FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 45, No. 1, 2007
pp. 51-56

Influence of maternal dexamethasone treatment on morphometric characteristics of pituitary GH cells and body weight in near-term rat fetuses

M. Manojlović-Stojanoski, N. Nestorović, N. Negić, B. Filipović, B. Šošić-Jurjević, M. Sekulić and V. Milošević

Institute for Biological Research "Siniša Stanković", Belgrade, Serbia and Montenegro

Abstract: Growth hormone (GH) and glucocorticoids have a powerful influence on controlling fetal growth, differentiation and maturation of numerous tissues. In the present study, the effect of maternal dexamethasone (Dx) treatment on GH cells and body weight in 19- and 21-day-old rat fetuses was investigated using immunocytochemical and morphometric methods. Pregnant female rats received daily injections of 1.0-0.5-0.5 mg Dx/kg b.w. on days 16-18 of pregnancy (experimental group), while the control group received an equal volume of saline. Dx treatment of pregnant rats enhanced immunostaining intensity and significantly increased ($p<0.05$) GH nuclear and cell volume, as well as volume density and number of GH cells per square millimeter in 19-day-old fetuses compared to the controls. In 21-day-old fetuses after maternal Dx administration, immunoreactivity, volume density and number of GH cells remained significantly increased ($p<0.05$). Dx treatment of pregnant rats resulted in marked body weight reduction of 21-day-old but not 19 days old fetuses in comparison with the corresponding controls. The presented results demonstrate that maternal Dx application has pronounced effect on morphometric parameters of GH cells of 19- and 21-day-old fetuses. Also, in near-term rat fetuses body weight was largely independent of pituitary GH cell activity.

Key words: Fetuses - Dexamethasone - GH cells - Pregnancy - Rat

Introduction

In fetal rats, initial pituitary growth hormone (GH) expression is detected on day 15 of gestation using a sensitive method like the reverse transcriptase-polymerase chain reaction (RT-PCR) [19]. In the following phase of GH cell development there occurs expression of Pit-1, a pituitary-specific transcriptional factor that mediates cell proliferation and differentiation into specific hormone producing cell types - thyrotropes, somatotropes or lactotropes [23]. During this period, the quantity of GH transcripts remains at an extremely low level. A marked increase in cell number and GH production occurs between days 18 and 19 of fetal development [27]. Afterwards, during the perinatal period, the characteristically high circulating GH level is the result of hypothalamo-pituitary system immaturity [2,28].

It has been considered that pituitary GH promotes and controls fetal development and body weight by stimulating the family of hepatic growth factors. Recent investigations showed that extrapituitary GH as well as local production of growth factors had great paracrine/autocrine influence on fetal developmental processes and differentiation. Expression of GH and GH receptor in a wide variety of tissues is established before the pituitary gland and circulatory system becomes functional [21,30]. In rats and mice a contribution of pituitary GH to growth, development and body weight has been demonstrated during the second week of life i.e. postnatally [18]. The influence of pituitary GH on normal growth and body weight in near-term fetuses, immediately after the GH cells become functional, is still difficult to understand and not well defined.

Adrenal glucocorticoids also have a powerful influence on growth, maturation and tissue remodeling during fetal development. Glucocorticoids accelerate the maturation of numerous organs essential for the maintenance of homeostasis immediately after birth [3,20]. In addition, glucocorticoids are indispensable for proper differentiation, expansion and function of certain pituitary cell lineages, especially GH cells [12,16].

Correspondence: M. Manojlović-Stojanoski, Institute for Biological Research "Siniša Stanković", Despota Stefana 142, 11060 Belgrade, Serbia and Montenegro;
e-mail: manojlo@ibiss.bg.ac.yu

The aim of our investigations was to assess the influence of Dx treatment of gravid females on morphometric parameters of pituitary GH cells and body weight in near-term fetuses and to examine the relationship between histological appearance and morphometric characteristics of pituitary GH cells and body weight in 19- and 21-day-old control fetuses and fetuses after maternal Dx administration.

Materials and methods

Animal treatment. Female and male Wistar strain rats, weighing approximately 250 and 400 g, respectively, were mated in the laboratory of the Institute for Biological Research, Belgrade, during the night. The morning on which sperm positive smears were obtained was declared gestation day 1. Pregnant females were housed individually under standard conditions (12:12 h light-dark cycle at 22 ± 2°C) and offered food and water ad libitum. On 16th day of pregnancy dams received subcutaneously 1.0 mg Dx/kg b.w., followed by 0.5 mg Dx/ kg b.w./day on 17th and 18th days of gestation. The control gravid females received the same volume of saline vehicle. On days 19 and 21 of gestation the rats were killed under ether anesthesia and the fetuses were removed and prepared for histological and morphometric measurements.

Experimental protocols were approved by the Local Animal Care Committee and conformed to the recommendations given in "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington D.C., 1996).

Light microscopy and immunocytochemistry. The pituitary glands, with part of the sphenoid bone or fetal heads, were excised and fixed in Bouin's solution for 48 h and embedded in paraffin. Three pituitary sections from the dorsal, medial and ventral parts, 5-μm thick, were deparaffinized in xylol and rehydrated in decreasing concentrations of ethanol before preparation for immunocytochemical staining.

Pituitary GH was localized immunocytochemically using the peroxidase-antiperoxidase complex (PAP) method of Sternberger [26]. Endogenous peroxidase activity was blocked by incubation in 9 mM hydrogen peroxide solution in methanol for 30 min at ambient temperature. Before application of specific primary antibodies, nonspecific background staining was prevented by incubation of the sections with normal nonimmune porcine serum diluted in phosphate-buffered saline (PBS; pH=7.4) for 60 min. Sections were then overlaid with the appropriate dilution (1:300) of specific rabbit anti-human GH antibodies (Dako, Glostrup, Denmark) for 24 h at room temperature [11]. After washing in PBS, sections were incubated for 60 min with the secondary antibody (swine-anti-rabbit IgG; Dako) for 45 min, rinsed again with PBS for 10 min and then incubated with rabbit PAP complex for 45 min. Antibody localization was visualized by immersing the sections in Tris-HCl-buffered saline (0.5 mol/l; pH=7.4) supplemented with 0.05% 3,3-diaminobenzidine tetrachloride (DAB; Serva, Heidelberg, Germany) and 9 mM hydrogen peroxide. Sections were thoroughly washed under running tap water, counterstained with hematoxylin and mounted in Canada balsam (Alkaloid, Skopje, Macedonia).

Morphometry. Volume densities (Vv) of the nuclei and cytoplasm of GH-immunopositive cells as well as the numerical density (Na) of their nuclei per μm^3 were measured in 50 test areas on each of three pituitary sections per pituitary gland, per fetus, at a magnification of 1000 ×, using the M_{42} multipurpose test system [31].

The number of nuclei of immunostained GH-producing cells was estimated using the formula of Weibel [31]. Since rat GH-positive cells are mononuclear, the numerical density of nuclei (Nv) corresponded to the number of cells per mm^3, according to the formula:

$$Nv = (k/\beta) \times (Na^{3/2}/Vv^{1/2})$$

On the basis of earlier karyometric studies, the shape coefficient β for pituitary cells was estimated to be 1.382 [9]. This relates Nv (number of cells counted per unit volume) to Na (number of cells counted per mm^2) and Vv (volume density) and depends on the axial ratio of nuclei. The volume density of GH-positive cells was expressed as a percentage of total pituitary cell volume.

Digital images were made using a Leica DM RB Photo Microscope (Leica, Wetzlar, Germany) equipped with a JVC TK 1280E Video Camera (Leica). Qwin software (Leica) was employed for the acquisition and analysis of the images.

Statistical analysis. All results were expressed as means for eight to ten animals per group ± SD. Data were tested for normality of distribution by the Kolmogorov-Smirnov test, whereas the homogeneity of variances was evaluated by the F-test. Student's t-test was used to compare the mean values. The minimum level of statistical significance was set at $p<0.05$.

Results

Histological analysis

In near-term fetuses, immunoreactive GH-producing cells of the anterior pituitary gland were usually rounded or ellipsoid. Immunopositive cells were scattered throughout the anterior lobe in both control groups. In 19-day-old fetuses, the intensity of staining varied between cells: numerous GH cells were weakly immunoreactive, while only a few GH cells showed intense immunostaining. After 48 h, GH cells became well differentiated with the characteristic cytoplasmatic ring-shaped area around the nuclei. Histological analysis also showed that the intensity of GH cell immunostaining increased throughout the duration of the examined period. An apparent increase in fetal GH cell number was seen in control fetuses from day 19 to day 21 of pregnancy (Fig. 1A and C).

All the examined 19-day-old fetuses from Dx-treated females had well differentiated GH cells at this stage of development with typical intensely, dark cytoplasmic staining around centrally located nuclei. The number and size of immunoreactive GH cells was increased 24 h after Dx treatment of gravid females in comparison with the corresponding control fetuses. The GH cells were evenly distributed throughout the gland with infrequent cluster formation. This increase in number and immunoreactivity of GH cells remained even 72 h after Dx application i.e. in 21-day-old fetuses compared with the control group (Fig. 1B and D).

Morphometric parameters

Morphometric parameters such as GH nuclear and cell volume, volume density and number of GH cells per unit area markedly increased in parallel with fetal development i.e. from gestational days 19 to 21 (Fig. 2).

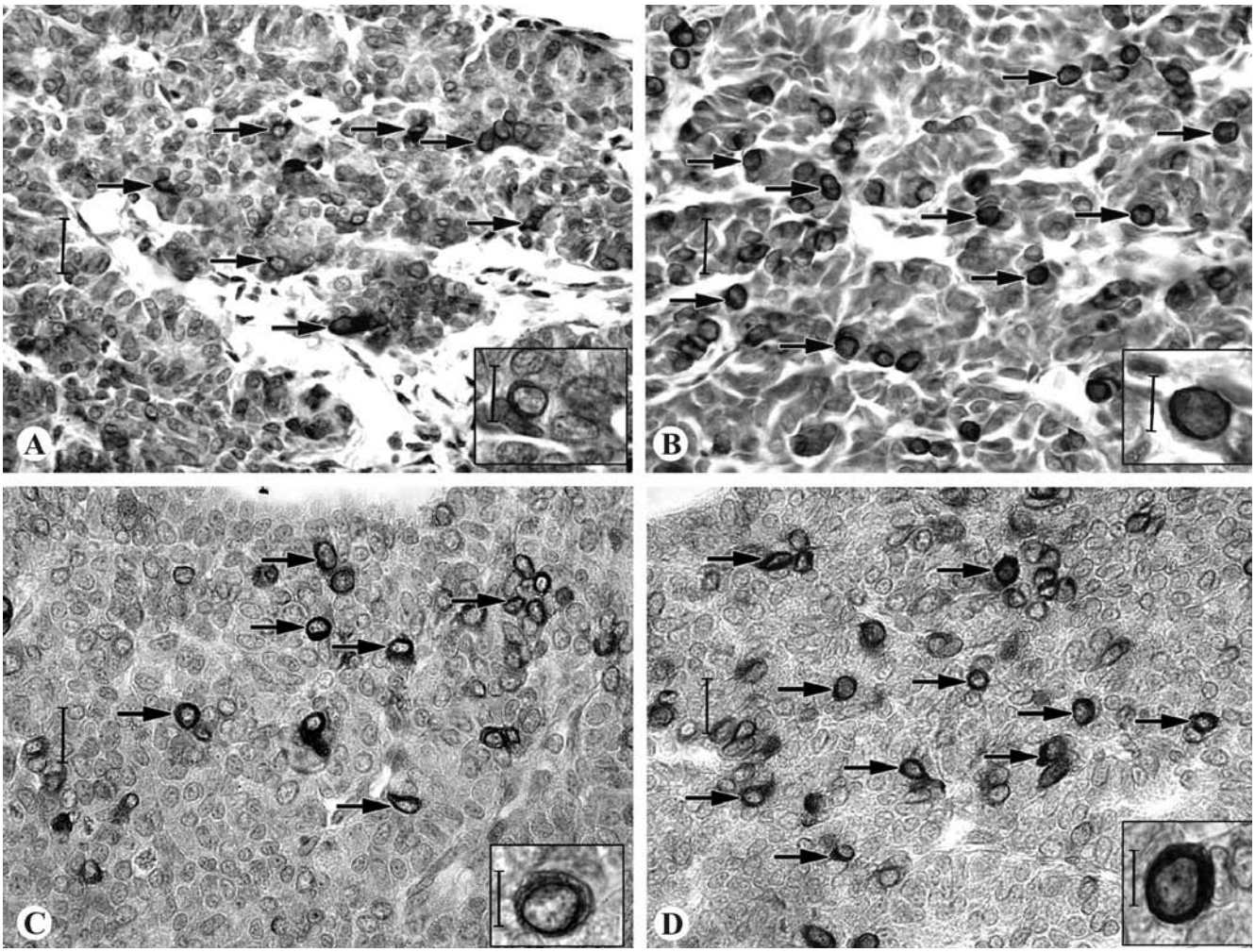

Fig. 1. Pituitary GH cells of control and experimental fetuses. **A**. Pituitary GH cells of control 19-day-old fetuses. Bar = 20 μm. **B**. Strong immunostaining of GH cells after maternal Dx treatment in 19-day-old fetuses. Bar = 20 μm. **C**. GH cells of control 21-day-old fetuses. Bar = 20 μm. **D**. Increased immunoreactivity and number of GH cells in 21-day-old fetuses after maternal Dx administration. Bar = 20 μm. Insets bar = 8 μm. Arrows: immunocytochemically labeled GH cells.

Dx treatment of pregnant rats resulted in significant increases in GH nuclear and cell volume in their 19-day-old fetuses, by 36% and 15%, respectively. The volume density as well as the number of fetal GH cells per square millimeter also showed a significant increase after maternal Dx administration when compared with 19-day-old control fetuses.

In 21-day-old fetuses, 72 hours after the last Dx application significantly higher values for GH cell volume density and their number per square millimeter were still observed in comparison with the corresponding control group. There were no significant differences for the GH nuclear and cell volumes between the experimental and control fetuses (Fig. 2).

Body weight

The body weights gradually increased in near-term control fetuses. Maternal exposure to Dx during days 16-18 of pregnancy did not affect the body weights of 19-day-old fetuses, but a significant reduction of body weight (24%) was noticed in 21-day-old fetuses in comparison with the corresponding control fetuses (Table 1).

Discussion

The present study demonstrated that maternal Dx administration did exert a stimulatory effect on all examined morphometric parameters of GH cells in the 19-day-old fetuses, and significant increases in volume density and number of GH cells per unit area remained in 21-day-old fetuses. Nevertheless, multiple Dx treatment of pregnant rats resulted in a significant reduction in body weight of 21-day-old but not 19 days old fetuses.

The marked increase in immunocytochemically labeled GH nuclear and cell volume from days 19 to 21 of fetal development was accompanied by a sig-

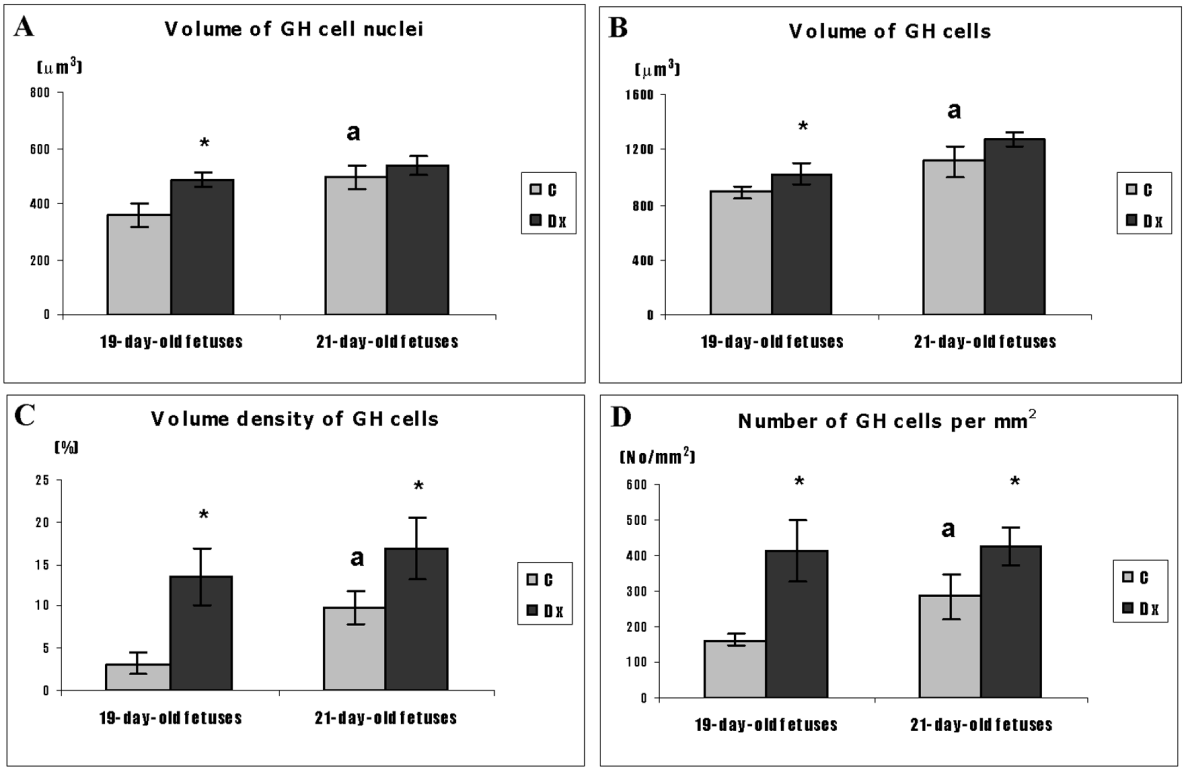

Fig. 2. Morphometric parameters of 19- and 21-day-old fetal GH cells after maternal treatment with Dx (Dx) - experimental group or saline-administered (C) - control group. **A**. Volume of GH cell nuclei. **B**. Volume of GH cells. **C**. Volume density of GH cells. **D**. Number of GH cells per unit area. Results are presented as means ±SD (n = 8); * $p<0.05$ vs. C; a $p<0.05$ vs. 19-day-old C.

nificant rise in volume density as well as in the number of GH cells per square millimeter. Morphometric measurement and histological analysis have demonstrated that fetal GH cells start to produce significant amounts of GH after day 19 of pregnancy, and develop into functional GH cells. These results are in agreement with the finding that pituitary GH content increased 58-fold from days 19 to 21 of fetal development [19]. The significant increase in the number of GH cells throughout the examined period might be explained by the findings of Taniguchi *et al.* [27]. These authors observed the first mitoses of immunopositive GH cells immediately after their appearance in the pituitary - on 19th fetal day. During subsequent days, numerous differentiated GH cells continue to proliferate thus forming the predominant subpopulation of all hormone-producing cells in the perinatal period [27].

Between days 18 and 19 of gestation, fetal GH cells appear spontaneously in parallel with the peak of activity of the fetal pituitary-adrenal system, i.e. with the increased glucocorticoid level in the fetal circulation [29]. Subsequent rapid functional maturation and the increase in the number of GH cells were associated with further rise in corticosterone during the last days of intrauterine development, demonstrating the indispensable role of circulating corticosteroids in GH cell onset and development [17,22]. On the contrary, decreased fetal circulating corticosterone due to metyrapone application significantly reduced the number of GH cells in the fetuses on day 19 of pregnancy [15].

Glucocorticoid passage through the placenta is highly controlled by the placental enzyme 11β-hydroxysteroid-dehydrogenase-II (11βHSD-II), which inactivates maternal corticosteroids to inert forms. Due to 11βSD-II activity, fetal glucocorticoid levels are much lower than maternal concentrations. Nevertheless, Dx treatment of gravid females affects fetuses, since Dx is a synthetic glucocorticoid that easily passes across the placenta, avoiding the enzymatic barrier, and reaches the fetal circulation [24]. In our 19-day-old fetuses, the elevated level of circulating corticosteroids induced the advanced histological appearance and increased size of GH cells in comparison to the controls. The enhanced immunostaining intensity and GH nuclear and cell volume appear to be the result of increased GH synthesis and storage in individual cells after maternal Dx exposure. Dx and corticosterone, the major circulating glucocorticoid in rats, were able to induce dose-dependent increases in GH mRNA *in vivo* [15] and *in vitro* within 24 h [13]. Moreover, Dx increased the percentage of total pituitary cells that secreted GH [17].

It has been established that the primary site of glucocorticoid action is the pituitary gland [13]. Gluco-

Table 1. Influence of maternal Dx administration on body weight in near-term fetuses

	C (g)	Dx (g)
19-day-old fetuses	1.39 ± 0.14	1.32 ± 0.13
21-day-old fetuses	3.62 ± 0.37 [a]	2.96 ± 0.29*

Results are given as means ±SD (n = 8); * $p<0.05$ vs. C; a $p<0.05$ vs. 19-day-old C.

corticoid receptor (GR) mRNA was detected in the adenohypophysis on 13th day of fetal development, and the intensity of the mRNA signal continuously increased from that time [5]. Increases in the number of fetal GH cells expressing GR between 17th and 18th days were confirmed by the results of immunocytochemical studies [14]. GR, acting as a transcriptional activator, is able to mediate glucocorticoid action, including Dx, during the fetal period of life, and therefore its appearance represents an important step in GH cell maturation.

Hypothalamic GH-releasing hormone (GHRH), a hypothalamic polypeptide, has a stimulatory effect on GH release and proliferation of pituitary GH cells. GHRH may play an active role in controlling the function of GH cells during the last few days of pregnancy [6]. The binding of GHRH to the pituitary GHRH receptor is an essential condition for the GH cell to respond in a regulated manner. Dx induced GHRH receptor mRNA expression and accumulation in the fetal rat pituitary gland [14] amplified the stimulatory influence of GHRH. As a consequence, Dx induced GH cells to synthesize and release more GH, leading to increases in GH cell size (nuclear and cell volume) as established in this work.

The present results demonstrate increased volume density and number of GH cells per square millimeter in 19-day-old experimental fetuses compared to control fetuses. Maternal Dx treatment induced the appearance of numerous immunopositive fetal GH cells, initiating and accelerating GH mRNA synthesis in immature GH cells, operating by an unidentified factor [16]. It was established that this factor is not Pit-1, which is known to be required for GH gene expression [13]. Corticosterone-induced GH cell differentiation involves GH expression in cells not expressing GH mRNA previously [17]. Moreover, Dx can induce GH progenitors to start GH synthesis one day earlier than in normal fetuses. *In vitro* findings suggested that incubation of pituitary gland with Dx for 24 h increased GH mRNA on fetal day 18 to a level nearly identical to that in intact 19-day-old fetuses [13]. The numerous *de novo* differentiated GH cells provoked by Dx treatment in 19-day-old fetuses represent a larger pool for further proliferation. During the next 48 h, from days 19 to 21 of fetal development, more GH cells underwent division in the experimental group than in the control group. A significant increase in GH cell number and volume density compared to the controls remained in 21-day-old fetuses after maternal Dx exposure. Increased GH cell number demonstrated by morphometry was also visible during microscopic examination of histological sections.

As mentioned before, functional status of fetal adrenal glands has a great impact on GH cell development. In 19-day-old fetuses, corticosterone production was not decreased by maternal Dx treatment, meaning that level of circulating corticosteroids of fetal origin was not altered under Dx influence [10]. At the same time, both size and number of GH cells were increased after exposure of gravid females to Dx. In 21-day-old fetuses an opposite situation occurred. Maternal Dx treatment significantly inhibited steroidogenesis in the inner zone of adrenal gland cortex, responsible for glucocorticoid synthesis, which led to marked decline in blood glucocorticoid level [4]. Taking into consideration that Dx concentration decreased in the fetal circulation with advancing fetal age, its stimulatory effect on GH cells declined, too. Therefore, significant differences in volumes of GH cells and nuclei between experimental and control groups disappeared 72 h after the last Dx dose.

There were no significant differences in body weight between control and experimental 19-day-old fetuses. However, marked intrauterine growth retardation due to maternal Dx exposure throughout gestation becomes visible in 21-day-old fetuses. As a rapidly growing and short gestation species, pregnancy in rats lasts for 22 days. The last third is a critical developmental stage when organogenesis and differentiation take place and at the same time represents the most vulnerable period for inducing changes [1]. By accelerating organizational events and maturation of the organs [7], Dx induced fetal weight loss 72 h after the last dose. Current results show that the most intensive increase in fetal body weight occurs from day 19 to 21 of gestation when a progressive 2.6-fold rise in body weight has been established. Dx applied for three days during the last trimester of pregnancy led to interruption of the rapid fetal growth near-term and consequently the most notable weight reduction occurred in 21-day-old fetuses or immediately before term. Several studies in humans as well as in animals have demonstrated that fetal exposure to glucocorticoid excess induces intrauterine growth retardation [24,25]. It is also acknowledged that glucocorticoid treatment during pregnancy reduces birth weight that may persist postnatally in humans, non human primates and rodents. The precise effects on body weight changes depend on the dose used and its timing during pregnancy [8].

The present results confirm that maternal Dx treatment from days 16 to 18 of pregnancy can influence in different direction morphometric characteristics of pituitary GH cells and body weight in 19- and 21-day-old fetuses. Dx exerted stimulatory influence on immunoreactivity, size and number (morphometric parameters) of pituitary GH cell in near-term fetuses. At

the same time, after maternal Dx exposure body weight was not significantly changed in 19-day-old fetuses and then marked weight loss occurred in 21-day-old fetuses compared to controls. It might be concluded that during the last days of pregnancy pituitary GH cell activity did not control fetal body weight.

Acknowledgements: This work was supported by the Ministry for Science and Environmental Protection of Republic Serbia, Grant no. 143007.

References

[1] Bakker JM, van den Dobbelsteen GPJM, Kroes H, Kavelaars A, Heijnen CJ, Tilders FJH, van Rees EP. Long-term gender-specific effects of manipulation during pregnancy on immune and endocrine responsiveness in rat offspring. J Neuroimmunol, 1998; 82: 56-63

[2] Collins BJ, Szabo M, Cuttler L. Differential desensitization response of the neonatal and adult rat somatotroph to growth hormone-releasing hormone and phorbol ester. Mol Cell Endocrinol, 1996; 117: 75-81

[3] Crowley P, Chalmers I, Keirse MJ. The effects of corticosteroid administration before preterm delivery: an overview of the evidence from controlled trials. Br J Obstet Gynaecol, 1990; 97: 11-25

[4] Hristić M, Kalafatić D, Plećaš B, Manojlović M. The influence of prolonged dexamethasone treatment of pregnant rats on the perinatal development of the adrenal gland of their offspring. J Exp Zool, 1997; 279: 54-61

[5] Kitraki E, Kittas C, Stylianopoulou F. Glucocorticoid receptor gene expression during rat embryogenesis. An in situ hybridization study. Differentiation, 1997; 62: 21-31

[6] Korytko AI, Zeitler P, Cuttler L. Developmental regulation of pituitary growth hormone-releasing hormone receptor gene expression in the rat. Endocrinology, 1996; 137: 1326-1331

[7] Langley-Evans SC. Fetal programming of cardiovascular function through exposure to maternal undernutrition. Proc Nutr Soc, 2001; 60: 505-513

[8] Lesage J, Del-Favero F, Leonhardt M, Louvart H, Maccari S, Vieau D, Darnaudery M. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. J Endocrinol, 2004; 181: 291-296

[9] Malendowicz LK. Sex differences in adrenocortical structure and function. I. The effects of postpubertal gonadectomy and gonadal hormone replacement in nuclear volume of adrenocortical cells in the rat. Cell Tissue Res, 1974; 151: 525-536

[10] Manojlović-Stojanoski M, Nestorović N, Negić N, Filipović B, Šošić-Jurjević B, Milošević V, Sekulić M. The pituitary-adrenal axis of fetal rats after maternal dexamethasone treatment. Anat Embryol, 2006; 211: 61-69

[11] Milošević V, Brkić B, Velkovski SD, Sekulić M, Lovren M, Starčević V, Severs WB. Morphometric and functional changes of rat pituitary somatotropes and lactotropes after central administration of somatostatin. Pharmacology, 1998; 57: 28-34

[12] Negić N, Nestorović N, Manojlović-Stojanoski M, Filipović B, Šošić-Jurjević B, Milošević V, Sekulić M. Multiple dexamethasone treatment affects morphometric parameters of gonadotrophic cells in adult female rats. Folia Histochem Cytobiol, 2006; 44: 87-92

[13] Nogami H, Inoue K, Kawamura K. Involvement of glucocorticoid-induced factor(s) in the stimulation of growth hormone expression in the fetal rat pituitary gland in vitro. Endocrinology, 1997; 138: 1810-1815

[14] Nogami H, Inoue K, Moriya H, Ishida A, Kobayashi S, Hisano S, Katayama M, Kawamura K. Regulation of growth hormone-releasing hormone receptor messenger ribonucleic acid expression by glucocorticoids in MtT-S cells and in the pituitary gland of fetal rats. Endocrinology, 1999; 140: 2763-2770

[15] Nogami H, Tachibana T. Dexamethasone induces advanced growth hormone expression in the fetal rat pituitary gland in vivo. Endocrinology, 1993; 132: 517-523

[16] Nogami H, Yokose T, Tachibana T. Regulation of growth hormone expression in fetal rat pituitary gland by thyroid or glucocorticoid hormone. Am J Physiol, 1995; 268: E262-E267

[17] Porter TE, Dean CE, Piper MM, Medvedev KL, Ghavam S, Sandor J. Somatotroph recruitment by glucocorticoids involves induction of growth hormone gene expression and secretagogue responsiveness. J Endocrinol, 2001; 169: 499-509

[18] Rodier PM, Kates B, White WA, Phelps CJ. Birthdates of the growth hormone releasing factor cells of the rat hypothalamus: an autoradiographic study of immunocytochemically identified neurons. J Comp Neurol, 1990; 291: 363-372

[19] Rodriguez-Garcia M, Jolin T, Santos A, Perez-Castillo A. Effect of perinatal hypothyroidism on the developmental regulation of rat pituitary growth hormone and thyrotropin genes. Endocrinology, 1995; 136: 4339-4350

[20] Rotenberg M, Gewolb IH. Reversal of lung maturational delay in the fetus of the diabetic rat using triiodothyronine or dexamethasone. Biol Neonate, 1993; 64: 318-324

[21] Sanders EJ, Harvey S. Growth hormone as an early embryonic growth and differentiation factor. Anat Embryol, 2004; 209: 1-9

[22] Sato K, Watanabe YG. Corticosteroids stimulate the differentiation of growth hormone cells but suppress that of prolactin cells in the fetal rat pituitary. Arch Histol Cytol, 1998; 61: 75-81

[23] Savage JJ, Yaden BC, Kiratipranon P, Rhodes SJ. Transcriptional control during mammalian anterior pituitary development. Gene, 2003; 319: 1-19

[24] Seckl JR. Glucocorticoid programming of the fetus; adult phenotypes and molecular mechanisms. Mol Cell Endocrinol, 2001; 185: 61-71

[25] Seckl JR, Meaney MJ. Glucocorticoid programming. Ann NY Acad Sci, 2004; 1032: 63-84

[26] Sternberger LA, Hardy PH Jr, Cuculius JJ, Meyer HG. The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem, 1970; 18: 315-333

[27] Taniguchi Y, Yasutaka S, Kominami R, Shinohara H. Proliferation and differentiation of pituitary somatotrophs and mammotrophs during late fetal and postnatal periods. Anat Embryol, 2001; 204: 469-475

[28] Torronteras R, Gracia-Navarro F, Elsaesser F. Control of growth hormone secretion from porcine fetal and neonatal pituitary tissue in vitro by growth hormone-releasing hormone, somatostatin, and insulin-like growth factor. Neuroendocrinology, 1997; 65: 117-128

[29] Watanabe YG, Haraguchi H. Immunohistochemical study of the cytogenesis of prolactin and growth hormone cells in the anterior pituitary gland of the fetal rat. Arch Histol Cytol, 1994; 57: 161-166

[30] Waters MJ, Kaye PL. The role of growth hormone in fetal development. Growth Horm IGF Res, 2002; 12: 137-146

[31] Weibel E. Stereological methods, Vol. 1. Practical methods for biological morphometry, Academic Press, New York, 1979; pp 1-415

Received: June 23, 2006
Accepted after revision: September 5, 2006