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Editors

Dr. Faruk DÜNDAR

Zhandos ALIMGEREY

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THE FEATURES OF PROLIFERATIVE PROCESSES IN THE THYROID GLAND OF THE WISTAR RAT'S YOUNG OFFSPRING AFTER INTRAUTERINE ACTION OF DEXAMETHASONE

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ABSTRACT

The urgency of the problem of thyroid disease in children and adolescents in Ukraine has existed for a long time. In clinical practice, synthetic glucocorticoids such as dexamethasone are used to accelerate fetal maturation in pregnant women at risk of preterm birth. In humans and other mammals, a surge of cortisol in the body causes structural and functional changes in the tissues of the fetus, preparing it for childbirth and extrauterine life, but they can have long-term consequences in the structural organization of organs remotely postnatally. Unfortunately, despite the large number of studies on the effects of glucocorticoids on the fetus, there is almost no data on the prenatal effect of dexamethasone on the processes of proliferation in the thyroid gland. In the experiment animals were divided into 3 groups (108 Wistar rats): I - intact rats; II - control - animals which, on the 18th day of the dated pregnancy transuterine, transdermal, subcutaneously in the interscapular area was injected with 0.9% saline in the amount of 0.05 ml; III - experimental group - animals, which injected the same type as a control with a solution of dexamethasone at a dose of 0.05 ml at a dilution of 1:40 intrauterinely on the 18th day of pregnancy (Ukrainian patent №112288). The thyroid gland with the tracheal area was removed on the 1-21 days of life. Immunohistochemical study was performed according to the protocol recommended for a particular antibody manufacturer. Used monoclonal antibodies ki-67 (Ki-67), Fox-1 Antibody (A-12) - to assess proliferative activity, the company Santa Cruz Biotechnology, Inc. (USA). The thyroid infant rats, which prenatally exposed to dexamethasone, is structurally represented by chaotically located follicles of different diameters with a predominance of large with desquamated cells in the lumen, and proliferative changes aimed at forming extrafollicular which is confirmed immunohistochemically by the presence of Ki-67 positive cells. Intracellularly, protein-synthesizing organelles of thyrocytes also proliferate, to which there is a clear cytoplasmic and nuclear reaction with Fox-1 antibodies. Proliferative processes in the thyroid of the experimental group are stabilized while maintaining the morphological structure of the hypofunctional type, and remain lower compared to the control and intact groups. Morphological signs of functional tension of the thyroid gland animals exposed prenatally to dexamethasone, which correlate with a decrease in proliferative activity, indicate a functional compensatory response of synthetic and hormone-producing function, but suppression of proliferative processes, despite the slight manifestations. The thyroid gland of morphological hypofunctional type after prenatal action of dexamethasone in young rats, indicates an adaptogenic compensatory response and morpho-functional immaturity of the organ during this period, which may be the basis for provoking the preservation of such morphogenetic factors under the influence of stressors.

Key words: thyroid gland, proliferation, dexamethasone, experiment, rats.

1. INTRODUCTION

The urgency of the problem of thyroid disease in children and adolescents in Ukraine has existed for a long time. Pathology of the thyroid gland over the past 20 years has been ranked first among endocrine diseases in children in Ukraine, and it forms the picture of the prevalence of all endocrine diseases, as it occupies a total of 58.0% of their structure, mainly due to disorders of organ morphogenesis and immune neuro-endocrine imbalance [2, 3]. The action of thyroid hormones is variously directed at all metabolic processes, functions of many organs and tissues, including fetal development, growth processes and tissue differentiation [10, 11]. The thyroid gland has a high sensitivity to exogenous and endogenous influences and the ability to morphologically rearrange tissue [5, 13, 14].

Dexamethasone is a potent synthetic glucocorticoid that suppresses the immune response and has long been used in treatment protocols for many metabolic and inflammatory pathological processes. Its effectiveness exceeds cortisol by 20-30 times. At the same time, it is widely used in the treatment of pregnant women at risk of premature birth, as it accelerates fetal maturation [7, 12]. Scientists have experimentally proved that as a result of the influence of glucocorticoids on the body of animals in postnatal ontogenesis of different ages in the thyroid gland were determined morphological signs that indicate a decrease in its functional activity [4, 6, 8]. Despite the large number of studies on the effects of glucocorticoids on the fetus [1, 9], there is almost no data on the prenatal effect of glucocorticoids on the processes of morphogenesis of the thyroid gland in the postnatal period. Thus, the question of prenatal influence of dexamethasone on the processes of thyroid morphogenesis in the postnatal period is morphologically unresolved, and requires further scientific clarification, which is an extremely important experimental basis for improving the tactics of neonatal and pediatric care by neonatologists, pediatricians, and pediatricians.

The aim of the work is to establish the peculiarities of the morphogenesis of the thyroid gland of rats in the milk period of postnatal ontogenesis in the norm and after the intrauterine action of dexamethasone.

2. MATERIAL AND METHODS

The study involved the thyroid glands of 108 white laboratory Wistar rats from 1 to 21 days of age. The animals were obtained from the vivarium of PE "Biomodelservice" in Kyiv. Rats were kept in a vivarium in acrylic cages with a volume of 300 cm³ for 4-5 animals each and free access to water on a standard diet. Before and during the experiment, the rats were in the same conditions: in the vivarium at t 20-25 ° C, humidity not more than 50%, the volume of air exchange (extraction-inflow) 8:10, in the light mode day and night. The animals were healthy in behavior and general condition. The conditions of care for animals complied with the norms of the "International Recommendations for Medical and Biological Research with the Use of Animals". The work also followed the rules and regulations established by the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 18.03.86) and the Law of Ukraine "On Protection of Animals from Cruelty" (from 21.02.2006 (3447- IV, edition of 09.12.2015, grounds 766-19). Animals were divided into 3 groups of animals in each group: Group I - intact rats; Group II - control - animals which, on the 18th day of a dated pregnancy transuterine, transdermal, subcutaneously in the interscapular area was injected with 0.9% saline in the amount of 0.05 ml; III - experimental group - animals, which during laparotomy, by intrauterine, transdermal subcutaneous injection in the interscapular area was injected with a solution of dexamethasone at a dose of 0.05 ml at a dilution of 1:40 intrauterinely on the 18th day of pregnancy (Ukrainian patent №112288) . In the experimental subgroups used the allowable,

generally accepted number of animals for statistical processing and obtaining reliable results - 6 animals.

The thyroid gland with the tracheal area was removed on 1, 3, 7, 11, 14, 21 days of life, 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 with a thickness of 4 μm were stained with hematoxylin and eosin for observation light microscopy and morphometry, histochemically with azan to determine the density of the colloid. The number of cells was counted in histological sections in a standardized field of view of the microscope at magnification x40 (number of cells per 40,000 μm^2), followed by calculation of the cell density per 1 mm^2 .

Immunohistochemical study was performed according to the protocol recommended for a particular antibody manufacturer. Used monoclonal antibodies ki-67 (Ki-67) - to assess proliferative activity, the company Santa Cruz Biotechnology, Inc. using the method of indirect staining with immunoperoxidase using conjugated HRP murine IgG-binding proteins, m-IgG κ BP-HRP, followed by incubation in a substrate of peroxidase and a mixture of chromogen DAB-3-diaminobenzidine tetrachloride and hemper dyeing enlightenment and conclusion in balm.

The result was regarded as positive in the precipitation of chromogen salts in the form of a specific reaction (nuclear, cytoplasmic reaction depending on the location of the antigen). The intensity of benzidine label deposition was evaluated in points according to the following gradation: "0" - no reaction, light - yellow color. "1" - weak reaction - light - brown color; "2" - moderate reaction - brown color. "3" - intense reaction - dark brown color. Intermediate shades denoted 0.5; 1.5 and 2.5 points, respectively.

In order to control the method, a series of studies was conducted using positive and negative samples, which served as standards.

Morphometry and photodocumentation of the studied objects were performed using a microscope "Primo Star" (Carl Zeiss, Germany) using an AxioCam camera, a set of morphometric studies was performed using Zeiss Zen (2011).

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.), Excel (Microsoft Office, USA). All research results were recorded in journals and protocols of primary documentation, as well as with the use of electronic media. The hypothesis about the normality of the distribution of the studied indicators was tested using the Shapiro-Wilk test. The median, lower and upper quartiles were calculated, and the data were presented as Me (Q1; Q3). Significance of differences between means was assessed using Student's parametric t-test at normal and was considered statistically significant at a confidence level of at least 95%. For all types of analysis, the differences were considered significant at $p < 0,05$.

3. RESEARCH AND FINDINGS

The morphological structure of the thyroid gland of newborn rats 1-3 days of life of all groups was represented by glandular parenchyma and connective tissue stroma with blood and lymphatic vessels and nerves. The study of thyroid micropreparations of animals experimentally prenatally exposed to dexamethasone revealed that the gland of animals of the first day of life was slightly more excited: the number of resorption vacuoles in the colloid, which was dense in total, increased. The height of thyrocytes in the first day of life was 6.35

(5.31; 6.87) μm , which was almost no different from the control. In the interfollicular intervals there were more follicles of colloidal type with ki-67-positive cells.

At this age, there is a large number of light thyrocytes in the wall of the follicles, it is ki-67-positive of the cell. The axis of proliferation is oriented parallel to the basement membrane of the follicle. If in the preparations of the thyroid gland of intact animals it was easy to find epithelial proliferative cell clusters, then in the glands of this period the animals of the experimental group they were almost absent. On the third day of life in the thyroid glands of animals of the experimental group was 6.37 (5.35; 6.92) μm , which was 1.1 times higher than the control value.

On the 7th day of postnatal life, the structure of the gland changed in the direction of increasing the manifestations of the process of blocking the excretion of thyroid hormones, but the synthesis and excretion of components into the follicle cavity was preserved. Follicles enlarged throughout the body, and large subcapsular follicles were even slightly deformed due to the appearance of intussusception directed into the cavity of the follicles. The average values of thyrocyte height of such follicles were inextricably reduced compared to the control and their median was 6.05 (4.85; 6.98) μm , but their cubic shape was preserved. At the sites of intussusception, the shape of the cells was cubic and single prismatic, ki-67-positive cells were visualized, resorption vacuoles were absent, and their number was much smaller compared to the thyroid glands of intact and control animals. One week after birth, the thyroid glands of experimental animals exposed to dexamethasone showed a decrease in the area of the thyroid epithelium. This is due to the fact that the height of the cells of the follicular epithelium becomes smaller, the cubic and flat shape of thyrocytes predominates, cylindrical cells are rare, mainly in small follicles. The increase in the area of the colloid compared to the control was 1.6 times (856 (797; 889.6) μm^2) due to the increase in the number of large and medium-sized follicles containing dense, dense colloid and desquamated cells, with no vacuolation of the colloid.

On day 11 of postnatal life, large follicles with flattened thyrocytes, desquamated cells, and dense colloid without resorption vacuoles were subcapsularly visualized in the thyroid glands of animals prenatally exposed to dexamethasone. In the part of the follicles lined with cubic thyrocytes, the colloid was absent. The number of microfollicles became smaller, and the proportion of proliferating ki-67-positive cells in the wall of the follicles decreased insignificantly compared to the previous period, but compared to the control of proliferating cells was 1.8 times less. In the wall of the follicle there were much more light thyrocytes, which usually contained a large structured nucleus, in which 2-3 nucleoli were visualized. The height of thyrocytes decreased compared to the control and was 6.03 (3.55; 7.01) μm and 6.72 (5.47; 6.93) μm , respectively.

On the 14th – 21th day, the thyroid gland of experimental animals was characterized by the fact that the mosaic consisted of large follicles with flattened thyrocytes. There were areas of parenchyma with clusters of small follicles. The number of light thyrocytes increased compared to the previous term and groups of intact and control, which indicates the differentiation of cells into active hormone-producing. Clearly, this process is a compensatory response to morphological manifestations of hypofunction of the thyroid gland, which correlated with the height of thyrocytes, the median values of which were 5.78 (3.38; 7.34) μm and 1.2 times less than the control value.

4. CONCLUSIONS

Morphological signs of functional tension of the thyroid gland in newborns exposed prenatally to dexamethasone, which correlate with a decrease in proliferative activity, indicate

a functional compensatory response by synthetic and hormone-producing function, but suppression of proliferative processes, despite. Morphogenesis of the thyroid gland by hypofunctional type after prenatal action of dexamethasone in young rats, indicates an adaptogenic compensatory response and morpho-functional immaturity of the body during this period, which may be the basis for provoking the preservation of such morphogenetic factors under stressors.

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