Steroids 84 (2014) 22–29

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Morpho-functional characteristics of rat fetal thyroid gland are affected by prenatal dexamethasone exposure

Milica N. Manojlović-Stojanoski *[,1], Branko R. Filipović [1], Nataša M. Nestorović, Branka T. Šošić-Jurjević, Nataša M. Ristić, Svetlana L. Trifunović, Verica Lj. Milošević

Institute for Biological Research "Siniša Stanković", University of Belgrade, 142 despota Stefana Blvd., 11060 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 9 September 2013
Received in revised form 24 February 2014
Accepted 4 March 2014
Available online 20 March 2014

Keywords:
Thyroid
Fetal development
C cells
Follicular cells
Dexamethasone
Stereology

ABSTRACT

Thyroid hormones (TH) and glucocorticoids strongly contribute to the maturation of fetal tissues in the preparation for extrauterine life. Influence of maternal dexamethasone (Dx) administration on thyroid glands morpho-functional characteristics of near term rat fetuses was investigated applying unbiased stereology. On the 16th day of pregnancy dams received 1.0 mg/Dx/kg/b.w., followed by 0.5 mg/Dx/kg/b.w. on the 17th and 18th days of gestation. The control females received the same volume of saline. The volume of fetal thyroid was estimated using Cavalieri's principle; the physical/fractionator design was applied for the determination of absolute number of follicular cells in mitosis and immunohistochemically labeled C cells; C cell volume was measured using the planar rotator. The functional activity of thyroid tissue was provided from thyroglobulin (Tg) and thyroperoxidase (TPO) immunohistochemical staining. Applying these design-based modern stereological methods it was shown that Dx treatment of gravid females led to a significant decrease of fetal thyroid gland volume in 19- and 21-day-old fetuses, due to decreased proliferation of follicular cells. The Tg and TPO immunohistochemistry demonstrated that intensive TH production starts and continues during the examined period in control and Dx-exposed fetuses. Under the influence of Dx the absolute number of C cells was lower in both groups of near term fetuses, although unchanged relation between the two populations of endocrine cells, follicular and C cells suggesting that structural relationships within the gland are preserved. In conclusion maternal glucocorticoid administration at the thyroid gland level exerts growth-inhibitory and maturational promoting effects in near term rat fetuses.

1. Introduction

The thyroid gland contains two major types of endocrine cells, follicular cells, which form thyroid follicles for storing thyroglobulin, and a minor population of C cells, which primarily produce calcitonin (CT). In contrast to follicular cells, C cells are heterogeneously distributed in the thyroid lobes [1]. The thyroid gland originates partly from the pharyngeal endoderm which differentiates into follicular (thyroid hormone (TH)-producing) cells. The second type of progenitors derive from the most caudal pharyngeal pouches and are subsequently incorporated into the thyroid bud where they differentiate into C cells [2].

In fetal rats, significant growth and rapid structural and functional development of the thyroid gland happen during the last third of gestation. The first appearance of follicles, iodine organification and thyroid hormonogenesis occur in parallel with a marked increase of thyroid-stimulating hormone (TSH) in the fetal circulation and expression of TSH receptors (TSHR) in thyroid tissue [3]. Since immunohistochemical appearance of TSH cells in the rat pituitary is recorded on day 17 of fetal development, and their number continues to grow until the second week of life [4], maturational changes in the thyroid gland lead to a significant increase in the blood concentration of TH during the perinatal period. During this transient window in time numerous tissues are sensitive to TH action which induces indispensable, permanent changes in their structure and functions. Iodothyronine deiodinase (D1, D2, D3) enzymes provide biologically active triiodothyronine (T3) to developing tissues by activating and/or deactivating systemic serum TH. Three types of deiodinases differ in tissue distribution, substrate specificity and sensitivity to inhibiting compounds [5]. Action of D2 and D3 preserves the safe level of T3 in the developing brain and the pituitary, while the activity of D3 in the utero-placental unit protects fetal tissues against high maternal thyroxine (T4) concentrations [5].

* Corresponding author. Tel.: +381 11 2078 323; fax: +381 1 2761 433.
E-mail address: manojlo@ibiss.bg.ac.rs (M.N. Manojlović-Stojanoski).
[1] M. Manojlović-Stojanoski and B. Filipović contributed equally to this work.

http://dx.doi.org/10.1016/j.steroids.2014.03.006
0039-128X/

Little is known about the activity of C cells in fetal rat thyroids. An earlier study detected CT in the thyroid gland of 17.5-day-old rat fetuses [6] and the rise in plasma CT levels between 19.5 and 20.5 days of gestation corresponded to increased C cell activity [7].

As glucocorticoids have a potent influence on maturation of fetal lung and other tissues they have been used for more than 40 years in human pregnancies at risk of preterm delivery. Antenatal glucocorticoids mimic the actions of the endogenous rise in plasma glucocorticoids, reducing complications, such as neonatal respiratory distress syndrome, and most importantly, neonatal mortality [8]. Synthetic glucocorticoids, such as dexamethasone (Dx), are poorly metabolized by placental 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), an enzyme which largely prevents maternal glucocorticoids from reaching the fetus, pass into the fetal circulation and shape fetal development [9]. Although elevated glucocorticoid levels during pregnancy are associated with in utero growth retardation, metabolic, cardiovascular and immune adaptations under the influence of glucocorticoids are fundamental to successful reaction to birth-related stress and the environmental challenges of the neonate [10,11]. Nevertheless, as an established programming concept, all these adaptations predispose organisms to diseases in adulthood [11].

Prenatal alterations in the maternal glucocorticoid environment reflect fetal, neonatal and adult hypothalamic–pituitary–thyroid (HPT) axis activity and function [12,13]. Maternal Dx administration influences TH level and peripheral deiodination of TH in ovine fetuses [12]. In addition, alterations in the maternal glucocorticoid milieu during pregnancy have been found to affect functioning of the HPT axis in both female and male adult rat offspring at all levels (hypothalamic TRH expression, pituitary TSH level, and thyroid hormonogenesis) [13,14]. Additionally, thyrocytes express glucocorticoid receptor (GR), and this signalling system contributes to differentiation of thyroid cells [15]. Thus, it was hypothesized that maternal glucocorticoid administration during pegnancy affects fetal thyroid gland. To address this hypothesis, our study aim was to determing structural changes of thyroid gland, as well as immunohistochemical properties of follicular and C cells in 19- and 21-day-old fetuses after maternal exposure to Dx.

2. Material and methods

2.1. Animals

Adult female Wistar rats, weighing 260 ± 10 g, bred in the laboratory of the Institute for Biological Research, Belgrade, were used. The animals were kept in a light (lights on 06.00–20.00 h) and temperature (22 ± 2 °C) controlled room. Rat chow and tap water were available ad libitum. Female rats were examined daily and only those showing regular 4-day cycles were included. The presence of spermatozoa in vaginal smears the morning after caging with a fertile male in the night of proestrus was indicative of pregnancy and this day was considered as day 1 of gestation. Dams were randomized into a control and an experimental group, each consisting of six animals. On day 16 of pregnancy experimental dams received 1.0 mg Dx (Dexamethasone phosphate – Krka, Novo Mesto, dissolved in 0.9% saline)/kg b.w. subcutaneously, 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. Exposure to tapering regime of Dx i.e., reduction of Dx doses has been used in order to minimize adverse effects of glucocorticoid administration. The applied doses of Dx are equivalent to doses used as anti-inflammatory therapy in clinical practice [16–18]. The same effects of subcutaneus and intramuscular application of Dx are established, so the consequences of these routes of Dx administration are comparable [18,19]. Fetuses from control and experimental females were sacrificed under ether narcosis on days 19 and 21 of gestation and they are referred to as 19-day-old and 21-day-old fetuses. Each group ($n = 6$) was formed from randomly selected male fetuses (based on ano-genital distance), taking into account that fetuses were taken from different mothers.

All animal procedures complied with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

2.2. Tissue preparation and immunohistochemistry

The laryngeal area, including the thyroid gland, was immediately excised, fixed in Bouin's solution for 48 h and dehydrated in increasing concentrations of ethanol and xylene. After embedding in Histowax (Histolab Product AB, Göteborg, Sweden), each tissue block was serially sectioned at 3 μm thickness on a rotary microtome (RM 2125RT Leica, Wetzlar, Germany). Sakura Tissue-Tek Accu-Edge Low-Profile microtome blades for extremely thin sectioning were used. The slices were placed on silica coated glass slides (SuperFrost Plus, Prohosp, Denmark).

CT-immunopositive thyroid C-cells and thyroglobulin (Tg)-immunopositive cells were localized using the peroxidase–anti-peroxidase (PAP) method. Endogenous peroxidase activity was blocked by incubation in 9 mM hydrogen peroxide solution in methanol for 15 min at ambient temperature. Nonspecific background staining was prevented by incubation of the sections with nonimmune, normal porcine serum diluted in phosphate-buffered saline (PBS; pH 7.4) for 60 min. For detection of CT in the C cells anti-human CT antiserum (Dakopatts, Copenhagen, Denmark) diluted 1:500 were incubated for 60 min and served as the primary antibody; for Tg detection rabbit-anti-human antibody diluted 1:500 was employed for 120 min (Dakopatts, Copenhagen, Denmark). After washing in PBS, sections were incubated for another 60 min with the secondary antibody, polyclonal swine-anti-rabbit IgG/HRP (Dako, Glostrup, Denmark), and rinsed again with PBS for 10 min. Antibody localization was visualized using 0.05% 3,3-diaminobenzidine tetrachloride (DAB) liquid substrate chromogen system (Dako). Sections were thoroughly washed under running tap water, counterstained with hematoxylin and mounted in DPX.

Thyroperoxidase (TPO) localization was also determined. After dewaxing and hydration the sections were exposed to microwaves (800 W) in 0.05 M citrate buffered saline (pH 6.0) for 10 min for antigen retrieval. The subsequent immunohistochemical procedure was described before. Mouse monoclonal antbody directed against human TPO (Santa Cruz Biotechnology INC, CA, USA; diluted 1:25) was applied as the primary antibody, and sections were incubated overnight, while polyclonal rabbit anti-mouse IgG/HRP (Dako, Glostrup, Denmark) was used as the secondary antibody.

2.3. Stereological measurements

All stereological analyses were carried out using a workstation comprising a microscope (Olympus, BX-51) equipped with a microcator (Heidenhain MT1201) to control movement in the z-direction (0.2 μm accuracy), a motorized stage (Prior) for stepwise displacement in the x–y directions (1 μm accuracy), and a CCD video camera (PixeLink) connected to a 19" PC monitor (Dell). The whole system was controlled by a newCAST stereological software package (VIS – Visiopharm Integrator System, version 2.12.1.0; Visiopharm; Denmark). The main objectives were planachromatic 10× dry lenses and a 100× oil lens. Control of the stage movements and the interactive test grids (uniformly spaced points test grids