EXPERIMENTAL BIOLOGY

Histophysiology of Ovaries and Adrenal Glands in Rats and Brain Development in Their Progeny: Correlation and Cluster Analysis

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Brain weight of 21-day-old rat fetuses positively correlates with the area of the glomerulosa zone, the ratio of this area to the area of the fasciculata-reticularis zone, activity of hydroxysteroid dehydrogenase in the glomerulosa zone, and the ratio of its activity in the glomerulosa and reticularis zones. In the one-day-old newborns these relationships were absent.

Key Words: *brain; progeny; adrenal glands; ovaries; correlations*

Hormones are essential factors affecting embryonic cerebral organogenesis [5,8-12]. Their effects on this process were predominantly studied under experimental conditions or pathological processes that considerably disturbed hormone concentration in the mother-placenta-fetus system. At the same time, the dependence of brain development in rat embryos on the maternal endocrine status under physiological conditions received less attention. Our aim was to study some features of this problem.

MATERIALS AND METHODS

Eleven 4-5-month-old rat dams and their fetuses (embryonic day 21, n=85) were studied together with 20 female rats of the same age taken 1 day after delivery and their 1-day pups (n=208). The females and litters were decapitated; in females, body weight and weights of ovaries and adrenal glands were measured, while in the pups the body and brain weights were determined. Immediately after decapitation the left adrenal gland and left ovary from dams were used to make cryostat slices crossing the central area of the organ. In these slices 3β-hydroxysteroid dehydrogenase (HSDH) was histochemically visualized under standard conditions [4] and enzyme activity was determined with the help of a Lyumam I-2 microscope (550 nm) in different zones of the adrenal cortex (25 adrenocorticocytes in each zone) and in the corpus luteum, atretic follicles, and theca interna of mature ovarian follicles. The ratio of enzyme activity in the glomerulosa zone (GZ) to that in the fasciculata and reticularis zones was calculated. The right adrenal glands from females and the brains from fetuses (2 male and 2 female from each litter) were fixed in Carnoy fluid and embedded in paraffin. Slices of the adrenals (7 μm thick) were stained with hematoxylin and eosin and brain slices were stained with 1% methylene blue. The slices crossing the central area of the adrenals were analyzed morphometrically [1]. The determined parameters were cross-section areas of the adrenal cortex and medulla, GZ, and the fasciculata-reticularis zone (FRZ), since there was no clear-cut boundary between the fasciculata and reticularis zones. In each case the ratio of GZ to FRZ was calculated. The thickness of the somatosensory cortex was measured with an MOB-15 ocular-micrometer;

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the neuron density in developing layer V was determined. For each litter the mean body weight and mean brain weight were determined. These parameters were used to search for correlation with morphometric indices of the adrenals and ovaries of dams. The paired and multiple correlation analysis and the cluster analysis were performed with the help of a Statistica-5 software.

RESULTS

The data on 21-day-old embryos agree with the well-known fact that brain weight positively correlates with fetal body weight [1,7]. The correlation coefficient between body weight and brain weight of individual fetuses was 0.55, while comparison of the litter-averaged data yielded r=0.77. In the 1-day-old pups the correlation coefficients were 0.81 and 0.89, respectively. In 21-day-old fetuses the correlation between brain weight and morphometric characteristics of the cortex was insignificant: r=13 for cortex thickness, and r=-0.13 for the number of neurons in a standard vision field. In 1-day-old pups these coefficients were 0.52 and -0.54, respectively; the correlation was significant (p<0.05). These findings indicate that at postnatal day 1 brain growth and the formation of brain cortex are coupled more tightly than in the prenatal period.

The correlation analysis reveal no significant relationships between fetal brain weight and HSDH activity in the steroid-producing ovarian structures and weigh of ovaries in mothers both at pregnancy day 21 and postnatal day 1 (Table 1). It can be explained by negligible effect of progesterone and estrogens on fetal body and brain weights and by the fact that during pregnancy these hormones are produced primarily by placenta but not ovaries [5]. In addition, maternal milk-supplied ovarian hormones play only a minor role during the first postnatal day.

A significant positive correlation was found between HSDH activity in adrenocorticocytes of GZ and the litter-averaged brain weight of 21-day-old fetuses. A strong positive correlation was found between brain weight and the ratio of HSDH activity in GZ to that in the reticularis zone (Table 1). Brain weight in fetuses significantly correlated with GZ cross-section area and CZ to FRZ ratio. These cytochemical and morphometric indices also correlated with the fetal body weight (Table 1).

The positive correlation between body and brain weights and HSDH activity in GZ cells can be ex-

TABLE 1. Paired Correlation Coefficients of Brain and Body Weights of 21-Day-Old Fetuses and 1-Day-Old Pups with Morphometric Indices of Adrenals and Ovaries in Their Mothers

Indices	Group of rats			
	21-old-day fetuses		1-day-old pups	
	brain weight	body weight	brain weight	body weight
Weight				
adrenal glands	-0.19	-0.51	0.16	-0.04
ovary	-0.15	-0.41	0.07	-0.14
HSDH activity:				
GZ	0.66*	0.67*	0.02	0.18
fasciculata zone	0.33	0.09	-0.2	-0.16
reticularis zone	-0.28	-0.32	-0.13	-0.15
GZ/fasciculata zone	0.38	0.53	0.27	0.36
GZ/reticularis zone	0.87*	0.94*	0.16	0.36
corpora luteum	-0.2	-0.3	-0.19	-0.25
atretic follicles	0.14	-0.06	-0.36	-0.32
theca interna	0.28	0.09	-0.39	-0.42
Cross-sectional area				
adrenal cortex	-0.18	-0.47	0.28	0.19
GZ	0.53*	0.26	-0.07	-0.4
FRZ	-0.29	-0.55*	-0.34	-0.4
GZ/FRZ	0.7*	0.73*	0.15	0.12
medulla	-0.3	-0.37	-0.38	-0.26

Note. *p<0.05.

plained by the ability of aldosterone (hormone of GZ) to stimulate anabolism [5] and directly affect the brain: prevention of neuronal death induced by adrenalectomy [13] and interaction with brain mineralocorticoid receptors [8]. When considering the correlation of brain and body weights with the ratio of HSDH activity in GZ to that in the reticularis zone, one can use the data on the negative correlation between body weight of 21-day-old fetuses and the concentration of androgens in mother's blood [12] that are partially produced in the reticularis zone [2,6]. In addition, it should be noted that proportion of various hormones produced in the adrenal cortex depends on its functional activity; hormonopoiesis in this gland is strongly affected by pregnancy [2,3,6,10].

It is noteworthy that the strongest correlations were revealed not between individual indices, but between their ratios (Table 1), which indicates the important role of not only individual hormones, but also their interactions.

Stress stimulation and ACTH injection activate hormone production predominantly in the adrenal FRZ and induce its hypertrophy, while GZ is less sensitive to these interventions [2,6]. In light of this, the high ratios of cross-sectional area of GZ to FRZ, as well as the ratio of HSDH activity in GZ to that in the fasciculata or reticularis zones indicate lower functional activity of the pituitary-adrenal system in pregnant rats, which correlates with greater fetal brain and body weight.

Although the studied indices of the adrenal cortex are related not only to brain weight, but also to body weight, the effect of adrenal indices on brain weight is not entirely determined by their influence on body weight. This follows from comparison of correlation coefficients: r=0.77 between brain and body weights; r=0.87 between brain weight and the ratio of HSDH activity in GZ to that in the reticularis zone. In addition to paired correlation coefficients we calculated the multiple correlation coefficient between the fetal brain weight and the ratio of cross-sectional areas of GZ to FRZ (R=0.9) and the ratio of HSDH activity in GZ to that in the reticularis zone (R^2=0.81). The values of brain weight predicted by Statistica-5 software from the "adrenal" indices were close to the experimental values (Table 2).

The cluster analysis divided all dams and their fetuses into 2 clusters that significantly differed in fetal body weight, ratios of HSDH activity in the specified zones and GZ to FRZ cross-sectional areas. In the first cluster (5 litters) the mean values were 210 mg, 1.7, and 0.295, respectively, while in the second cluster (6 litters) the indices were 188 mg, 0.82, and 0.17 (p<0.01).

In contrast to 21-day-old fetuses, in 1-day-old pups the correlation between brain weight and morphometric indices of mother's adrenals was considerably weaker and statistically insignificant (Table 1). This presumably results from the destruction of the mother-placenta-fetus system after delivery, considerable decrease of the effect of maternal hormones, changes in the mother's endocrine status in comparison with that at the end of pregnancy [5,10], and the effect of environmental factors on pups. On the other hand, a drastic decrease in the strength of the studied relationships after the first postnatal day may indirectly indicate a regular character of the relationships observed in 21-day pregnancy.

TABLE 2. Comparison of Brain Weight of 21-Day-Old Fetuses with Predicted Values Calculated from Histophysiological Parameters of the Adrenal Glands

Mean brain weight in litter, mg	
predicted	measured
191	197
201	204
226	224
188	168
191	194
197	195
209	209
198	203
211	212
187	193
181	183

Our findings attest to the essential role of mother's adrenal cortex in the regulation of fetal brain development during physiological pregnancy. The mechanisms underlying the revealed relationships require special investigation.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. O. I. Kirillov, *Stress-Induced Adrenal Hypertrophy* [in Russian], Moscow (1994).
3. S. V. Kuznetsova and R. V. Uchakina, *Fiziol. Chel.*, **24**, No. 1, 114-117 (1998).
4. Z. Lojda, R. Gossrau, and T. H. Shiebler, *Enzyme Histochemistry*, Berlin (1979).
5. V. B. Rozen, *Basic Endocrinology*, [in Russian], Moscow (1994).
6. B. Ya. Ryzhavskii, *Postnatal Ontogenesis of Adrenal Cortex* [in Russian], Novosibirsk (1989).
7. B. Ya. Ryzhavskii and S. I. Biryukova, *Byull. Eksp. Biol. Med.*, **116**, No. 12, 641-642 (1993).
8. E. R. De Kloet, N. Y. Rots, and A. R. Cool, *Cell. Mol. Neurobiol.*, **16**, No. 3, 345-356 (1996).

9. E. Gould, *Ann. New York Acad. Sci.*, **14**, No. 743, 73-92 (1994).
10. M. A. Magiakou, G. Mastorakos, D. Rabin, *et al.*, *J. Clin. Endocrinol. Metab.*, **81**, No. 5, 1912-1917 (1996).
11. S. D. Moffat, E. Hamson, J. C. Wickett, *et al.*, *Brain Res.*, **767**, No. 2, 297-304 (1997).
12. K. Tsutsui, *Zool. Sci.*, **9**, No. 6, 1226-1229 (1992).
13. C. Woolley, E. Gould, R. R. Sakai, *Brain Res.*, **9**, No. 1/2, 312-315 (1991).