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CHANGES IN CORTICOSTEROCYTES OF THE ZONA RETICULARIS AND CHROMAFFIN CELLS OF THE ADRENAL GLAND IN RATS DURING THE INHIBITION OF TESTOSTERONE SYNTHESIS

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Metabolic disorders, which are the main causes of many diseases and can be linked to the pathogenesis of other nosologies, remain relevant objects of scientific research. It has been proved that testosterone is an important biological regulator of metabolic processes in the male body. Modern studies are performed on the effect of testosterone deficiency on the state of neurons, testicles, liver and other body organs and tissues. The paper describes the main morphological changes of corticosterocytes of the reticular zone of the cortical substance and chromaffin cells of the medulla of the adrenal glands of white rats during central deprivation of testosterone synthesis through experimental blockade of gonadotropic hormones. It was established that inhibition of testosterone synthesis led to dystrophic changes and disruption of the synthetic function of corticosterocytes of the reticular zone, which was manifested at the microscopic level by the variability of morphometric indicators.

Key words: testosterone deprivation, corticosterocyte reaction, reticular zone, cortical substance, medulla, adrenal gland.

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ЗМІНИ КОРТИКОСТЕРОЦИТІВ СІТЧАСТОЇ ЗОНИ ТА МОЗКОВИХ ЕНДОКРИНОЦИТІВ НАДНИРКОВИХ ЗАЛОЗ ЩУРІВ НА ТЛІ ДЕПРИВАЦІЇ ТЕСТОСТЕРОНУ

Метаболічні порушення, що є головними причинами багатьох захворювань та можуть бути ланками патогенезу інших нозологій залишаються актуальними об’єктами наукових досліджень. Доведено, що тестостерон є важливим біологічним регулятором процесів обміну в чоловічому організмі. Проводяться сучасні дослідження щодо впливу дефіциту тестостерону на стан нейронів, яєчок, печінки та інших органів і тканин тіла. У роботі описані основні морфологічні зміни кортикостероцитів сітчастої зони кіркової речовини та ендокриноцитів мозкової речовини надниркових залоз білих шурів під час центральної депривації синтезу тестостерону шляхом експериментальної блокади гонадотропних гормонів. Встановлено, що пригнічення синтезу тестостерону призводило до дистрофічних змін та порушення синтетичної функції кортикостероцитів сітчастої зони, що на мікроскопічному рівні виявлялось варіабельністю морфометричних показників.

Ключові слова: депривація тестостерону, реакція кортикостероцитів, сітчаста зона, кіркова речовина, мозкова речовина, надниркова залоза.

The study is a fragment of the research project “Experimental and morphological study of the effect of cryopreserved preparations of cord blood and embryofetoplacental complex (EFPC), dipherelin, ethanol and 1 % methacrylic acid ether on the morpho-functional state of several internal organs”, state registration No.0119U102925.

Metabolic changes resulting from dysregulation at any level of the hypothalamic-pituitary-gonadal axis can be linked to the pathogenesis of several diseases [11]. Therefore, the gonadotropin-releasing hormone, a regulator of sexual and other endocrine glands, remains the object of modern scientific research [12, 15]. Some works study the quantitative composition of steroid hormone biosynthesis metabolites in biological fluids and their role in the development and progression of hormone-dependent diseases of the reproductive system [7].

Modern studies are performed on the influence of stress reactions and harmful factors on the structure of the adrenal glands [8, 9, 13]. Studying intranuclear mechanisms regulating adrenocortical function is a complicated issue of molecular endocrinology [3]. Changes in the adrenal glands against the background of an imbalance of thyroid hormones in the mother's body [4] and the relationship between the renin-angiotensin and hypothalamic-pituitary-adrenocortical systems during type 2 diabetes are widely studied [1].

It is known that oxidative stress is an important pathogenic link between organ and tissue damage [6, 14]. Some scientists are investigating the effect of antiandrogenic or estrogenic chemical disruptors on the course of lipid peroxidation in the genital organs of rats [5]. It has been proven that testosterone, an androgen and steroid hormone of the adrenal glands, has a common biochemical origin [10]. Therefore, the study of morphological changes of the adrenal glands against the background of oxidative stress caused by testosterone deficiency is relevant and promising.

The study of the effect of central testosterone deprivation on the zona reticularis of the adrenal cortex (ZRAC) and chromaffin cells (ChC) of the adrenal medulla is especially relevant. ZRAC cells synthesize substances that are precursors of testosterone, and the adrenal medulla (AM) produces hormones responsible for implementing stress reactions.

Modelling of pathological conditions during the study is carried out by administering pharmacological drugs. For example, triptorelin acetate is a synthetic analogue of gonadotropin-releasing hormone. It is widely used to treat various benign and malignant diseases of the reproductive system of men and women. During long-term use, this drug causes the development of chemical castration due to a decrease in the production of luteinizing (LH) and follicle-stimulating hormones [6, 14].

Correction of the reactive changes that occurred during the experiment in the examined organs and tissues takes place due to the use of drugs with an antioxidant effect, which can be a promising method of pathogen therapy and prevention of changes in the body caused by a decrease in testosterone [1, 2, 4, 14].

Thus, our study is designed to reveal the relationship between central testosterone deprivation and reactive changes in ZRAC and MO of adrenal glands.

The purpose of the study was to establish morphological changes in corticosterocytes of the zona reticularis of the adrenal cortex and chromaffin cells of the adrenal medulla during the inhibition of testosterone synthesis.

Materials and methods. The adrenal glands of 45 white male rats were studied. Rats were divided into 5 groups: 1 group – of 5 intact rats and 4 groups of 10 rats injected with Diphereline (IPSEN PHARMA, France, triptorelin acetate). The study material was sampled in terms of 1, 3, 6, and 9 months from the beginning of the experiment [6, 14].

Working with animals, we guided the national regulations: General ethical principles of experiments on animals (Ukraine, 2001), consistent with the requirements of the European Convention for the protection of vertebrates used for experimental and other scientific purposes (Strasbourg, 1985), Law of Ukraine No. 3447-IV of 21.02.2006 On the Protection of Animals from Cruelty and the Helsinki Declaration on Humane Treatment of Animals.

The histological method was used to study the morphological features of corticosterocytes of the zona reticularis (CZR) and chromaffin cells (ChC) of the adrenal medulla (AM).

Measurements and calculations of the mean diameter of the cytoplasm and the nucleus volume (NV) of the CZR were carried out, and the ChC nucleus volume and the density of the AM cords were determined by counting the number of ChC in the areas of the AM, the mean area of which was 36422.511±2082.09 μm². After preliminary photography of the sections at a magnification of ×400, measurements were made using the AimImage Examiner software.

Statistical methods were to determine the objectivity and reliability of the obtained results, and the morphometric analysis was carried out according to generally accepted statistical methods using the Microsoft Office Excel 2007 software.

Results of the study and their discussion. On histological preparations of intact rats' adrenal glands, the gland's structure does not differ from the norm. The adrenal gland is surrounded by a connective tissue capsule and consists of cortex and medulla. The glomerular zone is formed by small corticosterocytes with basophilic cytoplasm and rounded nuclei. Dark and light spongy corticosterocytes of the bundle zone form straight, radially directed cords. Light cells have vacuolated cytoplasm, and dark cells have basophilic cytoplasm.

CZR has smaller sizes compared to spongy corticosterocytes, the mean size (MS) of CZR is 13.63±0.472 μm, and the mean value of CZR NV is 174.57±1.27 μm^3. The cells form cords that go in different directions and anastomose with each other.

Changes in the values of the mean size of the CZR and the mean value of the CZR NV in the studied terms are shown in Table 1.

Table 1

Changes in the CZR MS and the mean values of CZR NV against the background of central testosterone deprivation

Terms of the study	CZR MS (in μm)	CZR NV (in μm3)
Intact group	13.63±0.472	174.57±1.27
1st month	17.29±0.432*	136.75±1.14*
3rd month	19.74±0.132*×	167.69±1.22*×
6th month	22.58±0.242*×	266.35±1.82*×
9th month	20.46±0.122*×	163.83±2.12*×

Notes: * $p<0.05$ compared to the intact group; × $p<0.05$ compared to the previous follow-up period.

By the 1st month of the experiment, a significant increase in CZR size and a significant decrease in CZR NV were detected compared to the indices of the intact group. On the histological preparations in the 3rd month of the experiment, a slight and reliable increase in the size of the CZR and a reliable increase

in the NV were found, compared to the indices for the 1st month of the experiment (2nd group). In the sections for the 6th month of the experiment, a moderate increase in CZR MS and a significant reliable increase in NV were found, compared to the indices for the 3rd month of the experiment (group 3). By the 9th month of the experiment, in histological preparations, a significant decrease in CZR MS and a significant decrease in NV were found, compared to the indices by the 6th month of the study.

Thus, there was a significant increase in the size of the cytoplasm and a decrease in the NV of corticosterocytes of the reticular zone for the 1st month of the experiment, compared to the indices of the intact group. The absence of signs of cellular atypia, the accumulation of lipid inclusions in the cytoplasm, and the appearance of corticosterocytes in which large lipid vacuoles push the nucleus to the periphery ("ring-shaped cells") indicate the phenomenon of fatty dystrophy (Fig. 1).

An increase in CZR MS and a significant increase in NV by the 3rd month of the experiment, compared with the indices by the 1st month of the experiment, cytoplasmic basophilia and the absence of vacuolization (fat inclusions) indicate activation of protein synthesis activity of cells (Fig. 2).

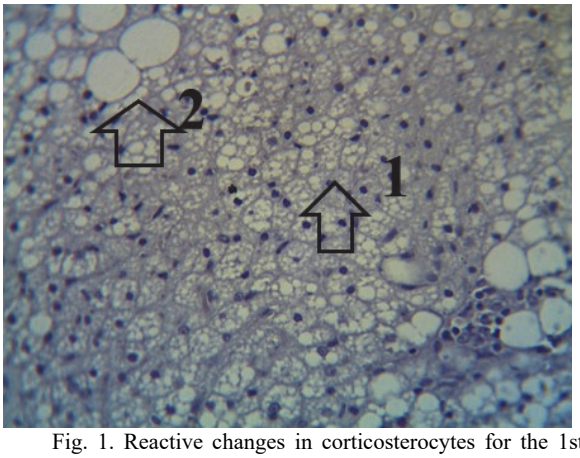

Fig. 1. Reactive changes in corticosterocytes for the 1st month of central testosterone deprivation, 1 – lipid inclusions in the cytoplasm of corticosterocytes, 2 – "ring-shaped cells". Staining: hematoxylin-eosin. Magnification: ×400

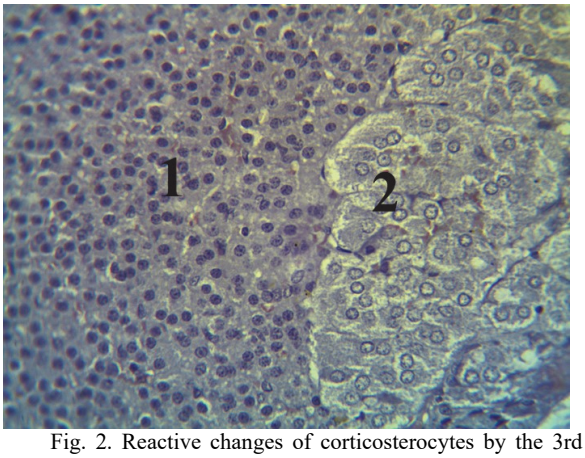

Fig. 2. Reactive changes of corticosterocytes by the 3rd month of central testosterone deprivation, 1 – reticular zone, 2 – medulla. Staining: hematoxylin-eosin. Magnification: ×400

A moderate increase in CZR MS, a significant and reliable increase in NV by the 6th month of the experiment compared to the indices in the 3rd month, a decrease in cytoplasmic basophilia, and the appearance of lipid inclusions indicate a gradual return of cells to an intact state (Fig. 3).

A significant decrease in the CZR MS of the reticular zone and NV by the 9th month of the experiment and moderate basophilia with minor lipid inclusions confirms the return of cells to the parameters of the intact group.

During the MR morphometric study, the small and large diameters of endocrinocyte nuclei and the number of cells in the studied areas with an mean area of 36422.511±2082.09 μm² were determined. With the help of the number of cells in the sections of the given area, we determined the density of the brain substance, that is, the predominance of the cellular component over the stromal component or vice versa. The small and large diameters were measured to count the NV of cells. The indicator of CZR NV and the ChC nuclei volume (ChC NV) was calculated using the formula for the volume of an ellipsoid:

$V = \pi/6 \times D \bullet d^2$, where V is the volume of the nucleus, d is the length of the small diameter of the nucleus, and D is the length of the large diameter of the nucleus.

The mean value of the indices of CZR NV and ChC NV according to the terms of the study is presented in Table 2.

Table 2

Indices of the mean value of CZR NV and the ChC NV (in μm³)

Research period	CZR NV	ChC NV
Intact group	174.57±1.27	420.82±1.52
1st month	136.75±1.14*	461.71±3.09*
3rd month	167.69±1.22*×	851.59±2.61*×
6th month	266.35±1.82*×	889.68±3.24*
9th month	163.83±2.12*×	851.16±1.84*

Notes: * $p<0.05$ compared to the intact group; × $p<0.05$ compared to the previous follow-up period.

Let's compare the indices of ChC NV for the 1st month of the experiment (2nd experimental group) with the indices of the intact group. We can see an increase in this index by 10 %. Comparing the ChC NV

index for the 3rd month of the experiment (group 3) to the indices of the 2nd group (1st month of the experiment), a significant increase in NV by 84 % was observed. A 4 % increase in the NV index occurred in the ChC by the 6th month of the experiment (group 4) compared to the indices of group 3. Whereas, already at the 9th month of the experiment (5th group), compared to the indices of the 4th group, there was a decrease in NV by 4 %. Thus, with the help of the diagram, a reliable and significant increase in the ChC NV is identified precisely for the 3rd month of central testosterone deprivation (Fig. 4).

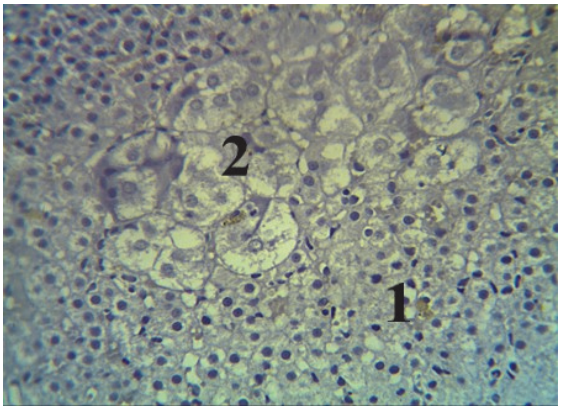

Fig. 3. Reactive changes of corticosterocytes by the 6rd month of central testosterone deprivation, 1 – reticular zone, 2 – medulla. Staining: hematoxylin-eosin. Magnification: ×400

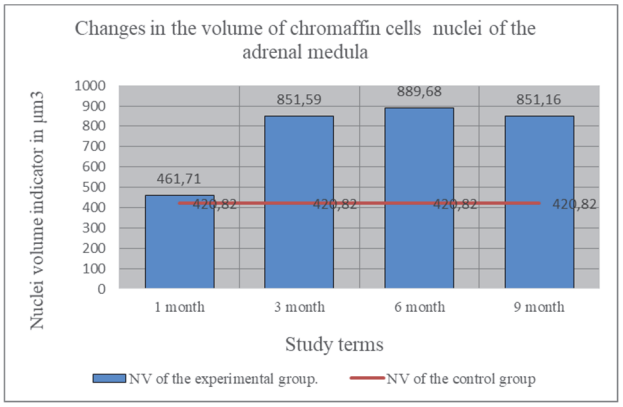

Fig. 4. Comparative characteristics of changes in the nuclei of endocrinocytes of the medulla.

An important index that was also studied was the calculation of the quantitative cellular composition of the studied areas of the medulla of the adrenal glands in all terms of the experiment. Quantitative characteristics of the ChC of the intact group were 14.7±2.89 in the area whose mean area was 36422.511±2082.09 µm². This index of the number of ChC in the 2nd group (1 month of the experiment) was 33.8±5.93, which indicates a significant and reliable increase of the parenchymal component already in the 1st month of the experiment. By the 3rd month (experimental group 3), the cellular composition of AM significantly increased to 49.3±5.55, compared to the intact and 2 experimental groups, which confirms significant reactive changes not only in the reticular zones of the adrenal gland, but also directly in the brain substance during central testosterone blockade. By the 6th month of the experiment (4th experimental group), the number of ChC significantly decreased by 30.5±2.53 compared to the previous period (3rd month of the experiment) and even with the indicators of the second group. Therefore, this index for the 6th month approaches the indices of the intact group. In the 9th month of the study (experimental group 5), compared to the previous period, an increase in the mean value of the quantitative composition of 43.07±7.07 was found, which indicates a new activation of ChC of the adrenal glands in this period.

After conducting morphological and morphometric studies of a significant number of indicators of the components of the adrenal cortex and medulla, visualizing the results in the form of tables and diagrams, it is possible to identify the main terms of development of pathological and reactive changes in the adrenal glands against the background of central testosterone deprivation. And in this way, it is possible to prove the relationship of the processes occurring in the gonads of the male reproductive system not only with the reactivity of the cortical substance of the adrenal gland but also with morphometric changes in its medulla.

Thus, the main positive peak moments of the reactivity of the cortical substance of the adrenal glands were observed from the 3rd to the 6th month of the experimental study. This was reflected in the activation of the protein-synthetic function of CZR. Accumulation of lipid inclusions in the CZR cytoplasm and the appearance of “ring-shaped cells” for the 1st month of the study was interpreted by us as a primary reverse reaction of CZR to the administration of triptorelin acetate. A decrease in cytoplasmic basophilia and a moderate number of lipid inclusions in the CZR indicates a gradual return of cells to an intact state from the 6th to the 9th month of the experiment.

As for the ChC, the peak increase of the mean index of NV by 84 % by the 3rd month of the experiment and the number of ChC to the index 49.3±5.55, compared to the data of the 1st month and the intact group (33.8±5.93 and 14.7 ±2.89, respectively), confirms the reaction of the medulla of the adrenal gland during central testosterone deprivation by administering the drug triptorelin acetate.

After analyzing scientific works related to the topic of our research, we found that these articles describe pathophysiological reactions and biochemical processes of oxidative stress [6, 14] in the adrenal glands against the background of thyroid hormone imbalance [4], type 2 diabetes [1] and administration of other chemicals [5]. Our study of changes in the adrenal glands against the background of oxidative stress

caused by testosterone deficiency is aimed at studying the morphological features and morphometric indicators of CZR and ChC.

Conclusion

1. The long-term effect of intramuscular administration of triptorelin acetate leads to reactive changes in the structure of corticosteriocytes of the reticular zone of the adrenal glands of rats starting from the 1st month of the study with a maximum at the 6th month of observation and a gradual statistically significant decrease of the indices to the values of the intact group from the 9th month, but without their complete recovery.

2. A significant increase in the number of corticosterocytes by 3 times and the volume of their nuclei by 84 % at the 3rd month of the experiment, with the maximum value at the 6th month, confirms significant reactive changes directly in the brain substance during central testosterone blockade.

3. We found a direct correlation between changes in the corticosterocytes of the reticular zone and the adrenal medulla of white rats against the background of testosterone deprivation, indicating the involvement of not only hypothalamo-pituitary dependent endocrine organs but also organs of the sympathoadrenal system.

Prospects for further research. It is planned to carry out a histochemical study of CZR and ChC to prove at the biochemical level the influence of testosterone on the reticular zone's metabolic processes and the adrenal medulla.

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