-pig placenta*

These measurements were made at 25–30 min of perfusion. Glutamate influx was calculated from the venous–arterial difference in perfusate concentration of glutamate $1\text{-}^{14}\text{C}$ radioactivity and perfusate flow rate, and efflux from this value and the net rate of release from the placenta. Values are the means $\pm$ S.E.M. from eight perfusions. Significance of difference from zero: * $P < 0.01$; ** $P < 0.001$.

	Umbilical arterial concentration (μM)	Umbilical venous–arterial concentration difference (μM)	Flux (nmol/min per g)		
			Net release	Influx	Efflux
Glutamine	797 ± 17	26 ± 21	10.3 ± 7.4	—	—
Glutamate	85 ± 18	$17 \pm 4^*$	$6.4 \pm 1.5^*$	$4.1 \pm 0.9^*$	$10.5 \pm 1.1^{**}$

placenta was 122 ± 34 (8) d.p.m./min per g wet wt., compared with an influx of glutamate $1\text{-}^{14}\text{C}$ radioactivity of 7921 ± 1098 (8) d.p.m./min per g. Thus, at a steady state, only $1.5 \pm 0.2\%$ of the C-1 of glutamate entering the placenta from the foetal medium found its way into the C-1 of glutamine transported from the placenta on the foetal side; 98.5% of it was therefore carried into other pathways along with glutamate from other sources.

Discussion

If our perfusion results reflect the situation *in vivo*, they show that, at normal steady-state foetal amino acid concentrations, there is a net outflow of glutamate from the guinea-pig placenta into the foetal circulation. This suggests that the guinea-pig foetus near term is a net user of glutamate, in contrast with the foetal lamb and human, which excrete it (Lemons *et al.*, 1976; Rooth & Nilsson, 1964; Hayashi *et al.*, 1978).

The overall maternal-to-foetal gradient of plasma glutamine concentration in the guinea pig (Table 1) is steeper than for any of the other amino acids (cf. Hill & Young, 1973), but the distribution of this amino acid between the maternal, placental and foetal compartments is qualitatively similar to that of most of the others. Glutamate is different, however, in that it has a low foetal concentration, and only a small overall uphill gradient across the placenta but an exceptionally high trophoblast concentration. Placental glutamate concentration appears also to be particularly high in the human (Phillips *et al.*, 1978), and values uncorrected for blood spaces indicate that the same applies in the sheep (J. Horn, D. Noakes & M. Young, unpublished work). The function of this glutamate is not known. The trophoblast membranes in the guinea pig must either concentrate glutamate to a very great degree or have limited permeability to glutamate accumulating from intracellular synthesis.

Eaton *et al.* (1979) have measured the influx of amino acids into the isolated guinea-pig placenta perfused on both the maternal and foetal sides, using a paired-tracer dilution technique. They found an influx of 5.6 nmol/min per g on the foetal side, very similar to the rate that we have found of about 4 nmol/min per g (Table 2), but they used a concentration 5–10 times greater than in our perfusions. These two results would be consistent if the transport system for glutamate influx were saturated at physiological concentrations. This is known to be the case for glutamate influx across the blood/brain barrier (Pardridge & Oldendorf, 1977). If, in addition, the carrier characteristics for placental efflux were the same as for influx, this would also explain the similarity of efflux rate to influx rate found here, in spite of the very much greater internal

concentration, without the need for an extremely asymmetric, highly energy-consuming, system. Being a strongly hydrophilic anion, glutamate would be expected not to penetrate the membrane readily by passive diffusion.

Although the permeability of the guinea-pig placenta to glutamate may be limited compared with other amino acids (Eaton *et al.*, 1979), our results also reveal a significant degree of glutamate exchange across the membrane on the foetal side of the trophoblast. Our values for influx and thus also for efflux, based on venous–arterial differences of perfusate glutamate radioactivity, are, if anything, underestimated because of possible backflux of label. This error is probably small, however, because of the diluting effect of the very high intracellular concentration.

The similarity of the intracellular specific radioactivities of glutamate and glutamine implies a rapid conversion of glutamate into glutamine in the placenta, but not necessarily net synthesis. A rapid interconversion is equally possible. Some of the C-1 labelling of glutamine may be due to 1-carboxyl exchange in pre-existing glutamine with $^{14}\text{CO}_2$ from glutamate oxidation, but this is likely to be small because negligible radioactivity was found in free CO_2 in the placentas after perfusion. The minimal out-flow of labelled glutamine from the placenta, despite efficient intracellular labelling, suggests that this latter pool of glutamine is separate from glutamine transferred from the mother.

It is not known whether, in the pregnant ewe or human, glutamine transported from the placenta is derived from foetal glutamate, although Holzman *et al.* (1979) suggest that in the sheep this source could represent only a small contribution to the total glutamine flow. However, our evidence, firstly that the net flow of glutamine in the guinea-pig is from the placenta to the foetus, and secondly that, even of the glutamate carbon entering the placenta by exchange from the foetal circulation, only a very small proportion appears in glutamine emerging from it, shows that in this species there is no physiological pathway from foetal glutamate to glutamine released from the placenta.

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