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Placental System A Amino Acid Transport is Reduced in Pregnancies With Small For Gestational Age (SGA) Infants but Not in Preeclampsia with SGA Infants

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Abstract

Preeclampsia and intrauterine growth restriction (IUGR) are both associated with abnormal remodeling of maternal spiral arteries perfusing the placental site. This would be expected to be associated with reduced fetal growth, yet only one third of infants of mothers with preeclampsia are growth restricted. Infants with IUGR have decreased concentrations of amino acids in their blood and system A amino acid transporter activity is reduced in their placentas. Since infants of preeclamptic pregnancies have increased circulating amino acids, we tested system A amino acid transport activity of placental villous fragments from pregnancies with small for gestational age (SGA) infants with and without maternal preeclampsia and from uncomplicated and preeclamptic pregnancies with normal sized infants. We confirm the reduced uptake of amino acids in SGA pregnancies without preeclampsia but report that placental amino acid uptake of SGA infants with maternal preeclampsia is not reduced and is identical to uptake by normal and preeclamptic pregnancies with normal weight infants.

Keywords

Placental transport; Amino acid; Preeclampsia; Intrauterine growth restriction

1. Introduction

Preeclampsia and intrauterine growth restriction (IUGR) share a similar abnormality of placental implantation [1]. In both conditions there is failed remodeling of the uterine spiral arteries supplying the placental site. Whereas in normal pregnancy the smooth muscle and elastic lamina are lost, accompanied by a striking increase in arterial luminal diameter, this adaptation is diminished or absent in preeclampsia and IUGR. Despite this alteration that would be predicted to strikingly reduce placental perfusion and nutrient delivery to the fetus, only one third of infants from preeclamptic pregnancies are growth restricted [2]. Examination of

the placenta including quantitative morphometric analysis indicates striking differences in villous development and fetoplacental angiogenesis between the placentas of IUGR infants and normal weight infants, whether the IUGR is accompanied by preeclampsia or not [3-7]. Placentas from preeclamptic pregnancies appear very similar to normal placentas with the exception of placentas from early onset preeclamptic pregnancies and preeclamptic pregnancies complicated by IUGR. The latter cases resemble IUGR without preeclampsia [7].

Members of our group previously reported that amino acid concentrations, established by others to be reduced in the blood of IUGR infants from pregnancies without preeclampsia [8-10], are increased in the blood of infants of preeclamptic pregnancies including those complicated by IUGR [11]. Amino acids are not only important for protein synthesis in the fetus but are estimated to supply 40% of fetal energy needs [12]. The system A amino acid transporter, also known as the Na⁺-dependent neutral amino acid transporter (SNAT), mediates transport of small neutral amino acids such as alanine, serine, glutamine and glycine in a Na⁺-coupled fashion [13]. System A transporter activity is reduced in the placenta of IUGR pregnancies without preeclampsia [13,14]. We asked whether amino acid transfer would be affected similarly in placentas from preeclamptic pregnancies. We examined system A amino acid transporter activity in placental villous fragments from uncomplicated pregnancies ($n = 9$), otherwise uncomplicated pregnancies with small for gestational age (SGA) infants ($n = 5$), and preeclamptic pregnancies with ($n = 5$) and without SGA ($n = 10$). Our goal was to test the hypothesis that there are fundamental differences in IUGR pregnancies with and without preeclampsia. This would be supported by normal placental uptake in placentas from preeclamptic pregnancies even with SGA infants.

2. Methods

Women were recruited as part of an ongoing study of preeclampsia approved by the University of Pittsburgh Institutional Review Board. Smokers were excluded. Preeclampsia was defined as gestational hypertension and proteinuria after 20 weeks gestation. Gestational hypertension was a systolic blood pressure ≥ 140 mmHg or diastolic ≥ 90 mmHg. Proteinuria was defined as $\geq 1+$ on a catheterized urine specimen or ≥ 2 on a voided specimen, or >300 mg of protein on a 24 hour urine collection, or a random urinary protein: creatinine ratio of >0.3 [15]. Our estimate of IUGR pregnancies was SGA defined as ≤ 10 th birth weight centile for gestational age adjusted for race and sex. Babies were considered to have asymmetric growth when the growth percentiles for baby weight were significantly less than for baby length and/or head circumference. In recognition of the potential discrepancy between SGA (which does not preclude constitutionally small fetuses) and IUGR (the failure of an infant to achieve its genetic growth potential), we use SGA for clinical data and IUGR when the concept of growth restriction is addressed.

All system A amino acid uptake measurements were performed using Tyrode's buffer as previously described [14]. ¹⁴C-Methylaminoisobutyric Acid (MeAIB; 50.5 mCi/mmol) was from NEN Life Science Products (Boston, MA).

Fresh placental tissue was obtained within 10 minutes after delivery. Biopsies were collected from the placenta from the central part of cotyledons that were free of infarcts at a site midway between the cord insertion site and the edge of the placenta.

Placental system A activities were measured as previously described [16]. Villous fragments (approximately 3 mm³) were pre-incubated in 1 ml volumes of Tyrode's: DMEM buffer for 60 minutes at 37°C. Subsequently, uptake of C¹⁴-MeAIB was measured in sodium-containing or sodium-free (for non-specific uptake) Tyrode's buffer containing C¹⁴-MeAIB (1.7 μ mol/L,

0.05 $\mu\text{Ci/ml}$) for 20–240 minutes at 37°C. The uptake of $\text{C}^{14}\text{-MeAIB}$ was stopped by washing villous fragments in ice-cooled sodium-free Tyrode's buffer two times for 20 seconds each. Total pmol of $\text{C}^{14}\text{-MeAIB}$ accumulated was normalized to protein content. System A activity was calculated by subtracting the uptake in sodium-free media from the uptake in sodium-containing media and expressed as pmol $\text{C}^{14}\text{-MeAIB/mg}$ protein. Assays were completed within 6 hours after delivery to decrease loss of functional integrity of the villous trophoblast.

The accumulation of lactate dehydrogenase into the media was measured after assay completion (LDH diagnostic from Sigma: St. Louis, MO), and the cell damage of villous fragment was estimated to compare cell viability among these four groups. LDH data were expressed as percentage of maximum releasable LDH upon cell lysis.

Data are presented as mean and standard error of the mean (SEM). Demographic data were compared by one factor analysis of variance with Tukey–Kramer post-hoc analysis or by chi square analysis for categorical data. Uptake was assessed by repeated measures analysis of variance. Post hoc analysis was done by: (1) reanalysis with pair wise comparison of individual diagnostic groups; and (2) reanalysis after exclusion of individual diagnostic groups. Significance was accepted at $p < 0.05$. Data were analyzed using JMP software, SAS Institute Incorporated (Cary, North Carolina).

3. Results

The demographic data (Table 1) indicated the expected differences in blood pressure between preeclamptic and other pregnancies. The SGA infants with or without preeclampsia were not different in birth weight or birth weight centile but were significantly smaller than the infants of preeclamptic women without SGA infants and infants from uncomplicated pregnancies. Gestational age at delivery of the SGA infants from preeclamptic pregnancies was not different than that of SGA infants from non-preeclamptic pregnancies but was less than for other infants. Other demographic data did not differ by group.

System A amino acid transport activity assessed by the uptake of $\text{C}^{14}\text{-MeAIB}$ was significantly lower in explants of placentas from SGA infants whose mothers did not have preeclampsia (Fig. 1) [13,14]. Analysis indicated a significant effect of time ($p < 0.001$) and diagnostic group ($p = 0.005$). Interestingly, the uptake in placental fragments from preeclamptic pregnancies with or without SGA infants was not different than the uptake by placentas from normal pregnancies. There was no difference by diagnosis if the SGA without preeclampsia group was excluded from the analysis ($p = 0.36$). Consistent with this, post hoc analysis revealed no significant differences between amino acid uptake of placentas from normal pregnancies, preeclamptic pregnancies without SGA or preeclamptic pregnancies with SGA infants ($p \geq 0.3$). Uptake by placentas from SGA pregnancies without preeclampsia was significantly reduced compared to all other groups ($p < 0.001$).

We examined LDH release to ascertain tissue viability. The concentration of LDH in the media after the assay was determined for 4–6 samples from each diagnostic group. Results were not different among the diagnostic groups (percent of maximum releasable: Control = 0.23 ± 0.01 , SGA = 0.24 ± 0.01 , Preeclampsia without SGA = 0.25 ± 0.02 , Preeclampsia with SGA = 0.24 ± 0.01 ; $p = 0.56$).

4. Discussion

In this study we report that system A amino acid transporter activity is not reduced in placentas of SGA infants from preeclamptic pregnancies. We confirm the reduced system A transporter activity previously reported with IUGR pregnancies without preeclampsia [13,14]. Although most prior studies of IUGR without preeclampsia have been with isolated microvillous

membrane vesicles, we confirm the same findings in intact placental fragments. In studies of protein restricted rats treated with a regimen that leads to IUGR, system A-mediated amino acid uptake was similarly reduced and preceded reduction in fetal weight suggesting that the change is causally relevant [17].

The finding of uptake in preeclampsia with normally grown infants equivalent to uptake in uncomplicated pregnancies with normally grown infants is compatible with the relatively normal morphology and absorptive surfaces in these placentas reported previously [7]. Morphological difference between placentas from uncomplicated pregnancies and preeclamptic pregnancies with normally grown fetuses delivered near term (as was the case with the patients in this study) are at most minor. However, the findings of normal amino acid uptake even in placentas of SGA fetuses of preeclamptic pregnancies would not have been predicted by morphological studies. In these studies, abnormalities in placentas from preeclamptic pregnancies with growth restricted infants closely resemble those of growth-restricted fetuses whose mothers do not have preeclampsia [3-7]. In pregnancies complicated by growth restriction with or without preeclampsia obtained after 34 weeks of gestation, absorptive surface and placental volume are similarly reduced below those of uncomplicated pregnancies. Although not statistically significantly different, the pregnancies with SGA infants and preeclampsia in our study delivered on average two weeks earlier than pregnancies with SGA alone. This is unlikely to explain differences since even with early onset preeclampsia, absorptive surfaces and placental volumes were similar to those of placentas from pregnancies with growth restriction without preeclampsia and less than in normal pregnancy [7]. Thus, the striking differences in system A amino acid transporter activity, normal in preeclamptic pregnancies even when associated with SGA, does not appear to be explained by currently available placental morphological studies including quantitative morpholometric studies. The reduced system A amino acid uptake in placentas from growth-restricted fetuses (without preeclampsia) is also not secondary to reduced viability as LDH release was similar in all diagnostic groups.

The birth weight centile for SGA infants with and without preeclampsia were nearly identical (5.7 ± 0.7 vs. 5.9 ± 0.8) as were placental weight and infant/placental weight ratios (Table 1). Given that the placentas of SGA infants without preeclampsia had significantly reduced system A amino acid uptake, as has been previously reported [13,14], it is likely that most of these infants were pathologically, not constitutionally, small. Interestingly, in reviewing clinical criteria, we found that objective evidence of IUGR, abnormal Doppler velocimetry or asymmetric growth profile, was present in 3 of 5 of the SGA infants of preeclamptic mothers and only 1 of 5 of the SGA infants whose mothers did not have preeclampsia. Thus, the normal amino acid uptake by the placentas of SGA infants of preeclamptic mothers was probably not due to less severe growth restriction in these infants.

This observation that growth restriction with preeclampsia is associated with normal system A amino acid transporter activity while uptake is reduced with SGA alone suggests that growth restriction in the two settings may be substantially different. Several of the metabolic changes of preeclampsia, including increased lipids [18] and insulin resistance [19,20] and increased amino acid concentration in infants of preeclamptic mothers [11] are consistent with an adaptive change in preeclampsia to increase nutrient availability that is not present in SGA without preeclampsia. In addition, leptin, which can increase placental system A activity [16], is increased in preeclampsia [21] and reduced with SGA without preeclampsia [22]. Jansson et al. have emphasized that placental nutrient uptake is regulatable [23]. We propose that IUGR in preeclampsia represents a response to a fetal signal stimulated by reduced placental nutrient availability that is not adequate to overcome this challenge. Conversely we posit that IUGR without preeclampsia is due to the absence of this signal.

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Fig. 1.

System A amino acid uptake in different diagnostic groups. Villous fragments were prepared from placentas from uncomplicated pregnancies (◆, $n = 9$), pregnancies complicated by SGA (▲, $n = 5$), or with preeclampsia with (●, $n = 5$) or without (□, $n = 10$) SGA infants. After preincubation for 60 minutes samples were incubated for the represented times with C¹⁴-MeAIB in the presence and absence of sodium. The data presented are the mean and SEM of sodium dependent uptake. Data analyzed as repeated measures ANOVA indicated a significant effect of time ($p < 0.001$) and diagnosis ($p = 0.005$). If results from the placentas of SGA infants without preeclampsia were excluded there was no difference by diagnosis ($p = 0.36$).

Table 1

Characteristics of patient groups

	Normal (<i>n</i> = 9)	Preeclampsia (<i>n</i> = 10)	SGA (<i>n</i> = 5)	Preeclampsia SGA (<i>n</i> = 5)
Maternal age	27.8 ± 1.9	27.3 ± 2.0	25.9 ± 1.0	28.5 ± 2.4
Maternal BMI (kg/m ²)	27.1 ± 2.3	25 ± 1.6	22.4 ± 2.4	26.6 ± 3.5
Maternal race (B and other/W)	3/6	3/7	4/5	0/5
Percent nulliparous	88	100	100	100
Blood pressure at delivery (mm Hg)	126 ± 2/71 ± 2 B	145 ± 2/90 ± 2 A	116 ± 2/63 ± 3 B	147 ± 5/94 ± 6 A
Blood pressure <20 weeks GA	113 ± 2/69 ± 1	113 ± 3/71 ± 2	112 ± 2/73 ± 2	117 ± 1/71 ± 2
Gestational weeks at delivery	39.0 ± 0.6 A	38.7 ± 0.6 A	38.3 ± 0.8 A, B	35.6 ± 0.8 B
Birth weight (g)	3466 ± 157 A	3134 ± 126 A	2451 ± 126 B	1934 ± 273 B
Birth weight centile	60.5 ± 7.4 A	36.9 ± 8.1 A	5.9 ± 0.8 B	5.7 ± 0.7 B
Placental weight (g)	542 ± 38 A	419 ± 41 A, B	298 ± 51 B	255 ± 51 B
Infant weight/placental weight	6.6 ± 0.4	7.7 ± 0.60	9.0 ± 0.8	7.8 ± 0.5
Route of delivery (cesarean section/ total)	2/9	2/10	1/5	0/5
Labor/total	7/9	10/10	4/5	5/5

Where ANOVA indicated differences, different letters indicate significant differences between groups whereas presence of the same letter indicates no difference.