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RESEARCH ARTICLE

Influence of Proteoglycanes on Reactive Changes of Cerebral Neurons under Stress

Alexey Rozhentcev[1]*, Mikhail Rozhentcev[1], Sergey Lobanov[2], Nailya Cemerkhanova[1], Olga Valiullina[1]

1. *Federal State Budgetary Educational Establishment of Higher Education «Bashkir State Agrarian University», Ufa, Russian Federation.*

2. *Federal State Budgetary Educational Establishment of Higher Education «Bashkir State Pedagogical University», Ufa, Russian Federation.*

***Corresponding Author:** **Alexey Rozhentcev**

Abstract

The article presents research data on the effect of proteoglycans on reactive changes in cerebellum neurocytes under the action of a stressor. We simulated hypobaric hypoxia as a stress factor affecting cerebellar neurons. This is due to the fact that hypoxic conditions are widespread and can occur both in injuries and in various diseases. The histological and histochemical methods were used to determine the effect that proteoglycans have on changes that occur in cerebellum neurocytes under the influence of a stressor. Our comprehensive studies have helped to establish that the introduction of proteoglycans in order to correct the effect of hypobaric hypoxia on cerebellum neurocytes has a positive effect on intracellular production and excretion of high molecular weight compounds. So, in the neurons of the cerebellum of rats of the experimental group, a smooth increase in the processes of physiological regeneration was noted. In addition, destructive changes in neurocytes were less pronounced compared with the control group. The ultrastructural studies of rat cerebellum nerve cells in the control group showed that under the action of hypoxia in the early stages there was an increase in the volume of intracellular structures, and then, with prolonged exposure – a decrease in their volume. This, in turn, has a negative effect on metabolic processes inside the neuron and, as a consequence, the growth of destructive changes. An experimental study showed the effectiveness of the use of proteoglycans for the correction of changes in cerebellum neurocytes under the influence of a simulated stress factor.

Keywords: *Hypoxia, Cerebellum, Neuron, Proteoglycans, stress factor, Ultra-structure.*

Introduction

The lifestyle of a modern person is inextricably linked with all kinds of gadgets (from the TV remote control to the garage door remote control). In view of this, a person develops a sedentary lifestyle; he becomes less active and susceptible to various diseases. And in order to realize his natural potential he needs to be healthy and active [1]. In addition, other negative factors act on each person [2, 6].

We attribute hypoxia to such negative factors that have a strong effect not only on the ultrastructure of tissues, but also on the body as a whole [7, 9].Hypoxia at all times of medical science has been and remains one of the main problems. Since hypoxic conditions can occur not only with hypoxia itself, but also with other diseases [10, 11].This implies the need for further accumulation and expansion of knowledge about the processes and related changes occurring in the body tissue under the influence of hypoxic conditions. The changes that occur in the body against the backdrop of existing hypoxic conditions are clearly detected at the cellular level. These changes are also observed in the nerve cells of the cerebellum [12].

Under the influence of such states, rearrangement of the ultrastructural elements of the cell occurs [13, 15].Depending on the duration of the effect of hypoxia on the body tissues, not only compensatory-adaptive rearrangements of the nerve cell ultrastructure can occur, but also various maladaptive ones [16, 19]. These changes, in turn, can last quite a long time, even after the cessation of hypoxia [20, 22].It should be noted that the correction of pathological changes that occur in the tissues of the body under the influence of hypoxic conditions, in some cases is not effective enough [23, 24, 14, 9].Based on the foregoing, the aim of our study is to determine the features of ultrastructural changes in the rat cerebellum with the introduction of proteoglycans against the background of simulated hypobaric hypoxia.

Material and Research Methods

Experimental surveys were conducted from 2018 to 2019. As an object of study, we selected sexually mature individuals of VISTAR rats. The experimental part of the study was carried out in accordance with applicable laws and ethical standards: State Standard P 53434–2009, "Principles of Good Laboratory Practice"; "International Guidelines for Biomedical Animal Research" (1985); "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1986); "Guidelines for the care and use of laboratory animals" (8^{th} edition 2011); "Order of the Ministry of Health of the USSR No. 755 of August 12, 1977 "On measures to further improve the organizational forms of work using experimental animals".

To identify the features of changes in the cerebellum neurocytes with the introduction of proteoglycans against the background of the action of the stressor, 2 groups of animals were used: experimental and control (19 and 15 rats, respectively). Both groups were exposed to simulated hypoxia: rats were placed in a pressure chamber for 30 minutes with a decrease in pressure inside the chamber to 100 mm Hg. Art. Then the rats had resuscitation. Then, 0.7 ml of glycosaminoglycan solution (12.5%) was intraperitoneally administered to animals in the experimental group.

In order to identify the dynamics of changes in the cerebral nerve cells in the ultrastructure under the influence of a stressor, the animals were withdrawn from the experiment at strictly defined time intervals during the first 7 days. There are six total time intervals: 1 hour, 6 hours, 12 hours, 24 hours, 72 hours, 168 hours. The removal of rats from the experiment was carried out using an overdose of anesthesia followed by decapitation. Then, during the first 8 to 10 minutes, the cranium opened, the whole brain was taken and placed in formalin (10% solution of neutral formalin in phosphate buffer at a pH of 7.2 - 7.6).In order to study the characteristics of cerebellar cells, their location, histological methods were used by staining standard sections with hematoxylin-eosin according to the method developed by G. A. Merkulov [25].

To study glycosaminoglycans, histochemical methods were used-staining of standard sections (prepared using an ultratome) with a thickness of 0.1-0.5 microns, methylene blue and toluidine blue [26].Using the LKB-III ultra tome, ultrathin sections with a thickness of about 700 angstroms were made, followed by contrasting with uranyl acetate and lead citrate according to Reynolds. The sections were studied in a JEM - 100 S transmission electron microscope (JEOL Ltd., Japan), at an accelerating voltage of 90 kV. The results of the studies were statistically processed in the Windows 10 operating system using the STATISTICA application (Stat Soft Inc., USA).

Results and Discussion

The hypoxic conditions (of various etiologies) as a stress factor have a significant effect on the condition of nerve cells, on the intensity of metabolic processes, on the number and composition of intermediate metabolic products that change the pH of the medium and a number of other changes. All this can be detected in the form of the appearance of areas of enlightenment around the neurocytes. A significant number of studies have been conducted to study the effect of hypoxic conditions on the developing brain. So McKillen et al. showed in their studies using a model of ischemic hypoxia in newborn rats, that nerve cells are very sensitive to the effects of hypoxia, and this, in turn, causes significant impairment in the development of an individual in the postnatal period.

Under the action of hypoxic conditions, damage to the neurons of the subplate occurs. These disorders are associated with periventricular leukomolation, which is caused by the selective death of neurons of subplasts [27].J. Volpe, in his work, draws attention to the fact that a large number of premature babies have significant impaired development of the nervous system (impaired cognitive abilities and motor functions).

The scientists usually associate this type of damage to the central nervous system with periventricular leukomolation (this is a special type of cerebral damage to white matter). The author indicates that with periventricular leukomolation, a neuronal / axonal disease is usually observed, which affects the white matter not only of the basal ganglia, brain, thalamus, brain stem, but also the cerebellum.

These pathological conditions, he writes, are clearly enough distinguishable to be combined into one concept of "Encephalopathy of Prematurity." "Encephalopathy of prematurity" is due to a whole complex of primary destructive disorders and secondary disorders of maturation [20].K. Segovia et al. note that disruption of the myelination process in chronic lesions of white matter is due to a combination of delayed degeneration of oligodendrocyte precursors and a halt in their maturation [14].

The data of their work indicate that a delay in the maturation of oligodendrocyte precursors may be decisive for more serious damage to the white matter in premature individuals with repeated recurrent ischemic hypoxia. Peña Cristina Barradas et al conducted research on the model proposed by Robinson. The main essence of this experimental model was to squeeze the uterine arteries for a certain period of time on the eighteenth day of pregnancy [19].

As a result, lesions were found in both the white and gray matter of the cerebellum tissues. The displacement of nerve tissue by glia cells was detected. The simulated ischemic hypoxia revealed a high level of iNOS, which can last up to four weeks after the experiment. The authors note that nitric oxide plays an important role in the maturation, migration, and synaptic plasticity of cells; however, its increased content in tissue cells caused by hypoxia contributes to their damage.

The experimental studies conducted by the scientists have found that, against the background of ischemic hypoxia, there is deterioration in the formation of oligodendroglia in the tissues of the cerebellum of animals.

The content of PDGFRA + cells decreases and nerve fiber myelination stops. The authors point out obvious damage to cerebellar cells under the action of ischemic hypoxia, however, the detected motor changes were not described and no data were presented. Azam Ramezani et al. in their studies demonstrated that the lack of methylenetetrahydrofolate reductase (MTHFR) in mice causes a delay in brain development and leads to significant cerebellar abnormalities, which manifests itself in a violation of the development of granular cells and the structure of neurons [16].

The authors showed that the administration of trimethylglycine (betaine) had a positive effect on the formation of the cerebellum in puppies. The use of betaine reduced the severity of destructive changes in the cerebellum, and in some cases limited the localization of these lesions to two anterior lobes. Singh D. K. in his works indicates that periventricular white matter is selectively vulnerable to the action of ischemic hypoxia in premature babies [28]. Moreover, in the perinatal period of development, the activation of amoeboid microglia cells occurs, which is associated with damage to the periventricular white matter, observed during the action of ischemic hypoxia.

In the developing brain, amoeboid microglia cells (AMC) exhibit surface receptors and antigens similar to monocytes derived from tissue macrophages. Thus, the periventricular white matter in which amoeboid microglia cells predominate is selectively vulnerable to hypoxia. Amoeboid microglia cells are very sensitive to various hypoxic conditions and immediately begin to produce an excessive amount of inflammatory cytokines, such as tumor necrosis factor α (TNF – α) and interleukin 1β (IL-1β), as well as glutamate, nitric oxide and reactive oxygen species, which in the compartment cause oligodendrocyte death and axon degeneration, as well as degeneration of the immature blood-brain barrier. This, in turn, leads to neonatal death or a long-term deficit in the neurodevelopment of an individual.

As noted in the work, similar processes occur in the tissues of the cerebellum under the action of hypoxia. This is manifested in Purkinje cell death when activated amoeboid microglia cells produced TNF-α and IL-1ß factors through their respective receptors. The data of electron microscopy studies helped to identify the dynamics of changes in clarity and contour disorders among ultrastructural elements of the cell. In an ultra-structural study, cerebellum neurocytes had relatively clear contours. Their cell membrane and membranes of intracellular structures in some areas lose the clarity of their contours and acquire a slight "blur". More pronounced "blurring" phenomena are observed among intracellular membrane structures (Fig. 1).It was found that the nerve cells of the granular layer of the cerebellum have different sizes and shapes. These neurocytes are characterized by light and dark forms, of varying degrees of osmiophilism, which also indicates that they are in different functional activity.

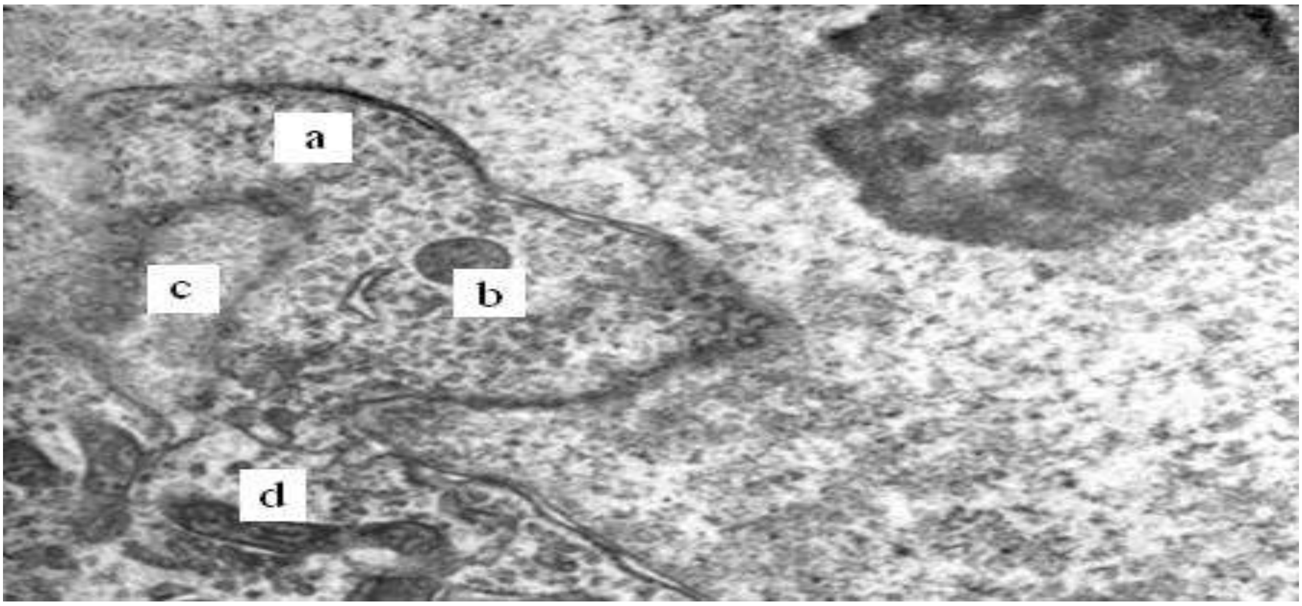

Figure 1: Cerebellar neuron. Experiment - 1 hour after hypoxia and correction with proteoglycans. Purkinje pear-shaped neuron. The nucleus - a, the nucleolus osmiophilic - b, the nuclear pores - c, and the mitochondria - d. EMF x 4500

The cell membrane of the cerebellar neurocytes is with a somewhat blurred outline. On the cell membrane, in its individual sections, a delicate flocculent material is found. The outlines of the nuclei of these neurocytes are also not even and have invaginations on their surface. The nuclei of some cells have taken the shape of an oval or bean-like shape. The membrane of the nuclei in some areas acquires slight loosening and conditionally less osmiophilism. The nuclear pores are detected on the nuclear membrane. In the perinuclear space, a fragile flocculent material is found, poorly perceiving the dye.

The nuclear substance is heterogeneous in composition. Chromatin in the nucleoplasm is not evenly distributed and is found along the periphery of the nucleus in the form of clots. Preferably, heterochromatin is located between the nuclear pores. The nuclear membranes of individual neurons have invaginations of different depths and sizes. Rarely is the cytoplasm of neurons which cells may contain single primary lysosomes of high electron density. These lysosomes are found near the mitochondria or near the Golgi complex. The Golgi apparatus contains all its inherent elements; it is located near the invagination of the nucleus.

In the lumen of the Golgi apparatus, fibrillar, granular material with a low electron density is detected. Nearby vesicles have the same electron density with a smooth-walled structure. The endoplasmic reticulum is structurally represented by granular (rough) and agranular (smooth) components. A flaky, fibrillar medium-electron density material is found in the lumen. On some membrane fragments of the granular part of the endoplasmic reticulum, the ribosomes are not evenly distributed. The smooth-contoured and pubescent vesicles of various electron densities are found near these fragments.

The ribosome cells contained in the cytoplasm are found in the form of polysomes of various sizes, some of which are fixed on the membrane of the endoplasmic reticulum.

Most mitochondria are represented by oval or round shapes. The outer mitochondrial membrane has relatively clear contours, and the inner membrane at this stage has an abundance of ruptures. In some mitochondria, relatively clear cristae are found. The mitochondria are represented by both large and small forms. The osmiophilism of the mitochondrial matrix of different mitochondria is not the same. In structure and form, mitochondria are heterogeneous.

After the sixth hour of the experiment, the introduced glycosaminoglycans during simulated hypoxia led to changes in cerebellum tissue, manifested by changes in the perception of dyes. In the blood vessels of the microvasculature, changes were also revealed during the studies. In the vessels of the capillary link, endothelial cells lining their lumen acquired a "swollen", "juicy" appearance. The cytoplasm of endotheliocytes contained a large number of pinocytotic vesicles. Among the vessels of the microvasculature, the phenomenon of hyperemia persists, in almost all its links. An ultrastructural study shows that in the walls of the capillaries forming their endotheliocytes, the phenomenon of pinocytosis is observed, the intensity of which increases in comparison with the early period. The changes that occur in the microvasculature under the influence of hypoxia are not the same.

This is manifested to a greater extent among the vessels of the venular link, in view of the fact that their diameter is more pronounced than that of arterioles. On the studied drugs, the phenomenon of dilatation of vessels is observed, i.e. alternation of sections of extensions and contractions. In some areas of rat cerebellum tissue, perivascular edema is observed. The assessment of the volume of intracellular structural elements of cerebellar neurocyte cells by the morphometric method revealed significant changes (Table 1, Fig. 2).

Table 1: Percentage of endogenous cellular structures of cerebellar neurocytes, correction with proteoglycans

	Control group	hours					
		1 hour	6 hours	12 hours	24 hours	72 hours	168 hours
Mitochondria	10.2	7.1	7.9	8.9	10.3	11.5	12.8
Golgi apparatus	19.7	5.5	6.1	7.3	8.5	9.8	11.1
Endoplasmic reticulum	23.4	9.3	10.3	11.5	12.4	13.0	13.8

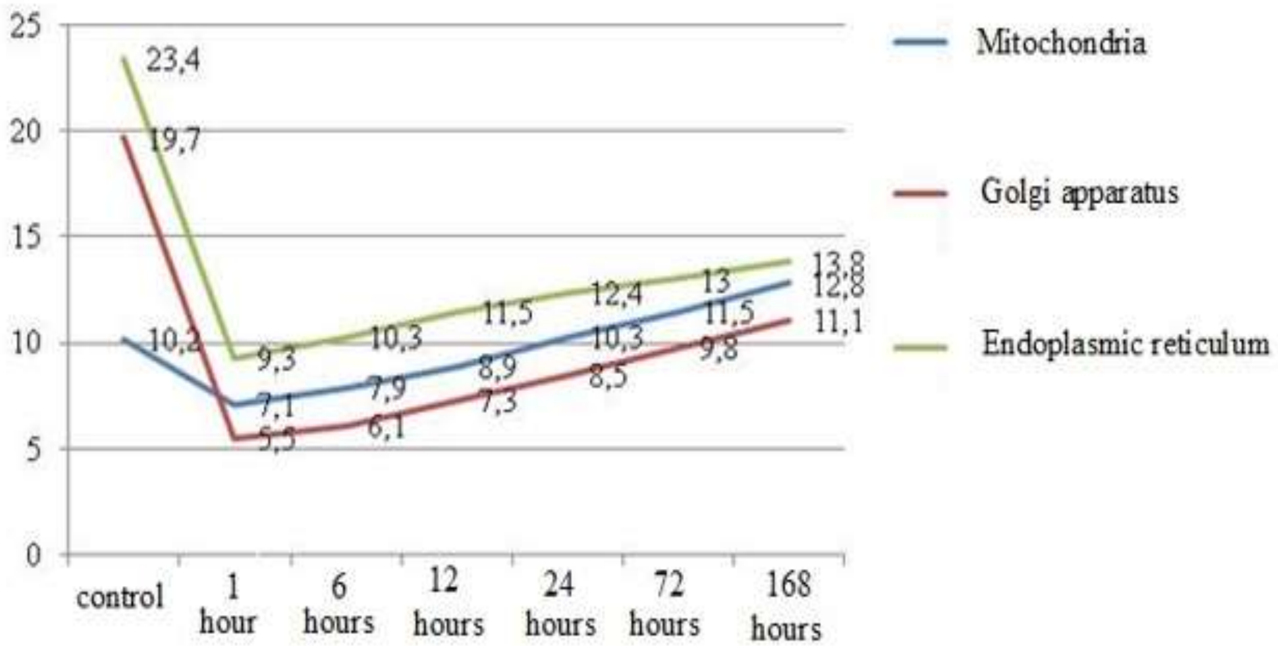

Figure 2: The percentage of endogenous cellular structures of cerebellar neurocytes during correction with proteoglycans

The structure of the cellular elements of the cerebellum also undergoes changes; they are traced throughout the experiment. The contours of the membranes of neurocytes remain slightly blurred; in some places they become fuzzy. In the nucleus of the cerebellar neurocyte, a nuclear membrane with pores is determined. In nuclei, nucleoproteids are mainly concentrated on the periphery. The mitochondrial membranes acquired a slight blur. In shape, the mitochondria acquired a swollen appearance, with poorly distinguishable cristae. In individual mitochondria, destructive changes are visible (Fig. 3).

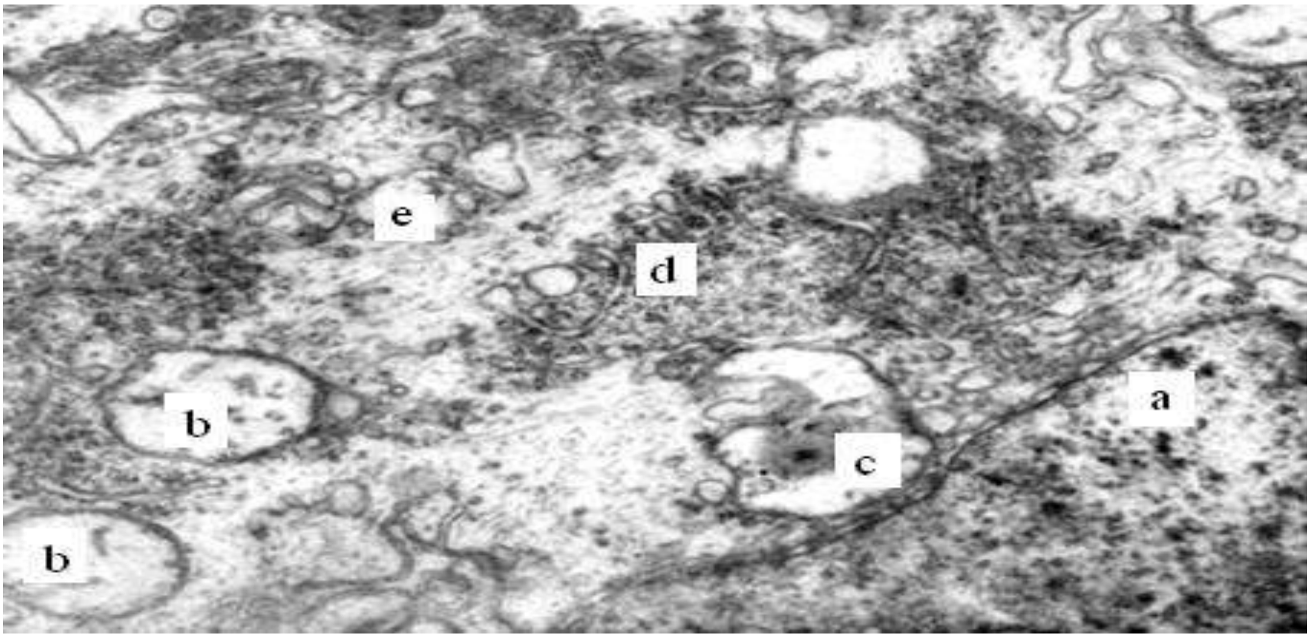

Figure 3: Nerve cell of the cerebellum. An experiment six hours after hypoxia and correction with proteoglycans. The nucleus - a, mitochondria of a swollen species with poorly distinguishable cristae - b, the expansion of the nuclear spaces - c, the rough endoplasmic reticulum - d, and vacuolization is e. EMF x 35,000

In the examined cerebellum neurocytes, single lysosomes of different electron density are found in the cytoplasm. Near the Golgi apparatus, the content of smooth-contour vesicles containing low electron density material increases. In the lumen of the Golgi apparatus, a delicate flocculent substance is detected. The endoplasmic reticulum retains its ultrastructure relatively well at the sixth hour of the experiment. Near it are vesicles of different shapes and sizes. The largest numbers of them are smooth contours. At the twelfth hour of the experiment, the membranes of the nerve cells in the cerebellum have relatively clear contours; around them is a material that is heterogeneous in composition. A detailed analysis of this material allows us to determine that it consists of small grains with a flocculent structure.

Vesicular organoids of various shapes, sizes and electron densities are found in the cytoplasm of cells; their size can vary within two to three times. In cerebellum neurocytes, such vesicular structures are found near the endoplasmic reticulum and Golgi apparatus. In the Golgi apparatus, flocculent material of medium electron density is detected. In the endoplasmic reticulum, a fine-grained flocculent material is found, most of which is located in the area of the smooth contour part. In the rough part of the endoplasmic reticulum, finely fibrillar material is detected. In the cytoplasm of neurocytes, mitochondria are located both one by one and in groups. At this stage, differences in shape and size appear in the mitochondria. An increase in the number of small mitochondria of high electron density is noted.

In the cytoplasm, the content of ribosomes, both free and fixed on the membranes of the endoplasmic reticulum, increases. Free ribosomes are most often grouped into clusters of different sizes. After the first and third days of the experiment with correction of glycosaminoglycans, the studies performed reveal the transformations of the structural components of cells and the extracellular matrix. The indications of ultra-structural studies indicate the preservation of pinocytotic vesicles in the endothelial cells of the vessels of the microvasculature, although their number decreases. The appearance of lysosomes is observed in cerebellum neurocytes. By single structures, they are found in different areas of the cytoplasm.

In some areas along the periphery of these lysosomes, in the form of local formations, foci of destruction are found. In individual lysosome cells located near mitochondria caused changes in their structure. On the seventh day of the experiment, the development of processes of transformation and renewal of intracellular structures is observed in the nerve cells of the cerebellum. In cells of cerebellar neurons, autolysis processes are observed simultaneously with these processes, which are manifested by the characteristic homogenization of individual regions of the cytoplasm. In tissues, the number of lysosomes of different electron density increases (Fig. 4).

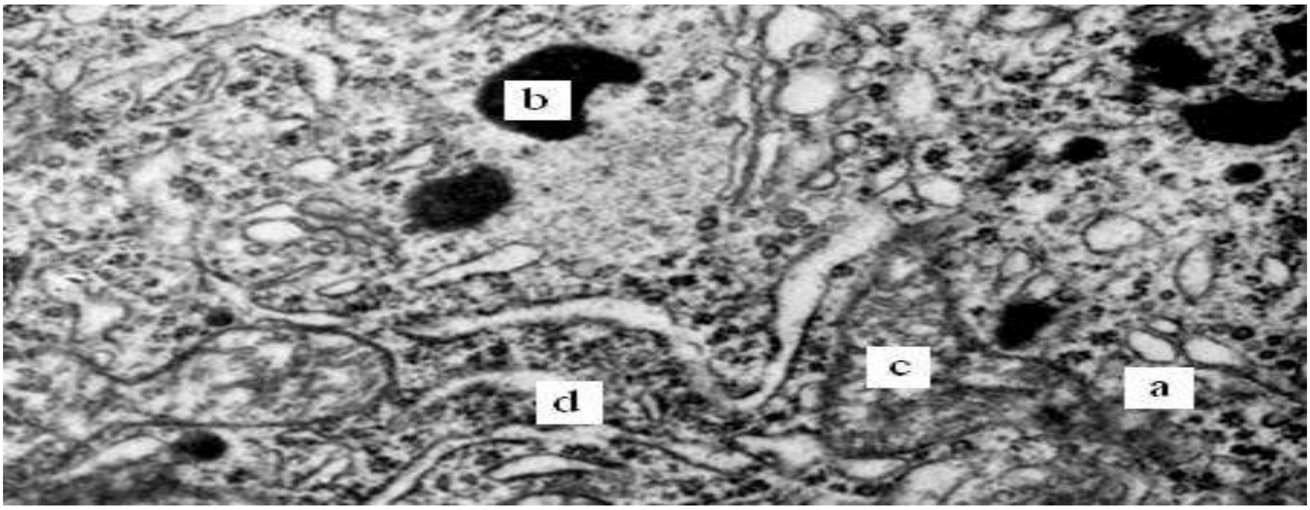

Figure 4: Nerve cell of the cerebellum. An experiment seven days after hypoxia and correction with proteoglycans. Ribosomes - a, lysosomes - b, degeneration site - c, endoplasmic reticulum - d. EMF x 45000

As can be seen from electron microscopic studies of the effects on the cerebellar nerve tissue by simulated hypoxia and subsequent correction by proteoglycans, changes occurring in the cerebellar nerve tissue occur in phases. Such transformations include the difference of neurocytes from each other with ultrastructural signs of their own intracellular and extracellular metabolism, which coincides with the data of other authors.

The Golgi apparatus and the endoplasmic reticulum of cells are characterized by an increase in the function of synthesis and secretion of intra- and extracellular structures and substances. The intensification of these processes is directly related to the detection, both on the cell surface and in the internal environment of the cell, of a flocculent, fine-grained and fine-fibrillar substance used by the cell for the construction of protein ultra-structures and intracellular proteoglycans.

These processes consume a large amount of energy, which leads to the activation of their mitochondria. It should also be noted that studies conducted during the experiment reveal an imbalance in the biosynthesis and secretion of protein - polysaccharide and protein - lipid compounds. Gradually, during the experiment in the cerebellar neurons, an increase in the processes of restoration of subcellular structures and the extinction of destructive transformations are observed. At the first stages, this is explained by maladaptation, and later on by the operation of compensatory-adaptive processes.

Conclusions

The use of a whole complex of electron microscopic research methods allowed us to determine the features of changes in the cerebellum tissues at the ultrastructural level with the introduction of proteoglycans against the background of the action of a simulated stressor. The modeling of hypoxia led to a change in the ultrastructure of cerebellar neurons and their composition, both in the control and in the experimental group.

A decrease in the osmophilicity of cell membranes and glycocalyx content was noted. In this case, a decrease in the content of ribosomes on the membranes of the endoplasmic reticulum was observed. In the endoplasmic reticulum itself, enlightenment of the matrix was observed, which was detected at different times of the experiment. With the pathological effect of hypobaric hypoxia in the early stages, an increase in the volume of intracellular structures of cerebellar neurocytes was noted, then there was a decrease in their volume.

However, it should be noted that maladaptive processes detected at the ultrastructural level in the experimental group are less pronounced compared to the control group. In the cerebellar neurons under these conditions, the phenomena of a gradual increase in the processes of physiological regeneration and a gradual attenuation of destructive changes were traced. These changes are manifested in the form of the inclusion of compensatory-adaptive mechanisms and processes of maladaptation. The experimental study showed that the use of proteoglycans positively affects adaptive processes occurring at the ultrastructural level of cell organization. Against the background of the introduction of a solution of proteoglycans, the synthesis of macromolecular compounds is improved.

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