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Case Report

Strangulation Asphyxia Causes Shrinkage and Hyperchromia of Neurons in the Cerebral Cortex of Rats

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Abstract

Acute oxygen starvation of the brain causes structural and metabolic disorders in neurons, leading to their death. When studying the brain of rats under conditions of strangulation asphyxia, modeled by compression of the trachea with a ligature, the presence of anoxic damage to neurons in the occipital lobe cortex was revealed. After 30 minutes of asphyxia, the neurons acquired an elongated shape, losing their roundness, without changing in size. With 60 minutes of asphyxia, there was a decrease in the area of neurons, which worsened by 24 hours of asphyxia, but there was no change in the shape of neurons. During the studied periods of asphyxia, an increase in the number of hyperchromic wrinkled neurons was observed with a decrease in the number of normochromic neurons and their complete disappearance by 24 hours of asphyxia.

Keywords: asphyxia; neurons; occipital cortex; brain; rats

Introduction

Acute oxygen deficiency can cause severe and often irreversible changes in brain tissue and even lead to its death [3]. Brain asphyxia occurs in many diseases, pathological conditions and exposure to environmental factors. In particular, oxygen deficiency can occur as a result of disruption of the passage of air through the respiratory tract due to the influence of external mechanical factors (mechanical asphyxia) [4]. The occipital lobe of the brain, which is responsible for the perception and processing of visual information and orientation in a new environment, deserves special attention. Hypoxia of this area of the brain can lead to loss of visual function - cortical blindness.

Previous studies have studied morphological changes in the cerebral cortex under conditions of total ischemia caused by decapitation [1, 2].

To date, the features of damage to neurons in the occipital lobe of the brain due to anoxia of respiratory origin caused by external mechanical factors have not been studied.

The goal is to study the features of morphological changes in neurons of the occipital lobe of the brain of rats with strangulation asphyxia.

Materials and methods of research

The study was conducted on outbred white rats (24 males, weight 240±20 g), divided into a control and three experimental groups with strangulation asphyxia, 6 animals in each group.

The control group consisted of sham-operated rats without strangulation asphyxia (group 1, n=6).

Strangulation asphyxia was modeled under thiopental anesthesia (iv, 50 mg/kg) by placing a ligature on the trachea 1.0 cm below the cricoid cartilage of the larynx for 30 minutes (group 2), 60 minutes (group 3) and 24 hours (group 4) [7].

The experiment was carried out in compliance with the requirements of Directive of the European Parliament and Council No. 2010/63/EU of 22.09.2010 on the protection of animals used for scientific purposes.

The brain was removed in the cold and fixed in Carnoy's fluid. Serial frontal paraffin sections 7 µm thick were prepared and stained with thionin using the Nissl method. The location of the occipital lobe cortex was determined using a stereotaxic atlas [6].

In each animal, 30 neurons of the fifth layer of the occipital lobe cortex were studied and their area, shape and degree of chromatophily were determined. Changes in the area and shape (form factor, elongation factor) of neurons were assessed using the ImageWarp image analysis program (Bitflow, USA) [5].

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

Research Results

In the animals of the experimental groups during the studied periods, morphological changes in the neurons of the occipital lobe of the brain were noted in the form of changes in the area (Table 1), the shape of neurons (Table 1, Fig. 1) and the intensity of staining of their cytoplasm.

Groups	Indicators		
	neuron area (μm2)	form factor (units)	elongation factor (units)
control	220,0 (175,5; 264,5)	0,9 (0,9; 0,9)	1,4 (1,2; 1,4)
SA 30 min	190,5 (145,5; 234,5)	0,6 (0,6; 0,6)*	2,3 (2,2; 2,4)*
SA 60 min	124,4 (123,4; 126,4)*#	0,6 (0,6; 0,6)*	2,4 (2,3; 2,4)*
CA 24 h	111,1 (109,2; 119,3)*#&	0,6 (0,6; 0,6)*	2,6 (2,5; 2,8)*#&

Note: – * – differences are significant ($p<0.05$) compared to the control group
– # – differences are significant ($p<0.05$) compared with 30 minute SA
– & – differences are significant ($p<0.05$) compared with 60 minute SA

Table 1: Indicators of size (area) and shape (form factor and elongation factor) of neurons in the occipital lobe of the brain of rats with strangulation asphyxia (SA), Me(LQ;UQ)

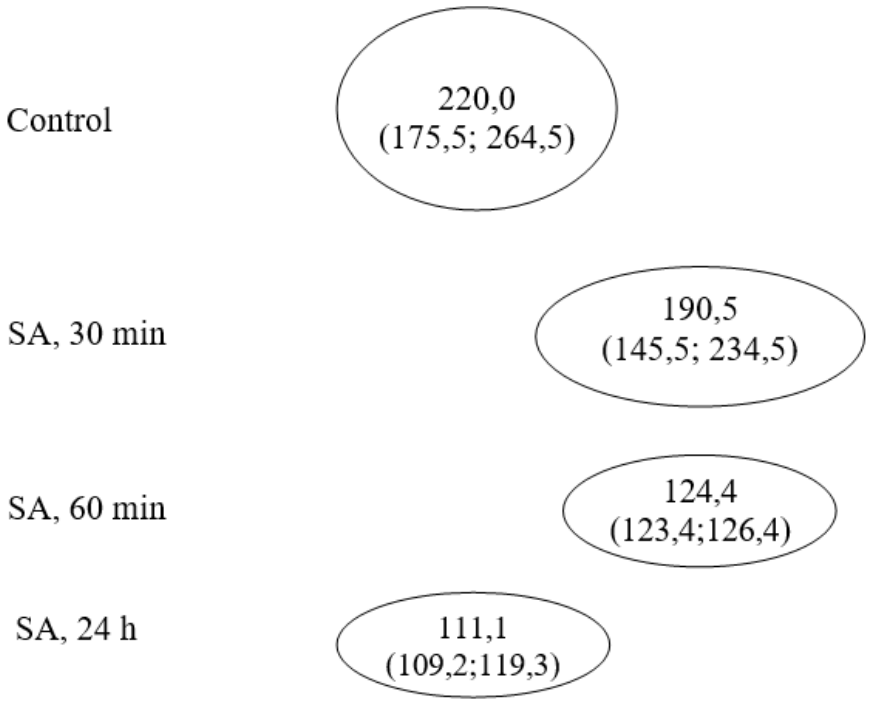

Figure 1: Graphic representation of the morphometric parameters of neurons (size, μm2 and shape) of the occipital lobe of the brain in rats with strangulation asphyxia (SA)

After 30 minutes of asphyxia, there was no change in the area of neurons compared to the value of the control group, but there was a decrease in the form factor by 29% ($p<0.05$) and an increase in the elongation factor by 68% ($p<0.05$), which indicates a change in the shape of neurons in the form of a loss of sphericity and an increase in their elongation.

After 60 minutes of asphyxia, the area of neurons decreased by 40% compared to the control group ($p<0.05$), at the same time the form factor decreased by 32% ($p<0.05$), and the elongation factor, on the contrary, increased by 74% ($p<0.05$).

Moreover, in rats with a 60-minute asphyxia, there was a decrease in the area of neurons compared to a 30-minute period of asphyxia by 35% ($p<0.05$), and there were no changes in the shape of neurons ($p>0.05$).
By 24 hours of asphyxia, the area of neurons decreased by 50% compared to the control group ($p<0.05$), the form factor decreased by 32% ($p<0.05$), and the elongation factor increased by 88% ($p<0 .05$), which reflects an increase in the elongation of perikarya.

In rats with 24-hour asphyxia, a decrease in the area of neurons was observed compared with 30 and 60-minute periods of asphyxia (a

decrease in area by 42% ($p<0.05$) and 11% ($p<0.05$), respectively), as well as an increase in the elongation of neurons (increase in elongation factor by 11% ($p<0.05$) and 8% ($p<0.05$), respectively, while the form factor did not change, $p>0.05$).

In contrast to the control group, where 95% of neurons were represented by normochromic cells, in the experimental groups there was a significant decrease in normochromic neurons and an increase in hyperchromic wrinkled neurons: up to 75% in the group of rats with 30-minute strangulation asphyxia ($p <0.05$), up to 80 % – in the group of rats with 60-minute strangulation asphyxia ($p<0.05$) and up to 64% – in the group of rats with 24-hour strangulation asphyxia ($p<0.05$), which are known to be markers of anoxia of nervous tissue [2 , 3]. By 24 hours of asphyxia, there was a complete disappearance of normochromic neurons and the appearance, along with hyperchromic wrinkled neurons, of hypochromic cells with pericellular edema (11%, $p<0.05$), as well as shadow cells (25%, $p<0.05$).

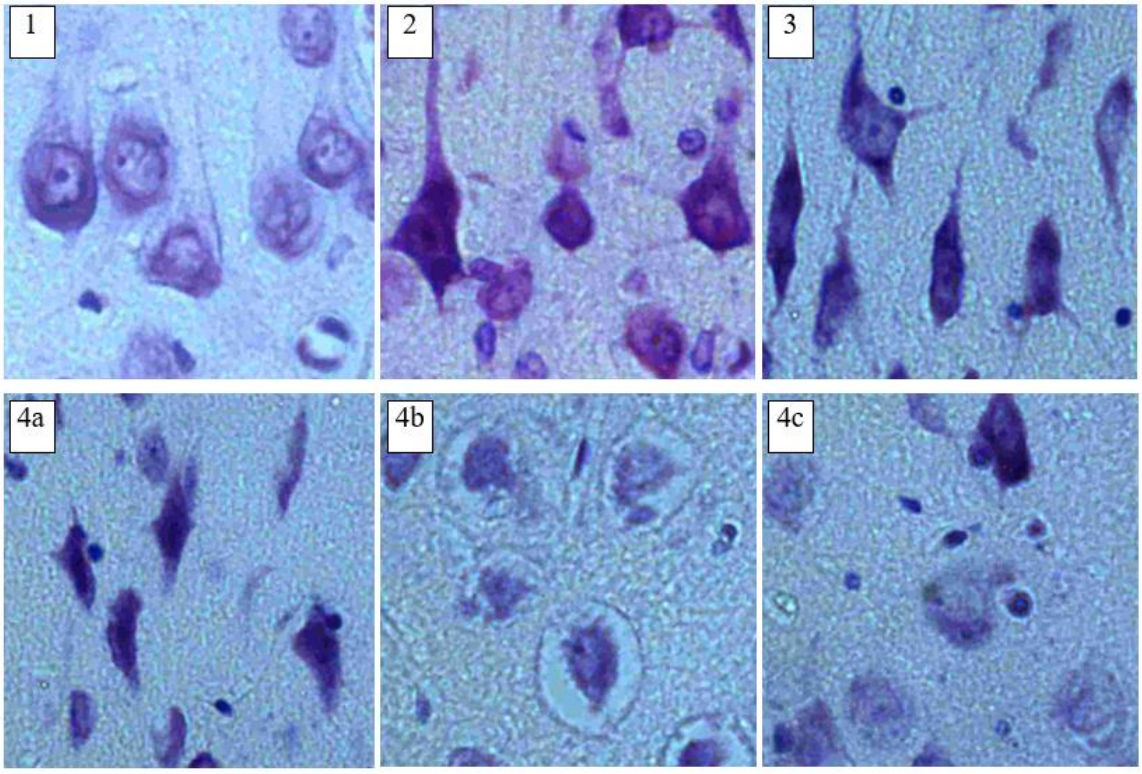

Figure 4: Neurons of the fifth layer of the occipital lobe cortex.
Digital microphotography. Nissl staining. In. lens x 40.
1 – control group (normochromic neurons);
2 – 30 min strangulation asphyxia (hyperchromic shriveled neurons);
3 – 60 min strangulation asphyxia (hyperchromic shriveled neurons);
4 – 24 hours strangulation asphyxia (a – hyperchromic shriveled neurons; b – hypochromic neurons with pericellular edema; c – shadow cells).

Conclusion:

Thus, strangulation asphyxia led to anoxic damage to the neurons of the occipital lobe cortex of rats, manifested in the form of changes in the size and shape of neurons, and the intensity of staining of their cytoplasm. After 30 minutes of asphyxia, the neurons acquired an elongated shape, losing their roundness; their sizes did not change. With 60 minutes of asphyxia, a significant decrease in the area of neurons was noted, which worsened by 24 hours of asphyxia, while no change in the shape of neurons was observed. During all periods of asphyxia studied, an increase in the number of hyperchromic wrinkled neurons was observed with a decrease in the number of normochromic neurons and their complete disappearance by 24 hours of asphyxia.

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