Placental Transfer of Analogs of Glucose and Amino Acids in Experimental Intrauterine Growth Retardation

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Summary

We have employed the model in which one uterine artery is ligated to study maternofetal transport and tissue uptake of glucose and amino acids in the intrauterine growth-retarded rat. On the 18th day of gestation, the artery supplying one uterine horn was ligated. Two days later the rats received [³H]2-deoxyglucose and [¹⁴C]α-aminoisobutyric acid iv. One hour later the growth-retarded and control fetuses were delivered by Cesarean section and appropriate blood samples were obtained. The growth-retarded fetuses had an average weight reduction of 27%, significantly increased placental to fetal weight ratio and brain to body ratio, and a significantly reduced liver to body ratio. Total radioactivity derived from tritiated deoxyglucose in whole fetal tissues, placenta, liver, and brain were significantly decreased in the intrauterine growth-retarded (IUGR) fetuses; this was also true per gram of tissue except for liver. Liver to plasma, brain to plasma, and whole fetal tissue to plasma ³H ratios were significantly increased in the IUGR group. The radioactivity derived from [¹⁴C]α-aminoisobutyric acid was significantly reduced in whole fetal tissues, placenta, liver, and brain in the IUGR fetuses whether expressed per whole organ or per gram of tissue. Significant differences in liver to plasma, brain to plasma, and whole tissue to plasma ¹⁴C ratios were not observed.

Speculation

The large reduction in maternofetal transfer of deoxyglucose and α-aminoisobutyric acid when uterine blood flow is reduced raises the possibility that one of the major causes of retarded growth *in utero* is a diminished supply of glucose and amino acids to the fetus. The fetal liver appears to be especially effective in increasing its extraction of 2-deoxyglucose from fetal plasma during this period of nutrient restriction. Since a major cause of fetal growth retardation in man is an impairment in maternal circulatory supply to the placenta, a similar mechanism of growth retardation may contribute to human IUGR. The possibility that nutritional supplementation may be of value in improving the growth conditions for the fetus when uterine blood flow is restricted would seem worthy of further investigation. More information is also required about the mechanisms responsible for the apparent improved extraction of glucose from plasma in IUGR.

In developed societies, a major cause of fetal malnutrition and growth retardation may be an impairment in maternal circulatory supply to the placenta (12). Glucose and amino acids are among the important nutrients supplied by the mother to the fetus (24). Since the fetus is entirely dependent upon the maternal circulation for nutrition, a significant reduction in uteroplacental blood flow might limit the supply of these critical substrates to the growing fetus, resulting in intrauterine malnutrition.

The available information on the qualitative and quantitative aspects of maternofetal transfer of these nutrients, as well as data concerning possible alterations in their tissue distribution during conditions of limited transfer are scanty.

In order to gain more insight into maternofetal transport and tissue uptake of glucose and amino acids in IUGR, we have employed the model devised by Wigglesworth (31), in which the artery to one uterine horn is ligated. Our goal in these initial studies was primarily to test the assumption that in this model uterine blood flow restriction limits the rate of glucose and amino acid transport to the placenta and fetus. To the best of our knowledge, this assumption has not been examined in any animal system where maternal flow restriction has been induced. In exploring placental transport and tissue uptake, further metabolism of the compounds in question should ideally be excluded. For this reason radioactive analogs of glucose and amino acids known to undergo little or no further intracellular alteration were used. The results of some of our initial studies are reported here.

MATERIALS AND METHODS

ANIMALS AND SURGICAL PROCEDURE

The experiment involved six pregnant Sprague-Dawley rats with accurately timed gestations (within 12 hr). They were caged individually and given free access to Purina laboratory pellets and tap water until the time of surgery. Experimental intrauterine growth retardation was induced by slight modification (18) of a previously described procedure (31). On the 18th day of gestation, a lower midline laparotomy was performed under ether anesthesia. The uterus was exposed and the uterine artery supplying one uterine horn was ligated with two silk ligatures. The artery of the opposite uterine horn was left untouched. Following surgical intervention the incision was immediately closed with silk sutures. Two days later, the pregnant rats received 25 mCi [³H]2-deoxyglucose (10 Ci/mM) and 1 μCi [¹⁴C]α-aminoisobutyric acid (AIB) (9 mCi/mM)/100 g of body weight iv. These compounds were obtained as high purity radiochemicals from Amersham/Searle and were not repurified before use. Sixty minutes after the injections, the 15 living IUGR and 27 control fetuses were delivered by Cesarean section. Heparinized blood samples were obtained from the maternal abdominal aorta just prior to removal of the fetuses and fetal blood samples were taken from deep neck incisions immediately after delivery.

The placenta, liver, and brain of each fetus, as well as the remaining fetal tissues were freed from surrounding membranes, lightly blotted, and promptly frozen, weighed, and homogenized in distilled water. The homogenates were carefully diluted to 10% tissue (w/w), centrifuged, and a 0.5-ml aliquot of the supernatant fluid was counted in 15 ml Aquasol. This method is virtually the same as that used by Young and Widdowson (33). Essentially, all

of the counts derived from the labeled substrates after digestion of selected homogenates with NCS solubilizer (Amersham/Searle) were found in the supernatants. For statistical analysis, the results from the normal or stunted newborns from each litter were pooled; averaged, and used as a single observation. Whole fetal tissue data given in the tables are the sum of analyses of liver, brain, and remaining carcass weights or radioactivities, excluding the placenta. Radioactive products were not further analyzed.

RESULTS

MORPHOMETRIC FINDINGS

These findings are summarized in Table 1 and confirm the validity of the model. In particular, average liver weight was much smaller in the IUGR fetuses, while the reduction in brain weight amounted to only 12%. The liver to body ratio was significantly smaller in the IUGR group, while brain to body ratio was significantly greater than in the controls, similar to results shown pre-

viously by Wigglesworth (31). This nonuniform fetal growth retardation is characteristic of type I IUGR, associated with maternal vascular disturbances.

PLACENTAL TRANSFER AND TISSUE UPTAKE OF [³H]-DEOXYGLUCOSE (TABLES 2-4)

The radioactivity of 2-deoxyglucose in fetal plasma as well as its mean fetal to maternal plasma ratio averaged 34% less in the IUGR group. Total radioactivity in whole fetal tissues, placenta, liver, and brain were also significantly decreased. When expressed per gram of tissue, the uptake of 2-deoxyglucose was significantly smaller in IUGR fetuses and placentas. Although brain uptake of 2-deoxyglucose/g was significantly smaller in the IUGR fetuses, liver uptake per gram showed an opposite trend, although the difference did not reach statistical significance. Liver to plasma ratio and brain to plasma ratios averaged 73 and 24%, respectively, greater in the IUGR group and whole fetal tissue to plasma ratios were also significantly higher.

Table 1. *Morphometric finding*¹

Weight (g)	IUGR	Control	P	Mean from control (%)
Total fetus	2.22 ± 0.37	3.03 ± 0.43	<0.001	-26.7
Placenta	0.476 ± 0.028	0.514 ± 0.038	>0.05	-7.4
Liver	0.125 ± 0.023	0.210 ± 0.033	<0.001	-40.2
Brain	0.125 ± 0.010	0.141 ± 0.013	<0.02	-11.5
Ratios				
Placenta/fetus	0.237 ± 0.034	0.182 ± 0.022	<0.01	+30.2
Liver/fetus	0.056 ± 0.003	0.069 ± 0.001	<0.001	-18.8
Brain/fetus	0.060 ± 0.005	0.049 ± 0.003	<0.01	+22.5

¹ Values are means ± SE. Statistical analysis was performed by Student's *t* test for paired samples.

Table 2. *Tissue radioactivity (total cpm)*¹

	IUGR	Control	P	Mean from control (%)
[³ H]2-deoxyglucose				
Fetus	480,839 ± 93,698	766,783 ± 126,622	<0.01	-41.1
Placenta	112,887 ± 7,337	139,998 ± 12,416	<0.01	-19.4
Liver	27,087 ± 4,917	37,833 ± 3,981	<0.01	-28.4
Brain	26,972 ± 5,187	36,400 ± 6,443	<0.02	-25.9
[¹⁴ C]AIB				
Fetus	9,425 ± 2,117	22,722 ± 4,776	<0.01	-58.5
Placenta	3,680 ± 306	5,992 ± 621	<0.01	-38.6
Liver	517 ± 85	1,101 ± 173	<0.01	-53.0
Brain	247 ± 22	454 ± 57	<0.01	-45.6

¹ Values are means ± SE. Statistical analysis was performed by Student's *t* test for paired samples.

Table 3. *Plasma and tissue radioactivity*¹

	IUGR	Control	P	Mean from control (%)
[³ H]2-deoxyglucose				
Maternal plasma	104,397 ± 9,861	104,397 ± 9861		
Fetal plasma	48,157 ± 7,514	73,755 ± 11,137	<0.01	-34.7
Fetus	206,333 ± 20,125	247,896 ± 21,932	<0.01	-16.8
Placenta	238,487 ± 12,553	275,911 ± 22,855	<0.05	-13.6
Liver	225,134 ± 29,185	191,833 ± 22,013	<0.05	+17.4
Brain	214,500 ± 40,216	256,479 ± 37,418	<0.05	-16.4
[¹⁴ C]AIB				
Maternal plasma	6,357 ± 715	6,357 ± 715		
Fetal plasma	3,966 ± 413	6,180 ± 557	<0.001	-35.8
Fetus	4,119 ± 408	7,388 ± 1073	<0.01	-44.3
Placenta	7,821 ± 630	11,920 ± 1400	<0.02	-34.4
Liver	4,225 ± 248	5,397 ± 581	>0.05	-21.7
Brain	2,006 ± 170	3,190 ± 259	<0.01	-37.1

¹ Results have been expressed as cpm/ml or cpm/g. Values are means ± SE. Statistical analysis was performed by Student's *t* test for paired samples.

Table 4. Ratios of radioactivity in plasma, fetus, placenta, and fetal liver and brain¹

	IUGR	Control	P	Mean from control (%)
³ H]2-deoxyglucose				
Plasma fetal/maternal	0.453 ± 0.039	0.690 ± 0.051	<0.01	-34.2
Fetus/plasma	5.095 ± 1.041	4.105 ± 1.024	<0.01	+24.1
Liver/plasma	5.257 ± 1.016	3.042 ± 0.811	<0.001	+72.8
Brain/plasma	4.643 ± 0.681	3.761 ± 0.534	<0.02	+23.5
¹⁴ C]AIB				
Plasma fetal/maternal	0.634 ± 0.045	0.987 ± 0.0414	<0.001	-35.8
Fetus/plasma	1.049 ± 0.090	1.234 ± 0.193	>0.05	-15.0
Liver/plasma	1.167 ± 0.170	0.919 ± 0.133	>0.05	+27.0
Brain/plasma	0.531 ± 0.064	0.545 ± 0.060	>0.05	-2.6

¹ Ratios are derived from individual data used for calculating results given in Table 3. Values are means ± SE. Statistical analysis was performed by Student's *t* test for paired samples.

PLACENTAL TRANSFER AND TISSUE UPTAKE OF AIB (TABLES 2-4)

The radioactivity of AIB in fetal plasma and its mean fetal to maternal ratio were reduced an average of 36% in the IUGR group. Total radioactivity in whole fetal tissues, placenta, liver, and brain was significantly smaller in IUGR fetuses.

When expressed per gram of tissue, fetal, placental, liver, and brain radioactivity was also significantly smaller in IUGR fetuses. Liver to plasma ratio was greater in the IUGR group, although the difference did not reach statistical significance. No trend toward differences was noted in brain to plasma ratios or whole fetus to plasma ratios.

DISCUSSION

Fetal nutrition depends not only on an adequate source of nutrients, but also on their efficient delivery to the fetus. A reduction in uteroplacental blood flow, which was the cause of experimental IUGR in this study, may play an important role in human IUGR as well. In certain conditions, such as preeclampsia (10), hypertension (2, 10), and supine hypotension (3), the usual circulatory adjustments required to ensure optimal maternal perfusion of the placenta site fail to occur.

Active transfer of glucose and amino acids across the placenta is well documented in several species (6, 8, 11, 14, 17, 23). To investigate whether a reduction in uteroplacental blood supply interferes with adequate transport of these nutrients, we have employed labeled analogs in this study. Although 2-deoxyglucose is a most suitable analog for use in studies of glucose transport (1, 13, 28-30), we are unaware of its previous use in examination of maternofetal glucose transfer. It differs from glucose in the replacement of the hydroxyl group on the second carbon atom by a hydrogen atom, and they share a common transport system. 2-Deoxyglucose is converted to 2-deoxyglucose-6-phosphate by hexokinase, but this product is not metabolized further by glycolysis or the hexose monophosphate shunt, nor it is known to be incorporated into glycogen. It appears to be a very poor substrate for rat liver glucose-6-phosphatase (2, 3, 10, 33). AIB was selected as an analog of the naturally occurring amino acids because it is not metabolized in mammalian tissues, but is transported by the same system which utilizes alanine, glycine, and a variety of other amino acids (5, 25).

Joyce and Young (15) and Krauer *et al.* (16) have shown by *in situ* perfusion of the guinea pig placenta that transfer rates of glucose and AIB are related to the maternal blood flow. Furthermore, a single spontaneous guinea pig runt fetus accumulated less than normal quantities of AIB that had been injected into the maternal circulation at the end of gestation (32). There is no published data of which we are aware documenting the status of fetal nutrient transfer in flow-restricted cases of IUGR in any species, except for umbilical artery ligation in the sheep (22). In the IUGR rat model employed in this study, as well as in a substantial proportion of human pregnancies associated with IUGR, fetal growth impairment is associated with restricted uter-

oplacental blood supply. Our findings in the experimental model prove that there is a large reduction in the maternofetal transfer of 2-deoxyglucose and AIB when uterine blood flow is reduced. This result provides objective documentation of the impaired transfer previously only assumed in this widely used animal model of IUGR.

Neonatal hypoglycemia occurs predominantly in newborns whose birth weights are low for gestational age (7, 19). Toward the end of gestation large glycogen reserves are built up in the fetal liver in all mammalian species which have been examined (27). In contrast, low levels of hepatic glycogen have been found in IUGR human infants at necropsy (9, 26). In experimentally induced IUGR rat fetuses, a reduction of liver glycogen content was also observed (30, 31). The reduced transfer of glucose across the placenta demonstrated in this study may play an important role in the reduced blood glucose concentrations reported in IUGR rat fetuses immediately following delivery (4, 20, 21), as well as in the diminished glycogen reserves associated with retarded fetal growth.

Interestingly, the IUGR fetal tissues demonstrated an increase in extraction of 2-deoxyglucose from plasma, which was observed in the whole fetus, liver, and brain. Liver appears to be especially effective in this regard, since uptake of 2-deoxyglucose per gram of tissue was not decreased and even showed a trend toward increase in IUGR livers. However, such possible adaptation was not effective in normalizing the uptake of 2-deoxyglucose in other tissues, including brain. There was also a trend toward enhanced liver extraction of AIB from fetal plasma in the IUGR group, but this trend was not significant. No such trend was noted in the whole fetus or brain. The mechanism and physiologic significance of these apparent changes in tissue accumulation of glucose and amino acid analogs in IUGR require further elucidation. Although some of these changes may be explained by a reduced level of the natural substrates in extracellular pools compared with the labeled compounds sharing common transport systems with them, the interorgan differences observed indicate that the full explanation of these alterations is more complex.

CONCLUSION

In initial studies in the rat using the experimental IUGR model associated with restricted uterine blood supply, we have tested the assumption that there is a reduction in maternofetal nutrient transfer to IUGR fetuses. By using a radioactive 2-deoxyglucose and AIB, reduced transfer was demonstrated to placenta, fetal plasma, whole fetus, liver, and brain. Thus, the retarded growth *in utero* in this model can reasonably be explained, at least in part, by diminished supply of amino acids and glucose to the fetus. The reduced transfer of glucose across the placenta may play an important role in the diminished glycogen reserves and neonatal hypoglycemia associated with retarded fetal growth. Fetal tissues, particularly the liver, adapt to the reduced glucose supply by enhanced extraction of glucose from the circulation.

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