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IMPACT OF HYPOXIC PRECONDITIONING ON THE ALTERATIONS IN PHOSPHATE-ACTIVATED GLUTAMINASE ACTIVITY IN THE BRAINS OF OFFSPRING

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Abstract:

The hypoxic preconditioning model is widely used in experimental studies to identify mechanisms for increasing the tolerance of organisms to subsequent hypoxic exposure. Glutaminase is the main enzyme that synthesizes glutamate, which has two important physiological roles: in the postnatal period, it acts as an excitatory neurotransmitter, and during the prenatal period, it regulates neurogenesis, synaptogenesis, and the survival of nerve cells. In our work, in 4 experimental groups, we investigated the effect of hypoxic preconditioning performed during days 16–21 of pregnancy on glutamate synthesis in the brains of 17-day-old and 6-month-old offspring. It was found that prenatal hypoxia led to a pronounced increase in the enzyme activity in various brain structures in early postnatal ontogenesis, while a decrease was observed in adult animals. In contrast, exposure to acute hypoxia resulted in a more significant increase in glutaminase activity in the brains of adult animals. Prenatal fetal hypoxic preconditioning caused a weakening effect on the increase in enzyme activity in 17-day-old rat offspring and a down-regulation in 6-month-olds, compared to the group that suffered acute hypoxia in the postnatal period. Thus, a neuroprotective adaptive-compensatory effect of prenatal preconditioning has been demonstrated, which can be associated with both the physiological and excitotoxic effects of glutamate.

Keywords: hypoxic preconditioning, prenatal period, offspring, brain, phosphate-activated glutaminase.

INTRODUCTION

The study of how various stressors during the intrauterine period affect further brain development during postnatal ontogenesis is of considerable interest to neurobiologists. The effects of prenatal hypoxia can appear in adult animals in the form of short- and long-term impairments of cognitive function and delayed reflex development [3, 4, 5]. The disturbances in learning, memory, and behavior observed in animals are associated with the processes occurring in the maturing brain during the period of exposure to an adverse factor. Oxygen deficiency negatively affects the survival, proliferation, migration, and differentiation of neural progenitor cells, synaptic plasticity, and wiring of neuronal networks [15]. Mediators and modulators such as glutamate, GABA, serotonin, and others take an active part in these processes. Glutamate, its receptors, and its transporters are actively involved in the

formation of neural wiring in the brain [10]. It is important to note that many neurotransmitters, before their future role as synaptic transmitters, have a neurotrophic role and are implicated directly in the growth and differentiation of neurons during brain maturation. The neurotrophic and neurotoxic properties of excitatory amino acids attract particular attention to the study of the glutamatergic system during brain development, since alterations in this system may be involved in pathological processes in the brain [6].

Also, hypoxic preconditioning of the fetus can reduce or prevent brain damage caused by hypoxia in subsequent early postnatal life. The study of neuroprotection pathways activated by hypoxic preconditioning of the fetus is necessary to understand the mechanisms of brain adaptation [2].

The work aimed to study the effect of hypoxic fetus preconditioning on the glutaminase (EC 3.5.1.2) activity in the mitochondria of various brain structures of rat offspring. Compare the results obtained with the controls and data after hypoxia suffered in the prenatal and postnatal periods.

MATERIAL AND METHODS

Experiments were performed on Wistar rats. The 1st Group (Controls) consisted of the 17-day-old (eye-opening time) and 6-month-old (adults) offspring obtained from the females kept in the chamber at a normal oxygen concentration. The 2nd Group included offspring of rats subjected to hypoxia on prenatal days 16–21 after reaching the age of 17 days and 6 months. Hypoxia for this group was created daily for 1 h in a pressure chamber ventilated with a mixture of 5% O_2 and 95% N_2 gases measured by a gas meter. The 3rd Group included 17-day-old and 6-month-old rats subjected to acute hypoxia with a mixture of 5% oxygen and 95% nitrogen gases for 1 h. 4th Group included rat pups subjected to hypoxic preconditioning (12% O_2 and 88% N_2) for 1 h daily during prenatal days 16-21 and acute hypoxia (5% O_2 and 95% N_2) for 1 h on the 17 and 180 postnatal days. Glutaminase activity was determined by the method [9]. The mitochondrial fraction was isolated by differential centrifugation [1]. The total protein was measured by the Bradford method [8]. All experiments were conducted in compliance with bioethical rules for the treatment of laboratory animals. Statistical processing was performed in MS Excel using the Student's t-test.

RESULTS AND DISCUSSION

The results showed that in the rat brain, the highest activity of glutaminase was observed in cortical areas (Fig. 1 and 2). The specific activity of glutaminase increased in the mitochondrial fraction of various cortical brain regions by 47–105% and in the cerebellum by 120% in 17-day-old rats who suffered prenatal fetal hypoxia. The group of rats subjected to acute hypoxia noted an even higher up-regulation of the enzymatic activity of glutaminase in brain structures. The offspring of rats that suffered fetal hypoxic preconditioning showed a less pronounced increase in glutaminase activity following acute hypoxia, compared with the group of rats exposed only to acute hypoxia (Fig. 1).

It should be noted that in 17-day-old rat pups following prenatal hypoxia, a pronounced up-regulation of glutaminase activity was observed in cortical regions, especially in the visual cortex, as well as in the cerebellum and hippocampus, compared to 6-month-old rats.

In 6-month-old rats that suffered hypoxia during the fetal stage, multidirectional changes in the activity of glutaminase were observed in the studied brain structures (Fig. 2). Acute hypoxic impact caused an up-regulation in glutaminase activity in almost all investigated brain regions in 6-month-old rats, and the severity of the increase was predominantly higher than in 17-day-old rats. The 6-month-old rats from the hypoxic preconditioning group showed down-regulation of enzymatic activity in response to acute hypoxic exposure compared with the group of acute postnatal hypoxia. So prenatal hypoxic preconditioning caused a down-regulation of glutaminase activity following the impact of acute oxygen deficiency.

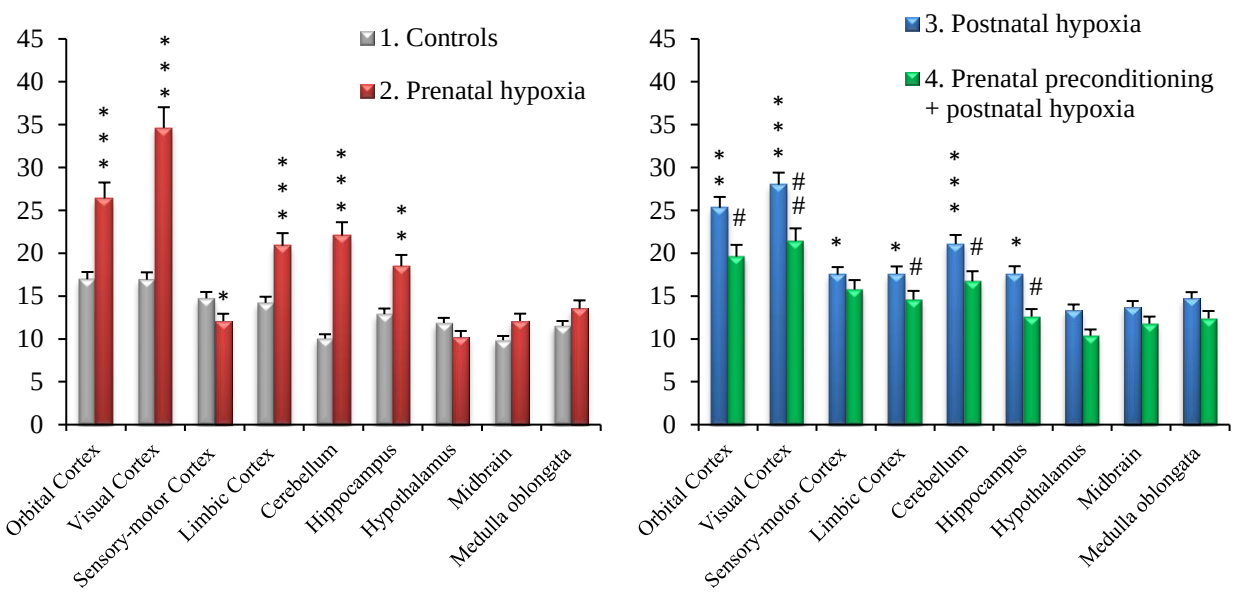

Figure 1. Comparative diagram of the effects of prenatal hypoxia suffered during the fetal period of prenatal development (on the left), acute hypoxia, and prenatal hypoxic preconditioning (on the right) on specific glutaminase activity in mitochondria of different brain regions of 17-day-old rats (μmol $N\text{-}NH_3$ / h · mg, M ± SEM; n = 8).

Note: * - p<0.05, ** - p<0.01, *** - p<0.001 compared to controls.
- p<0.05, ## - p<0.01 compared to the 3rd group.

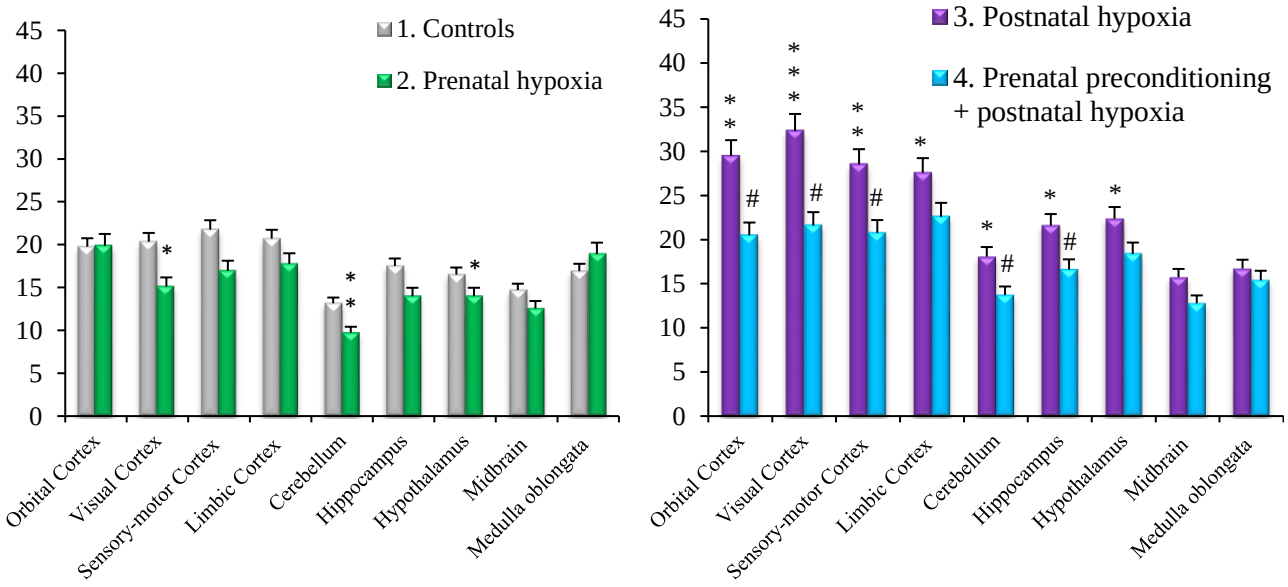

Figure 2. Comparison of the impact of prenatal hypoxia exposed during the fetal period of prenatal ontogenesis (on the left), acute hypoxia, and prenatal hypoxic preconditioning (on the right) on the specific activity of glutaminase in mitochondria of various brain areas of 6-month-old rats (μmol $N\text{-}NH_3$ / h · mg, M ± SEM; n = 8).

Note: * - p<0.05, ** - p<0.01, *** - p<0.001 compared to controls.
- p<0.05 compared to the 3rd Group.

The alterations observed can be explained considering the leading role of glutaminase in the maturation of nerve cells during the prenatal period of development [6] and its role in the synthesis of glutamate, which performs neurotransmitter function in 2/3 of the brain excitatory synapses, as well as the excitotoxic effect leading to degeneration and apoptosis of neurons [11]. Under the influence of hypoxia, the synaptic apparatus of neurons of various cortical and subcortical brain structures of young rats is damaged [1]. It should be noted that hypoxia in the embryonic brain realizes its specific effect through the activation of the hypoxia-inducible factor (HIF) signaling pathway, which is an important link in the formation of physiological systems during embryonic development. HIF, interacting with neuronal genes, leads to a complex change in the development of the CNS [5, 13, 14]. At the same time, HIF can have a protective effect in response to increased glutamate levels and glutamate neurotoxicity [16].

Hypoxic preconditioning has been shown to alter glutamine synthetase, related to glutamate metabolism as well as lactate dehydrogenase, which converts pyruvate to lactate [7, 12]. Our results are consistent with the suggestion that prenatal hypoxic preconditioning significantly increases the resistance of the brain to hypoxic impact [10].

CONCLUSION

Thus, 17-day-old and 6-month-old offspring subjected to hypoxic preconditioning during the prenatal period showed a weakening of elevated glutaminase levels caused by acute hypoxic exposure. This change in glutaminase activity is aimed at reducing the excitotoxicity of glutamate, but at the same time, it can affect the processes of neurotransmission.

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ВЛИЯНИЕ ГИПОКСИЧЕСКОГО ПРЕКОНДИЦИОНИРОВАНИЯ НА ИЗМЕНЕНИЕ ФОСФАТ-АКТИВИРУЕМОЙ ГЛУТАМИНАЗНОЙ АКТИВНОСТИ В ГОЛОВНОМ МОЗГЕ ПОТОМСТВА

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Модель гипоксического прекондиционирования широко используется в экспериментальных исследованиях для выявления механизмов повышения толерантности организмов к последующему гипоксическому воздействию. Глутаминаза является основным ферментом, синтезирующим глутамат, который выполняет две важные физиологические роли: в постнатальном периоде – в качестве возбуждающего нейромедиатора, а в пренатальном периоде – участвует в регуляции нейрогенеза, синаптогенеза и выживании нервных клеток. В нашей работе в 4 экспериментальных группах мы исследовали влияние гипоксического прекондиционирования, проводимого на 16–21 день беременности, на синтез глутамата в мозге 17-дневных и 6-месячного потомства. Установлено, что пренатальная гипоксия приводила к выраженному повышению активности ферментов в различных структурах головного мозга в раннем постнатальном онтогенезе, тогда как у взрослых животных наблюдалось ее снижение. Напротив, воздействие острой гипоксии приводило к более значительному увеличению активности глутаминазы в мозге взрослых животных. Пренатальное гипоксическое прекондиционирование плода вызывало

ослабляющий эффект на повышение активности фермента у 17-дневного потомства крыс и снижение у 6-месячных крысят по сравнению с группой, перенесшей острую гипоксию в постнатальном периоде. Таким образом, продемонстрирован нейропротекторный адаптивно-компенсаторный эффект пренатального прекондиционирования, который может быть связан как с физиологическим, так и с эксайтотоксическим действием глутамата.

Ключевые слова: гипоксическое прекондиционирование, пренатальные период, потомство, мозг, фосфат-активируемая глутаминаза

HIPOKSİK PREKONDİSİONLAŞMANIN NƏSİLLƏRİN BAŞ BEYİNDƏ FOSFAT-FƏALLAŞDIRICI QLUTAMİNAZA FERMENTİNİN FƏALLIĞINA TƏSİRİ

Leyla Bağdad qızı Qədirova

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Hipoksik prekondisionlaşma modeli, orqanizmin sonrakı hipoksiyaya qarşı tolerantlığını artıran mexanizmləri müəyyənləşdirmək üçün eksperimental tədqiqatlarda geniş istifadə olunur. Qlutaminaza qlutamatı sintez edən əsas fermentdir. Qlutamat iki mühüm fizioloji rol oynayır: postnatal dövrdə oyandırıcı neyrotransmitter kimi, prenatal dövrdə isə neyrogenezini, sinaptogenezini və sinir hüceyrələrinin sağ qalmasını tənzimlənməsində iştirak edir. İşimizdə, 4 eksperimental qrupda, boğazlığın 16-21-ci günlərində həyata keçirilən hipoksik prekondisionlaşmanın 17 günlük və 6 aylıq nəsillərin beynində qlutamat sintezinə təsirini tədqiq edilmişdir. Müəyyən edilmişdir ki, prenatal hipoksiya müxtəlif beyin strukturlarında erkən postnatal ontogenezdə fermentin aktivliyinin nəzərəçarpacaq dərəcədə artmasına səbəb olmuşdur, yetkin heyvanlarda isə onun azalması müşahidə edilmişdir. Kəskin hipoksiya isə əksinə yetkin heyvanların beynində qlutaminazanın aktivliyinin daha əhəmiyyətli artmasına səbəb olmuşdur. Prenatal hipoksik prekondisionlaşma olunmuş heyvanlar qrupunda kəskin hipoksiyaya məruz qalmış heyvanlarla müqayisədə 17 günlük siçovul nəslində fermentin aktivliyinin artmasına zəiflədici təsir göstərmiş və 6 aylıq nəslində azalmasına səbəb olmuşdur. Beləliklə, prenatal prekondisionlaşmanın neyroprotektiv adaptiv-kompensator effekti qlutamatın həm fizioloji, həm də eksitotoksik təsiri ilə əlaqələndirilə bilər.

Açar sözlər: hipoksik prekondisionlaşma, prenatal dövr, nəsil, beyin, fosfat-fəallaşdırıcı qlutaminaza

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