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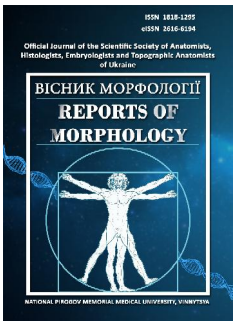

Morphological changes of sexually mature rat's pineal gland and cerebellar cortex under long-term exposure to heavy metal salts

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Pollution with heavy metal salts is an important environmental problem today, having an adverse effect on public health. The endocrine system maintains homeostasis in the body. The purpose of the work is to study the morphological changes of the cerebellar cortex and epiphyses of sexually mature male rats under the condition of long-term exposure to the body of a complex of heavy metal salts. The morphological changes in the cerebellar cortex and epiphysis of sexually mature male rats under the condition of long-term exposure to heavy metal salts was studied. Animals of the experimental group were simulated microelementosis by adding to drinking water a mixture of heavy metal salts for 60 days: zinc ($ZnSO_4x7H_2O$) - 5 mg/l, copper ($CuSO_4x5H_2O$) - 1 mg/l, iron ($FeSO_4$) - 10 mg/l, manganese ($MnSO_4x5H_2O$) - 0.1 mg/l, lead ($Pb(NO_3)_2$) - 0.1 mg/l and chromium ($K_2Cr_2O_7$) - 0.1 mg/l. Morphological, morphometric and statistical research methods were used. Long-term (60-days) intake of heavy metal salts mixture in the body of experimental animals leads to the development of the general adaptation syndrome, the stage of chronic stress "subcompensation" in the pineal gland. Morphological changes in the organs had a nonspecific polymorphic character, such as a sharp violation of hemodynamics, a violation of the morphology of the vascular wall, the state of pinealocytes and Purkinje cells, the development of tissue hypoxia, processes of apoptosis and reactive astrogliosis as a response to the action of a damaging agent. The pineal gland of the experimental animals showed signs of indole production, but the evacuation of hormones (including melatonin) into the vascular bed was hampered due to the violation of the morphology of the vascular wall and the cell membrane of pinealocytes. This led to a deficiency of this hormone in the body of the experimental animals, which negatively affected the adaptive processes in the cerebellar cortex in response to the action of the stress agent. Compensatory and adaptive processes in the pineal gland and cerebellar cortex had signs of functional stress. Adaptive processes were observed both in a small number of pinealocytes and in Purkinje cells, as well as an active adaptive glial reaction in both organs.

Keywords: *pineal gland, heavy metals, cerebellar cortex, reactive astrogliosis.*

Introduction

Pollution by salts of heavy metals is an important environmental problem today, which negatively affects the health of the population. Such a negative impact determines the development of various pathologies, disruption of the body's homeostasis and morphological transformations in various tissues [24, 25], since each trace element is potentially toxic in case of excessive exposure [5]. Various human diseases and toxicity are often associated with oxidative stress [5].

To date, the human pineal gland is the least studied endocrine gland, which occupies one of the central places in the endocrine regulation of the vital activity of all organs and systems, carries out adaptive reactions of the body to the changing conditions of the external environment. It is known that the hormone melatonin (indole metabolite of the amino acid tryptophan, which is mainly produced by the pineal gland) is the strongest natural inhibitor of free radical processes in the body [5, 23].

Melatonin is a neuropeptide that is synthesized by the pineal gland and with the help of which the pineal gland participates in the organization and regulation of cyclic processes [19]. It is believed that the pineal gland secretes

the hormone in the form of two separate portions: one, at a low concentration, enters the blood and connects with peripheral organs, and the other, at a higher concentration, enters the cerebrospinal fluid and binds to brain receptors [29]. The pineal gland, with the help of melatonin, controls the endocrine, nervous and immune systems, integrates the systemic response to adverse factors affecting the body's resistance [9]. At the same time, another property of melatonin was discovered - optimization of brain activity and at the same time counteracting pathological processes that cause its disturbances. Improvement of brain activity under the influence of melatonin is associated with several mechanisms: antioxidant effect, weakening of glutamate neurotoxicity, activation of neuron growth factor and limitation of apoptosis of nerve cells. Melatonin provides protection of brain cells by decomposition of hydrogen peroxide into water and oxygen, by utilization of free hydroxyl radicals, activation of the natural system of antioxidant protection, by activation of superoxide dismutase and catalase [3, 13]. In addition, melatonin neuroprotection is based on the limitation of various forms of neurotoxicity (glutamate, nitric oxide, p-amyloid peptide, metals, etc.), as well as synchronization of biorhythms, changes in endocrine status [4].

Thus, it has been proven that a number of diseases of the nervous system are directly related to melatonin deficiency: Parkinson's disease, Alzheimer's disease. It should also be added that melatonin reduces the neurotoxicity of glutamate in brain tissues, the aggressiveness of nitric oxide, activates neuron growth factors, and at the same time limits the apoptosis of nerve cells [19].

Biological activity of melatonin in most cases is mediated by interaction with specific membrane and nuclear receptors. Membrane melatonin receptors (MT1, MT2) coupled to G proteins have a high affinity for their ligand and are localized in the suprachiasmatic nucleus (SCN) and other nuclei of the hypothalamus, hippocampus, cerebral cortex, and cerebellum [27].

To date, it is known about environmental factors that can cause negative changes in various structures of the cerebellum: bisphenol A (contained in plastic dishes), alcohol, methylazoxymethanol salts, hyperdynamia, hypoxia, hypodynamia, radiation [17], the sweetener aspartame [2, 11], methyl chloride, methyl bromide, thiophene [14, 16]. However, the study of histopathomorphological changes in the cerebellum under the influence of various negative factors showed that the most pronounced changes are observed in Purkinje neurons [12, 26]. Melatonin is also known to prevent radiation-induced reduction in Purkinje cell volume and number [18].

To date, it has been established that a violation of the melatonin-producing function of the pineal gland can be both congenital and acquired during life, as a result of the action of various negative factors of the external and internal environment. The reaction of pinealocytes to a certain influence depends not only on the intensity and duration of its action, but also on the hormonal saturation of the body, against the background of which this influence is carried out [19].

The aim of the work is to study morphological changes in the cerebellar cortex and epiphyses of sexually mature male rats under the condition of long-term exposure to a complex of heavy metal salts.

Materials and methods

Animals

The experiment was performed on 12 white sexually mature male rats weighing 200-250 g, aged 7-8 months, which were divided into 2 groups (the control and the experimental ones). Animals of the both groups were kept in the normal vivarium conditions, where the equal keeping conditions, nutrition, proper care and natural light (day/night) were maintained, with a constant ambient temperature (20-22°C). The animals had free access to drinking water. The study was carried out in the autumn-winter period.

Experimental microelementosis model

The experimental group included of rats, which for 60 days of drinking water mixture with heavy metal salts: zinc ($ZnSO_4x7H_2O$) - 5 mg/l, copper ($CuSO_4x5H_2O$) - 1 mg/l, iron ($FeSO_4$) - 10 mg/l, manganese ($MnSO_4x5H_2O$) - 0.1 mg/l, lead ($Pb(NO_3)_2$) - 0.1 mg/l and chromium ($K_2Cr_2O_7$) - 0.1 mg/l. The selected concentration of salts in the mixture was due to the presence of such concentrations of these salts in the soil and drinking water of Ukraine some regions according to literature sources [24, 25].

Removing animals from the experiment

Groups of experimental animals were removed from the experiment after previous thiopental anesthesia (at the dose of 30-40 mg/10 g body weight) on the 60th day of the experiment, in compliance with national and international standards on bioethics. All animal studies were conducted in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals for Experimental and Scientific Purposes (Strasbourg, 1986) and the "General Ethical Rules for Animal Experiments" approved by the First National Congress of Bioethics (Kyiv, 2001, Ukraine), Protocol No. 4 of 06/03/2020 Commission of Bioethics of Sumy State University. The subject of the study is the pineal gland and cerebellum cortex of experimental and control animals.

Extraction technique and histological studies

To study the morphological changes in the structural components of the pineal gland and cerebellum cortex conventional procedures of microanatomical (histological, histochemical and cytochemical) study method were used. Hematoxylin-eosin staining was used to assess the morphological state of the pineal gland, according to Van Gieson and Einarson. Studies of the cerebellar cortex were performed on histological preparations stained with hematoxylin-eosin and acridine-orange. Assessment of the cerebellum cortex and pineal gland's morphological state was performed by a number of microscopic indices: state

of stromal and parenchymal components, state of the vascular bed, changes in blood rheology, state of pinealocytes, astrocytic glia, Purkinje cells. General morphological and morphometric analysis was performed using the "Leica DM 500" light-optical microscope and with x4, x10, x40 lenses, binoculars 7 and 10; fluorescent microscope "Mikmed-2". Lenses 40, 90, 100; glasses - 7 and 10, immersion medium - non-fluorescent oil. Photo documentation of the results obtained was performed with a digital video camera "Leica DM IC C50 HD Camera". "Leica Application Suite LAS EZ version 20.0 [Build: 292] Copyright @ 2010" software was used.

Statistics

Processing of digital results was performed by applied statistical methods using the Microsoft Word Excell 2010 text editor with AtteStat 12.0.5 application. Reliability of the difference between the experimental and control data of morphometric parameters was assessed using the Student's test, the probability of error less than 5 % ($p<0.05$) was considered sufficient.

Results

After 60 days of the experiment, the epiphysis of the experimental animals had an oval shape, preserved its anatomical integrity and connection with the vascular plexus. Heavy metal salts caused noticeable negative changes in all structural components of the gland: stromal, vascular and parenchymal.

The length of the gland increased by 5.6 % (1.186±0.061 mm; $p>0.05$), the width by 2.7 % (0.838±0.072 mm; $p>0.05$) in comparison with the indicators of control animals.

Long-term administration of heavy metal salts to the body of rats caused vascular congestion. The vessels of the epiphysis, especially the capsule and subcapsular zone were dilated, significantly full of blood, with the initial stages of violation of the rheological properties of blood (stasis and initial stages of blood coagulation). The vascular wall thickened, especially in the large supplying vessels of the subcapsular zone. The lumen area of blood vessels increased by 2.25 times ($p<0.001$) relative to the indicators of control animals (Table 1). Around the vessels of the subcapsular zone, a rather pronounced active glial reaction was observed in the form of reactive astrogliosis, which is an adaptive reaction of neuroglia to the action of heavy metal salts [10]. In addition to the local, a general diffuse glial reaction was also observed (Fig. 1). Vascular engorgement of the gland was accompanied by swelling of the intertrabecular connective tissue component (Fig. 2), capillary engorgement in some areas was accompanied by their spasm in others. The capsule of the gland was significantly thickened (see Fig. 2), its thickness was 2.5 times greater than that of the control animals ($p<0.05$) (see Table 1).

Parenchyma of the pineal gland of the experimental animals was slightly spongy. Moderate disruption of the cytoarchitectonics of cellular trabeculae were observed. The

Table 1. Results of the pineal gland structural components' morphometric study in sexually mature rats under the heavy metal salts impact (X±CD).

Index	Groups of laboratory animals	
	Rats of the control group, n=6	Rats of the experimental group, n=6
Large diameter of pinealocyte nuclei, mm	3.831±0.192	5.182±0.246**
Small diameter of pinealocyte nuclei, mm	2.733±0.441	3.621±0.272
Cross-sectional area of pinealocyte nuclei, mm^2	7.270±1.240	25.18±3.09***
Large diameter of pinealocyte bodies, mm	6.019±0.931	8.817±0.498*
Small diameter of pinealocyte bodies, mm	4.374±0.383	4.613±0.142
Cross-sectional area of pinealocyte bodies, mm	23.04±1.83	51.62±3.05***
Area of the pinealocyte cytoplasm, mm^2	15.77±0.39	26.38±1.63***
Nuclear cytoplasmic ratio	1:0.461±0.224	1:0.142±0.213
Optical density of the nucleus, RU	110.1±1.1	83.17±5.39***
Optical density of cytoplasm, RU	132.6±1.3	85.61±2.86***
Vessels area, mm^2	61.27 ±0.67	137.8±1.6***
Absolute number of pinealocytes	113.7±1.5	71.51±1.83***
Absolute number of astrocytic glia cells	42.25±1.48	92.04±3.29***
Glyocyto-neuronal index	0.374±0.122	1.227±0.579
The average diameter of the caryon	3.228±0.374	4.327±0.711
Capsule thickness, mm	1.537±0.716	3.887±0.561*

Note: reliable compared to the control - * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

nuclei and cytoplasm of pinealocytes underwent morphological changes. Pinealocytes with signs of indoleamine production prevailed on samples stained by Einarson's gallocyanin. Light pinealocytes with lightened, vacuolated cytoplasm and oval, enlarged nuclei were detected only in certain fields of view. The increase in terms of heavy metal salts entering the body of rats caused polymorphic rearrangements of the chromatin of pinealocyte nuclei. Thus, a sufficient number of cells with deformed, irregularly shaped, homogeneous nuclei were visualized in the preparations. However, some cells had hypertrophied nuclei, with lightened chromatin meshwork, chromatin margination, and hypertrophied, hyperchromic nucleolus on the background of lightened karyoplasm. In addition, the preparations contained a sufficient number of cells with fine- and coarse-grained condensation of chromatin, which were diffusely located in the enlightened nucleus in the form of blocks of various sizes.

The general morphometric indicators of the nucleus and cytoplasm of pinealocytes also underwent changes. Linear indicators, as well as the area of nuclei and bodies of

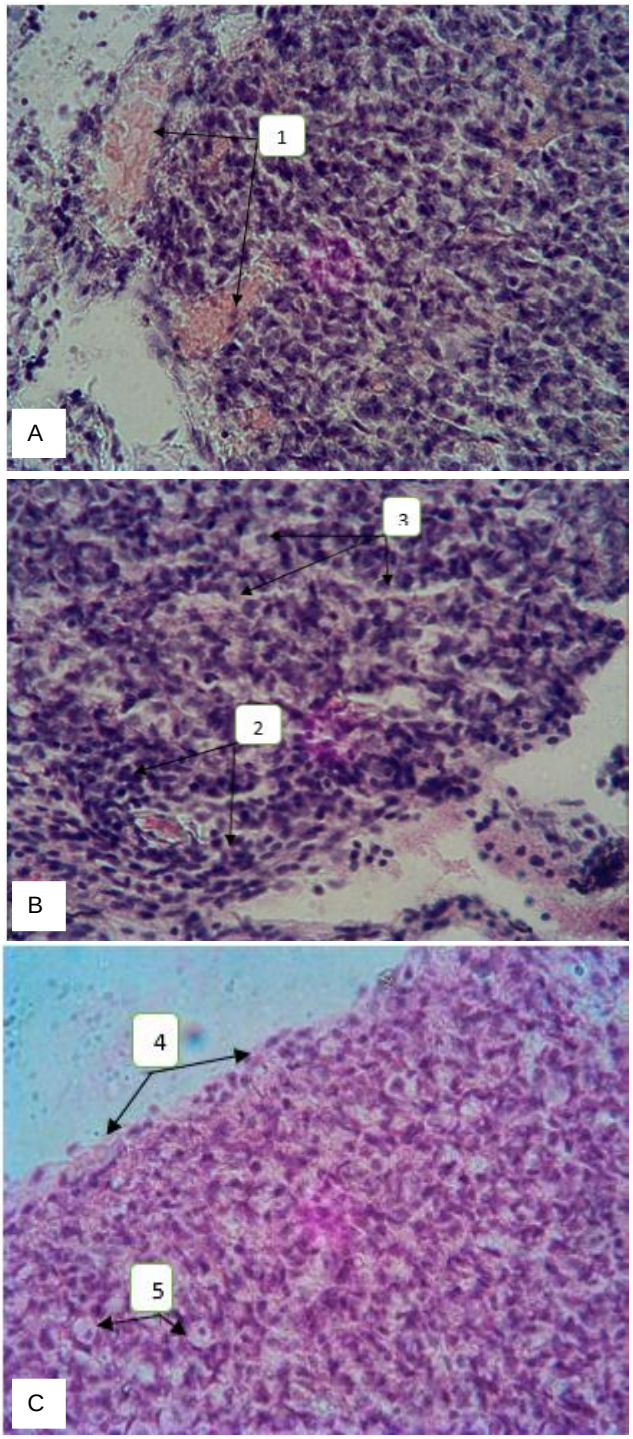

Fig. 1. Morphological rearrangements of the structural components in the pineal gland of experimental (A, B) and control (C) animals under the conditions of 60-day exposure to of heavy metal salts: A: 1 - plethora of the subcapsular zone vessels with signs of the blood rheological properties impairment; B: 2 - reactive astrogliosis; 3 - expansion of intertrabecular spaces; C: 4 - connective tissue capsule; 5 - light pinealocyte. Hematoxylin-eosin staining. x400.

pinealocytes underwent a dynamic increase in comparison with the indicators of control animals. The large and small diameters of pinealocyte nuclei increased, respectively, by 35.2 % ($p<0.01$) and 32.6 % ($p>0.05$), and the large and small diameters of pinealocyte bodies increased, respectively, by 46.5 % ($p<0.05$) and 5.5 % ($p>0.05$) in comparison with indicators of control animals. The cross-sectional area of nuclei, cytoplasm, and bodies of pinealocytes increased, respectively, by 3.5 times ($p<0.001$), 2.2 times ($p<0.001$) and 67.4 % ($p<0.001$) relative to the indicators of control animals. The average diameter of the karyon increased by 34.0 % relative to the control animals ($p<0.001$). The general indicators of the nuclear-cytoplasmic ratio were 1:0.142 and decreased relative to the control animals by 69.5 % ($p>0.05$). The optical density of nuclei and cytoplasm of pinealocytes decreased, respectively, by 24.4 % ($p<0.001$) and 35.5 % ($p<0.001$) compared to control animals. Long-term ingestion of heavy metal salts into the body of rats caused apoptotic rearrangements of pinealocytes and a decrease in their absolute number by 37.0 % ($p<0.001$), while the absolute number of astrocytic glia cells, on the contrary, increased by 2.2 times ($p<0.001$). The glial-neuronal index increased and exceeded the indicators of control animals by 3.3 times ($p>0.05$) (see Table 1).

In the experimental animals of the 60-day period of the experiment, morphological changes in the cerebellar cortex had a pronounced and progressive character. The number of neurons with reversible morphological changes decreased and, at the same time, the number of dystrophically changed cells, in which the processes were irreversible, increased.

As in the pineal gland, hemodynamic disorders, among which hypoxic changes predominated, were the primary morphological changes in the cerebellar cortex. The vessels of the cerebellar cortex (mainly the venous channel) were dilated and filled with cellular elements of blood, with the development of a morphological pattern of stagnant phenomena. Violation of the rheological properties of blood

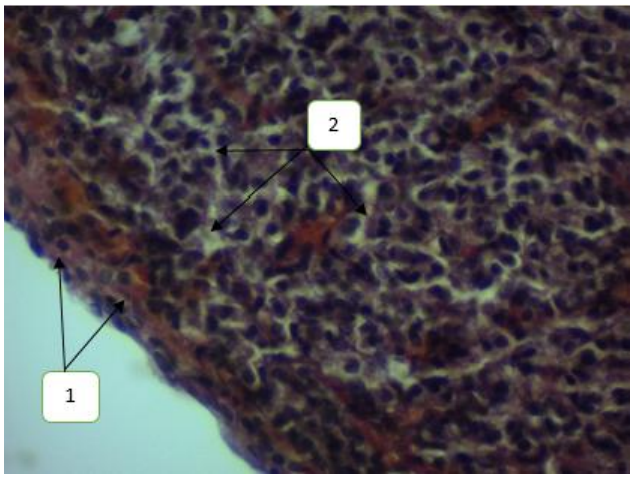

Fig. 2. Morphological changes in the structural components of the pineal gland under conditions of 60-day exposure to heavy metal salts: 1 - thickening of the gland capsule; 2 - growth of the connective tissue component of the stroma. Van Gieson staining. x400.

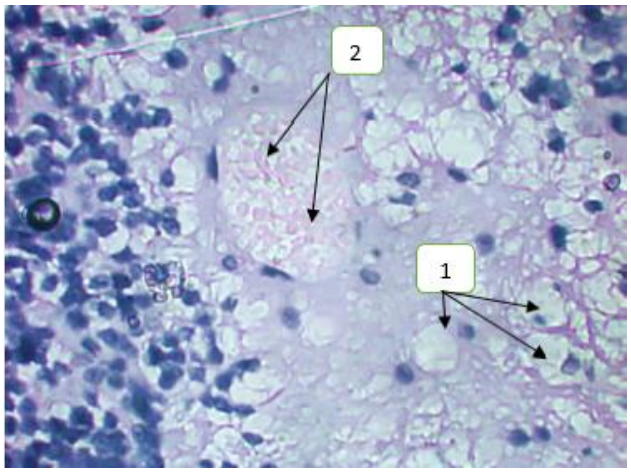

Fig. 3. Cerebellar cortex of experimental group rats after 60-day exposure to heavy metal salts. 1 - sponginess of the substance of the cerebellar cortex; 2 - venous congestion. Hematoxylin-eosin staining. x400.

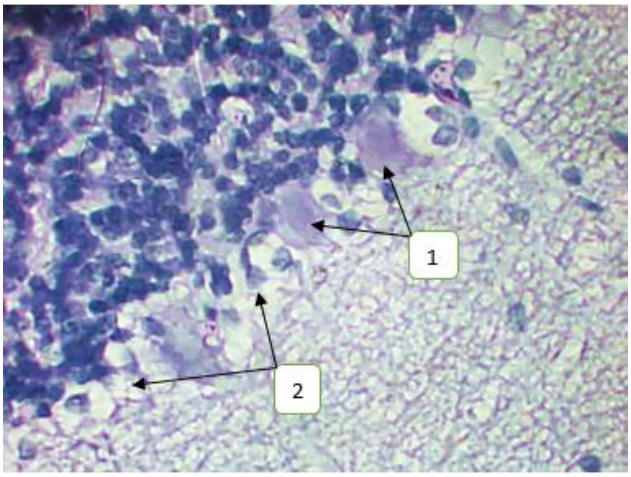

Fig. 4. Cerebellar cortex of an experimental rat after 60-day exposure to salts: 1 - karyocytolysis and karyolysis of Purkinje cells; 2 - area of partial loss of Purkinje cells. Hematoxylin-eosin staining. x400.

in the form of stasis and initial stages of sweetening of blood cells was observed. Hemocapillaries were unevenly filled with blood, dystonic (in some areas they were expanded, in others - spasmodic). The wall of hemocapillaries showed signs of impaired permeability, which was accompanied by the release of blood and plasma elements into the perivascular space with the formation of perivascular edema. After the 60-day period of the experiment, sponginess of the parenchyma of the cerebellar cortex and disruption of the cytoarchitectonics of the cells of the cerebellar cortex were noted. Zones of desolation (lacunae) were formed in the granular layer (Fig. 3).

The state and morphological features of the Purkinje cells of experimental animals were studied, taking into account their special vulnerability to various negative influences [12, 26]. Salts of heavy metals caused in Purkinje cells rather pronounced and widespread various morphological changes characterized by polymorphism.

Acute swelling of a part of Purkinje cells and their nuclei with the formation of pericellular edema was observed. Blurredness and roundness of cell contours, lightness of the cytoplasm were noted. The nuclei were hypochromic, indistinct, enlarged, in some places eccentrically located and had a well-contoured, hypertrophied and hyperchromic nucleolus, which was located in the center of part of the nuclei. In individual neurons, the nucleus occupied almost the entire cytoplasm. Chromatin margination was noted in some nuclei.

Along with the cells in a state of acute edema, there was a small number of neurons in a state of hypoxic changes (homogenizing splitting of Purkinje cells) and deeply changed cells with the formation of cells - shadows. Neurons with signs of homogenizing splitting of Purkinje cells had pale, homogeneous cytoplasm, the tigroid of which was in a state of partial or complete chromatolysis. Nuclei were sharply hyperchromic. Nuclei were not contoured, or, in individual cells, were hyperchromic and hypertrophied. In some places, Purkinje cells were surrounded by gliocytes.

In the cerebellum of the experimental animals, during the 60-day period of the experiment, neurons that were in a state of karyocytolysis and karyolysis with the formation of shadow cells prevailed. Attention was drawn to multiple foci of loss of Purkinje cells with preservation of the molecular and granular layers of the cerebellar cortex (Fig. 4).

At this time of the experiment, an increase in glial reaction was noted, especially around pathologically changed cells and vessels, in the form of hyperplasia processes (satellitosis and neuronophagy). Neurons with a pronounced process of karyocytolysis, which turned into shadow cells, underwent neurophagy with the formation of numerous foci of loss of Purkinje cells.

According to the data of morphometric studies, the indicators of the large diameter of the bodies of pear-shaped neurons increased by 9.7 % ($p>0.05$). The linear indicator of the small diameter of neuron bodies increased by 7.6 % ($p>0.05$), the outer perimeter of neuron bodies increased by 17.5 % ($p<0.01$). The cross-sectional area of neuron bodies increased by 31.7 % ($p<0.01$). The cross-sectional area of the cytoplasm of neurons increased by 27.6 % ($p<0.01$). The morphometric indicators of the state of the nucleus at this time of the experiment acquired a slight stabilization relative to the indicators of the control animals. Thus, the morphometric index of the large diameter of the nuclei of pear-shaped neurons decreased by 1.8 % ($p>0.05$), and the small one by 7.0 % ($p>0.05$), the outer perimeter of the nuclei was smaller than the indicators of control animals by 12.6 % ($p>0.05$). The cross-sectional area of the nuclei of neuron bodies exceeded the indicators of control animals by 39.0 % ($p<0.05$). The nuclear-cytoplasmic ratio was 1:0.552±1.119 and increased by 10.0 % ($p>0.05$) compared to the indicators of control animals (Table 2).

When studying the cytochemical state of Purkinje cells of experimental animals (acridine orange staining), a decrease

Table 2. Results of a morphometric study of Purkinje cells in the mature rats cerebellar cortex under the heavy metal salts impact.

Index	Groups of laboratory animals	
	Rats of the control group, n=6	Rats of the experimental group, n=6
Large diameter of the neuron's bodies, mm	6.418±0.412	7.036±0.258
Small diameter of the neuron's bodies, mm	4.012±0.076	4.311±0.132
Large diameter of the neuron's nuclei, mm	1.105±0.083	1.079±0.031
Small diameter of the neuron's nuclei, mm	0.793±0.101	0.741±0.012
Cross-sectional area of neurons, mm^2	406.9±31.5	536.1±19.9**
Cross-sectional area of neuron's nuclei, mm^2	135.8±18.4	190.0±1.2*
Cytoplasmic area of neurons, mm^2	271.1±13.2	346.1±18.7**
Nuclear-cytoplasmic index	1:0.51±1.086	1:0.552±1.119
The outer perimeter of neuron bodies, mm	39.95±1.71	46.93±0.87**
The outer perimeter of the neurons nuclei, mm	3.182±0.173	2.783±0.082
Normochromic neurons, %	48.63±1.83	31.63±1.72***
Hyperchromic neurons, %	46.48±0.94	30.04±1.53***
Hypochromic neurons, %	4.867±1.289	38.33±0.31***

Note: reliable compared to the control - * p<0.05; ** p<0.01; *** p<0.001.

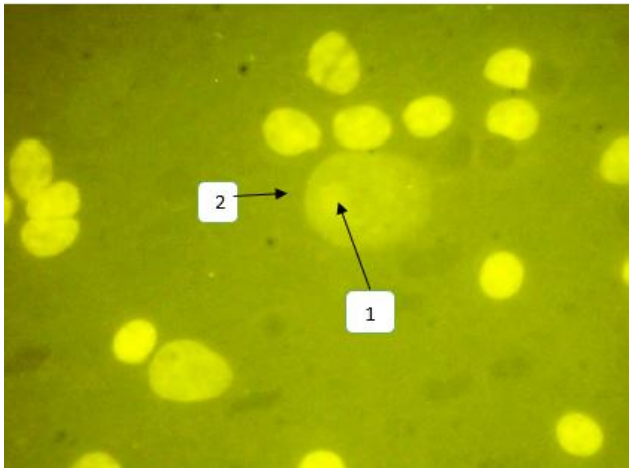

Fig. 5. Cerebellar cortex after 60-day exposure to heavy metal salts: Disappearance of RNA structures in the nucleus (1) and cytoplasm (2) of Purkinje cells. Acridine orange staining. x900.

in the content of RNA structures in the cytoplasm of part of the cells, an increase in the size of the nucleoli, an increasing condensation of the chromatin network of the nuclei, and destruction of the nuclei were observed. The level of saturation with RNA structures of the cytoplasm of neurons decreased, which was reflected in a decrease in the degree of saturation of the cytoplasm of neurons with red dusting (Nissl's substance) (Fig. 5). Evaluation according to the Keplow

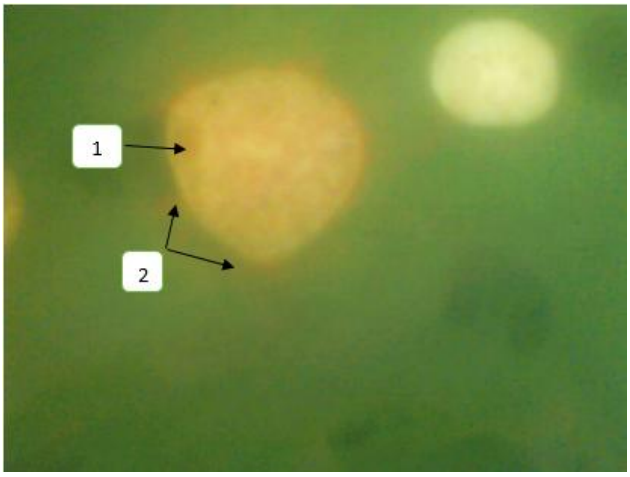

Fig. 6. Rat cerebellar cortex after 60-day exposure to heavy metal salts: Sufficient level of RNA structures in the nucleus (1) and cytoplasm (2) of Purkinje cells. Acridine orange staining. x900.

formula: + and ++ conditional units, which indicated an average and low degree of RNA saturation of Purkinje cells. Against the background of neurons with a medium and low degree of luminescence of RNA structures in the cytoplasm, there was a small number of cells with a high degree of luminescence of RNA structures (++++), which indicated the development of reparative processes in these cells.

Nuclei had increasing condensation of chromatin in the form of multiple clumps with chromatin margination. In cells with a small content of RNA structures in the cytoplasm, and the vast majority of them, nucleoli in the nuclei were weakly contoured or not at all detected, luminescence was noted with a bright yellow or pale red glow, depending on their saturation with RNA structures. This indicated an insufficient number of RNA structures in the nuclei. In single neurons that had sufficient saturation of cytoplasmic RNA structures (+++), the nucleoli also had a sufficient number of these structures, were hypertrophied, with the presence of bright red luminescence (+++) and sometimes shifted to the inner karyolemma of the nucleus (Fig. 6).

The number of hyperchromic neurons decreased by 35.4 % (p<0.001) and normochromic neurons by 34.9 % (p<0.001) compared to the control. Compared with control animals, hypochromic neurocytes increased their number by 7.9 times (p<0.001) (see Table 2).

Discussion

It is common knowledge that the neuroendocrine system is a regulator of all vital processes of the body. At the same time, this system is subject to the primary threat of intoxication and destabilization, the consequences of which are extremely dangerous for vital functions [7]. At the current stage of the development of natural knowledge, the interest of many researchers is focused on the problem of stress, especially its role in the processes of adaptation of the body to endogenous and exogenous influences, as well as in the occurrence of some diseases. According to modern data

[7], the pineal gland is an organ that combines the processes of adaptogenesis and immunogenesis, participates in the initiation of stress reactions and determines the sequence of disorders in the body at different stages of stress development.

Experimental and clinical data indicate a close connection of the pineal gland with the cerebellum [27]. Thus, the hormone of the pineal gland melatonin, which has an anti-stress, antioxidant effect, suppresses the formation of nitric oxide, the initiation of apoptosis, regulates circadian rhythms [22], has membrane melatonin receptors in various structures of the brain, including the cerebellum [27]. In our opinion, a comprehensive study of morphological features of the pineal gland and cerebellar cortex of sexually mature rats under conditions of 60-day exposure to a complex of heavy metal salts is interesting. The study of organometric indicators of the pineal gland during the 60-day period of the experiment indicated a slight hypertrophy of the organ in comparison with the indicators of control animals, which indicated the development of adaptive processes in response to the action of heavy metal salts.

Morphological changes in the epiphysis and cortex of the cerebellum were related to changes in all structural components of the studied organs: stromal, parenchymal, and vascular, which was confirmed by their morphological, morphometric, and cytochemical indicators. At the same time, both in the pineal gland and in the cerebellar cortex, the morphological changes had a non-specific polymorphic character. The basis of the occurrence of destructive changes in the structural components of the pineal gland and the cerebellar cortex was hypoxia, which is caused by both direct and indirect (mediated) effects of heavy metals on the vascular endothelium [28]. Thus, both in the pineal gland and in the cerebellar cortex, the pathogenetically morphological basis for negative changes was circulatory disorders, namely, vascular congestion (mainly venous), violation of the permeability of the vascular wall with the formation of perivascular edema, sponginess, and edematous processes, both in the cerebellar cortex, as well as in the pineal gland.

At the same time of the experiment, the rheological properties of blood in the form of stasis and the initial stages of blood clotting were disturbed in the bloodstream of both organs. Most likely, disorders of the rheological properties of blood were caused by the direct action of heavy metal salts on the capillary endothelium. At the same time, there was a violation of the functions of intracellular organelles, transport, metabolic, synthetic and adhesive functions of the endothelium. And as a result, there was a violation of hemorheology and microcirculation (changes in the number of active functioning capillaries, stasis, erythrocyte sludge, microthrombosis, paresis and vascular dystonia) [21, 28]. In addition, about 90 % of lead ions (which are part of the studied complex of heavy metal salts) entering the blood are bound by erythrocytes [20]. The absence of a blood-brain barrier in this organ can be considered an additional link of hemodynamic disturbances in the pineal gland [1].

The described morphological disorders in the vascular bed of the pineal gland indicated the gradual development of chronic hypoxia in the organ, which led to the further development of sclerotic changes due to hypoxia of collagen synthesis by fibroblasts [6]. Sclerotic changes, edema, and blood circulation disorders negatively affected the state of pinealocytes, including their secretory activity and the processes of releasing hormones into the blood due to impaired permeability of the vascular wall and disruption of the plasmalemma membrane of pinealocytes.

After a 60-day period of exposure to heavy metal salts on the pineal gland, morphologically, indole production prevailed in the gland, and the nuclear apparatus of part of the glandulocytes showed signs of hypertrophy of both the nucleus and the nucleolus, with an increase in the number of the latter. This undoubtedly indicated a significant stress on the controlling properties of the nucleus on synthetic processes in cells.

Morphometric indicators (area of nuclei, cytoplasm of pinealocytes and the average diameter of their karyon) remained increased compared to the indicators of control animals and confirmed the results of light-optical studies. The optical density of chromatin nuclei, on the contrary, decreased by 24.4 %, which according to Bulyk R. E. et al. [8]: "increased nuclear volume and low optical density of nuclear chromatin staining indicates the activation of genetic material and is regarded as an increase in cell function". But part of the pinealocytes still had morphological signs of chromatin margination, which are harbingers of apoptosis. Confirmation of the initial stages of apoptotic rearrangements in pinealocytes is a further decrease in their absolute number by 37.0 % in comparison with the indicators of control animals. That is, an increase in the synthetic activity of a part of pinealocytes against the background of a decrease in their absolute number indicated a general high load on the gland and, according to Gubina-Vakulyk G. I. [15], had a compensatory nature in connection with a decrease in the number of pinealocytes under conditions of chronic exposure to the complex salts of heavy metals. All this indicates a gradual decrease in reserve mechanisms of physiological adaptation in the pineal gland of experimental animals.

In response to the action of heavy metal salts, an active glial reaction was detected in the peripheral areas of the gland, which can be considered an active adaptive reaction of neuroglia to the action of heavy metal salts, and during the 60-day period of the experiment, the reaction of astroglia intensified, and both diffuse and local astrogliosis were detected around the vessels of the subcapsular zone, with the formation of "couplings". Such morphological formations, in our opinion, contributed to delaying the diffusion of heavy metal salts through the vascular wall into the parenchyma of the pineal gland. The formed perivascular astroglial proliferates [15], may indirectly testify to more intense processes of pinealocyte apoptosis in response to the action of a damaging agent. However, other properties

of astrocytic neuroglia in glial proliferates cannot be eliminated. In our opinion, they are aimed at achieving water-ion homeostasis in the gland by improving the trophicity of pinealocytes, their barrier function, preventing the penetration of heavy metals into the parenchyma of the gland. It is impossible to overestimate the contribution of astrocytes in the protection of the gland parenchyma from oxidative stress by synthesizing the gaseous gliotransmitter hydrogen sulfide (H_2S), which has the properties of a synaptic modulator and neuroprotector, protecting the organ from oxidative stress [10]. An increase in the number of glial elements in the pineal gland definitely has a certain compensatory and adaptive value, especially in the processes of transfer of RNA, amino acids and growth factors to pinealocytes [10]. Morphological changes in the body were confirmed by their morphometric indicators. Thus, a gradual dynamic increase in the absolute number of astrocytic glial cells by 2.2 times and the gliocyto-neuronal index by 3.3 times was observed in comparison with the indicators of control animals.

Thus, long-term (60-day) administration of a mixture of heavy metal salts to the body of experimental animals led to the development of a general adaptation syndrome and the stage of "subcompensation" of chronic stress in the pineal gland and the body as a whole.

Therefore, the morphological changes in the pineal gland had a non-specific polymorphic character in the form of a sharp violation of hemodynamics in the organ, the morphology of the vascular wall, the development of tissue hypoxia, a delay in the release of hormones into the blood as a result of a violation of the permeability of the cell membrane and vessel wall, processes of accelerated apoptosis of a part of pinealocytes, reactive astrogliosis in response to the action of a damaging agent.

Along with negative changes in the pineal gland, compensatory and adaptive processes with signs of functional stress were also noted. A slight hypertrophy of the organ and pinealocytes, an increase in the average diameter of the karyon, an active adaptive glial reaction and activation of synthetic processes in the part of pinealocytes were revealed.

In our opinion, the delay in the release of indole-containing hormones of the pineal gland (including melatonin) into the blood had negative consequences for endocrine regulation and preservation of homeostasis in the cerebellar cortex. Although, this aspect of the regulation of adaptive mechanisms in the cerebellum can be considered an addition to other, no less significant.

Morphological changes of Purkinje cells were quite distinct and widespread, had a versatile, non-specific, polymorphic character, and some cells had an irreversible character. The morphological indicators of the cells of the cerebellar cortex were characterized by the development of swelling due to a decrease in the relative density of cells and their nuclei. The trend towards an increase in all morphometric indicators of the bodies of pyriform neurons remained. Morphometry of the state of the nucleus in this term of the experiment showed signs of slight stabilization relative to the parameters of the control animals. Thus, the morphometric indicators of the nuclei of pear-shaped neurons decreased unreliably. At the same time, the cross-sectional area of the nuclei of neurons exceeded the indicators of intact animals by 39.0 %.

The number of neurons with reversible morphological changes decreased and, at the same time, the number of dystrophically changed cells, in which the processes were irreversible, increased. This is evidenced by the gradual and increasing inhibition of protein synthesis in the cytoplasm of neurons (chromatolysis), as well as the increase in reactive changes in neurons (the state of the nucleus and nucleoli, which were in a state of deep irreversible changes (rhexis, lysis), the formation of heterochromatin lumps in the chromatin of the nucleus, the decrease amount of RNA in nucleoli). Thus, the number of hyperchromic neurons decreased by 35.4 % compared to the control, and the number of normochromic neurons decreased by 34.9 %. Hypochromic neurocytes, in comparison with intact animals, increased their number by 7.9 times.

A fairly high level of RNA structure content was still preserved in some neuron nuclei, and their hyperplasia was observed. This, in our opinion, can be explained both by a compensatory and adaptive increase in the synthesis of RNA structures and proteins in individual cells (adaptive and adaptive changes), and by inhibition of the transition of substances from the nucleolus to the nucleus and further into the cytoplasm (this can lead to inhibition of synthesis protein in the nucleus and in the cytoplasm).

In the conditions of the experiment, glial hyperplasia was noted in the cerebellar tissue with the development of the process of satellitosis and neuronophagy around deeply altered neurons. Taking into account the works of a number of authors [21], hyperplasia of glia under the influence of Pb, due to its high plastic and adaptive properties to the surrounding microenvironment, should be considered as the ability to maintain high functional activity of the neuron-glia system and neuron-glia-capillary relationships. In addition, the glial reaction of the cells can be a sign of the compensatory and protective reaction of the cerebellar tissue. Forming a protective barrier around blood vessels, glial proliferates are thus likely to be able to prevent the penetration of the toxin into the brain substance.

Conclusions

1. Prolonged (60 days) ingestion of a complex of heavy metal salts into the experimental animals body led to the development of a general adaptation syndrome in the epiphysis and cerebellar cortex, the stage of "subcompensation" of chronic stress.

2. Morphological changes in the organs had a non-specific polymorphic character, such as a sharp violation of hemodynamics, a violation of the morphology of the vascular wall, the state of pinealocytes and Purkinje cells, the development of tissue hypoxia, processes of apoptosis

and reactive astrogliosis as a response to the action of a damaging agent.

3. Although the pineal gland of the experimental animals showed signs of indole production, the evacuation of hormones (including melatonin) into the vascular bed was hampered due to a violation of the morphology of the vascular wall and the cell membrane of pinealocytes. Most likely, this led to a deficiency of this hormone in the body of the experimental animals, which negatively affected the adaptive processes in the cerebellar cortex in response to the action of the stress agent.

4. Along with negative changes in the pineal gland and cerebellar cortex, compensatory and adaptive processes with signs of functional stress took place. Adaptive processes were observed both in a small number of pinealocytes and in Purkinje cells, as well as an active adaptive glial reaction in both organs. The signs listed above can probably indicate the possibility of regenerative processes in the studied organs after the termination of the negative effect of the complex of heavy metal salts.

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МОРФОЛОГІЧНІ ПЕРЕБУДОВИ ШИШКОПОДІБНОЇ ЗАЛОЗИ ТА КОРИ МОЗОЧКА СТАТЕВОЗРІЛИХ ЩУРІВ ПРИ ТРИВАЛОМУ ВПЛИВІ СОЛЕЙ ВАЖКИХ МЕТАЛІВ

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Забруднення солями важких металів є актуальною екологічною проблемою сучасності, яка негативно впливає на здоров'я населення. Ендокринна система підтримує гомеостаз в організмі, в тому числі й у нервовій системі. Метою роботи є дослідження морфологічних змін кори мозочка та епіфізів статевозрілих самців-щурів за умови тривалого впливу на організм комплексу солей важких металів. Досліджено морфологічні зміни кори мозочка та епіфізів статевозрілих самців щурів за умов тривалого впливу солей важких металів. Тваринам дослідної групи моделювали мікроелементоз шляхом додавання до питної води суміші солей важких металів протягом 60 діб: цинку ($ZnSO_4x7H_2O$) - 5 мг/л, міді ($CuSO_4x5H_2O$) - 1 мг/л, заліза ($FeSO_4$) - 10 мг/л, марганцю ($MnSO_4x5H_2O$) - 0,1 мг/л, свинцю ($Pb(NO_3)_2$) - 0,1 мг/л і хрому ($K_2Cr_2O_7$) - 0,1 мг/л. Використовували морфологічні, морфометричні та статистичні методи дослідження. Тривале (60 діб) надходження до організму піддослідних тварин комплексу солей важких металів призводило до розвитку в епіфізі та корі мозочка загального адаптаційного синдрому, стадії "субкомпенсації" хронічного стресу. Морфологічні перебудови в органах мали неспецифічний поліморфний характер, як то різке порушення гемодинаміки, порушення морфології судинної стінки, стану пінеалоцитів та клітин Пуркіньє, розвиток тканинної гіпоксії, процесів апоптозу та реактивного астрогліозу як відповідь на дію пошкоджуючого агенту. В епіфізі піддослідних тварин виявлені ознаки індол-продукції, але евакуація гормонів (в тому числі мелатоніну) до судинного русла була уповільнена внаслідок порушення морфології судинної стінки та клітинної оболонки пінеалоцитів. Це призводило до недостатності цього гормону в організмі піддослідних тварин, що негативно впливало на адаптивні процеси у корі мозочку у відповідь на дію стресорного агенту. Компенсаторно-пристосувальні процеси в епіфізі та корі мозочка мали ознаки функціонального напруження. Спостерігалися адаптивні процеси як у незначної кількості пінеалоцитів, так і у клітинах Пуркіньє, а також активна адаптивна гліальна реакція в обох органах.

Ключові слова: *шишкоподібна залоза, важкі метали, кора мозочка, реактивний астрогліоз.*