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ADAPTIVE REARRANGEMENTS OF STRUCTURAL COMPONENTS IN THE ADULT RATS' ADENOHYPOPHYSIS AFTER LONG-TERM EXPOSURE TO HEAVY METAL SALTS

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The article presents the results of the morphofunctional rearrangements'study of the adenohypophysis' structural components of sexually mature rats after long-term exposure to heavy metal salts and an adaptive period of 90 days. General morphological, morphometric, immunohistochemical and statistical research methods were used. A long-term period of adaptation to the heavy metal salts' exposure significantly improved the condition of the adenohypophysis'structural components: the swelling of the gland and the expressiveness of the connective tissue component decreased, the condition of the rheological properties of blood, histoarchitectonics, the condition of adenocytes and their secretory activity improved, the level of expression of HSP90 in the cytoplasm of adenocytes increased to strongly positive, disappeared processes of cyst formation, the tension of compensatory and adaptive processes decreased. The above-mentioned morphofunctional rearrangements had a positive effect on the processes of the hormones'evacuation into blood, the course of the general adaptation syndrome and the restoration of homeostasis in the organ and the body as a whole.

Key words: general adaptation syndrome; pituitary gland, anterior; metals, heavy; homeostasis; adrenocorticotropic hormone

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АДАПТИВНІ ПЕРЕБУДОВИ СТРУКТУРНИХ КОМПОНЕНТІВ АДЕНОГІПОФІЗУ СТАТЕВОЗРІЛИХ ЩУРІВ ПІСЛЯ ТРИВАЛОГО ВПЛИВУ СОЛЕЙ ВАЖКИХ МЕТАЛІВ

У статті представлені результати дослідження морфофункціональних перебудов структурних компонентів аденогіпофіза статевозрілих щурів після довготривалого впливу солей важких металів та адаптивного періоду на протязі 90 діб. Використовувались загальні морфологічні, морфометричні, імуногістохімічні та статистичні методи дослідження. Довготривалий термін адаптації до дії солей важких металів суттєво покращив стан структурних компонетів аденогіпофіза: зменшився набряк залози та виразність сполучнотканинного компонента, покращився стан реологічних властивостей крові, гістоархітектоніка, стан аденоцитів та їх секреторна активність, підвищився рівень експресії HSP90 в цитоплазмі аденоцитів до сильно позитивного, зникали процеси кістоутворення, зменшилося напруження компенсаторно-адаптивних процесів. Вищевказані морфофункціональні перебудови позитивно вплинули на процеси евакуації гормонів у кров, перебіг загального адаптаційного синдрому, відновлення гомеостазу у органі та організмі в цілому.

Ключові слова: загальний адаптаційний синдром; гіпофіз, передній; метали, важкі; гомеостаз; адренокортикотропний гормон.

The work is a fragment of the research projects "Morphological aspects of experimental pathology of internal organs and musculoskeletal system", state registration No. 0123U101135 and "Development of a method for diagnosing and predicting the course of tumors using cell adhesion molecules of cancer-embryonic antigen and cyclooxygenases", state registration No. 60.

One of the main problems of modern society is the progressive pollution of the environment by heavy metals, which poses a great threat to living organisms [6, 7]. The rate of generation and distribution of heavy metals in the environment has increased significantly compared to the last century. Heavy metals are found in the environment, food, consumer goods and are endocrine disruptors [9]. They imitate natural hormones, inhibit the action of hormones or change the normal regulatory function of the endocrine system. EDCs can alter the action of hormones in multiple ways. EDCs can effect on hormone synthesis in the endocrine gland [10]. As per U.S. Environment Protection Agency (EPA), EDCs is defined as ''an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones those are present in the body and are responsible for interfering the homeostasis, reproduction, and developmental process. About 85,000 chemicals are manufactured, of which more than thousands are considered as EDCs [5]. The hormones of the pituitary gland's anterior lobe are central to the body's homeostasis, participate in the initiation of the stress reaction, limiting its further development, preventing adverse effects on the body. The pituitary gland and adrenal glands are the morphological substrate of the stress-realizing systems of the body, ensuring the development of compensatory and adaptive processes in response to extreme exposure [3]. In modern scientific literature, there are data on the negative impact of some heavy metal salts on the pituitary gland. The effect of cadmium Cd, chromium Cr VI, arsenic [1, 8] and lead [1, 3, 4] on the hypothalamic-pituitary system and adenohypophysis of rats was studied. Cr VI accumulates in the pituitary and hypothalamus, and decreases serum prolactin levels in vivo but observed no effects on LH levels. Cr VI induced apoptosis evidenced by nuclear fragmentation and caspase 3 activation. The anterior pituitary gland can be a target

of Cr VI toxicity in vivo and in vitro, thus producing a negative impact on the hypothalamic-pituitary-gonadal axis and affecting the normal endocrine function. Cabilla and others found in their study that cadmium, chromium (VI) and arsenic in the pituitary gland increased lipid peroxidation and the expression of several markers of oxidative stress. Cell death was mainly due to caspase-dependent apoptosis. These results showed that these heavy metals have a deleterious effect on hypothalamic-pituitary physiology, altering hormone release and promoting cell death [8]. The influence of lead had a negative effect on the secretion of sex hormones by the pituitary gland [1, 3, 4, 14]. Today, an important environmental problem in some northern regions of Ukraine is the accumulation of salts of heavy metals (zinc, chromium, lead, manganese, copper and iron) in the soil, water and air, which is observed in different combinations depending on the region and causes a negative impact on the health of the population [11].

The purpose was to find out the morphofunctional rearrangements and changes in the structural components of the pituitary gland of sexually mature rats after long-term exposure to a complex of heavy metal salts (zinc, copper, iron, manganese, lead, and chromium).

Material and methods. The experiment was performed on 12 white mature male rats weighing 200–250 g at the age of 7–8 months, which were divided into 2 groups (control and experimental). The control group included rats that were kept in vivarium conditions similar to those of experimental animals. At the same time, a constant temperature regime was observed, as well as a natural day/night regime. As food, the control animals received granular mixed fodder, drinking – ordinary drinking water. The study was conducted in the autumn-winter period. Animals were kept and manipulated in accordance with national and international bioethics standards. The experimental group included rats that, after a 90-day period of adding a complex of heavy metal salts to the drinking ration: zinc (ZnSO4 7H2O) – 5 mg/l, copper (CuSO4 5H2O) – 1 mg/l, iron (FeSO4) – 10 mg/l, manganese (MnSO4 5H2O) – 0.1 mg/l, lead (Pb (NO3) 2) – 0.1 mg/l and chromium (K2Cr2O7) – 0.1 mg/l, during 90 days were in the adaptive period, used ordinary drinking water.

Groups of experimental animals were removed from the experiment after previous thiopental anesthesia (at the rate of 30–40 mg / 10 g of body weight) on the 180th day of the experiment (Protocol No. 8 of 17/11/2020 of the Bioethics Commission of Sumy State University). The subject of the study was the pituitary gland of experimental and control animals. For morphological studies of the pituitary gland, the organ was removed, histological preparations were made, stained with hematoxylin-eosin and Mason-Goldner were stained according to the original method [11]. The histological examination was performed in the period from 2020 to 2023 at the Department of Pathological Anatomy of Sumy State University, the Department of Anatomy of Pavel Josef Shafarik University in Košice (Slovakia) and the Department of Pathological Anatomy, Forensic Medicine and Pathological Physiology of the Dnipro State Medical University. The absolute number of different types of adenocytes was counted in a grid by experimenters in various random fields of the adenohypophysis view (at least 5 fields from each control and experimental animal).

To calculate the level of expression of receptors for antibodies, used a semi-quantitative method. Determination of the expression of the heat shock protein marker 90 (Hsp90) was performed using an antibody panel “Thermo scientific”, USA: rabbit polyclonal antibody to the Hsp90 protein with a titer of 1:200 according to the manufacturer's recommendations. Evaluation of Hsp90 marker expression was performed by the number of stained nuclei and cytoplasm of gland cells. The result was expressed as a percentage and evaluated on a scale in the case of a positive reaction: low positive (1 point), moderately positive (2 points) and strongly positive (3 points) reaction, taking into account the number of cells and the intensity of their color. The number of HSP90-positive cells was counted in a grid by experimenters in various random fields of view of the adenohypophysis (at least 10 fields from each control and experimental animal). The functional state of the pituitary was evaluated by determining the adrenocorticotropic hormone ACTH (pg/ml) in the serum of peripheral blood of experimental animals (by the ELISA method). A set of reagents from the Siemens was used on the Immulite 1000 Siemens Healtheare Global Immune Chemiluminescent Analyzer. General morphological analysis was performed using a light optical microscope Zeiss Primo Star with lenses x4, x10, x40, glasses 7 and 10. For the morphometric study of micropreparations, the “SCPR-2017-Zen 2 lite” software was used with photo-documentation of the results by means of “axiocam ERC 5S Zeiss” digital camcorder. Statistical processing of the obtained data was performed by parametric method of variation statistic using the software package STATISTIKA v. 10 (“StatSoft Inc.”, USA). Data are presented as the mean (X) ± standard deviation (SD), using the Student's t-test. The error probability of less than 5 % ($p \leq 0.05$) was considered sufficient.

Results of the study and their discussion. 90 days of adaptation to a long-term exposure to heavy metal salt complex contributed to the development of positive changes in most structural components of

the adenohypophysis. The length of the pituitary gland increased by 13.7 % (P>0.05) in comparison with the indicators of control animals and by 23.3 % (P>0.05) in relation to the indicators of animals during the 30-day period of adaptation. The width of the pituitary gland decreased by 23.8 % (P<0.01, t=3.46) compared to the indicators of control animals and increased by 34.1 % (P<0.01, t=3.52) compared to the indicators of animals 30-day adaptation period. The thickness of the pituitary capsule significantly increased compared to control animals by 78.3 % (P<0.01, t=3.73) (Table 1).

Table 1

Indices of experimental and control rats' adenohypophysis after adaptation to long-term exposure to heavy metal salts complex (X ± SD)

Indices	Groups of laboratory animals	
	Rats of the control group, n=6	Rats of the experimental group, n=6
Pituitary length, mm	7.2±0.24	8.19±3.03
Pituitary width, mm	6.39±0.33	4.87±0.29
Capsule thickness, μm	2.26±0.33	4.03±0.34**
Vessel's area, μm^2	343.04±6.68	425.36±9.7***
Chromophobic cells	58.21±2.84	60.43±1.56
Chromophilic acidophiles	33.32±1.06	34.09±0.17
Chromophilic basophils	8.47±0.63	5.48±2.74
ACTH (pg / ml)	571.5±2.73	568.5±2.86

Note: ** p ≤ 0.01*** p≤0.001

A slight decrease in the value of the fibrous component of the connective tissue in the stroma of the gland was observed. Parenchymal and stromal edema, as well as the area of intertrabecular spaces decreased, vascular congestion disappeared, and the state of blood rheological properties significantly improved. Only in individual capillaries and large vessels, violations of the rheological properties of blood in the form of aggregation of erythrocytes to the vascular wall and processes of sweetening in the form of "coin columns" were still visualized. Manifestations of excessive collagenization of the vessel wall disappeared (Fig. 1).

The lumen area of blood vessels increased by 24 % (P<0.001, t=6.99) compared to the indicators of control animals and by 89.6 % (P<0.001, t=16.02) compared to the indices of animals with a 30-day period of adaptation. The histoarchitectonic of the epithelial trabeculae improved as a result of the reduction of edema of connective tissue trabeculae and vascular plethora blood. In the parenchyma of the gland, the processes of cyst formation practically disappeared and the secretory properties of the organ were somewhat restored. A gradual restoration of the cellular composition of the experimental rat's adenohypophysis was observed. Thus, in the composition of epithelial trabeculae, an increase in the number of chromophils cells and, at the same time, a decrease in the number of chromophobic cells were found in comparison with the indices of the animals of the previous 30-day period of adaptation.

Basophilic adenocytes were localized in groups of medium and large sizes in different parts of the adenohypophysis parenchyma. When microscopic preparations were stained by Masson-Goldner, the cytoplasm of basophils cells had an intense blue color, which indicated an increase in their synthetic activity (Fig. 2).

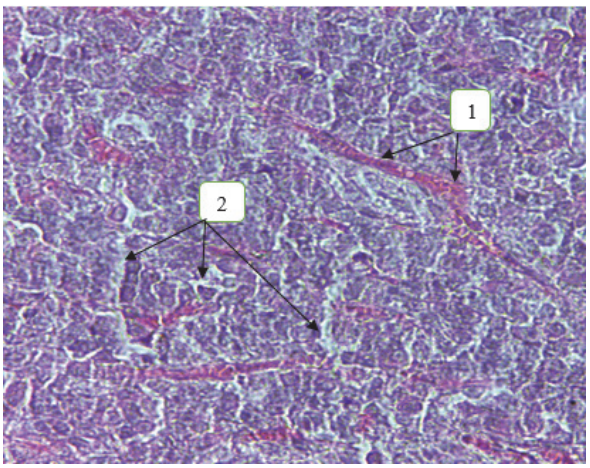

Fig. 1. Morphological rearrangements in the experimental rat pituitary gland under the condition of 90-day adaptation to heavy metals salts: 1 – vascular plethora blood; 2 – edema tissue trabeculae Staining: by hematoxylin-eosin. × 400x.

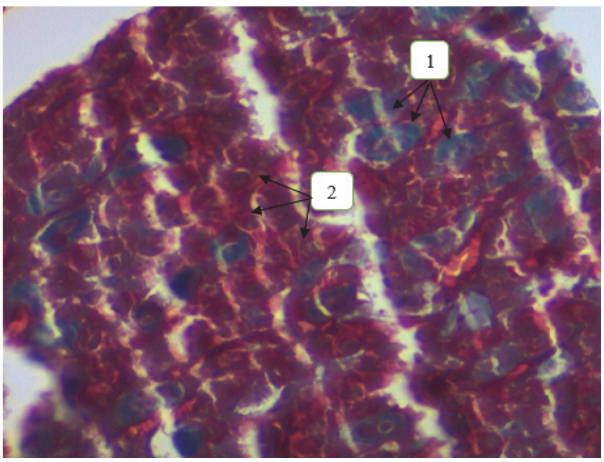

Fig. 2. Cellular composition of the experimental rat's adenohypophysis after 90-day adaptation to 90-day exposure to heavy metal salts, 1 – chromophilic basophils; 2 – chromophilic acidophiles. Staining: by Mason-Goldner. × 400x.

The number of basophils decreased by 35.4 % (P>0.05) compared to the indices of control animals and at the same time increased by 17.8 % (P>0.05) compared to the indices of animals of the 30-day period of adaptation. The number of acidophilus increased by 2.3 % (P>0.05) compared to the indices of control animals and by 12.9 % (P<0.001, t=6.34) compared to the indices of animals with a 30-day period of adaptation. The number of chromophobes increased by 3.8 % (P>0.05) compared to the indices of control animals and at the same time decreased by 7.2 % (P<0.05, t=2.92) compared to the indices of animals with a 30-day period of adaptation.

On the 90th day of adaptation to the influence of heavy metal salts, the morphological features of adenocytes changed. Cells with hypochromic cytoplasm and oval, hypertrophied nuclei were found in the parenchyma of the gland. The chromatin network of the nuclei was lightened, with a well-contoured nucleolus against a background of lightened karyoplasm. Part of the nuclei had finely dispersed, diffuse condensation of chromatin and its margination to the karyomembrane. The disappearance of adenocytes with signs of cytoplasmic vacuolization and balloon dystrophy was observed.

On the 90th day of adaptive changes to the long-term effects of heavy metal salts in the experimental animals' adenohypophysis no significant differences were found in the expression level of Hsp90α in adenocytes in comparison with the indicators of animals with a 30-day period of adaptation. In the cytoplasm of adenocytes, a moderate (2 points) and strongly positive (3 points) level of expression for Hsp90 remained. Hsp90-positive cells were diffusely located in the parenchyma and had a high level of staining (++ and +++). The number of low-positive adenocytes in the adenohypophysis of experimental animals decreased by 63.35 % (P<0.001, t=154.51) compared to control animals. The number of moderately positive HSP90 adenocytes increased by 18 % (P<0.001, t=5.69) in the adenohypophysis of experimental rats compared to control animals. In the adenohypophysis of experimental rats, compared with control animals, the number of strongly positive HSP90 adenocytes increased by 57 % (P<0.001, t=44.01) (Table 2).

Table 2

Expression level of HSP90 in adenohypophysis adenocytes of control and experimental rats after adaptation to long-term exposure to heavy metal salts complex (X ± SD)

HSP90 expression	Control group total number 6 animals	Experimental group total number 6 animals
Low positive 1	63.35±0.41	-
Moderately positive 2	36.65±0.18	43.22±1.14***
Strong positive 3	-	56.78±1.29***

Note: *** p≤0.001

Functional changes on the part of the experimental animals' pituitary gland under the condition of a 90-day period of adaptation to the chronic effect on the body of a combination of heavy metal salts were manifested in the restoration of homeostasis in the pituitary axis of the experimental rats. There was a decrease in stress on the side of ACTH secretion by the pituitary gland. At the same time, the level of ACTH in the blood plasma of experimental rats practically reached the indicators of control animals.

Prolongation of the adaptation's terms to long-term exposure to heavy metal salts contributed to the development of positive changes in most of the structural components of the experimental animals' adenohypophysis. Signs of compensatory hypertrophy of the organ were revealed both in comparison with the indices of control animals and animals of 30 days of adaptation. There was a decrease in edema phenomena, an improvement in the rheological properties of the blood and a decrease in the value of the fibrous component of the connective tissue in the gland's stroma, but the pituitary capsule still remained significantly thickened. In contrast to the previous, 30-day period of adaptation, manifestations of excessive collagenization of the vessel wall disappeared, which undoubtedly had a positive effect on the blood supply of the organ and regenerative processes in the gland. However, the area of the lumen of blood vessels still remained increased, which can be considered as further manifestations of compensatory mechanisms in the body in response to hypoxic changes caused by long-term exposure to heavy metal salts. Such morphological changes in the adenohypophysis on the 90th day of adaptation undoubtedly had a positive effect on the trophism and course of regenerative adaptive processes in the adenohypophysis, histoarchitectonics and cellular composition of the parenchyma. At the same time, the processes of cyst formation disappeared, the state of cellular trabeculae improved, the number of chromophilic basophils increased by 17.8 % and acidophilus by 12.9 % in comparison with the indices of animals with a 30-day period of readaptation [11].

In our opinion, this indicated the recovery of the organ's secretory properties. After all, in our opinion, the development of cysts in the adenohypophysis of experimental animals is associated with the membrane-toxic effect of heavy metal salts, hypoxic changes in the organ [7, 12], hormonal imbalance in

the body [11, 13], processes of the oxidant stress development, increasing the number of free radicals. The disappearance of cysts in the adenohypophysis on the 90th day of adaptation undoubtedly indicated an improvement in the condition of the membranes of adenocytic membranes and the vascular wall, an increase in the synthetic activity of the organ, and an improvement in the processes of hormone evacuation into the vascular bed. This can be confirmed by the increase in the expression of HSP90 in the cytoplasm of 57.14 % of adenocytes to a strongly positive level and the achievement of the level of ACTH in the blood serum of experimental animals compared to control rats. An increase in the expression of HSP90 in the cytoplasm of adenocytes can be considered one of the mechanisms of protection of cells and organs. According to the literature, production of heat shock protein (HSP) by cells makes these cells more resistant to further extreme conditions, developing resistance to further stress. After all, the renaturation of proteins damaged during stress is an integral part of stress resistance. Hsp90 has been shown to play an important role in protein quality control by directing damaged proteins to the 26S proteosome for degradation or to other chaperones (particularly Hsp70) for renaturation. Under stressful conditions that lead to the accumulation of proteins in cells with a disordered conformation, Hsp90 is believed to partially switch to their refolding [2]. This fact indicates the involvement of Hsp90α in active processes of adaptation of adenohypophysis cells to long-term exposure to heavy metal salts.

Morphological signs of adenocytes testified to the acceleration of restorative processes in their structure and improvement of their functional activity: the cytoplasm of adenocytes lost signs of vacuolization, balloon dystrophy, and vacuolar degeneration. Adenocyte nuclei had signs of hypertrophy, in contrast to the 30-day adaptation period, nucleoli appeared in the nuclei. However, part of the nuclei still had a shallow, diffuse condensation of chromatin and its marginalization to the karyomembrane, which can be considered posthypoxic phenomena.

Conclusion

Thus, a comprehensive study of the structural components of the adenohypophysis of experimental animals with a 90-day period of adaptation to the long-term effect of heavy metal salts indicated the development of a number of positive adaptive-regenerative morphofunctional changes in the organ, aimed at restoring homeostasis, improving the state of membranes, metabolic processes, improving the secretory activity. This, of course, had a positive effect on the course of the general adaptation syndrome in the body of experimental rats after long-term exposure to heavy metal salts.

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