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EXPERIMENTAL ARTICLES

The Effects of Severe Hypoxia and Hypoxic Postconditioning on the Glutathione-Dependent Antioxidant System of the Rat Brain

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Abstract—In this study we investigated the effects of severe hypobaric hypoxia (SH) and severe hypobaric hypoxia accompanied by postconditioning using mild hypobaric hypoxia (PostC) on the glutathione-dependent antioxidant system in the rat hippocampus and neocortex. SH (3 h, 180 mmHg, 5% O_2) led to oxidative stress that was associated with a decrease in the total glutathione level, as well as in antioxidant capacity. PostC (2 h, 360 mmHg, 10% O_2) led to incomplete recovery of the total glutathione level and up-regulated glutathione peroxidase activity. In the neocortex, SH did not lead to the development of posthypoxic pathology. A small decrease in total glutathione, glutathione peroxidase activity, and antioxidant capacity on the 1st day after SH was corrected by the 2nd day. In contrast, glutathione reductase activity decreased by the 4th day after exposure to SH. PostC led to a consistent decrease in the total glutathione level but normalized glutathione reductase activity. We found that the studied brain structures develop a specific response to SH. In the hippocampus, SH led to oxidative stress, whereas the neocortex was not affected by exposure to SH. Partial differences between brain areas are based on better antioxidant defense of the neocortex in comparison with the hippocampus. PostC corrects posthypoxic pathology in the hippocampus with involvement of the glutathione-dependent antioxidant system. In the neocortex, PostC did not lead to a significant biochemical response.

Keywords: glutathione-dependent antioxidant system, antioxidant capacity, hippocampus, neocortex, severe hypoxia, postconditioning by mild hypobaric hypoxia

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INTRODUCTION

The most important condition for the normal functioning of the brain is the maintenance of the physiological oxygen concentration. Molecular oxygen is a strong oxidizer and is therefore necessary in numerous processes, in particular, in the reactions of energy metabolism and in the synthesis and disintegration of biologically active substances, for example, eicosanoids and catecholamines. On the other hand, the same characteristics of oxygen makes it potentially dangerous to brain cells, because during one-electron reduction it may serve as a source of reactive oxygen species (ROS). In addition to the well-known systems of ROS generation, the brain has alternative systems that are associated, for example, with disruption of the integrity of synaptic membranes and lead to increased release of excitotoxic compounds [1, 2].

Depending on the concentration, ROS perform regulatory functions or may participate in the development of pathology. Positive effects predominate at their low concentrations and include the regulation of various physiological processes [2].

Due to the peculiarities of its metabolism and lipid composition, the brain is very sensitive to excessive amounts of ROS. Owing to the high concentration of polyunsaturated fatty acids, the membranes of neuronal cells are the most susceptible to lipid peroxidation (LPO) [2]. LPO products can damage other macromolecules, in particular proteins, which form Schiff bases when interacting with oxo-groups.

In physiological conditions, ROS formation is balanced by their utilization by antioxidant systems. One of the key antioxidant systems of the brain is the glutathione-dependent system. Along with the system of thioredoxin, it is the most ancient and universal system [3]. The glutathione-dependent system consists of glutathione and glutathione-dependent enzymes including glutathione peroxidases and reductase. The group of glutathione peroxidases performs detoxification reactions of both ROS and the products of their interaction with macromolecules, for example, lipid

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hydroperoxides [4]. Glutathione reductase restores oxidized glutathione using reduced NADP. In addition to the reactions of the ROS utilization, the glutathione-dependent antioxidant system also takes part in maintenance of the cellular redox status and regulation of cellular signaling [4–6].

Increased ROS generation may lead to the development of pathological effects, which are combined under the term oxidative stress. Oxidative stress is the process of excessive ROS generation, which is accompanied by damage to macromolecules, a decrease in antioxidant activity, and a shift in the cellular redox status to the oxidized side, which often leads to cell death [7].

A large amount of data has been accumulated that confirm the contribution of oxidative stress to pathological processes, in particular, to the effects of severe hypoxia/ischemia and subsequent reoxygenation [8]. Previously, using the model of severe hypobaric hypoxia (SH) in rats (3 h, 180 mmHg, 5% O_2) we revealed a change in the LPO intensity in response to severe hypobaric hypoxia, which was accompanied mainly by apoptotic cell death in the hippocampus [8, 9]. In addition to oxidative stress, well-studied processes of the ischemic cascade contribute to the development of pathology. Eventually, rats subjected to severe hypoxia demonstrate impaired stress response, as well as a behavioral deficit [9].

The phenomenon of postconditioning (PostC), which may potentially be used to prevent development of post-hypoxic pathologies, is being actively studied. Post-conditioning in the broadest sense is the presentation of an extreme treatment at a moderate intensity to individuals who have experienced a serious damaging exposure to stimulate the endogenous protective and regenerative potential. In the brain, this method was first performed as ischemic PostC (iPostC) [10]. In addition to iPostC, currently there are a number of techniques, including distant iPostC [11, 12], acidic PostC, and PostC mediated by the introduction of narcotics [13]. A method for PostC using mild hypobaric hypoxia (MHH PostC) (2 h, 360 mm Hg, 10% O_2) was developed in the Pavlov Institute of Physiology. It differs from all the above techniques in its non-invasiveness. In addition, a specific feature of this technique is the possibility of its delayed use, within 1 day after the damaging treatment. The most pronounced neuroprotective effect is provided by three episodes of MHH PostC with a 1-day interval between sessions. Rats exposed to SH in combination with MHH PostC are characterized by normal LPO processes, absence of cell death, and corrected behavioral and hormonal deficits [9, 14]. However, in contrast to iPostC, there are few data on the mechanisms of neuroprotection by MHH PostC. In particular, there are insufficient data on the regulation of antioxidant defense mechanisms. It has been shown previously for iPostC that during the first 24 h after exposure, the activity of catalase and superoxide dismutase is increased, which subsequently returns to the control values in the studied brain structures (hippocampus, cortex, and striatum) of rats [15]. An increase in the content and activity of these enzymes has also been demonstrated for the iPostC of the spinal cord after pathological ischemia [16]. For the glutathione-dependent antioxidant system, iPostC corrected the level of total glutathione after a significant decrease which was caused by global ischemia in the hippocampus of rats at 1.5 h after reperfusion. However, these results do not answer the question of the long-term dynamics of this parameter [17].

Hence, we studied the effects of SH and SH in combination with MHH PostC on the state of the glutathione-dependent antioxidant system and general antioxidant activity in the hippocampus and sensorimotor cortex of the rat brain during 4 days after SH.

MATERIALS AND METHODS

Animals. This study was performed with Wistar adult male rats weighing 200–250 g from the Collection of laboratory mammals of different taxonomic affiliation of the Pavlov Institute of Physiology RAS, which is supported by the program of bio-resource collections of Federal Agency of Scientific Organizations of Russia. The animals were grown in standard conditions of the vivarium of the Pavlov Institute of Physiology with free access to water and food.

Reagents. All reagents were obtained from Sigma Aldrich (United States) unless otherwise specified.

Hypobaric hypoxia. Severe hypobaric hypoxia (SH) was created using a hypobaric chamber of the flow type; the pressure was 160–180 mmHg; the duration of the exposure was 3 h. The chambers were blown off with air every 20 min in order to maintain a normal gas ratio and avoid hypercapnia [18].

Post-conditioning was performed by three exposures to a moderate hypobaric hypoxia (MHH PostC) at 360 mmHg for 2 h with 24 h intervals between sessions. The first session was performed 24 h after SH, as it was previously shown that this is the most effective MHH PostC regimen [19].

Decapitation of animals was performed with a guillotine 1, 2, and 4 days after SH and 1 day after the first and third MHH PostC sessions, which corresponds to days 2 and 4 after SH. After this, the brain was taken out; the hippocampus and sensorimotor cortex were isolated.

The animals of the control group that were not exposed to hypoxia underwent corresponding procedures to staying in the hypobaric chamber.

Isolation of the cytosolic fraction. The hippocampus or neocortex were homogenized on ice in a glass–glass system (Potter's homogenizer) in 2 mL of solution that contained 0.3 M sucrose, 1 mM EDTA, and 0.2 M Tris-HCl (pH 7.4). Centrifugation was performed for 10 min at 1000 *g* and 4°C. The supernatant

was transferred to clean tubes and centrifuged again for 20 min at 2000 *g* and 4°C. The resulting supernatant contained the cytosolic fraction [20]; it was aliquoted and frozen at –80°C.

Measurement of the total protein content. The amount of total protein in the samples was measured photometrically immediately before each experiment using a Biophotometer plus (Eppendorf, Germany) photometer from the Resource Center of Observatory of Environmental Safety of the Scientific Park of St. Petersburg State University in accordance with the standard three-wave protocol (240, 280, and 340 nm). The sample (20 μL) was diluted to 1 mL with distilled water and the optical density of the solution was measured.

Measurement of the total glutathione content. To measure the amount of total glutathione, 80 μL of the sample was diluted with an equal amount of 2M $HClO_4$ and incubated for 5 min at room temperature. The samples were then centrifuged at 5000 *g* for 5 min; the supernatant was transferred to clean tubes and neutralized with 2 M KOH. The samples were incubated for 5 min at room temperature and subjected to repeated centrifugation at 5000 *g* for 5 min; 25 μL of the supernatant was diluted with a reaction mixture containing 0.19 mg/mL NADPH (AppliChem., Germany) and 25 ng/mL DTNB (5.5 M-dithiobis-2-nitrobenzoic acid) (0.1 M phosphate buffer, 1 mM EDTA, pH 7.4). Yeast glutathione reductase was added to a final activity of 6 U/mL and the absorbance was measured at 412 nm on a SPECTROstar Nano spectrophotometric plate reader (BMG Labtech, Germany) [21].

The concentration of the total glutathione was expressed in nmol/mg protein. For each experimental group, $n = 4$. The experiment was performed twice.

Determination of glutathione peroxidase activity. The technique is based on the measurements of a decrease in the optical density of the NADPH solution at 340 nm. A reaction mixture that contained (final concentrations) 4 mM GSH (reduced glutathione), 2 mM NaN_3 (sodium azide), glutathione reductase 20 U/mL, 0.4 mM NADPH, and 0.25 mM H_2O_2 was added to 50 μL of the sample. The optical density of the solution was measured at 37°C on the SPECTROstar Nano spectrophotometric plate reader [22].

Glutathione peroxidase activity was expressed as the amount of consumed NADPH per min per mg protein. For each experimental group, $n = 4$. The experiment was performed twice.

Determination of glutathione reductase activity. 80 μL of the sample was diluted with a reaction mixture that contained (final concentrations) 0.4 mM NADPH and 4 mM GSSG (oxidized glutathione) (0.2 M phosphate buffer, 2 mM EDTA, pH 7.0). The absorbance was measured at 340 nm and 37°C on a SPECTROstar Nano spectrophotometric plate reader [23].

Glutathione reductase activity was expressed as the amount of consumed NADPH per minute per the amount of total protein (pmol NADPH/(min*mg protein)). For each experimental group, $n = 4$. The experiment was performed twice.

Determination of the total antioxidant activity of cells. A 7.5 nM fluorescein solution (in 10 mM phosphate buffer, pH 7.4) was incubated with the sample for 30 min at 37°C. A solution of the ROS generator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added to a final concentration of 30 mM [24]. The fluorescence intensity was measured using a FLUOstar Omega spectrophotometric plate reader (BMG Labtech, Germany) (Ex 485 nm, Em 520 nm). Antioxidant activity was assessed as luminous intensity normalized to the amount of protein. For each experimental group, $n = 4$. Each experiment was repeated twice.

Statistical analysis of the results. Statistical analysis of the results was performed using scripts in the free statistical language R (https://cran.r-project.org/) in the Resource Center of Observatory of Environmental Safety of the St.Petersburg State University Scientific Park. The bootstrap method was used to analyze and plot the graphs. The results are expressed in the form of box-and-whisker plots, which show the mean as a box, the 68% confidence interval for the mean (approximately ± the standard error of the mean), and the whiskers, the 95% confidence interval (approximately ± two times the standard error of the mean). Evaluation of the statistical significance was performed using the nonparametric Kruskal–Wallis test. The results were considered as significant at $p < 0.05$. As a post-hoc procedure, the Dunn's test was used for multiple samples at a significance level of $\alpha = 0.05$.

RESULTS

In the control groups, the level of total glutathione in the neocortex was slightly below the level in the hippocampus; however, the dynamics of this parameter in response to SH differed strongly for these structures. The amount of total glutathione in the hippocampus decreased significantly 1 day after SH and remained unchanged throughout the experiment. Triple MHH PostC resulted in some increase in the level of total glutathione, although its level still did not reach the control values (Fig. 1a).

However, the content of total glutathione in the neocortex was slightly decreased 1 day after SH and then restored to control values. Rats that were exposed to SH in combination with MHH PostC, in contrast, showed a decrease in the level of total glutathione in the neocortex throughout the entire experiment compared to animals exposed to SH (Fig. 1b).

The concentration of total glutathione in a cell directly depends not only on its synthesis but also on metabolism. Hence, we studied the activity of

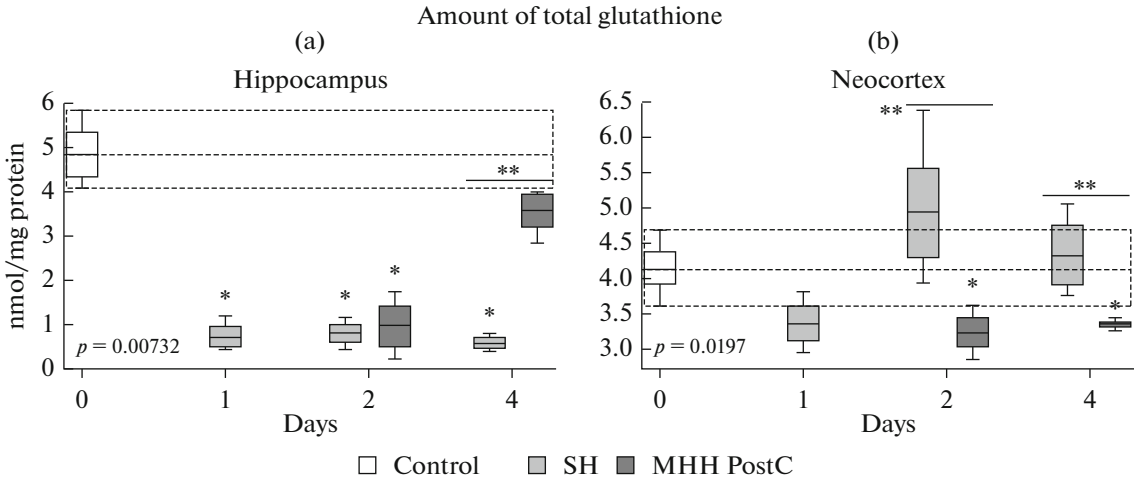

Fig. 1. The effects of severe hypoxia and severe hypoxia in combination with postconditioning with mild hypobaric hypoxia on the level of total glutathione in the cytoplasmic fraction of the hippocampus (a) and neocortex (b) 1, 2, and 4 days after the SH session and 1 day after the first and third MHH PostC session. The abscissa shows the time; the ordinate shows the glutathione concentration in nmol per mg of protein. White boxes, control ($n = 4$); light-gray boxes, a group exposed to SH ($n = 4$); dark gray boxes, a group exposed to SH in combination with MHH PostC ($n = 4$). The results are expressed in the form of box-and-whisker plot, which shows the mean; the box is the 68% confidence interval for the mean (approximately ± standard error of the mean); the whiskers show the 95% confidence interval (approximately ± two times the standard error of the mean). The P value was estimated using the nonparametric Kruskal–Wallis test; the results were considered significant at $p < 0.05$. As a post-hoc procedure, the Dunn test was used; *, significant differences compared to control values; **, significant differences between groups at respective time points at a significance level of $a = 0.05$.

enzymes of glutathione metabolism, that is, glutathione reductase and peroxidase.

The activity of glutathione peroxidase in the hippocampus of rats subjected to SH did not change throughout the experiment. However, three but not a single MHH PostC sessions resulted in a significant increase in the activity of this enzyme compared to the control and the group exposed to SH (Fig. 2a).

In the neocortex of rats subjected to SH, an increase in glutathione peroxidase activity occurred 1 day after SH and was followed by a decrease to control values for at least 4 days after exposure. The activity of the glutathione peroxidase enzymes was inversely correlated with the total glutathione during SH (Figs. 1b and 2b). MHH PostC did not lead to significant changes in glutathione peroxidase activity in the neocortex throughout the experiment (Fig. 2b).

The activity of glutathione reductase did not change under experimental conditions in the rat hippocampus (Fig. 3a). In contrast, the neocortical activity of glutathione reductase showed a tendency to decrease to the 4th day after exposure to SH. MHH PostC maintained this indicator at control values throughout the experiment (Fig. 3b).

The glutathione-dependent antioxidant system is essential for antioxidant defense of brain cells, which, however, includes other low-molecular compounds, proteins, and enzymes. We used the ORAC assay to assess the overall antioxidant activity of the cytosol, which reflects the antiradical capacity, mainly against peroxyl and hydroxyl radicals [25].

The total antioxidant activity of the cytosolic fraction of the hippocampus gradually decreased to the 4th day after SH (70% of the control). A single MHH PostC resulted in an increase in total antioxidant activity to 150% of the control, while subsequent MHH PostC sessions resulted in a significant decrease in this index to the level corresponding to SH (Fig. 4a).

In the neocortex, the total antioxidant activity significantly increased 24 h after SH but then returned to the control values. MHH PostC did not affect this index throughout the experiment (Fig. 4b).

DISCUSSION

Our results indicate that the total glutathione content was decreased for at least 4 days after exposure to SH (Fig. 1a), which may indicate a deficiency of the glutathione-dependent antioxidant system in the hippocampus of rats subjected to SH. It is possible that total glutathione is oxidized in the glutathione peroxidase reaction and/or released from the cell to maintain the normal GSH/GSSG ratio [26]. The study of the activity of one of the enzymes of the GSH metabolism glutathione peroxidase showed that its activity in the hippocampus does not undergo changes after SH and slightly increases in response to three but not a single round of MHH PostC (Fig. 2a). Previously, we showed a decrease in the content of one of the late LP products, Schiff bases, in the rat hippocampus in response to MHH PostC [14]. Given that GP uses GSH to eliminate the earlier LP product, lipid

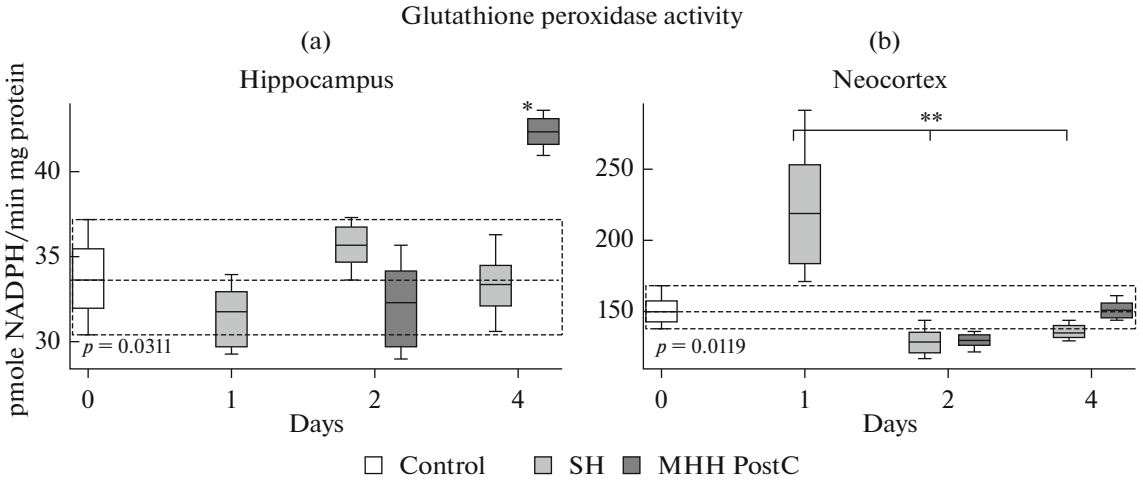

Fig. 2. The effects of SH and SH in combination with MHH PostC on glutathione peroxidase activity in the hippocampus (a) and neocortex (b) 1, 2, and 4 days after the SH session and 1 day after the first and third MHH PostC sessions. The abscissa is time; the ordinate shows the change in the concentration of NADPH in pmol per mg of protein per minute. White boxes, control ($n = 4$); light-gray boxes, a group exposed to SH ($n = 4$); dark gray boxes, a group exposed to SH in combination with MHH PostC ($n = 4$). The results are shown in the form of box-and-whisker plots, which represent the mean; the box gives the 68% confidence interval for the mean (approximately ± the standard error of the mean); the whiskers give the 95% confidence interval (approximately ± two times the standard error of the mean). The *P* value was estimated using a nonparametric Kruskal–Wallis test; the results were considered as significant at $p < 0.05$. As a post-hoc procedure, the Dunn test was used; *, significant differences compared with control values; **, significant differences between groups at respective time points at a significance level of $a = 0.05$.

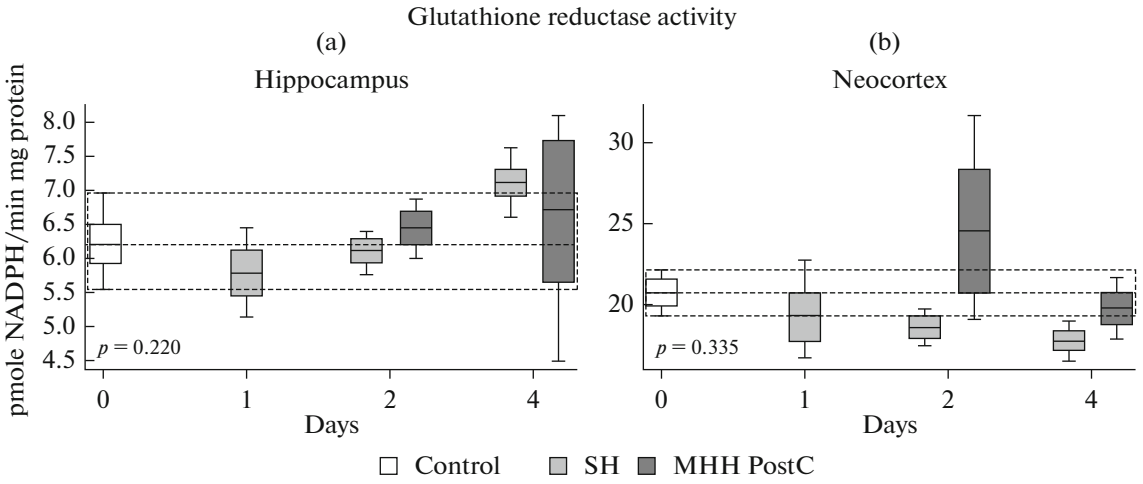

Fig. 3. The effects of SH and SH in combination with MHH PostC on glutathione reductase activity in the hippocampus (a) and neocortex (b) 1, 2, and 4 days after the SH session and 1 day after the first and third MHH PostC sessions. The abscissa is time; the ordinate shows the change in the concentration of NADPH in pmol per mg of protein per minute. White boxes, control ($n = 4$); light-gray boxes, a group exposed to SH ($n = 4$); dark gray boxes, a group exposed to SH in combination with MHH PostC ($n = 4$). The results are shown in the form of box-and-whisker plots, which represent the mean; box is the 68% confidence interval for the mean (approximately ± standard error of the mean); the whiskers give the 95% confidence interval (approximately ± two times the standard error of the mean). The *P* value was estimated using the nonparametric Kruskal–Wallis test; the results were considered as significant at $p < 0.05$.

hydroperoxides, it may be assumed that the increased activity of the enzymes of this group contributes to the normalization of LP, and at the same time may explain the insufficient recovery of the total glutathione level in response to MHH PostC. Due to low catalase activity of the brain, the glutathione peroxidase family plays an important role in protecting cells in both the adult and the developing brain [27, 28]. A homozygous knockout of the gene that encodes the membrane-bound isoform of the enzyme (GPx4) leads to early embryonic death of animals, which reflects its exceptional importance in mechanisms for

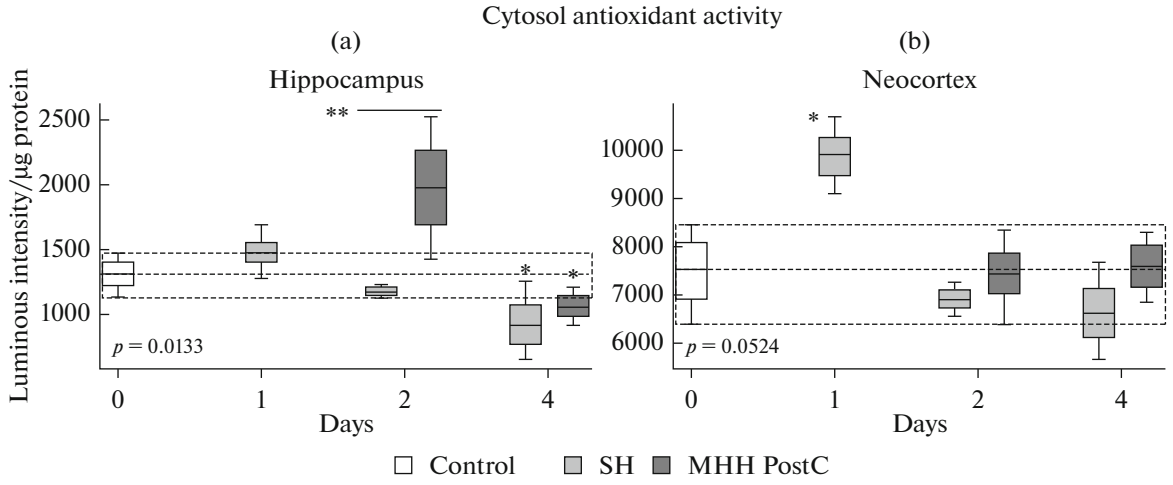

Fig. 4. The effects of SH and SH in combination with MHH PostC on cytosolic antioxidant activity in the hippocampus (a) and neocortex (b) 1, 2, and 4 days after the SH session and 1 day after the first and third MHH PostC sessions. The abscissa is time; the ordinate is the luminous intensity normalized per μg of protein. White boxes, control ($n = 4$); light-gray boxes, a group exposed to SH ($n = 4$); dark gray boxes, a group exposed to SH in combination with MHH PostC ($n = 4$). The results are shown in the form of box-and-whisker plots, which represent the mean; the box gives the 68% confidence interval for the mean (approximately ± standard error of the mean); the whiskers give the 95% confidence interval (approximately ± two times the standard error of the mean). The *P* value was estimated using the nonparametric Kruskal–Wallis test; the results were considered as significant at $p < 0.05$. As a post-hoc procedure, the Dunn test was used; *, significant differences compared with control values; **, significant differences between groups at respective time points at a significance level of $a = 0.05$.

protection against oxidative stress not only in adulthood but also during embryonic development [29].

Another enzyme that is responsible for maintenance of the reduced form of glutathione in cells is glutathione reductase. The activity of this enzyme did not change under experimental conditions (Fig. 3a). Note that the course of the glutathione reductase reaction depends on the amount of NADPH, which is mainly synthetized in reactions of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase and the 6-phospho-gluconate dehydrogenase reaction) [26, 30].

On the other hand, it may also be assumed that under the SH conditions, processes of glutathione biosynthesis are disturbed. The biosynthesis of GSH de novo occurs in the cytoplasm during two ATP-dependent reactions. A decrease in the amount of glutathione may be due to the lack of substrates for its synthesis at the early stages after SH and to a decrease in the activity of the enzymes of this process due to the lack of ATP at late stages. The lack of substrates may be caused by hypoxia-mediated depolarization of neuronal cell membranes and an increased level of extracellular glutamate, because the key substrate for the synthesis of glutathione in astrocytes, cystine, is transported through the cell membrane of astrocytes via the antiport with glutamate [26]. MHH PostC corrects the level of total glutathione but does not return it to the control level.

The total antioxidant capacity of the cytosolic fraction of the hippocampus, which is measured as antiradical activity against peroxyl and hydroxyl radicals, increases slightly on the 1st day after SH and then gradually decreases to the 4th day after this experimental exposure. This may indicate a gradual depletion of the reserves of antioxidant protection in the hippocampus under the experimental conditions. In response to a single MHH PostC, this index rises and then decreases to the level observed after SH. It is possible that in conditions of a reduced level of glutathione (Fig. 1a), antioxidant defense enzymes perform a basic protective function. This is indirectly confirmed by our data on an increase in the level of mRNA of some enzymatic antioxidants, such as catalase and Mn-SOD, in response to mild hypoxia. However, translation is triggered only during the pro-oxidative state, which occurs after a single but not three rounds of MHH PostC [31].

The data we obtained indicate that oxidative stress does not develop in the neocortex of rats that were subjected to SH. Moreover, at 1 day after reoxygenation there is an increase in glutathione peroxidase activity and the overall antioxidant activity of the cortex, which is associated with a normal level of total glutathione (Figs. 2b and 4b). This may indicate the activation of enzymes of glutathione biosynthesis, which ensure its sufficient level in the post-hypoxic period. By the 4th day after SH, glutathione reductase activity gradually decreases (Fig. 3b), which also does not seem sufficient for the development of oxidative stress in the late post-hypoxic period.

Due to the absence of pathology in the neocortex, MHH PostC has no corrective function. In contrast, MHH PostC leads to a decrease in the level of total

glutathione (Fig. 1b) and glutathione peroxidase activity 1 day after the first session of MHH PostC compared with the group subjected to SH (Fig. 2b). However, the total antioxidant activity does not decrease (Fig. 4b), which can partly be explained by the reduction of glutathione reductase activity to control values (Fig. 3b), which probably contributes to replenishment of the pool of reduced glutathione.

It may be assumed that the resistance of the rat neocortex to damaging hypoxia and reoxygenation is determined by the increased basal activity of antioxidant systems in comparison with the hippocampus. The glutathione peroxidase activity of the neocortex is 4.5 times higher than the activity in the hippocampus (Fig. 2), glutathione reductase is 3.5 times higher (Fig. 3), and total antioxidant activity is 5.5 times higher (Fig. 4). It is known that astrocytes play an essential role in maintenance of the functioning of the glutathione-dependent antioxidant system in neurons due to a relatively higher level of glutathione synthesis. According to the available literature data, the ratio of the number of neurons and glial cells does not differ significantly in the hippocampus and neocortex of adult rats [32], which is confirmed by a similar basal level of glutathione in these structures (Fig. 1). It follows from these data that the differences in the antioxidant defense of the hippocampus and the neocortex are not due to neuron–glial interactions in the glutathione system. In addition to the glutathione-dependent antioxidant system, the catalase, superoxide dismutase, and thioredoxin systems, as well as molecular antioxidants, also function in the brain. The basal activities of superoxide dismutase and catalase do not differ between the neocortex and the hippocampus. However, it was shown in studies using the global ischemia model that the preconditioning leads to a comparable increase in the activities of these enzymes [33]. Taken together with the data presented in this study, it may be assumed that there are differences in the mechanisms that provide functioning of the ROS utilization systems between the hippocampus and the neocortex. These differences are present at the level of regulation of the enzymes of oxidation and reduction of glutathione but are not related to its synthesis.

CONCLUSIONS

We revealed differences in the response of the hippocampus and neocortex of rats to hypoxic stress. In the hippocampus, in contrast to the neocortex, severe hypoxia causes the development of oxidative stress associated with a significant decrease in the amount of total glutathione. Postconditioning with moderate hypobaric hypoxia had a correcting effect on the glutathione-dependent antioxidant system of the hippocampus of rats that were subjected to severe hypoxia.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interest. The authors declared no conflicts of interest.

Ethical approval. The experiments were performed following the requirements formulated in the Directives of the Council of the European Community (86/609 / EEC) on the use of animals for experimental research. The protocols of experiments were approved by the Commission for the Humane Treatment of Animals of the Pavlov Institute of Physiology.

Informed consent. This article does not contain any studies with human participants performed by any of the authors.

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