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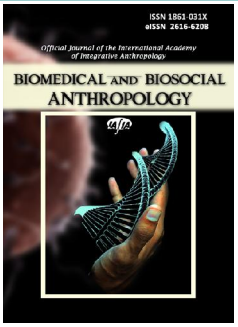

The effect of prenatal action of dexamethasone on morphological changes of the thyroid gland stromal compartment in juvenile rats

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The thyroid gland is important for the normal functioning of the body, and is the largest endocrine organ, which among the endocrine glands will be the first in the process of embryogenesis. In recent decades, the prevalence of thyroid pathologies of various origins among the world's population has reached critical proportions. The use of glucocorticoids during pregnancy remains a debatable issue in obstetrics today, as they can both positively and negatively affect the processes of organ morphogenesis and be the cause of pathological conditions in the postnatal period. It is known that the entry of cortisol from mother to fetus through the placenta is controlled by enzymes produced by the latter. However, synthetic glucocorticoids, such as dexamethasone, can freely cross the blood-placental barrier and cause changes in postnatal immunity and disease in the future. The thyroid gland, having a relatively "simple" basic structure depending on the level of activity and the period of ontogenesis, exhibits various forms of morphological organization. Ideas about the structure and function of the thyroid gland were formed in the course of centuries of its study. Modern experimental and technical capabilities of the study of the body provided information about the structural and functional features of the thyroid gland and the numerical relationships of its structures at different levels of the organization. The aim of the study was to establish the features of the morphogenesis of the rats' thyroid gland from 60 to 120 days of life after prenatal exposure to dexamethasone. Statistical analysis of the results was performed using a personal computer based on the Windows XP operating system using the statistical package "Statistica for Windows 6.0" (StatSoftInc., Serial number №AXXR712D833214FAN5), Excel (Microsoft Office, USA). Methods of variation statistics were used. All research results were recorded in journals and protocols of primary documentation, as well as with the use of electronic media. Significance of differences between groups was assessed using Student-Fisher t-test for a confidence level of at least 95 % ($p < 0.05$). During the study it was found that in the juvenile period the morphological structure of the rats' thyroid gland of intact and control groups is finally formed, in the parts of which the peripheral and central parts with rather high sclerosing index (6.20 and 6.46, respectively) can be microscopically distinguished due to prevalence. the percentage of parenchymal component (60.82±1.13 % and 61.44±0.71 %) above the stroma (9.86± 1.02 % and 9.53±0.94 %). The study of material obtained from animals prenatally exposed to dexamethasone showed a violation of the morphogenesis of histoarchitectonics of the thyroid gland with the formation of a lobular structure of its particles due to an increase in stromal component (23.63±0.88 %), which was expressed in a decrease in sclerosis index (2.364), but there was a compensatory increase in the percentage of follicular epithelium on the 120th day of life (55.87±0.79 %) compared with the 90th day (49.24± 1.25 %), without morphological signs of functional disorders.

Key words: *thyroid gland, dexamethasone, morphogenesis, experiment, stromal component, rat.*

Introduction

To date, the range of use of steroids is very wide [1, 2]. In obstetrics, there is a method of prevention of primary pulmonary atelectasis in premature infants by administering dexamethasone to a pregnant woman, which

transplacentally stimulates the production of surfactant in the lungs of the fetus. Long-term possible negative consequences have not been studied enough [3, 6, 12]. There are clinical observations, based on which we can say about the possible teratogenic effects, the increased risk of developing in the offspring of endocrine pathology, cardiovascular system, pancreatic diseases, even the negative impact on brain development [9-11, 13]. There is an assumption that the negative effect of glucocorticoids is realized through genes, which in turn affect the regulation of the synthesis of these hormones and the production of some mediators (dopamine, acetylcholine, norepinephrine) [16, 18, 21]. At present, the question of the use of glucocorticoids during pregnancy remains controversial, the question of the duration of the therapeutic effect has not yet been resolved. Glucocorticoids have a strong influence on the growth and maturation of fetal organ systems, but excessive action of exogenous glucocorticoids can slow fetal growth and change developmental processes in sensitive tissues [4, 5, 19, 20].

In recent decades, the prevalence of thyroid pathologies of various origins among the world's population has reached critical proportions. According to the American Association of Clinical Endocrinologists (AACE), about 13 million people in the United States, or 4.78 % of the population, have clinically undiagnosed thyroid dysfunction [8, 14].

The problem of morphofunctional homeostasis of the thyroid gland in ontogenesis under the influence of various factors covers the processes of morphogenesis of the body, compensatory-adaptive mechanisms, the activity of proliferative processes [7, 15, 17, 22].

The aim of the study: to determine the effect of prenatal action of dexamethasone on the structural transformation of the rats' thyroid gland from the 60th to the 120th day of postnatal development.

Materials and methods

The material for the study was the thyroid gland of Wistar rats aged 60 to 120 days of the postnatal period, 6 animals in each subgroup (54 objects in total). Three groups of animals were studied: I gr. - intact animals (norm); II gr. - control, animals which were injected with 0.9 % NaCl solution on the 18th day of the dated pregnancy; III gr. - experimental group, rats, which in the antenatal period on the 18th day subcutaneously in the interscapular area was injected with a solution of dexamethasone in the amount of 0.05 ml at a dilution of 1:40. For antenatal administration of dexamethasone and saline to pregnant rats under aseptic conditions under general anesthesia, a midline laparotomy was performed, followed by intrauterine, transdermal subcutaneous injection of solutions to each fetus separately. Animals were removed from the experiment on the 60th, 90th and 120th day of life. The thyroid gland was fixed in a 10% solution of neutral buffered formalin during the day. The objects were filled into paraffin blocks by the conventional method. Histological sections 4 ?m thick were stained with hematoxylin-eosin for observation light microscopy and morphometry, according to the Van Gieson method for visualization of stromal components of the thyroid gland.

According to morphometric indicators of the relative content of follicular epithelium and stroma, the sclerosing index was calculated:

SI = Se / Ss,

where SI is the sclerosing index, Se is the relative area of the epithelium, Ss is the relative area of the stroma.

Photo-documentation of the studied objects was performed using a Carl Zeiss microscope "Primo Star" using an AxioCam camera, a set of morphometric studies was performed using the Zeiss Zen program (2017).

Statistical analysis of the results was performed using a personal computer based on the Windows XP operating system using the statistical package "Statistica for Windows 6.0" (StatSoftInc., Serial number №AXXR712D833214FAN5), Excel (Microsoft Office, USA). Methods of variation statistics were used. All research results were recorded in journals and protocols of primary documentation, as well as with the use of electronic media. Significance of differences between groups was assessed using Student-Fisher t-test for a confidence level of at least 95 % ($p < 0.05$).

Results

The histological structure of the thyroid gland is of great interest to scientists, and the study of its morphofunctional structure confirms that this organ is very favorable to various factors, which is why the change in the structure of the thyroid gland is of great importance. In the rats' thyroid gland, there is a clear division of the parenchyma into peripheral and central areas. In the peripheral area, the follicles are larger, C cells are absent, and in the central area, the follicles are small with a large number of C cells (Fig. 1a).

Histological study of the animals' thyroid gland 60th day of life after prenatal action of dexamethasone on the body showed that the formation of the morphological structure of the gland differs from that of animals of the control and intact groups. Due to the increase in the stromal fibrous component, there was a tendency to form a lobular morphological type of thyroid gland in animals of the experimental group (Fig. 1b), in contrast to the two comparison groups, which maintained the division of organ tissue into central and peripheral parts, indicating the formation of parenchymal morphological type of the thyroid gland structure (see Fig. 1a).

The percentage of stromal-parenchymal parameters (Tab. 1) underwent mostly changes from the stroma (3.4% more in the experimental group than the control). In terms of staining intensity, the collagen content in the connective tissue layers was higher in the thyroid gland of animals prenatally exposed to dexamethasone, compared with the

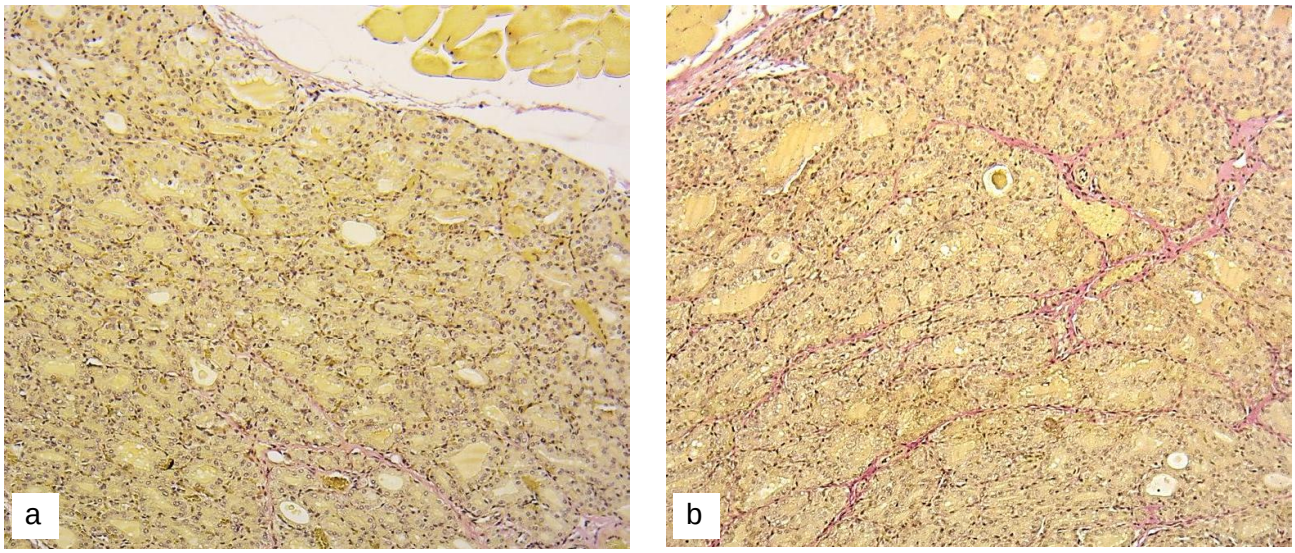

Fig. 1. Rats thyroid gland of the 60th day of life of control (a) and experimental (b) groups. Staining by the Van Gieson method. Magnification x200.

Table 1. The relative content of the structural elements of the rat`s thyroid gland in the juvenile period of life.

Animals age	Animals group	The relative content of the thyroid structural components, %			Sclerosis index
		Follicular epithelium	*Colloid*	*Stroma*	
60 days	I	65.93±1.34	23.81±0.93	9.637±0.744	6.841
	II	66.31±0.92	23.25±0.44	9.828±0.641	6.747
	III	61.75±0.84*	24.42±0.61	13.28±0.86*	4.650*
90 days	I	66.24±0.82	22.33±0.74	10.24±0.32	6.469
	II	66.45±1.16	21.61±0.33	9.603±0.521	6.920
	III	49.24±1.25*, **	30.16±0.58*, **	19.56±0.78*, **	2.517*, **
120 days	I	60.82±1.13**	27.91±0.92**	9.86±1.022	6.168
	II	61,44±0.71**	28.46±0.47**	9.531 ±0.943	6.446
	III	55.87±0.79*, **	20.34±1.23*, **	23.63±0.88*, **	2.364*, **

Note: * - significant difference from control, p<0,05; ** - significant difference in comparison with the previous term p<0.05. I - intact group of animals, II - animals of the control group, III - animals of the experimental group prenatally exposed to dexamethasone.

control. But despite almost the same indicators of parenchymal elements in all study groups, the index of sclerosis in the thyroid gland of the experimental group was 1.4 times lower than in animals of the control group, indicating an increase in the content of the stromal component of the organ.

On the 90th day of life there was a moderate swelling of the interparticle stroma, there was dilation of blood vessels. The content of collagen fibers, as well as mast cells, fibroblasts and fibrocytes in the thyroid gland of animals of the experimental group (Fig. 2a) increased in comparison with similar indicators in the control and intact groups. The follicles were mostly medium and large. It should be noted the high level of colloid accumulation in the lumens of the follicles, as well as its sparseness and low color intensity in the experimental group of animals Fig. 2b).

In an experimental study, it was found that at 120th days of animal life, the structural organization of the thyroid gland of animals of the intact and control groups was identical, but significantly different from that of animals that received prenatal dexamethasone. In animals of intact and control groups, the morphological organization of thyroid structures was a parenchymal type of structure and it was possible to distinguish the central and peripheral parts of the particles, although clear boundaries between them were not visualized, and the criterion was the size of follicles on the periphery are larger in size) (Fig. 3a). The relative indicators of stroma content and sclerosing index did not differ statistically significantly relative to the previous study period in the above groups (see Tab. 1). In the experimental group of animals that were prenatally exposed to dexamethasone, the lobes of the thyroid gland had a lobular type of structure (Fig. 3b). Despite this morphological picture and the increase in the percentage of stroma compared to the previous period by 4.07 % and compared to the control by 14.099 %. The sclerosing index decreased slightly (1.064

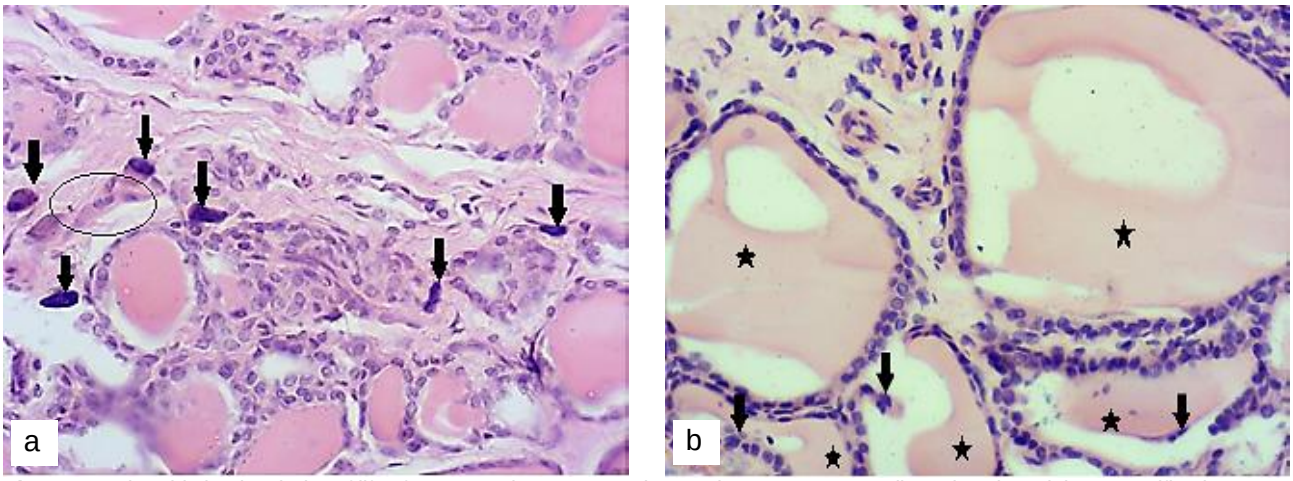

Fig. 2. Rats thyroid gland 90th day of life after prenatal exposure to dexamethasone. Hematoxylin and eosin staining. Magnification x400. a - arrows indicate mast cells, circle - clusters of fibroblasts; b - * - colloid in follicles without parietal vacuolation, with signs of desquamation (indicated by arrows).

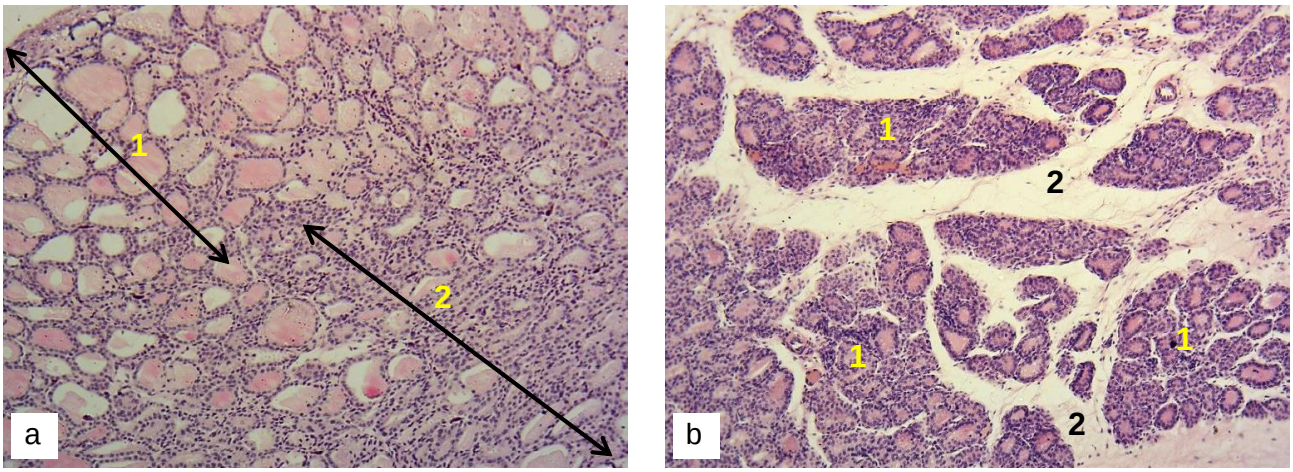

Fig. 3. Rats thyroid gland of 120 days of life in normal (a) and after prenatal action of dexamethasone (b). Hematoxylin and eosin staining. Magnification x200. a - 1 - the peripheral part of the thyroid gland, 2 - the central part of the thyroid gland; b - 1 - lobules of the thyroid gland, 2 - connective tissue septums.

times) compared to the previous period due to an increase in the percentage of follicular epithelium relative to the previous period, but compared to the control group, this figure was almost 3 times lower (see Tab. 1).

Discussion

According to the study, dexamethasone, which enters the blood-placental barrier, affects the formation of connective tissue in the thyroid gland and the formation of its morphological type in the postnatal period. There are conflicting data in the literature on the effect of glucocorticoids on connective tissue and collagen formation [1, 9, 12]. Thus, most studies aimed at studying the effect of glucocorticoids on connective tissue in the postnatal period indicate their inhibitory effect on connective tissue proliferative processes [10, 11]. However, a group of scientists studying the effects of glucocorticoids in the mother-fetus system found an increase in the distribution density of fibroblasts in the dermis of newborn rats and an increase in the number of fibroblast mitoses, and later similar data were obtained in the liver [19-21]. Studies have shown that glucocorticoids can stimulate the synthesis of collagen and non-collagen proteins in the cultivation of vascular smooth muscle cells [5, 14]. Scientists in a study of histopathological changes in the heart after neonatal treatment with dexamethasone found an increase in collagen in the heart of rats for 50 days after birth [7]. Thus, the data obtained are a continuation of the study of the prenatal effect of dexamethasone on the morphogenesis of organs and connective tissue in the thyroid gland, which has a similar trend in the study of a number of parenchymal organs.

Conclusions

1. During the study it was found that in the juvenile period the morphological structure of the rats' thyroid gland of intact and control groups is finally formed, in the lobes of which the peripheral and central parts with rather high sclerosing index due to the predominance of the percentage of

parenchymal component can be microscopically distinguished.

2. A study of material obtained from animals prenatally exposed to dexamethasone showed a violation of the morphogenesis of histoarchitectonics of the thyroid gland with the formation of the lobular structure of its particles due to an increase in stromal component, which was expressed in a decrease in sclerosing index, but compensatory increase without morphological signs of functional disorders.

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