Response of placental amino acid transport to gestational age and intrauterine growth retardation

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Fetal N requirements increase with gestational age. During the final trimester, human fetal weight dramatically increases from approximately 1 kg at 27 weeks to 3-5 kg at term, exhibiting a growth rate of 30-35 g/d (Schneider, 1996) and an amino acid requirement of 40-60 mmol/d (Smith, 1986). The ultimate source of amino acid-N for the fetus is the maternal circulation. Any evaluation of fetal amino acid requirements, however, must take into account the requirements and metabolism of maternal amino acids by the placenta. The close interrelationship between placental and fetal amino acid absorption and metabolism is exemplified by the high NH₃ production (Holzman et al. 1977) and glutamine synthetase (EC 6.3.1.2; Diersks-Ventling et al. 1971) activity of the placenta. Placental cytotrophoblasts and syncytiotrophoblasts are known to catabolize large amounts of glutamate by both amino transferase and deamination pathways (Broeder et al. 1994). This metabolism has led to the concept that placenta is the 'functional fetal liver', until the fetus develops these metabolic capacities (Remesar et al. 1980; Battaglia, 1992; Hay, 1995).

The present review describes the expression of amino acid transport proteins that mediate the transfer of cationic amino acids, glutamine and glutamate into and across the placenta. Knowledge of how the normal pattern of placental amino acid transport expression is regulated may facilitate the design of nutritional strategies to address pathological states of fetal development and N metabolism associated with intrauterine growth retardation (IUGR). IUGR, which may be defined as birth weight less than the 10th percentile for gestational age, affects a large number of births annually in the USA (Goldre, 1989). The deleterious effects of IUGR are not limited to the perinatal period, and may present increased risks for diabetes, coronary disease and stroke (Barker, 1994).

In primates and higher rodents, the chorio-allantoic placenta is of the haemochorial type in which maternal blood comes into direct contact with the placental villous surface. In human subjects (haemomonochorial placenta), the barrier to nutrient passage between maternal and fetal circulations is the syncytiotrophoblast (Smith et al. 1992), with its apical (microvillus), maternal-facing, and basal, fetal-facing, plasma membrane subdomains. In rats (haemotrichorial placenta), the apical membrane of the layer II syncytiotrophoblast and the basal membrane of the layer III syncytiotrophoblast of the chorio-allantoic placenta represent the structural and functional barriers to substrate passage between maternal and fetal circulations respectively (Davies & Glasser, 1968; Metz, 1980). The structure of the haemochorial placentas of human subjects and rats differ from the epithelio-chorial and syndesmo-chorial placentas found in the sheep, pig and cow, in which nutrients derived from the maternal circulation must traverse maternal uterine tissue before reaching the fetal circulation (Munro, 1985). These differences in placental structure must be considered when comparing experimental models of placental amino acid absorption.

Mediated amino acid transport by the placenta
Cationic amino acids

Lysine and arginine demonstrate high fetal:maternal concentrations in many species (Yuulevich & Sweiry, 1985). Cationic amino acid transport across the placenta has been documented in both human subjects and rats (Wheeler & Yuulevich, 1989; Furesz et al. 1991, 1995; Eleno et al. 1994; Malandro et al. 1994), and the mRNA that encode proteins associated with systems y+ (cationic amino acid transporter 1; Albritton et al. 1989), b0L, (neutral and basic amino acid transporter; Tate et al. 1992; Bertran et al. 1992), and y+L (heavy chain of the 4F2 surface antigen; Wells et al. 1992; Fei et al. 1995; Novak et al. 1997) have been detected by Northern blot analysis. Na+-independent system y+ activity (White & Christensen, 1982; White, 1985) has been described in human and rat placenta (Furesz et al. 1991, 1995; Malandro et al. 1994). A Na+-dependent system B0L-like activity is present in the rat placental apical membrane, but not in the basal membrane, nor in the apical or basal membranes derived from human placenta (Furesz et al. 1991, 1995; Malandro et al. 1994). Rat and human placentas also contain a leucine-inhibitable Na+-independent cationic amino acid transport activity. Originally thought to be consistent with system b0L (Van Winkle et al. 1988; Furesz et al. 1991;...
Malandro et al. 1994), this activity now appears to be system y^+L (Devenet al. 1992; Eleno et al. 1994; Novak et al. 1997).

Glutamine

Glutamine is the most abundant amino acid in both fetal and adult serum (Cetin et al. 1988; Economides et al. 1989). In the fetus, glutamine is extensively metabolized as a substrate for synthesis of purines and pyrimidines and oxidized for metabolic energy (Windmueller & Spaeth, 1974). Utero-placental uptake of glutamine exceeds delivery to the fetus, indicating that the placenta also metabolizes glutamine (Liechty 1990). In hepatocytes, system N (Kilberg et al. 1980) is responsible for the Na^+-dependent transport of glutamine, histidine and, to a lesser extent, asparagine. Although the presence of a system N-like activity in human microvillous membrane vesicles was reported (Karl et al. 1989), Novak & Beveridge (1997) have suggested that this activity may instead reflect transport by system y^+L. In the latter study, glutamine transport was greater in the maternal-facing (apical) membranes than in fetal-facing (basal) plasma membranes (Novak & Beveridge, 1997).

Glutamate

Although a 'non-essential' amino acid, glutamate plays an important role in placental and fetal metabolism. In vitro, the human placenta is capable of absorbing 49% of the glutamate present in fetal perfusate and is thought to metabolize 80% of that absorbed (Schneider et al. 1979). In an ovine in vivo model, the placental trophoblast has been shown to extract nearly 90% of circulating fetal glutamate in a single circulatory passage (Vaughn et al. 1995), to metabolize rapidly absorbed glutamate by decarboxylation and oxidation (Moore et al. 1994), and to return about 6% of the absorbed glutamate to the fetus as glutamine by the action of placental glutamine synthetase (Battaglia, 1992). This synthesized glutamine, along with the glutamine absorbed from the maternal circulation, is released into the fetal circulation where it is utilized by the fetal liver, and other tissues, as a source of N. In the liver, the primary site of fetal glutamine metabolism, there is rapid conversion of plasma glutamine to glutamate (Vaughn et al. 1995). Collectively, these processes illustrate the importance of glutamate absorption by the placenta and define a fetoplacental 'glutamate-glutamate cycle' (Schneider et al. 1979; Vaughn et al. 1995), which acts to shuttle amino acid-N to the fetus in the form of glutamine and to return glutamate to the placenta. Much of the returned glutamate is oxidized for metabolic fuel, thus sparing glucose for use by the fetus (Moore et al. 1994; Takata et al. 1994). Glutamate also may be used to generate NADPH for placental fatty acid and steroid synthesis (Moore et al. 1994; Vaughn et al. 1995). Another important consequence of placental glutamate uptake from the fetal circulation may be the protection of the fetus from potentially neurotoxic levels of glutamate (Broeder et al. 1994).

Absorption of glutamate is thought to occur by concentrative transport mechanisms, because the milli-molar placental concentrations of anionic amino acids far exceed the micromolar concentrations detected in maternal and fetal blood (Dierks-Venting et al. 1971; Philippa et al. 1978; Schneider et al. 1979). Five complimentary DNA (GLAST1, GLT1, EAAC1, EAAT4, EAAT5) thought to encode proteins capable of Na^+-dependent, D-aspartate-inhibitable glutamate–aspartate transport activity, termed system X^+_AG (Gazzola et al. 1981), have been cloned (Kanai & Hediger, 1992; Pines et al. 1992; Storeck et al. 1992; Fairman et al. 1995; Arzila et al. 1997). The detection of mRNA for all glutamate transport proteins except EAAT5 in human placenta (Arzila et al. 1994; Fairman et al. 1995; Nakayama et al. 1996) suggests that they contribute to the system X^+_AG activity in placental vesicles (Moe & Smith, 1989; Hoeltzli et al. 1990). In the rat, over the last trimester, we have observed an increase in the steady-state mRNA levels for GLAST1, GLT1, EAAC1 and EAAT4, and determined that Na^+-dependent glutamate transport across the apical and basal plasma membrane of labyrinth tissue during the last trimester is primarily a function of system X^+_AG activity, in a manner that was consistent with a differential expression of GLAST1, GLT1 and EAAC1 proteins (Malandro et al. 1996; Matthews et al. 1997).

The previously mentioned studies address only transport processes at the syncytiotrophoblast layer. Maternal–fetal interactions, however, also occur elsewhere within the placenta. Maternal blood directly bathes spongiotrophoblast basophilic cells, glycogen cells and junctional giant cells (Davies & Glasser, 1968). Each of these cell types is thought to have specific functions, perhaps the best described of which is the production of endocrine hormones by the junctional giant cells of the rat and mouse placenta (Lee et al. 1988; Faria et al. 1991; Yamaguchi et al. 1994; Soares et al. 1996). Consistent with these findings, we have observed unique patterns of expression for GLT1, GLAST and EAAC1 proteins, throughout the chorioallantoic rat placenta (Matthews et al. 1997).

Patterns of increased placental amino acid transport with gestational age in the rat

Fetal maternal serum arginine and glutamine values increase with gestational age (Economides et al. 1989; Bernardini et al. 1991), which suggests that the capacity for concentrative transfer of these nutrients by the placenta increases with gestational age. To determine potential mechanisms responsible for this phenomenon, the relative increase in the activity of amino acid transport systems that recognize cationic and neutral substrates was determined in the apical and basal membranes of the rat syncytiotrophoblast (Table 1). The type and membrane-specific distribution of transporter activity at day 20 in the rat are similar to that observed for the term human placenta (Smith et al. 1992; Moe, 1995), except for the presence of Na^+-dependent system B^0,+ in the apical membrane (Furesz et al. 1991, 1995). All amino acid transport systems monitored displayed an increased transport capacity near term (day 20), as compared with the beginning of the third trimester (day 14). The concomitant and marked increase in apical Na^+-

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dependent system $B_{0}^{+}$ and Na$^{+}$-independent systems $y^{+}$ and $y^{+}L$ will facilitate an increased flux of cationic amino acids from the maternal circulation into the placenta. The subsequent high levels of cytosolic cationic amino acids will permit their downhill transfer across the basal membrane into the fetal circulation, thus meeting the increased demands of both tissues for these essential amino acids. Analogously, increases in the apical membrane transport capacity of both systems A and $B_{0}^{+}$, along with the increase in system $y^{+}L$ capacity in the basal membrane, will ensure the potential for an increase in the translocation of glutamine (and other small neutral amino acids) into the placenta and fetus. Collectively, these observations suggest that the increased fetal: maternal serum cationic and neutral amino acid values during the final trimester of gestation, the period of greatest growth by the rat and human placenta and fetus (Schneider, 1996), is facilitated by an increased capacity for amino acid transport across both plasma membranes.

The fetal: maternal serum glutamate value also increases with gestational age (Economides et al. 1989; Bernardini et al. 1991). Recently, we have observed a small increase in system $X_{AG}$ activity from day 14 to day 20 on the basal membrane of rat placenta and, in contrast, a large increase in system $X_{AG}$ activity on the apical membrane (Matthews et al. 1997). When considered in terms of the glucose-sparing effect of placental glutamate absorption and oxidation, this observation suggests that the placenta primarily increases its supply of oxidizable glutamate by increasing its capacity to absorb maternal-derived glutamate.

**Effect of a low-protein diet (intrauterine growth retardation pregnancy) on placental amino acid transport**

Maternal factors that contribute to IUGR in human subjects include pathological conditions, alcohol, cocaine, tobacco, and malnutrition (Sastry et al. 1989). In IUGR pregnancies of unknown aetiology, the uptake of lysine by placental slices (Yamaguchi et al. 1978) and the rate of system A-mediated 2-amino isobutyric acid transport (Dicke & Henderson, 1988) was diminished in placental tissue. Although these in vitro investigations support the in vivo observation of impaired uptake of 2-amino isobutyric acid from the maternal to the fetal circulation (Sybulski & Tremblay, 1967), fetal serum levels of alanine, a primary system A substrate, are slightly higher in small-for-gestational-age fetuses than in appropriate-for-gestational-age fetuses (Economides et al. 1989). Diminished placental blood flow in human subjects is often associated with IUGR (Bracero et al. 1989); therefore, although protein malnutrition results in smaller fetuses with poor developmental outcomes (Rosso, 1980), the relative contribution of diminished utero-placental blood flow vs. specific effects on placental amino acid transport mechanisms has not been established.

In rats, maternal malnutrition-induced IUGR has been associated with (a) reduced blood flow in the placenta (Rosso & Kava, 1980), (b) reduced rates of amino acid transfer to the fetal circulation (Rosso, 1975), and (c) reduced fetal and placental weights (Rosso, 1980). Alterations in the coordinated expression of transporters on the apical and basal domains of the placental syncytiotrophoblast may critically reduce the trans-epithelial flux of amino acids across the placenta and/or through the metabolic cycles that exist between the placenta and fetus. The effects of low-protein-diet-induced IUGR on the capacity of placental amino acid transport systems has been examined using a rat model (Table 2; Malandro et al. 1996). System A transport capacity was decreased by 55% on the apical plasma membrane and 50% on the basal plasma membrane subdomains of labyrinth trophoblasts of placentas isolated from IUGR dams. The capacity for system ASC-mediated transport of neutral amino acids, and system $B_{0}^{+}$ for neutral and cationic amino acids, was not altered. However, neutral and cationic amino acid transport mediated by system $y^{+}L$ (Novak et al. 1997) was reduced by approximately 70% on the basal membrane. System $y^{+L}$ activity also was reduced (20%) on the apical membrane, which was consistent with the concomitant decrease in total steady-state cationic amino acid transporter mRNA isolated from the whole chorio-allantoic placenta. In basal, but not apical, plasma membranes, Na$^{+}$-dependent glutamate uptake and EAAC1 mRNA were decreased (about 50%) in placentas isolated from IUGR dams (Malandro et al. 1996).

There was a reduction in fetal serum glutamate concentration and system $X_{AG}$ activity in the basal placental membrane in the IUGR pregnancies (Malandro et al. 1996). In contrast, maternal serum glutamate concentrations and the activity of system $X_{AG}$ in the apical membrane was unchanged (Malandro et al. 1996). These results may be indicative of substrate-controlled transporter localization. The specific mechanisms that sense serum amino acid availability and transduce this information into differential plasma membrane subdomain expression in the placenta have not been described. Amino acid-dependent

Table 1. Development of cationic and neutral amino acid transport capacity in rat placenta*

<table>
<thead>
<tr>
<th>Gestational age (d)</th>
<th>Apical membrane</th>
<th>Basal membrane</th>
<th>Reference</th>
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<td>$B_{0}^{+}$</td>
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<td>$y^{+}$</td>
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<tr>
<td>$y^{+}L$</td>
<td>+/−</td>
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<td>+</td>
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* $A$, Na$^{+}$-dependent uptake of 2-(methylamino)isobutyric acid; $B_{0}^{+}$, Na$^{+}$-dependent uptake of arginine; $y^{+}$, leucine-resistant Na$^{+}$-independent arginine uptake; $y^{+}L$, leucine-inhibitable Na$^{+}$-independent arginine uptake.

*The absence (−) or presence (+) of transport activity measured in the indicated day of gestation of rat placenta. The presence of transport activity, within a transporter system, is qualitatively scaled from − to ++.
changes in gene expression have been documented for a number of proteins, including amino acid transporters (Shay et al. 1990; Hyatt et al. 1997), although hormone signalling is also a likely regulator of amino acid transporters in IUGR pregnancies (Warshaw, 1990).

**Hormones and growth factors that are likely to mediate the effects on intrauterine growth retardation on placental amino acid transport**

The development of null-mutation mouse models has allowed investigation of the potential role of specific hormones and growth factors in IUGR-mediated alteration of normal placental and fetal development. Deficiency of maternal epididymal growth factor (EGF) causes severe IUGR in rats (Kamei et al. 1993), and null mutation of the EGF receptor causes placental disruption in homozygous fetuses (Sibilia & Wagner, 1995; Threadgill et al. 1993). Conversely, administration of EGF to pregnant rats has little effect on fetal size (Ali et al. 1990; Jansson & Skarland, 1990), and serum levels of EGF have not been clearly associated with IUGR. EGF is not produced by the fetus through most of gestation (Raaberg et al. 1988; Snead et al. 1989); knockout of transforming growth factor α, which is thought to interact with the EGF receptor during gestation, has little effect on fetal development (Mann et al. 1993).

In contrast, growth hormone (GH) and GH receptor are present in the fetus (Strosser & Mialhe, 1975; Garcia-Aragon et al. 1992; Gluckman et al. 1992), and decreased fetal levels of GH in human subjects may cause mild IUGR (Gluckman et al. 1992). However, infusion of rat dams with an antibody against GH-releasing hormone increases fetal weights, in association with elevated insulin-like growth factor (IGF)-1 and -2 levels (Spatola et al. 1991). Further confounding our understanding of the relationship between IUGR and GH are the observations that high doses of supplemental GH given to rat dams fed on an energy-restricted diet were associated with reduced maternal wasting and exacerbated fetal growth retardation (Chiang & Nicoll, 1991), and that transgenic mice, which constitutively secrete a large amount of GH, produce growth-retarded fetuses (Naar et al. 1991).

Insulin, also, is a critical fetal growth factor and fetal pancreatectomy causes profound growth retardation (Fowden et al. 1989). Null mutation of the fetal insulin receptor substrate-1 gene, involving in signalling from both the insulin receptor and IGF-1 receptor, produced profound IUGR (Araki et al. 1994), as did homozygous nonsense mutation of the human insulin receptor (Krook et al. 1993). Surprisingly, null mutation of the mouse insulin receptor produces little or no effect on intrauterine growth, which suggests that the growth-promoting effects of insulin in the mouse are mediated, at least in part, through the IGF-1 receptor (Accili et al. 1996; Joshi et al. 1996). Therefore, IGF-1, IGF-2 and the IGF-1 receptor must be considered as potential effectors of IUGR, as the mRNA for all three are expressed in the developing rat placenta (Pescovitz et al. 1991; Zhou & Bondy, 1992; Redline et al. 1993).

IGF-1 mRNA expression peaks within the rat placenta at approximately day 10 of gestation, and is either absent or in low quantities after day 14 (Pescovitz et al. 1991; Redline et al. 1993). Placental IGF-2 expression begins at approximately 10 d of gestation, rising to a maximum and stable level by day 15 (Pescovitz et al. 1991; Zhou & Bondy, 1992; Redline et al. 1993). Whereas maternal serum IGF-1 levels peak at mid-gestation and then fall by approximately 50%, maternal serum IGF-2 levels are low or undetectable throughout gestation (Gargasky et al. 1990). Fetal IGF-1 concentrations are correlated with fetal growth in the human subject (Lassarre et al. 1991; Leger et al. 1996).

Most *in vivo* studies have involved the administration of pharmacological doses of the hormone or growth factor to the mother; therefore, effects of fetal secretion are largely unknown. In addition, it is difficult to rule out secondary effects of the infused hormone or growth factor; for example, the regulatory effects of GH or EGF on IGF-1 production (Chernausek et al. 1991; Rotwein et al. 1993). Null mutations of growth factors or hormones and receptors of these ligands help to clarify these issues. Null mutations of IGF-1, IGF-2, and IGF-1 receptor (which mediates fetal effects of IGF-1 and, to a significant degree, IGF-2) result in severe IUGR (DeChiara et al. 1990; Baker et al. 1993; Liu et al. 1993; Lopez et al. 1996). IGF-2-knockout mice also have small placentas (DeChiara et al. 1990). Using null-mutation mouse models, our initial investigations indicate that IGF-2 and IGF-1 receptor differentially affect the expression profiles of specific anionic amino acid transporters through transcription and post-translation events (DA Novak, JC Matthews, MJ Beveridge, A Efstratiadis, E Dialynas, A Bartke and MS Kilberg, unpublished results).

In summary, the study of placental amino acid transport regulation is in its infancy. Some of this information is contradictory. The results of whole-tissue flux studies, using stable isotopes, has generated new theories of maternal–placental–fetal amino acid metabolism. Using newly-developed molecular reagents, several laboratories

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<th>Transport system...</th>
<th>A</th>
<th>ASC</th>
<th>B(^{\text{y}+})</th>
<th>y(^L)</th>
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<th>X(_{\text{AG}})</th>
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<tr>
<td>Apical</td>
<td>Decreased(^\ast)</td>
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A, Na\(^{\text{+}}\)-dependent uptake of 2-(methylamino)isobutyric acid; ASC, Na\(^{\text{+}}\)-dependent uptake of serine in the presence of 2-(methylamino)isobutyric acid; B\(^{\text{y}+}\), Na\(^{\text{+}}\)-dependent uptake of arginine; y\(^L\), leucine-inhibitable Na\(^{\text{+}}\)-independent arginine uptake; y\(^\ast\), leucine-resistant Na\(^{\text{+}}\)-independent arginine uptake; X\(_{\text{AG}}\), Na\(^{\text{+}}\)-dependent uptake of glutamate.

There was a significant difference when compared with control, \(\ast P < 0.05\).
have begun to determine the cellular distribution and relative abundance of amino acid transporter mRNA and the proteins responsible for these activities in the placenta. Likewise, studies using null-mutation and transgenic mice to mimic IUGR and other pathological states have begun to describe the hormonal or growth factor control over expression of amino acid transport in placental tissue during normal and disease states.

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References


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Nutrient regulation of gene expression


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