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DEXAMETHASONE TREATMENT DURING PREGNANCY INFLUENCES THE NUMBER OF TSH CELLS IN RAT FETUSES

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Abstract — Glucocorticoids and thyroid hormones control many aspects of fetal development. Using immunohistochemistry and stereology, in the present study we investigated the effects of dexamethasone (Dx) administration during pregnancy on pituitary TSH cells of 19-day-old fetuses. Doses of 1.0, 0.5, and 0.5 mg Dx/kg bw/day were given to the dams on three consecutive days starting on day 16 of gestation. Administration of Dx to pregnant rats induced a significant decline of fetal TSH cell number per unit of area and their volume density in comparison with the corresponding controls. Our results showed that maternal Dx administration inhibited multiplication of TSH cells in 19-day-old fetuses.

Key words: TSH cells, pregnancy, fetuses, rat, dexamethasone

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INTRODUCTION

Thyrotropin-releasing hormone (TRH), produced in the hypothalamus, is the major stimulator of thyroid-stimulating hormone (TSH) synthesis and release from the anterior lobe of the pituitary. This exerts a positive input to the thyroid hormones (TH), thyroxine (T4) and biologically active triiodothyronine (T3). By negative feedback at the hypothalamic and pituitary level, TH are the most important physiological regulators of the serum TSH level (Mitsuma et al., 1990).

During fetal development of the rat pituitary gland, TSH-immunoreactive cells first appear in the pars tuberalis, but their proliferation and differentiation are not controlled by the transcriptional factor Pit-1 (Sakai et al., 1999). In the rat fetal pars distalis, TSH mRNA was detected on day 15 of gestation (Brown et al., 2000); thereafter the thyrotrophs, immunocytochemically recognized in 16.5-17.5-day-old fetuses, were under the determinant role of Pit-1 (Sakai et al., 1999). However, thyrotrophs were few in 17.5-day-old rat fetuses, a rapid increase occurring during the second week after birth (Taniguchi et al., 2001).

Secretion TSH into fetal circulation first occurs on days 17-18, causing an immediate increase in TSH receptor mRNA in the thyroid. Acting through its specific receptor, TSH plays an important role in thyroid gland differentiation, growth, and function (Brown et al., 2000). Functioning of the pituitary-thyroid axis before birth is of critical importance, as thyroid hormones are essential for many aspects of fetal development, growth, and cellular metabolism (Morreale de Escobar et al., 1993; Silva, 1995). Thus, thyroid hormones are involved in the regulation of normal CNS maturation processes such as neurogenesis, neural cell migration, dendritic and axon growth, synaptogenesis, gliogenesis, myelination, and neurotransmitter synthesis (Alvarez-Dolado et al., 2000; Bansal et al., 2005); gonadal development (Francavila et al., 1991); and respiratory enzyme synthesis and heat production (Bhargava et al., 2007).

Glucocorticoids also exert powerful influence on the intrauterine development of fetal organ systems. Glucocorticoid receptors are found intracellularly in almost all tissues (Kitraki et al., 1997). Glucocorticoids affect fetal brain development, lung

maturity and surfactant phosphatidylcholine synthesis, induction of glucogenic hepatic enzymes, and maturation of rat renal mitochondria, providing effective oxidative phosphorylation (Barker, 1995; Prieur et al., 1998; De Kloet, 2004). These actions ensure an adequate response to environmental stimuli and maintenance of homeostasis in the newborns. Steroid administration during pregnancy and in premature infants therefore became the standard way to prevent life-threatening complications such as respiratory distress syndrome (Fitzerald et al., 1998).

The aim of our study was to investigate the influence of maternal treatment with the synthetic glucocorticoid dexamethasone (Dx) during the period of gestation when the fetal pituitary-thyroid axis is establishing its function on some histological and morphometric characteristics of pituitary TSH cells in 19-day-old fetuses.

MATERIALS AND METHODS

Animals

Female and male Wistar strain rats, weighing approximately 250 and 400 g, respectively, were mated in the laboratory at 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*. Dams were randomized into two groups: a control and an experimental group, each consisting of six animals. On day 16 of pregnancy, the experimental dams received subcutaneously 1.0 mg Dx (dexamethasone phosphate - Krka, Novo Mesto, Slovenia, dissolved in 0.9% saline)/kg b.w., followed by 0.5 mg Dx/kg b.w./day on days 17 and 18 of gestation. The control gravid females received the same volume of saline vehicle. On day 19 of gestation, the females were sacrificed under ether narcosis 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).

Tissue preparation

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. Serial 5-μm-thick tissue sections were deparaffinized in xylol and decreasing series of ethanol. Three pituitary sections from the dorsal, medial, and ventral parts were prepared for immunocytochemical staining. Pituitary TSHβ was localized immunocytochemically using the peroxidase-anti-peroxidase (PAP) complex method of Sternberger et al. (1970). Primary rabbit anti-rat TSHβ antiserum was kindly provided by Dr. A.F. Parlow, NIH, Bethesda, MD, USA.

Morphometrical and stereological measurements

All stereological analyses were carried out using a workstation comprising a microscope (Olympus, BX-51) equipped with a CCD video camera (PixeLink) connected to a 19" PC monitor (Dell). The whole system was controlled by the newCAST stereological software package (VIS – Visiopharm Integrator System, version 2.12.1.0, Visiopharm, Denmark). The main objectives used were planachromatic 4x and 20x dry lenses.

Firstly, the number of cells per unit of area was determined. At the monitor, a final magnification of 150x allowed easy and accurate recognition of tissue boundaries. After defining adenohypophyseal boundaries on the examined pituitary sections, the area in μm^2 was determined automatically using the Mask tool of newCAST software. Then exact counting of TSHβ immunostained cells was carried out at a final magnification of 750x (at the monitor) and the number of cells was expressed per unit of area.

Volume density was estimated employing a point grid with 16 points. After defining adenohypophyseal boundaries, the whole structure was overlaid with the point grid. The number of points falling within immunolabeled TSH cells divided by the total number of points hitting the reference space, i.e., marked adenohypophyseal tissue, represents the volume density of TSH cells.

The Rotator tool was used for estimation of the cell volume of 150-200 cells with nuclei per animal.

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

Statistical analysis

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

RESULTS

Histological analysis

The presence of TSH cells was detected in the pituitary pars distalis in 19-day-old control fetuses. These cells were characterized by large rounded or elongated nuclei surrounded by cytoplasm, the immunopositivity of which varied from intensive to pale. Immunocytochemically labeled TSH cells exhibited different shapes, rounded or polygonal, while cytoplasmic processes were occasionally present (Fig. 1). They were uniformly distributed among the parenchymal cells and around the capillary network. Groups of two to three cells were seen on histological sections rarely. After maternal Dx administration the histological appearance of fetal TSH cells was not altered significantly in relation to the controls, considering cell immunopositivity and size (Fig. 1).

Morphometric and stereological analysis

The volume of TSH cells in 19-day-old fetuses was not significantly changed after Dx treatment of pregnant rats. Administration of Dx to rats during the last week of pregnancy led to a significant decrease of TSH cell number per unit of area ($p<0.05$) in 19-day-old fetuses in comparison with the control group. Also, maternal Dx application induced a significant decline of fetal TSH cell volume density ($p<0.05$) compared to the controls (Fig. 2).

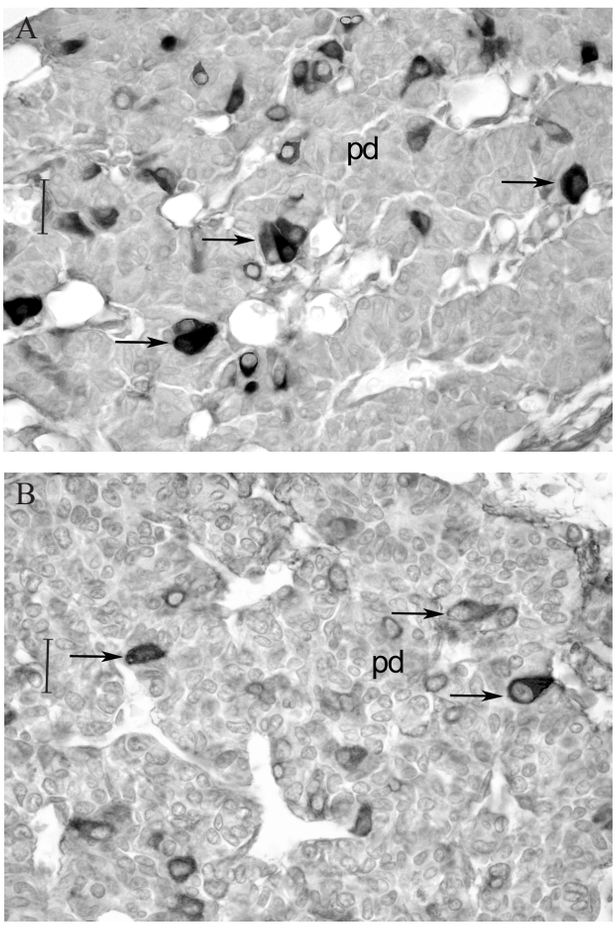

Fig. 1. A. Pituitary gland of 19-day-old fetuses with numerous immunocytochemically labeled TSH cells in the pars distalis (pd) located around a capillary and among other cells B. After maternal Dx administration, a decrease in the number of immunocytochemically labeled fetal TSH cells is evident. Abbreviations: pd) pars distalis; →) immunocytochemically labeled TSH cells; bar = 20 μm.

DISCUSSION

The results presented here indicate that fetal exposure to excess glucocorticoid levels *in utero* leads to a decreased number of fetal pituitary TSH cells per unit of area and a decline in their volume density in 19-day-old fetuses. The gravid female rats were treated with Dx from days 16 to 18 of pregnancy, a period when all types of fetal pituitary hormone-producing cells start to function.

Access of maternal endogenous glucocorticoids (corticosterone in rats) to the fetus is limited owing

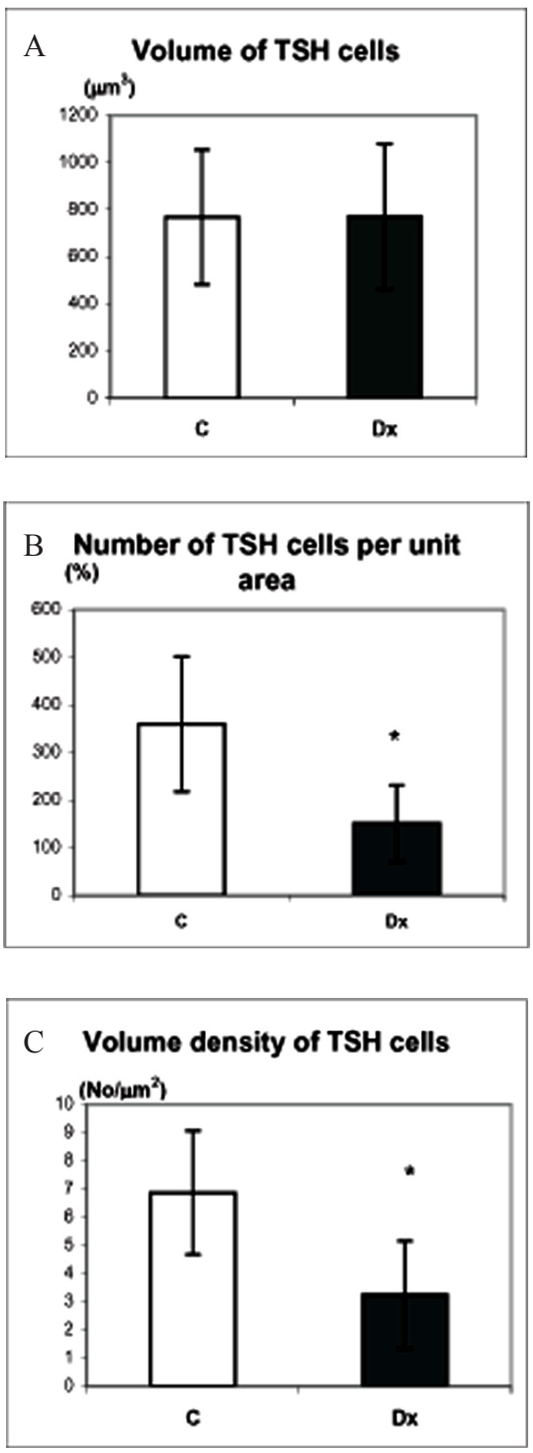

Fig. 2. Morphometric parameters for 19-day-old fetal TSH cells after maternal treatment with Dx (Dx, experimental group) or saline (C, control group). A. Volume of TSH cells. B. Number of TSH cells per unit of area. C. Volume density of TSH cells. Results are presented as means ± S.D. (n = 6); * p <0.05 vs. C.

to the activity of placental 11β-hydroxysteroid-dehydrogenase type 2 (11βHSD2), which oxidizes active corticosteroids to inactive ketone products. The synthetic glucocorticoid Dx is not metabolized by placental 11βHSD2, so it enters the fetal circulation and can influence many aspects of fetal development (Seckl, 2001).

During pituitary gland development, the number of TSH cells increases as a consequence of precursor cell differentiation and further division of already existing TSH cells. Taniguchi et al. (2001) demonstrated the proliferation of differentiated thyrotrophs in fetal rats by double immunostaining with ant-BrdU and anti-rTSH. They concluded that this proliferation occurred during the late fetal period. Our results showed that the number of fetal TSH cells is under the influence of Dx, too. The decreased number of TSH cells per unit of area and lower volume density of TSH cells in 19-day-old fetuses indicated that, after passing through the feto-placental barrier, Dx inhibited the proliferation of both precursors committed to a TSH destiny and differentiated TSH cells entering mitosis. It is known that during fetal development Dx also exerts strong antiproliferative action on different cell types, such as neurons in the developing brain (Matthews, 2000), pituitary ACTH cells (Manojlović-Stojanoski et al., 2006), and insulin-producing cells (Shein et al., 2003), as well as causing overall fetal growth impairment in humans, rats, and sheep (Jobe et al., 1998).

Using triple immunofluorescent staining for the α glycoprotein subunit βTSH and proliferate marker PCNA, Pope et al. (2006) also showed that differentiated TSH cells retained the ability to divide in human and mouse fetuses. This is probably the reason why the antiproliferative action of Dx on fetal TSH cells becomes pronounced and measurable. On the contrary, the same authors demonstrated that differentiated gonadotrophs were non-dividing cells during the fetal period.

Induction of programmed cell death by glucocorticoid treatment might also contribute to the decrease in number of TSH cells and their volume density that was established 24 h after maternal

exposure to Dx in our experimental conditions. Exposure to Dx induced a short burst of apoptosis that began a day or two after the start of treatment and lasted for 24 h or less in rat male adult pituitary glands (Nolan et al., 2004). Apoptosis increased in precursors, i.e., in hormonally undifferentiated pituitary cells, after Dx application (Nolan and Levy, 2006).

We found that exposure to Dx *in utero* did not influence cell volume or immunostaining intensity of TSH cells in 19-day-old fetuses, indicating that hormone synthesis and release were not affected by the treatment.

Repeated courses of maternal glucocorticoid therapy were approved and have become standard practice in cases of preterm delivery to minimize the frequency of respiratory problems and perinatal death. Investigations using animal models and clinical practice are focused on the short- and long-term consequences of such treatment (Bakker et al., 2001; Kapoor et al., 2008). The programming concept implied that changes caused by alterations to the intrauterine environment, e.g., fetal exposure to excess glucocorticoid, might be a basis for susceptibility to later chronic diseases (Barker, 1995). Further investigations are necessary to establish whether the decrease of TSH cell number in 19-day-old fetuses found here influences developmental processes with later long-term consequences or is reversed by fetal homeostatic mechanisms allowing normal functioning of the pituitary-thyroid axis.

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ТРЕТМАН ДЕКСАМЕТАЗОНОМ ТОКОМ ГРАВИДИТЕТА УТИЧЕ НА БРОЈ TSH ЋЕЛИЈА КОД ФЕТУСА ПАЦОВА

МИЛИЦА МАНОЈЛОВИЋ-СТОЈАНОСКИ, НАТАША НЕСТОРОВИЋ, НАТАША НЕГИЋ, БРАНКА ШОШИЋ-ЈУРЈЕВИЋ, СВЕТЛАНА ТРИФУНОВИЋ, ВЕРИЦА МИЛОШЕВИЋ и МИЛКА СЕКУЛИЋ[4]

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Глукокортикоиди и тироидни хормони контролишу бројне аспекте развоја фетуса. Користећи имуноцитохемију и стереолошке методе испитивали смо ефекте примене дексаметазона (Dks) током гестације на TSH ћелије хипофизе фетуса пацова старих 19 дана. Дозе од 1.0, 0.5 и 0.5 мг Dks/кг тм/дан примењиване су гравидним женкама три узастопна дана, почев од 16. дана гестације. Третман Dks код гравидних женки узрокује значајно смањење броја TSH ћелија фетуса по јединици површине и њихове волуменске густине у поређењу са одговарајућим контролама. Наши резултати показују да матернална примена Dks инхибира мултипликацију TSH ћелија код фетуса старих 19 дана.