

## **CHANGES OF THE ACTIVITY OF GLUTAMINE SYNTHETASE IN AN EXPERIMENTAL MODEL OF HYPOXIC PRECONDITIONING**

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**In the present work, the effect of hypoxic preconditioning on the activity of glutamine synthetase in the brain structures of old rats subjected to severe hypoxia was studied. The study of metabolic changes, in particular, some enzymes of glutamate metabolism, in the brain under conditions of oxygen deficiency and during hypoxic training is an actual problem, the solution of which makes it possible to determine possible ways to protect the nervous tissue from hypoxic damage and increase its stability. Hypoxia does not always induce cell death; under certain conditions, hypoxic exposure has a neuroprotective effect. Hypoxic preconditioning increases the resistance of neurons to the effects of more severe forms of hypoxia. In particular, the endogenous neuroprotective mechanism is triggered, the activity of glutamine synthetase increases, and, as a consequence, the neurotoxicity of glutamate decreases.**

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**Keywords:** hypoxic preconditioning, glutamine synthetase, neurotoxicity, brain, hypoxic model.

### **INTRODUCTION**

One of the actual problems of modern medicine and biology is the search for effective ways to increase the body's resistance to damaging factors. One such method is preconditioning. The method is based on the use of moderate subthreshold effects, which increase the non-specific resistance of the organism due to the activation of its endogenous genetically programmed defensive mechanisms. After preconditioning, cells become resistant to the effect of a stimulus of suprathreshold intensity.

Among the variety of stress factors, hypoxia is basic to the pathogenesis of many neurological disorders, cardiovascular, pulmonary, oncological diseases and other pathologies.

Severe hypoxia creates a serious threat to the human organism; depending on the dose used, it can provoke the onset or contribute to the progression of neurological disorders such as Alzheimer's disease and other dementias, Parkinson's disease, Huntington's disease, strokes, amyotrophic lateral sclerosis, and other age-associated neurodegenerative diseases [4, 9]. Increased vulnerability to hypoxia with aging is of particular importance in the development of age-related neurodegenerative disorders [10, 19].

The brain is an oxygen-dependent organ, which makes it the most vulnerable to hypoxia. Hypoxic exposure changes the normal balance of neurotransmitters. Hypoxia, by activating energetically disadvantageous anaerobic glycolysis, leads to excess glutamate emission and cell apoptosis. Glutamate excitotoxicity is

one of the causes of neurodegenerative disorders of the brain [8]. Glutamate (Glu) is the main excitatory neurotransmitter in the brain, and the glutamate-glutamine metabolic cycle between neurons and astrocytes is necessary to prevent neuronal excitotoxicity. The ATP-dependent enzyme glutamine synthetase (GS) is a key enzyme of the glutamate-glutamine cycle that catalyzes glutamine synthesis from glutamate and ammonia and regulates the homeostasis of glutamate. In the brain, GS is located exclusively in astrocytes, and its activity is highest in the cerebral cortex, cerebellum, and hippocampus. GS in the adult brain has a neuroprotective effect and, during embryogenesis, plays an important role in brain development [1, 2, 16].

Currently, of particular interest is the study of the neuroprotective effect of moderate forms of hypoxia. The phenomenon of "induced hypoxic tolerance" of the brain is that short-term and repeated exposition to mild or moderate hypoxia triggers cellular and physiological adaptation, increasing the resistance of neurons to severe hypoxic injuries [14, 21, 24].

The key role of the glutamatergic system in the development of hypoxic brain pathology is beyond doubt and forms the basis for the concept of excitotoxicity of severe hypoxia. At the same time, the participation of this system in the formation of hypoxic tolerance induced by preconditioning has not been studied enough.

The aim of the work was: 1) to study the activity of glutamine synthetase in some brain structures of old rats subjected to severe hypoxic hypoxia. 2) study of glutamine synthetase activity in some brain structures of old rats subjected to hypoxic preconditioning and subsequently undergoing severe hypoxic hypoxia.

## **MATERIALS AND METHODS**

This study was performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

*Animals:* old white Wistar rats were used in this experiment and were divided into the

following groups: 1) control old rats; 2) old rats, that had undergone severe hypoxic hypoxia; 3) old rats subjected to hypoxic preconditioning and subsequently severe hypoxic hypoxia.

*Hypoxic models:* severe hypoxia was created in a pressure chamber (5% O<sub>2</sub>) for 30 min per day for 7 days. Hypoxic preconditioning was created in a pressure chamber (12% O<sub>2</sub>) for 60 min per day for 14 days.

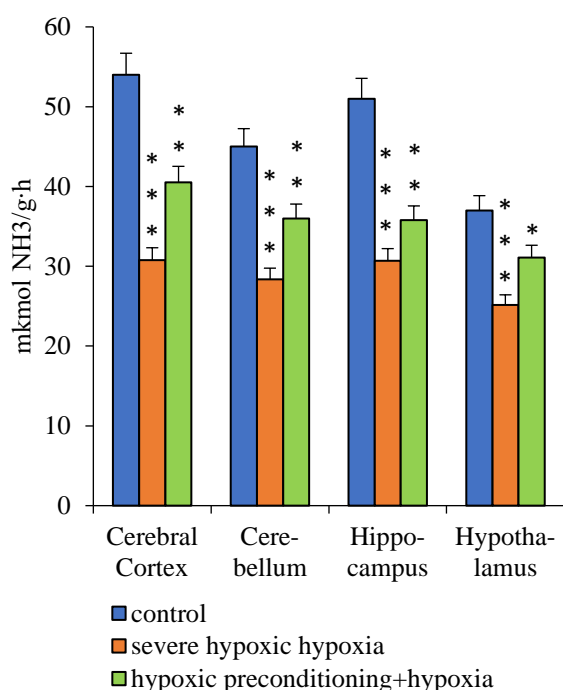
*Preparative methods:* all groups of rats were euthanized by decapitation after intraperitoneal injection of a mixture of Calyptol and Xylazine as narcosis. The brain was extracted from the skull and divided into areas: the cerebral cortex, cerebellum, hippocampus and hypothalamus. The brain tissue was homogenized using a teflon homogenizer in 0.32M cold sucrose solution. The activity of the enzyme glutamine synthetase was determined in the mitochondrial fraction obtained by differential centrifugation [18].

*Statistical analysis:* In statistical processing, Student's t-test was used to assess differences between the control and experimental groups. A p-value of 0.05 was considered statistically significant.

## **RESULTS AND DISCUSSION**

According to the results of the study, the activity of glutamine synthetase in rats subjected to severe hypoxia significantly decreases in all brain structures compared to the control: in the cerebral cortex - by 43%, the cerebellum - by 37%, the hippocampus - by 41%, the hypothalamus - by 32% (Fig. 1). Severe hypoxia damages the brain by disrupting the oxygen supply to the brain parenchyma, which is necessary for oxygen-dependent molecular processes. Hypoxic-mediated pathological conditions probably accelerate brain aging and provoke neurodegeneration. It is possible that the decline in resistance to hypoxia may be a basic aspect of brain aging [19]. Numerous studies have shown the significant role of glutamate excitotoxicity in the pathogenesis of neurodegenerative processes in the brain as a result of hypoxic damage [11]. Glutamine synthetase, performing the role of a

neuroprotectant, neutralizes the neurotoxicity of high concentrations of glutamate in normal conditions by converting glutamate into glutamine and binding ammonia [6]. But under hypoxic conditions, when structural and functional changes and neuronal damage occur, as well as the change in the plasticity of neural networks, glial cells, retaining their ability to capture glutamate by the energy of glycolysis, cannot fully neutralize the excess glutamate due to decreased levels of glutamine synthetase. Since astroglial glutamine synthetase is an endogenous protective mechanism against glutamate neurotoxicity, the reduction of GS activity has been suggested as a mechanism mediating neurotoxicity in neurodegenerative diseases [15, 23]. Therefore, increased expression of glutamine synthetase in astrocytes promotes the protection of neurons from the toxic effect of excess glutamate, which can be used in the search for new approaches in the treatment of neurodegenerative processes.



**Figure 1.** Changes of glutamine synthetase activity in old rats subjected to severe hypoxia and hypoxic preconditioning+hypoxia. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to control.

In preconditioned animals, the test for severe hypoxia demonstrated an increase in

glutamine synthetase activity in brain structures ( $p < 0.01$ ) relative to animals subjected to severe hypoxia without previous preconditioning. However, relative to the control data, the activity of the enzyme in preconditioned animals decreased: in the cerebral cortex - by 25%, the cerebellum - by 20%, the hippocampus - by 27% and the hypothalamus - by 16%. In particular, changes in GS level may affect glutamate uptake and glutamine production in astrocytes. On the other side, preliminary hypoxic training contributes to a less pronounced decrease in glutamine synthetase activity under conditions of severe hypoxia. In this case, perhaps, preconditioning increases the endogenous neuroprotective mechanism, reducing the hypoxia-mediated excitotoxicity of glutamate [13]. Additionally, preconditioning increases the body's resistance to hypoxia, which can counteract brain aging and neurodegenerative processes [7, 12].

Studies show that moderate hypoxia can relieve psychological stress and depression, as well as partially stimulate neurogenesis in the hippocampus [14, 22]. There is evidence of cognitive enhancement in hypoxia-preconditioned elderly adults [3, 20]. Hypoxic training has a positive effect on patients with multiple sclerosis and Huntington's disease [5]. Research is underway on the use of hypoxic preconditioning in patients with Alzheimer's disease [17]. Despite the reduced adaptive capacity of the elderly, however, their tolerance to moderate hypoxia is usually preserved, and subsequent adaptation can potentially be used therapeutically.

## CONCLUSION

Consequently, in old rats subjected to severe hypoxia, a more pronounced decrease in the activity of glutamine synthetase in brain structures was observed compared with preconditioned animals. In view of the fact that the brain is a highly plastic organ, moderate hypoxic stress initiates an increase in the resistance of the nervous tissue with the participation of numerous molecular mediators and the involvement of metabolic and enzymatic

alternative pathways. Ultimately, the survival of neurons improves. In particular, the endogenous neuroprotective mechanism is triggered, the activity of glutamine synthetase increases, and, as a consequence, the neurotoxicity of glutamate decreases. Therefore, increased expression of glutamine synthetase in astrocytes promotes the protection of neurons from the toxic effect of excess glutamate, which can be used in the search for new approaches in the treatment of neurodegenerative processes. Stimulation of brain tolerance to hypoxia through the use of preconditioning is a promising method for preventing hypoxic brain injury.

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## ИЗМЕНЕНИЕ АКТИВНОСТИ ГЛУТАМИНСИНТЕТАЗЫ НА ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ ГИПОКСИЧЕСКОГО ПРЕКОНДИЦИОНИРОВАНИЯ

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В представленной работе исследовано влияние гипоксического preconditionирования на активность глутаминсинтетазы в структурах головного мозга старых крыс, подвергнутых тяжелой гипоксии. Изучение метаболических изменений, в частности некоторых ферментов глутаматного обмена, в головном мозге в условиях кислородной недостаточности и при гипоксической тренировке является актуальной задачей, решение которой позволяет определить возможные пути защиты нервной ткани от гипоксических повреждений и повышения ее устойчивости. Гипоксия не всегда индуцирует гибель клеток, при определенных условиях гипоксическое воздействие оказывает нейропротективный эффект. Гипоксическое preconditionирование повышает устойчивость нейронов к воздействию более тяжелых форм гипоксии. В данном конкретном случае запускается эндогенный нейропротекторный механизм, повышается активность глутаминсинтетазы, снижается нейротоксичность глутамата.

**Ключевые слова:** гипоксическое preconditionирование, глутаминсинтетаза, нейротоксичность, мозг, гипоксическая модель

## HİPOKSİK PREKONDİSİONLAŞMANIN EKSPERİMENTAL MODELİNDƏ QLUTAMİNSİNTETAZANIN FƏALLIĞININ DƏYİŞİLMƏSİ

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Təqdim edilən işdə ağır hipoksiyaya məruz qalmış qoca siçovulların beyin strukturlarında qlutaminsintetazanın fəallığına hipoksik prekondisionlaşmanın təsiri öyrənilmişdir. Beyində oksigen çatışmazlığı şəraitində və hipoksik məşq zamanı metabolik dəyişikliklərin, xüsusən də qlutamat və enerjinin metabolizminin bəzi fermentlərinin öyrənilməsi çox aktualdır, bunun həlli beyin toxumasının hipoksiyadan qorunma yollarını müəyyənləşdirməyə və onun rezistentliyini artırmağa imkan verir. Hipoksiya hər zaman hüceyrənin ölümünə səbəb olmur, müəyyən vəziyyətlərdə hipoksiya neyroprotektiv təsir göstərə bilər. Hipoksik prekondisionlaşma neyronların daha ağır hipoksiyanın təsirlərinə qarşı müqavimətini artırır. Bu xüsusi halda endogen neyroprotektiv mexanizm aktivləşir, qlutaminsintetazanın fəallığı artır və qlutamatın neyrotoksikliyi azalır.

**Açar sözlər:** hipoksik prekondisionlaşma, qlutaminsintetaza, neyrotoksiklik, beyin, hipoksik model

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