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Comparative Characteristics of Histological Changes in Rats Brain Hippocampal Neurons with Anoxia of Respiratory and Ischemic Genesis

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RESEARCH ARTICLE

ABSTRACT

Acute oxygen deficiency serves as the basis for a variety of pathological processes in many diseases and environmental factors. When studying the hippocampus of rats under conditions of its total ischemia and mechanical asphyxia, the presence of structural changes in both studied periods (after 30 and 60 minutes) was revealed: a decrease in the area and a change in the shape (loss of sphericity and an increase in elongation) of cells, as well as a change in the degree of chromatophilia, which manifested itself a significant decrease in the number of normochromic neurons with a simultaneous increase in the number of hyperchromic and hyperchromic wrinkled neurons. At the same time, total cerebral ischemia led to a more significant decrease in the area of hippocampal neurons after 30 minutes of anoxia, while with mechanical asphyxia, changes in shape were noted to a greater extent in the form of a loss of sphericity and an increase in elongation. By 60 minutes of anoxia, there was no aggravation of changes in the size and shape of hippocampal neurons in total cerebral ischemia. At the same time, with mechanical asphyxia, there was a slight decrease in the area of neurons without changing their shape.

KEYWORDS
Anoxia, Neurons, Hippocampus, Total ischemia, Mechanical asphyxia

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INTRODUCTION

Anoxia as an extreme degree of acute oxygen deficiency leads to severe irreversible brain damage or even to its death [1-3]. Acute oxygen starvation is caused by various internal and external factors. Oxygen deficiency can primarily be the result of insufficient blood supply to the brain substance or the impact of an external mechanical factor (mechanical asphyxia) [4]. Previous studies have established the nature of changes in the parietal and occipital cortex of the brain in anoxia of circulatory and respiratory genesis. [5,6].

The hippocampus of the brain deserves special attention, which is associated with the importance in the life of the body and the severity of the consequences that develop when it is damaged. The hippocampus provides spatial orientation, plays an important role in olfactory reactions, in the provision of learning and memory processes. In rats, when the hippocampus is damaged, learned passive avoidance worsens due to the fact that they are not able to localize objects in space, while the animals do not cope well with switching skills. The most characteristic disorder in rats after hippocampectomy is the difficulty in developing delayed conditioned reflexes. [7].

A feature of anoxia of respiratory genesis, in contrast to anoxia of ischemic genesis, is the preservation of the heartbeat for a certain time. However, there is no oxygenation of the blood. Due to the lack of data on the consequences of the cessation of blood flow and respiration on the state of the hippocampus, it is advisable to conduct studies comparing neuronal changes in the hippocampus during mechanical asphyxia and total cerebral ischemia.

The aim is to compare histological changes in rat hippocampal neurons during anoxia caused by mechanical asphyxia and total cerebral ischemia.

MATERIALS AND RESEARCH METHODS

The study was carried out on outbred white rats (30 males, weight 240±20 g), divided into 5 groups (n=6) in compliance with the requirements of the Directive of the European Parliament and of the Council No. scientific purposes.
The experiments were performed on 2 models of cerebral hypoxia: mechanical asphyxia and total ischemia. The control group consisted of sham-operated rats (group 1). Simulation of mechanical asphyxia was performed by tying the trachea of rats below the cricoid cartilage with a ligature for 30 minutes (group 2) and 60 minutes (group 3). Simulation of total cerebral ischemia was performed by decapitation of rats with material sampling 30 minutes (group 4) and 60 minutes (group 5) after decapitation.

Surgical manipulations were performed under conditions of intravenous anesthesia (sodium thiopental, 40 mg/kg). The brain was removed and fixed in Carnoy's fluid, after which serial frontal paraffin sections 7 μm thick were made and stained according to the Nissl method. The location of the pyramidal layer of the field CA1 of the hippocampus was established using a stereotaxic atlas [8].

In each animal, 30 neurons of the pyramidal layer of the field CA1 of the hippocampus were studied with the determination of their size and shape [9]. Changes in the area and shape of neurons (form factor, elongation factor) were assessed using the ImageWarp image analysis program (Bitflow, USA). In histological preparations, various types of neurons were determined by the degree of staining of their cytoplasm (chromatophilia) [9].

The obtained quantitative continuous data were processed using the methods of nonparametric statistics, the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). The data are presented as Me(LQ;UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. Differences between the indicators of the control and experimental groups were considered significant at $p<0.05$ (Mann-WhitneyU-test) [10].

RESULTS

In animals of the experimental groups, structural changes occurred in the neurons of the pyramidal layer of the CA1 field of the hippocampus of the brain, which manifested themselves in changes in the size, shape of neurons, and the intensity of staining of their cytoplasm (Table 1).

Groups	Indicators		
	Square (micron ^{2})	**Form factor (units)**	**Elongation factor (units)**
Control	76,2 (72,9; 79,5)	0,9 (0,9; 0,9)	1,2 (1,1; 1,3)
Mechanical asphyxia 30 min	67,1* (62,7; 71,5)	0,7* (0,6; 0,7)	2,1* (2,0; 2,1)
Mechanical asphyxia 60 min	59,3* # (56,9; 61,6)	0,6* (0,6; 0,7)	2,2* (2,1; 2,3)
Otal cerebral ischemia 30 min	49,0* (47,0; 52,0)	0,6* (0,5; 0,6)	1,8* (1,8; 1,8)
Otal cerebral ischemia 60 min	54,0* (50,0; 60,0)	0,6* (0,5; 0,6)	1,8* (1,8; 1,9)
Note: * – differences are significant (p<0,05) compared with the control group # – differences are significant (p<0,05) compared with the group "mechanical asphyxia 30 min"			

Table 1: Dimensions and shape of neurons in the pyramidal layer of field CA1 of the hippocampus of the brain of rats with anoxia.

After 30 minutes of mechanical asphyxia, the area of neurons in the hippocampus decreased by 12% (p<0,05) compared to the control group, the form factor decreased by 27% (p<0,05), and the elongation factor increased by 76% (p<0,05), which reflects the loss of sphericity and, at the same time, an increase in their elongation.

In rats with total cerebral ischemia, by 30 minutes of the ischemic period, the area of neurons decreased by 36% (p<0,05), while the form factordecreased by 33% (p<0,05), and the elongation factor increased by 50% (p<0,05), which reflects a more significant decrease in the area of neurons than with mechanical asphyxia and a lower degree of loss of neuronal sphericity.

After 60 minutes of mechanical asphyxia, the neuronal area decreased by 22% ($p<0,05$) compared to the control group, the form factor decreased by 29% ($p<0,05$), and the elongation factor increased by 82% ($p <0,05$). When comparing experimental groups with 30 and 60 minute mechanical asphyxia, a decrease in the area of neurons by 12% ($p<0,05$) was revealed, while there was no change in the shape of neurons ($p>0,05$).

By 60 minutes of total cerebral ischemia, there was no aggravation of changes in the size and shape of neurons compared to the 30-minute period ($p>0,05$). In the control group, up to 94% of the rat hippocampal neuron population consisted of normochromic cells, while the remaining neurons were hyperchromic (5%) and hyperchromic wrinkled (1%) cells (Figure. 1, 2).

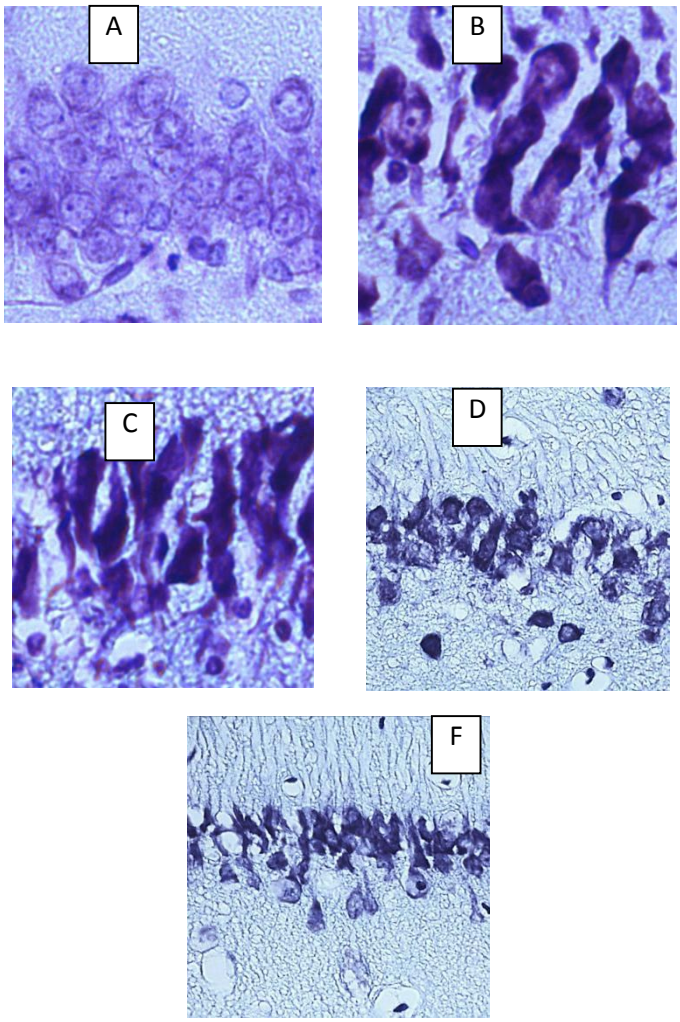

Figure 1: Neurons of the pyramidal layer of field CA1 of the rat hippocampus. Digital micrograph. Nissl stain

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A – control group (normochromic neurons); B – control group (normochromic neurons); C – control group (normochromic neurons) Magnification x 40 D – after 30 minutes of total cerebral ischemia (the predominance of hyperchromic and hyperchromic shriveled neurons); F – after 60 minutes of total cerebral ischemia (the predominance of hyperchromic and hyperchromic shriveled neurons). Magnification x 20.

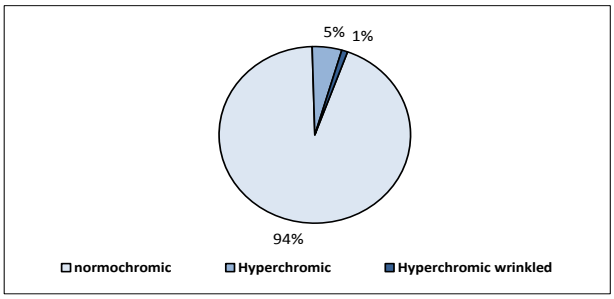

Figure 2: The ratio of neurons with different degrees of chromatophilia of the cytoplasm of the pyramidal layer of the field CA1 of the hippocampus of the brain of rats of the control group.

Hyperchromic and hyperchromic shriveled neurons predominated in both studied time periods in mechanical asphyxia and total cerebral ischemia: about 79% in groups of rats with 30-minute anoxia; about 98% – in groups of rats with 60 minutes of anoxia. So, in mechanical asphyxia and total cerebral ischemia, chromatophilia changes were unidirectional and were noted equally ($p>0.05$), which manifested itself in a significant increase in hyperchromic and hyperchromic shriveled neurons (Figure 3, 4).

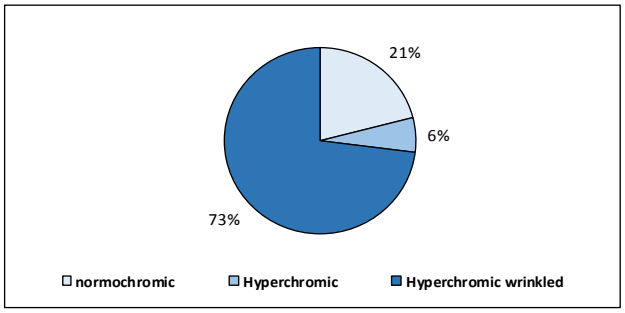

Figure 3: The ratio of neurons with different degrees of chromatophilia of the cytoplasm of the pyramidal layer of the field CA1 of the hippocampus of the brain of rats after 30 minutes of anoxia caused by mechanical asphyxia and total cerebral ischemia.

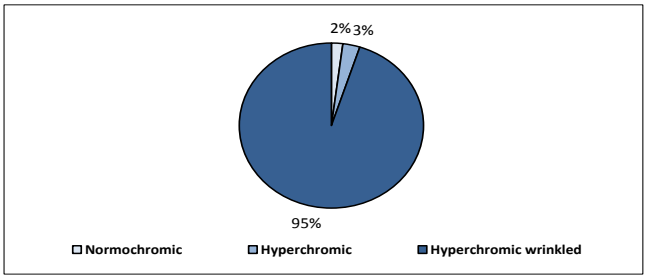

Figure 4: The ratio of neurons with different degrees of chromatophilia of the cytoplasm of the pyramidal layer of the field CA1 of the hippocampus of the rat brain after 60 minutes of anoxia caused by mechanical asphyxia and total cerebral ischemia.

DISCUSSION AND CONCLUSION

Thus, the study of the consequences of anoxia for the neurons of the pyramidal layer of field CA1 of the hippocampus of the rat brain under conditions of mechanical asphyxia and its total ischemia revealed the presence of unidirectional structural changes in both studied periods (after 30 and 60 minutes): a decrease in area and a change in shape (loss of sphericity and an increase in elongation) of cells, as well as a change in the degree of chromatophilia, which was manifested by a significant decrease in normochromic neurons with a simultaneous increase in hyperchromic and hyperchromic wrinkled neurons.

At the same time, total cerebral ischemia led to a more significant decrease in the area of hippocampal neurons (by 27%, $p<0,05$) compared with mechanical asphyxia after 30 minutes of anoxia. At the same time, shape changes in the form of a loss of sphericity and an increase in elongation were noted to a greater extent with mechanical asphyxia (a decrease in the form factor by 27%, an increase in the elongation factor by 76%, $p<0,05$). By 60 minutes of anoxia, there was no aggravation of changes in the size and shape of hippocampal neurons in total cerebral ischemia. At the same time, there was a slight decrease in the area of neurons (by 12%, $p<0,05$) without changing their shape during mechanical asphyxia.

These differences may be due to the preservation of cardiac activity during mechanical asphyxia for a short time.With mechanical asphyxia and total cerebral ischemia, similar changes in chromatophilia were recorded, which manifested itself in a significant increase in the number of hyperchromic and hyperchromic shriveled neurons.

Structural disorders of neurons in the hippocampus of the brain of rats with mechanical asphyxia were unidirectional in nature with previously obtained results on changes in the occipital and parietal cortex. In contrast to the occipital and parietal cortex, morphological changes were noted earlier and were more pronounced, namely, a decrease in the area and a change in the shape of neurons in combination with an increase in the degree of chromatophilia. [5,6].

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