Sexual responses to intrauterine stress: body and brain growth

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Summary

The aims of this work were to analyse body and brain growth as produced by deficiencies in the uteroplacental blood supply, and to evaluate sexual responses to intrauterine stress. Intrauterine growth retardation (IUGR) was experimentally induced in pregnant rats by partial obstruction in both uterine vessels at 1,7 and 14 gestational days. The dysfunctions in the placental circulation retarded both somatic and cerebral growth, depending on the period of gestational stress and the sex. Brain weight had a relatively greater resistance than body weight, which is called a "brain sparing" mechanism. The body and brain sexual dimorphism in control pups was inhibited in IUGR pups. This study shows that prenatal stress exposition might modify growth and sexual dimorphism at birth.

Key words: IUGR; Adaptation; Birth weight; Brain; Sexual dimorphism; Rats.

Introduction

Fetal growth is determined by genetic and environmental factors. The latter include both those relative to the maternal environment and those that are a result of the maternal-environmental interaction [1]. Intrauterine growth retardation (IUGR) is frequently associated to high prenatal and perinatal morbidity and mortality, and the causes are not completely known [2, 3]. Uteroplacental insufficiency is one of the main factors involved in IUGR. This term includes any condition which either limits or prevents the normal fetal-maternal exchange (nutrients, gases, water, etc.) due to structural defects or to reduced placental blood flow [4]. According to Harding and Johnston [5] nutrition is a key factor in fetal growth regulation, in which the interaction among fetus, placenta, and the maternal organism, plays a very important role.

Many experimental models have been applied in order to determine fetal growth responses to intrauterine stress and the underlying adaptive mechanisms [4]. As an example, the uterine vessel ligation developed by Wigglesworth can be cited [6]. He observed that maternal malnutrition and uterine isquemia could produce similar effects on fetal growth patterns. The fetus, however, has an important adaptive capacity, allowing an accelerative neurological maturity in some cerebral regions [7]. These observations agree with Warshaw [8] who proposed IUGR as an adaptive fetal response rather than pathology.

However, it is not well known if gestational age and/or fetal sex are involved in differences in the adaptive responses evoked by reduction in the placental blood flow. The aims of this work were to analyse body and brain growth as produced by deficiencies in uteroplacental blood supply during three gestational periods, and to evaluate sexual responses to intrauterine stress.

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Materials and Methods

Two hundred and six Wistar rats born and raised in the Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA-UNLP) were employed. The animals were kept free of pathogens and treated according to institutionalised guidelines [9]. Fifty females (200-250 g of body weight) and ten males were mated daily. The start of pregnancy was assumed by the presence of spermatozoa in the vaginal smear. Pregnant rats were allotted on individual steel boxes and received stock diet and water ad libitum.

The experimental groups were: Control (C) (N=28): constituted by pups born from normal pregnant rats. The IUGR offspring came from dams in which both uterine vessels were partially bent [10]. According to the gestational period in which this procedure was practiced the following subgroups were: IUGR1 (N=29): at day 1 of pregnancy *(Early period)*; IUGR7 (N=30): at day 7 of pregnancy *(Middle period)*; and IUGR14 (N=29): at 14 days of pregnancy *(Late period)*. The last group called Sham-operated (Sh), was constituted by offspring born from dams submitted to a laparoscopic surgery but the uterine vessels were not bent in order to isolate the effects of the surgery. The subgroups were Sh1 (N=30): laparotomy at day 1 of pregnancy; Sh7 (N=30): laparotomy at day 7 of pregnancy; and Sh14 (N=30): laparotomy at day 14 of pregnancy.

At birth, the newborn rats were weighted and then sacrificed. The brains were extracted and weigthed after removing the olfactory bulbs because our purpose was to analyse the main components of the central nervous system. The body and brain weight were recorded on a Mettler H80 scale (0.1 mg of precision).

The between-group comparisons and isolated factors are detailed in Table 1. Data were processed by single and multifactorial analysis of variance (ANOVA), and - in significant cases - the LSD post hoc test. For graphical comparisons, mean values were standardized by the percent of relative differences between means (RDM%), according to the formula: RDM= 100* (XSh-XIUGR / XSh), where X is the mean value of a measurement in each group [11].

Table 1. — *Comparisons and factors.*

Comparison	Factor	Substrate
C - Sh	surgery	males and females
Sh1 - IUGR1	intrauterine stress	early period - males and females
Sh7 - IUGR7	intrauterine stress	middle period - males and females
Sh14 - IUGR 14	intrauterine stress	late period - males and females
IUGR1 - IUGR7	early-middle period	IUGR - males and females
IUGR1 - IUGR14	early-lateperiod	IUGR - males and females
IUGR7 - IUGR 14	mid-late period	IUGR - males and females

C = control. IUGR = intrauterine growth retarded.
Sh = sham-operated

Results

The analysis of variance between control-sham-operated and sham-operated-IUGR showed, in both sexes, significant F values in body and brain weight (Table 2).

In males, body weight was significantly greater in sham-operated than in IUGR in all the gestational periods. The females, at variance, had significant F values during the early and middle pregnancy and non-significant during the late period. The brain weight in males, as the body weight, showed significant differences in the three periods, while in females there were significant differences only for the last two gestational periods (Table 3).

The between sex analysis in control and sham-operated groups showed significant differences in both variables, males being greater than females. However, there were non-significant differences between IUGR males and females (Table 4).

Table 2. — *Effects of surgery and growth retardation.*

Comparison	Body weight F	Brain weight SF
C - Sh	4.23**	3.36*
Sh - IUGR	16.00**	14.92**

C = control. IUGR = intrauterine growth retarded.
Sh = sham-operated. *p < 0.05. **p < 0.01.

Table 3. — *Differences between treatments (Sh-IUGR).*

Variable	Early period	Middle period	Late period
Body weight			
males	9.1**	9.1**	10.9**
females	22.9**	21.7**	1.9
Brain weight			
males	15.2**	4.2*	13.5**
females	4.1	13.9**	4.3*

*p < 0.05. **p < 0.01.

Table 4. — *Differences between sexes.*

Group	Body weight	Brain weight
C	5.06*	7.19*
Sh1	9.09**	15.18**
Sh7	9.10**	4.15*
Sh14	15.18**	5.39*
IUGR1	0.00	0.80
IUGR7	1.54	2.38
IUGR 14	0.00	0.93

*p < 0.05. **p < 0.01.

Discussion

During pregnancy, each organ or system has its own growth rate [12, 13, 14]. It is known that most of the modifications of fetal growth are induced by environmental factors. It has been estimated that the environment contributes to about 62-72% of body weight at birth [15, 16].

Several authors have observed that insufficiencies of placental blood flow during pregnancy alters body weight growth [6, 11, 15, 17-22). In such studies however, the obstruction of placental circulation was noted in only one gestational period, and the sex of the pups was not taken into account. In the present study reduced body weight was also found. In males, the greater growth delay was produced by stress during early pregnancy (16%), followed in decreasing intensity by the middle (13%) and the late periods (12%). In females, the body weight was more affected by intrauterine stress during the middle (14%) and early pregnancy (11%), but it was not affected in the late period (Figure 1A).

The brain weight was also reduced. In males, the effect during the early period (8%) was greater than the middle and late periods (6% and 5%, respectively). In females, the brain weight was more delayed during the middle

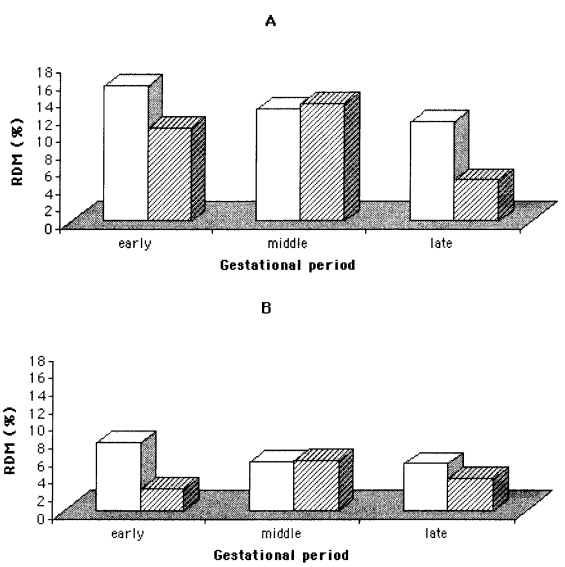

Figure 1. — Relative differences between means (RDM%) in body weight (A) and brain weight (B). White bars: males; striped bars: females.

(6%) followed by the late period (4%), while the early stress had no effect (Figure 1B). These results lead us to suppose that the uteroplacental dysfunctions such as reduced blood supply delay both somatic and cerebral growth, depending on the period in which the stress is applied as well as to the fetal sex. The relatively greater resistance of brain weight to intrauterine stress can be attributed to a "brain sparing" phenomena. It reflects a functional mechanism of protection of the nervous system compared to others tissues and organs.

According to Tanner [23] sexual dimorphism in some metrical traits begins in utero, reaching complete expression with the adolescent growth spurt. Sexual dimorphism results from differences in time and growth rates between sexes [24]. Because of this, the nature of such differences and the factors affecting its expression are highly linked to growth and development [25]. In humans, Hadders-Algra and Touwen [26] reported that IUGR males show a greater vulnerability of growth than IUGR females. Previous studies using the same experimental model [10], found that body weight as well as craniofacial dimensions are dimorphic in normal newborn rats, but not in IUGR rats. Our results agree with Oyhenart *et al.* [10], meaning that the sexual dimorphism in body and brain weight was inhibited by intrauterine stress, no matter the period of pregnancy. The underlying mechanisms of such differential responses can be explained by the hypothesis of "better growth canalization in females" proposed by Tanner [27], which means that under the same kind of stress, females are usually less affected than males.

Conclusions

Intrauterine growth retardation in body and brain growth is clearly associated to the gestational period of stress. The adjustments found in females demonstrate that there are sexual differences in the adaptation to an adverse prenatal environment. These differences modify the normal expression of sexual dimorphism at birth. The inhibition of sexual dimorphism results in a greater susceptibility in males than in females and it is independent of the period of stress.

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