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Partitioning of glutamine synthesised by the isolated perfused human placenta between the maternal and fetal circulations☆

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ABSTRACT

Introduction: Placental glutamine synthesis has been demonstrated in animals and is thought to increase the availability of this metabolically important amino acid to the fetus. Glutamine is of fundamental importance for cellular replication, cellular function and inter-organ nitrogen transfer. The objective of this study was to investigate the role of glutamate/glutamine metabolism by the isolated perfused human placenta in the provision of glutamine to the fetus.
Methods: Glutamate metabolism was investigated in the isolated dually perfused human placental cotyledon. U–^{13}C-glutamate was used to investigate the movement of carbon and ^{15}N-leucine to study movement of amino-nitrogen. Labelled amino acids were perfused via maternal or fetal arteries at defined flow rates. The enrichment and concentration of amino acids in the maternal and fetal veins were measured following 5 h of perfusion.
Results: Glutamate taken up from the maternal and fetal circulations was primarily converted into glutamine the majority of which was released into the maternal circulation. The glutamine transporter SNAT5 was localised to the maternal-facing membrane of the syncytiotrophoblast. Enrichment of ^{13}C or ^{15}N glutamine in placental tissue was lower than in either the maternal or fetal circulation, suggesting metabolic compartmentalisation within the syncytiotrophoblast.
Discussion: Placental glutamine synthesis may help ensure the placenta's ability to supply this amino acid to the fetus does not become limiting to fetal growth. Glutamine synthesis may also influence placental transport of other amino acids, metabolism, nitrogen flux and cellular regulation.
Conclusions: Placental glutamine synthesis may therefore be a central mechanism in ensuring that the human fetus receives adequate nutrition and is able to maintain growth.

1. Introduction

Glutamine and glutamate fulfil important metabolic functions underpinning cellular growth [1,2]. During gestation the growing fetus will require substantial amounts of these amino acids and evidence from animals suggests that placental synthesis of glutamine helps meet fetal demand. However the extent to which placental glutamate and glutamine metabolism occur in human placenta and its importance to fetal nutrition is not well understood.

Studies in the isolated perfused human placenta suggest that glutamate is converted to another amino acid, probably glutamine, as it crosses the placenta [3]. This is in keeping with studies in sheep and other species which show that the placenta synthesises glutamine from glutamate [4–7]. In sheep, production of glutamine has been shown to incorporate nitrogen from branched chain amino acids [8]. Furthermore, in sheep placental–fetal glutamine–glutamate cycling has been demonstrated suggesting that metabolic interaction between the placenta and other fetal organs is important in providing the fetus with the nutrients it requires [4].

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However, despite their importance for provision of amino acids to the fetus these processes are not well characterised in humans and as we have demonstrated previously it cannot be assumed that metabolic processes occurring in other species also occur in humans [9].

Fetal nutrition is not simply about the amount of nutrients reaching the fetus, the balance of nutrients is also important. The relative availability of specific amino acids may determine the pace and the nature of fetal growth and development. Thus the relative composition of amino acids transferred to the fetus may be as important as their quantity. For instance, high rates of cellular replication in the rapidly growing fetus create an enhanced demand for nucleotide synthesis and hence the preferential utilisation of glutamine, an absolute requirement for actively dividing cells [10]. Glutamine is now regarded as a conditionally essential amino acid and fetal development may be one of those times when its provision is essential [11].

Placental synthesis may provide an important source of the conditionally essential amino acid glutamine to the growing fetus. The aim of this study is therefore to investigate the role of placental glutamate–glutamine metabolism in the provision of glutamine to the human fetus.

2. Methods

Human placentas were collected from daytime normal term deliveries from uncomplicated pregnancies at the Princess Anne Hospital in Southampton. Ethical approval from the Southampton and Southwest Hampshire Regional Ethics Committee (REC approval number 308/03/w).

2.1. Perfusions

Placentas were perfused using the methodology of Schneider et al. [12], as adapted in our laboratory [13,14]. Placentas were collected within 30 min of delivery and placed on ice for transport to the laboratory where fetal side perfusion was established within approximately 30 min of collection. The fetal and maternal circulations were perfused with Earle's bicarbonate buffer ((EBB) (mm): 1.8 $CaCl_2$, 0.4 $MgSO_4$, 116.4 NaCl, 5.4 KCl, 26.2 $NaHCO_3$, 0.9 NaH_2PO_4, 5.5 glucose, containing 0.1% bovine serum albumin, and 5000 IU L^{-1} heparin, equilibrated with 95% O_2 – 5% CO_2) at 6 and 14 ml/min, respectively. Perfusion of the fetal circulation was established first, and, if fetal venous outflow was ≥95% of fetal arterial inflow, the maternal arterial perfusion was established 15 min later. Perfusion was performed for 5 h and samples were collected from the maternal and fetal veins every hour. At the end of this perfusion period the maternal and fetal circulations were washed out with EBB for 15 min, the cotyledon trimmed of non-perfused areas (perfused areas become white) and the cotyledon frozen for analysis of intracellular amino acids. In 6 placentas where fetal side recovery did not reach 95% maternal and fetal perfusion was performed for 15–20 min to wash out blood and the tissue was trimmed of non-perfused areas and frozen for analysis of baseline intracellular amino acid levels.

Four experimental protocols for placental perfusion with stable isotope labelled amino acids were performed as described in Table 1. ^{15}N-leucine was added to the maternal circulation as the placenta *in vivo* takes up leucine from the maternal circulation and releases leucine into the fetal circulation. ^{15}N-glutamate was added to both maternal and fetal circulations as the placenta takes up glutamate from both the maternal and fetal circulations.

The U–^{13}C-glutamate (97–99 atoms percent excess), ^{15}N-leucine (98 atoms percent excess) and ^{15}N-glutamate (98 atoms percent excess) were all obtained from Cambridge Isotope Laboratories, MA, USA. Perfusion of ^{13}C-glutamate was performed at near physiological concentrations while the ^{15}N amino acid perfusions were at approximately double physiological concentrations. Perfusion with stable isotope labelled glutamate or leucine was performed for 5 h and samples were collected from the maternal and fetal veins every hour. At the end of this perfusion period the maternal and fetal circulations were washed out with EBB for 15 min and the cotyledon collected for analysis of intracellular amino acids.

2.2. Intracellular amino acids

Frozen cotyledons were pulverised and a sample homogenised in 3 volumes of ice cold EBB. The homogenate was centrifuged and the supernatant stored at −80 °C until analysed by High Pressure Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GCMS) as described below.

2.3. Analysis of amino acids by gas chromatography mass spectrometry

Samples were mixed with an equal volume of 1 mol/L acetic acid, applied to a column containing Dowex 50X8 (H+) cation exchanger (Sigma Aldrich Chemical Co., Gillingham, UK) and washed with 10 volumes of distilled water. The amino acids were eluted in 3 mol/L ammonium hydroxide, dried and derivatised at 80 °C for 1 h with a mixture of equal volumes of N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide and acetonitrile. GCMS measurements were carried out with an Agilent 6890/5973 system (Agilent Technologies, Wokingham, UK) on a BPX5 30m × 250 μm column in electron impact mode. Isotopomer abundances were calculated based on the principles described by Wolfe [15].

2.4. Analysis of amino acids by HPLC

Amino acid concentrations were measured by HPLC with fluorescence detection using nor-valine as an internal standard [16]. Perfusate protein was removed by centrifugation following addition of an equal volume of 6% sulphosalicylic acid containing 100 μmol/L nor-valine (Sigma, UK). Immediately following automated pre-column derivatization of the supernatant with *o*-phthaldialdehyde/3-mercaptopropionic acid at pH 9.2 for 100 s at room temperature a 20 μL sample was injected into the HPLC system (Gilson HPLC, Anachem, Luton, UK). A binary solvent system was used to separate the amino acid derivatives on a Supelcosil C18 (25 × 0.46 cm, 5.0 μm) column (Sigma Aldrich, Gillingham, UK). Solvent A consisted of 100 mmol/L di-sodium hydrogen phosphate adjusted to pH 6.2 with propionic acid, methanol and tetrahydrofuran in ratio 460:40:5; Solvent B consisted of water, methanol, acetonitrile (Fisher Scientific, Loughborough, UK) in ratio 4:3:3. The eluted components were measured by fluorescence (λ_{ex} = 335 nm, λ_{em} = 455 nm). The amino acid:internal standard (nor-valine) peak area ratio was calculated and samples quantified by comparison to the area ratios of known amino standards. The coefficient of variation of the amino acid analysis was 2–5%.

2.5. Analysis of keto acids by HPLC

Concentrations of the keto acids in the maternal and fetal venous outflow and placental tissue homogenates were measured by HPLC using a modified method of Pailla [17]. Briefly, 0.5 ml samples and standards were deproteinated by mixing with an equal volume of 6% w/v sulphosalicylic acid containing 15 μmol/L ketovaleric acid. An aliquot of the supernatant was mixed with o-phenylenediamine in hydrochloric acid, heated at 80 °C, cooled, extracted into ethyl acetate and reduced to dryness under nitrogen. The residue was dissolved in methanol and a 50 μL sample was analysed by HPLC (Beckman System Gold, Beckman–Coulter Ltd., High Wycombe, UK) using a Supelcosil C18 (25 × 0.46 cm, 5.0 μm) column.

The eluted components were measured by fluorescence (λ_{ex} = 350 nm, λ_{em} = 410 nm). The response ratio of each keto acid peak was calculated as the area ratio to the internal standard peak. Concentrations of the individual keto acids were calculated by comparison to the area ratios of known keto acid standards. The limit of detection for the keto acids was 0.08 μmol/L for pyruvate, 0.60 μmol/L for α-ketoisocaproate, 0.03 for α-ketoisovalerate and 0.04 for α-keto-β-methylvalerate.

Table 1
Experimental parameters and uptake of labelled amino acids.

Protocol	Maternal artery flow 14 ml/min	Fetal artery flow 6 ml/min	Perfused tissue weight (g)	Labelled amino acid uptake at 5 h (nmol/min/g)	*n*
1. Fetal ^{13}C-glutamate	Buffer only	U–^{13}C-glutamate 100 μmol/L (≈physiological)	27.5 (6)	13.3 (2.07)	5
2. Maternal ^{15}N-leucine	^{15}N-leucine 146 μmol/L (≈2 × physiological)	Buffer only	39.3 (8.3)	16.7 (2.1)	5
3. Fetal ^{15}N-glutamate	Buffer only	^{15}N-glutamate 200 μmol/L (≈2 × physiological)	38.8 (10.1)	14.8 (4.5)	4
4. Maternal ^{15}N-glutamate	^{15}N-glutamate 200 μmol/L (≈2 × physiological)	Buffer only	34.0 (8.3)	17.5 (3.5)	4

Data are mean (SEM).