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Placental Amino Acid Transport Systems and Fetal Growth Restriction – A Workshop Report

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INTRODUCTION

Two circulations perfuse the placental barrier, the maternal or uterine on one side and the fetal or umbilical on the other. This barrier consists of two specific membranes, the maternal facing, microvillous (MVM) or apical membrane and the fetal facing or basal membrane (BM). As human gestation advances the surface area of the microvillous membrane increases. Though late in pregnancy, this increase is not of comparable rate to the growth rate of the fetus, supporting the concept that during later pregnancy the increasing physiological needs of the fetus are achieved by multiple functional changes in the total placental exchange capacity. One component of this capacity is amino acid transport. Both placental membranes have a range of active energy dependent amino acid transport systems [1]. These systems act in a coordinated manner to transport amino acids from both circulations into the trophoblast, as well as out of the trophoblast, into circulation. In addition to surface area, many other factors also influence overall activity. Maternal circulating concentrations of amino acids also play a major role in regulating umbilical uptake, where the transport of any given amino acid from placenta to fetus is considered a function of both its own maternal concentration and the maternal concentration of inhibitory amino acids [2]. Additionally, electrical gradients exist over the placenta, across which transport systems operate, indicative of the energy requirement of these systems [3]. It is important to remember that the trophoblast acts not only as a conduit for amino acids between the two circulations, but also has a high metabolic rate, utilizing amino acids for its own metabolic functions, which include hormone and steroid production as well as waste removal from fetal circulation [4].

To understand the overall functional capacity of the placental barrier, attempts to consider all of the above components need to be made, especially when investigating how adverse situations may impact upon the developmental and functional capacity of the placenta. Specifically, in the situation of fetal growth restriction (FGR), there is in vivo and in vitro data to demonstrate significant changes in the placental surface area/permeability constants [5], its amino acid transport activity in vitro [6–8], and in vivo [9–12]. The FGR placenta has decreased total villous surface area, indicating that morphometric changes in vascularization may contribute to the overall reduction in placental diffusional transport capacity. Thus, both decreased surface area for diffusional exchange for compounds such as oxygen and reductions in the number of specific nutrient transporters and their activities may contribute to a global reduction in nutrient transport in FGR pregnancies.

The purpose of this workshop was to deal with a range of work representing aspects of amino acid transport systems, including their locations, their substrates and their activities, from both in vitro and in vivo settings. However, rather than act as a forum for mini reviews, this workshop was designed for presenters to put forward experimental inconsistencies in some of these aspects for general discussion and input from the gathered group. The aim, at the end of the workshop, was to have a consensus of where specific placenta amino acid transporters may be located, what their substrates and their individual activities may be, how they might interact with one

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another and finally how these specifics may be altered in the situation of FGR.

HUMAN INFUSION STUDIES – DIFFERENCES IN CERTAIN AMINO ACID UPTAKES (A. M. MARCONI)

In pregnancy, for the majority of amino acids, concentrations are higher in fetal circulation than in maternal circulation. In FGR, the maternal concentrations of most essential amino acids are significantly higher than in appropriate-for-gestational-age pregnancies, closer to fetal concentrations. This observation, coupled with lower fetal amino acid concentrations in FGR, trending towards maternal concentrations, leads to significantly lower fetal–maternal differences [13]. These significantly lower fetal–maternal concentration differences occur independently of the degree of severity [13]. It has been of clinical interest to see if in human pregnancies complicated by FGR, the maternal intravenous infusion of amino acids can increase fetal amino acid concentrations and umbilical uptake. In a maternal infusion experiment using a solution of amino acids, the umbilical vein concentrations of valine, methionine, isoleucine, leucine, phenylalanine, arginine, serine, glycine, and proline were elevated [11]. Umbilical venoarterial differences of amino acid per mole of oxygen, as a in vivo measure of amino acid uptake, for leucine, isoleucine, methionine, arginine, glycine, serine, and proline were elevated, but not for lysine, histidine, threonine, valine, and phenylalanine [11]. These data suggest that the umbilical uptake of several of the essential amino acids may be reduced in FGR pregnancies compared with AGA pregnancies. Indeed, it has been demonstrated that the fetomaternal enrichment ratios of leucine and phenylalanine are significantly reduced in vivo in FGR pregnancies [10]. Additionally these human data highlight the importance of amino acid competition effects. In pregnant sheep studies, the transport of any given amino acid from placenta to fetus is a function of both its own maternal concentration and the maternal concentration of inhibitory amino acids [2]. These effects may also exist in the human placenta and if so may explain why in the human FGR pregnancy, leucine and say arginine uptake appear to be enhanced at the expense of valine, lysine, histidine and other essential amino acids. Discussion of these data focused around the interactions of the apical y^+ and the basal y^+L transport systems and their transport properties, and possible inhibition characteristics, highlighting the need to maybe redesign the amino acid infusion to take into account possible transport system and amino acid inhibitory effects. The infusate should aim at improving fetal nutrition to produce percent concentration increases in maternal concentrations that either are of similar value or are biased in favor of the amino acids with weak inhibitory power. Finally, the discussion also dealt with the heterogeneous nature of FGR and the need to carefully characterize FGR groups in considering certain treatments, especially as there have been conflicting reports in the literature concerning the value of supplementation. Attention to uniformly classifying FGR groups is needed as different placental function, based on the severity of FGR, may result in different amino acid transport function and ultimate umbilical supply to the developing fetus.

THE VALUE OF ISOFORM IDENTIFICATION – QUESTIONS OF THE ROLES OF INDIVIDUAL ISOFORMS WITHIN THE TROPHOBLAST (C. H. SMITH)

In isolated placental membranes, system y^+ activity is present in both MVM and BM, though the fraction of y^+ uptake is greater in MVM [14]. Activity in isolated BM is essentially completely inhibited by neutral amino acids and sodium, which suggests that it is likely to be inactive in utero. The cloning of trophoblast hCAT-1 and hCAT-2B, the components of system y^+ activity, has allowed molecular and cellular investigation of mechanisms responsible for these membrane differences [15]. Expressed in *Xenopus* oocytes, hCAT-1 is completely inhibited by increasing concentrations of neutral amino acids such as alanine, whereas hCAT-2B activity is only partially inhibited. These results suggest that hCAT-1 and -2B may be primarily expressed in trophoblast BM and MVM, respectively [16]. Transfection studies using the MDCK epithelial cell model support this concept of differential distribution of the isoforms between the two epithelial membranes [17]. In summary, these investigations highlight the need to take into account isoform characteristics and expression in understanding overall transport system function in the two trophoblast surface membranes. By way of conclusion discussion was expanded to consideration of the protein chain location, function and regulation of some of the other documented placental amino acid systems, A, L, ASC, y^+L and $b^{0,+}$.

REGULATION OF PLACENTAL AMINO ACID TRANSPORT SYSTEMS IN FGR – RESPONSE TO ADAPTATION FAILURE OR INSULT (J. D. GLAZIER)

The transport systems A, L and y^+L play a major role in transporting amino acids into and out of the placenta. Each system has defined protein associations, monomeric or heterodimeric, different substrates and sodium requirements [4,18]. Monomeric systems differ from heterodimeric systems in that monomeric systems are functional as a single protein in the cell membrane, while a heterodimeric system is one that to be functional requires the co-expression of a light chain and a heavy chain. Currently there are two well documented heavy side chains, the 4F2HC and the rBAT [19]. System A is a monomeric system, and consists of three different isoforms SNAT 1 (ATA-1), SNAT 2 (ATA-2) and SNAT 4 (ATA-3), in both the human and rat placenta. Each isoform of system A displays different affinities for the short chain neutral amino acids, including alanine, glycine and glutamine and requires sodium for its activity. In comparison, system L is a hetero-

dimeric system and has two light chains associated with its activity, LAT-1 and LAT-2. These proteins, in concert with the heavy chain, 4F2HC, have a broad specificity for neutral amino acids with bulky side chains including the essential amino acids, leucine, isoleucine, tryptophan, and phenylalanine. Unlike system A, sodium is not required for their activity. The y^+L also is a heterodimeric system transporting both neutral and cationic amino acids through the expression of one of its light chains, y^+LAT-1 or y^+LAT-2. It is interesting to note that while sodium is required for the transport of the neutral amino acids, it is not required for the cationic amino acids arginine and lysine. These three systems exist on both membranes and have been studied in a range of unfavorable situations, including FGR [6,7,20] and situations of hypoxia [21], where reductions in activity are reported. One of the interesting observations of these studies is that the major deficits of activity in FGR is not a change in affinity, but reductions in the V_{max} of these systems of between 30 and 60% [6,7]. Possible explanations of this reduced activity include induced changes in mRNA transcription, translational efficiency, possible alterations in the expression of protein particularly in aspects of trafficking and recruitment from intracellular compartment. Finally the question of a possible decreased catalytic turnover might also be considered where there may be post-translational modifications and the existence of as yet unreported polymorphisms.

AMINO ACID TRANSPORT SYSTEM FUNCTION – DIFFERENCES BETWEEN IN VIVO AND IN VITRO OBSERVATIONS (D. A. NOVAK)

The amino acid transport system A is found in both the apical and basal membranes of the trophoblast, and as mentioned earlier represents a monomeric transport system, comprised of three isoforms, SNAT-1, SNAT-2 and SNAT-4. When pregnant rats are fed a low-protein (5% casein), isocaloric diet when compared with dams pair-fed a control (20% casein) diet, diminished placental/fetal growth is observed. Additionally, a significant reduction in nutrient transfer to the fetus is suggested as the fetomaternal serum amino acid ratios are significantly reduced [22]. Supporting this observation, in vitro apical transport is reduced by approximately 50% and basal membrane transport by 70%, and the expression of SNAT-2 mRNA is diminished by 60% as compared to controls (unpublished data). In other experiments, where a slightly less restrictive diet is fed (8% casein), system A activity is still impaired. This impairment is measured in vivo as a reduction in the fetal accumulation of MeAIB (a system specific substrate, particularly for SNAT-2), following a maternal injection near term. Interestingly, the permeability of the placenta appears to be unaltered as the fetal/placental and fetal/serum inulin ratios are unaltered, suggesting that these observed changes are related to changes in transport system number and or activity. Unexpectedly, quantitative PCR results from these animals show an almost two-fold increase in SNAT-1 and SNAT-2 mRNA and no change in SNAT-4. However, when apical vesicle uptake studies are conducted, the 8% protein placenta have significantly reduced sodium dependent activity by approximately 30%, in line with the original in vivo observations of a reduced transport to the fetus. These studies together with the 5% protein diet experiments, demonstrate diminished placental/fetal growth and apical membrane system A mediated uptake, despite a difference in the level of protein restriction. However, SNAT-2 mRNA expression is diminished in the 5% group, but enhanced in the 8% group, despite a similar phenotype. Discussion in the workshop moved to possible reasons for this difference covering such possibilities as the interaction of starvation regulation genes analogous to Gln3 [23], regulating members of the system A family and the concept that regulation of system A isoforms may be stress regulated, though with different responses between the three isoforms.

FGR RELATED CHANGES IN TRANSPORTER SYSTEMS – SEVERITY OF FGR (T. R. H. REGNAULT)

One of the recent observations of studies in FGR is the heterogeneous nature of FGR. This is particularly important when considering possible treatments, as it is becoming apparent that not one magic bullet will assist all FGR outcomes and that treatments need to be designed for specifically tight groupings of FGR. In the ovine hyperthermic stress induced model of FGR, a sub-set of FGR has been observed [12]. This group of animals has been exposed to environmental stress, that normally reduces placental and fetal weights by 40—60% [24], though for these animals, while placenta and fetal weights are reduced, they are only reduced by approximately 25%. In these moderately FGR (mFGR) animals, umbilical oxygen, glucose and the essential amino acid uptakes are not different from control animals, whilst the severe FGR (sFGR) animals have significant reductions for all these substrates. Possible explanations for this difference appear in two areas. Firstly, the placental diffusional exchange capacity of the sFGR fetuses is significantly reduced, compared to control and mFGR fetuses, suggesting changes in placental permeability and surface area, acting as an impediment to control value uptakes per unit of fetal weight. Secondly, when examining the mRNA expression of system L light chain components, LAT-1 and LAT-2, it is observed that while concentrations in sFGR are not different from control placentae, mFGR concentrations are significantly elevated. This suggests that in the sFGR placenta there may be an up-regulation of specific transport systems, maybe to attempt overcome the reduction in calculated diffusional exchange capacity, though this is unable to reverse the total decreased amino acid uptake observed in these animals. The other fact highlighted is that in the mFGR placenta, there may be a successful up-regulation of transport system mRNA, to maintain umbilical amino acid uptake. Further studies considering histological analysis of these placentae, together

with possible in vitro placenta functional studies will greatly help the interpretation of these data, and assist in our understanding of the possible different placental outcomes of FGR pregnancies.

PLACENTAL SYSTEM A AND FGR – IS DOWN-REGULATION A ROBUST MARKER FOR FGR? (T. JANSSON)

Clinically, the identification of babies subjected to intrauterine under-nutrition is critical since FGR is associated with perinatal complications as well as adult disease. Birth weight is a relatively poor indicator of the adequacy of intrauterine nutrition and defining FGR on birth weight only is unreliable. Although the introduction of repeated umbilical Doppler flow measurements and intrauterine fetal weight estimates have improved the diagnosis of FGR the need for additional diagnostic tools remains. The recent demonstration that FGR is characterized by specific alterations in a number of placental nutrient transporters may offer a novel approach for diagnosis. In particular, decreased activity of system A has been suggested to represent a marker for FGR since system A activity has been shown to be down-regulated in microvillous plasma membranes isolated from FGR placentas in several studies [7,25,26]. However, some observations suggest a rather complex relationship between placental system A activity and fetal growth. First, within the normal range of birth weights MVM system A activity is inversely correlated to birth weight, i.e., a smaller baby has a higher placental system A activity [27]. Second, in the study of Jansson et al. [26] MVM system A was found to be reduced only in preterm FGR placentas whereas term FGR was characterized by unaltered transport activity. Third, MVM system A activity in accelerated fetal growth in association to diabetes has been reported to be either increased [28] or decreased [29]. In line with this latter study, preliminary data were presented to suggest that also in certain animal models down-regulation of placental system A may be a marker for accelerated fetal growth rather than FGR. The system A transporter is highly regulated. It is suggested that placental system A activity in vivo is affected by factors like maternal nutrition, ethnicity, smoking, diabetes control and body-mass-index that may interact in a complex fashion resulting in a placental system A activity that is not always correlated with fetal growth. It is concluded that although measurements of placental transporter activity and expression may represent a sensitive marker for FGR it is possible that other transport systems in addition to system A have to be assessed.

CONCLUSIONS

Each presenter put forward experimental inconsistencies in aspects of placental amino acid transport, with an emphasis upon transport in FGR, for general discussion and input from the gathered group. While the time permitted did not allow for in depth consensuses to be reached on many of the discussed topics, the workshop did provide a unique opportunity for investigators to be reminded of the types and complexities of the different systems studied in this field of research. As a result of these discussions it was agreed to establish a Placental Amino Acid Transport Systems Web page, where information and ideas can be publicly available.

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