GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effects of 2,4-Dimethoxyphenyl Biguanide on Glutathione System Activity in Rat Tissues in Brain Ischemia–Reperfusion

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We studied the effects of dimethoxyphenyl biguanide on glutathione peroxidase and glutathione reductase activities, level of reduced glutathione in rat tissues, and activity of some NADPH-regenerating enzymes (glucose-6-phosphate dehydrogenase and NADP-isocitrate dehydrogenase) under conditions of ischemia–reperfusion of the brain. Administration of this biguanide derivative under pathological conditions led to a decrease in the analyzed parameters (elevated under conditions of ischemia–reperfusion) in the serum and brain tissue. The results attest to less pronounced mobilization of the glutathione system (compared to pathological state) due to antioxidant and protective properties of 2,4-dimethoxyphenyl biguanide under conditions of ischemic tissue damage associated with oxidative stress.

Key Words: *2,4-dimethoxyphenyl biguanide; brain ischemia-reperfusion; glutathione antioxidant system; NADPH providers*

Ischemic brain damage is the leading cause of mortality and reduced quality of life in the population. The greatest contribution to the development of ischemic pathologies is made by the cascade of metabolic changes with disturbances in energy metabolism and intensification of free radical oxidation of biological substrates served as the main elements [1,3]. Under these conditions, the important adaptive role is played by antioxidant systems of the body, particularly by glutathione system responsible for deactivation of H_2O_2 and organic peroxides acting as prooxidants. Effective functioning of this system requires NADPH supply, which may involve the key enzyme of pentose phosphate pathway glucose-6-phosphate dehydrogenase (G-6-PDH; EC 1.1.1.49) and NADP-isocitrate dehydrogenase (NADP-IDH; EC 1.1.1.42) [6].

In light of high prevalence and severity of free-radical pathology, the possibility of using antioxidant compounds for the development of new drugs acting under conditions of activation of free radical oxidation is an important problem. In this respect, guanidine derivatives are of great interest, because they possess wide spectrum of biological activities. Some of them inhibit cellular oxidative reactions, particularly oxidative glycosylation of proteins, thus exhibiting antioxidant activity [8,10]. There are also data that guanidine derivatives, for example mercaptoethyl guanidine, metformin, *etc*., can act as free radical scavengers and inhibit processes of their formation, *e.g.* reactions catalyzed by NADPH oxidase and NO synthase [1,4].

The objective of this study was to investigate the effects of 2,4-dimethoxyphenyl biguanide on activity of

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glutathione peroxidase (GP, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2), G-6-PDH, NADP-IDH and the level of reduced glutathione in rat tissues under conditions of cerebral ischemia-reperfusion. Antiischemic activity of this compound was predicted by computer prediction of biological activity using PASS software.

MATERIALS AND METHODS

Experiments were performed on albino male rats weighing 150-200 g. The animals were divided into 3 groups: group 1 (n=8), controls (sham operated animals); group 2 (n=8), ischemia–reperfusion; and group 3 (n=9), ischemia–reperfusion+2,4-dimethoxyphenyl biguanide. Brain ischemia in the experimental animals was induced by 30-min occlusion of the common carotid arteries [3]. Reperfusion was achieved by removal of the occlusions. Blood flow resumption was controlled visually. After 3 days, the rats were narcotized and sacrificed. The blood was taken from the heart and the brain was removed routinely. Sham operated animals were used as the control.

Guanidine derivative, 2,4-dimethoxyphenyl biguanide, was synthetized at the Department of Organic Chemistry, Chemical Faculty, Voronezh State University. Control over the reaction path, qualitative and quantitative analyses of reaction mixtures, identification and structural analysis of obtained compounds was carried out by methods of thin-layer chromatography, mass-spectrometry, chromatography-mass-spectrometry, IR-, ^{1}H NMR-spectrometry, and element analysis. This compound was dissolved in 0.9% NaCl and administered intraperitoneally in a dose of 25 mg/kg animal body weight 2 times per day for 3 days.

To obtain homogenate, weighed sample of the whole rat brain was homogenized in 3 volumes of cold extraction medium (50 mM Tris-HCl, pH 7.8 with 1 mM EDTA, 1% β-mercaptoethanol) and centrifuged at 5000g for 10 min. Concentration of reduced glutathione was determined by spectrophotometry at λ=412 nm using Elman reagent [2]. Enzyme activity was determined on an SF-56 spectrophotometer at λ=340 nm. GP activity was measured using coupled enzyme reaction in spectrophotometric medium of following composition: 50 mM potassium-phosphate buffer (pH 7.4) with 1 mM EDTA, 0.12 mM NADPH, 0.85 mM reduced glutathione, 0.37 mM H_2O_2, and 1 U/ml GR. Control probe contained no reduced glutathione. GR activity was measured in spectrophotometric medium containing 50 mM potassium-phosphate buffer (pH 7.4) with 1 mM EDTA, 0.16 mM NADPH, and 0.8 mM oxidized glutathione. G-6-PDH activity was measured using 50 mM Tris-HCl buffer (pH 7.8) with 3.0 mM glucose-6-phosphate, 0.25 mM NADP, 1.0 mM $MnCl_2$. The medium for NADP-IDH activity measurement consisted of 50 mM Tris-HCl buffer (pH 7.6-7.8) with 1.5 mM isocitrate, 2 mM $MnCl_2$, 0.25 mM NADP, and 0.1 mM EDTA. The reaction was initiated by adding the enzyme. The enzyme quantity catalyzing the formation of 1 μmol reaction product or conversion of 1 μmol substrate over 1 min at 25°C was taken for 1 unit of enzymatic activity (U). Total protein concentration was established using Lowry method [9]. Experiments were carried out at least in 2 analytical replications. Obtained data was treated using statistical tests [5]. Statistically significant differences at p<0.05 are discussed.

RESULTS

The content of reduced glutathione in rat brain increased 2-fold during ischemia–reperfusion (Fig. 1), while GP and GR activities in times terms of U/g of wet brain weight increased by 3.3 and 4.2, respectively, in comparison with the control (Fig. 2). Under these conditions, serum concentration of reduced glutathione increased by 2.2 times (Fig. 3) and GP and GR activities (in U/ml) increased by 1.9 and 3.4 times, respectively (Fig. 3). Moreover, specific activity of these enzymes was also significantly increased (Figs. 2 and 3) [6]. This was interrelated with intensification of glutathione system functioning in response to oxidative stress, induced by disturbances in brain blood flow.

Administration of 2,4-dimethoxyphenyl biguanide to animals with ischemia-reperfusion decreased the content of reduced glutathione in the brain tissue and serum by 1.3 and 1.8 times compared to the corresponding parameter under pathological conditions (Fig. 1). At the same time, we observed a 1.1- and 1.4-fold decrease in specific GP activity and 1.3- and 1.5-fold decrease in GR activity in the brain tissue

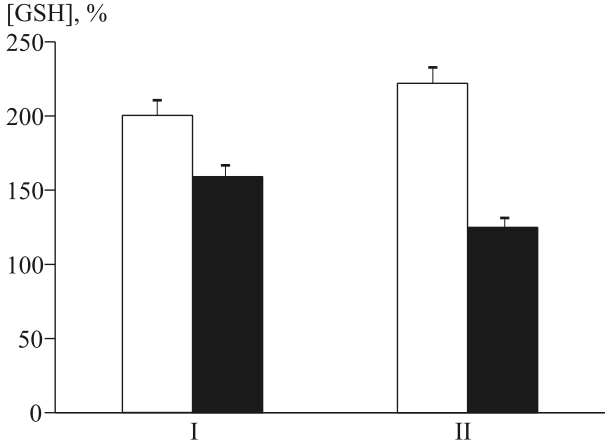

Fig. 1. Reduced glutathione level in rat brain (I) and serum (II). Here and in Figs. 2 and 3: light bars: animals with brain ischemia-reperfusion, dark bars: animals with brain ischemia-reperfusion receiving 2,4-dimethoxyphenyl biguanide. 100%: glutathione level in the control (0.225 mM in the brain and 0.272 mM in the serum).

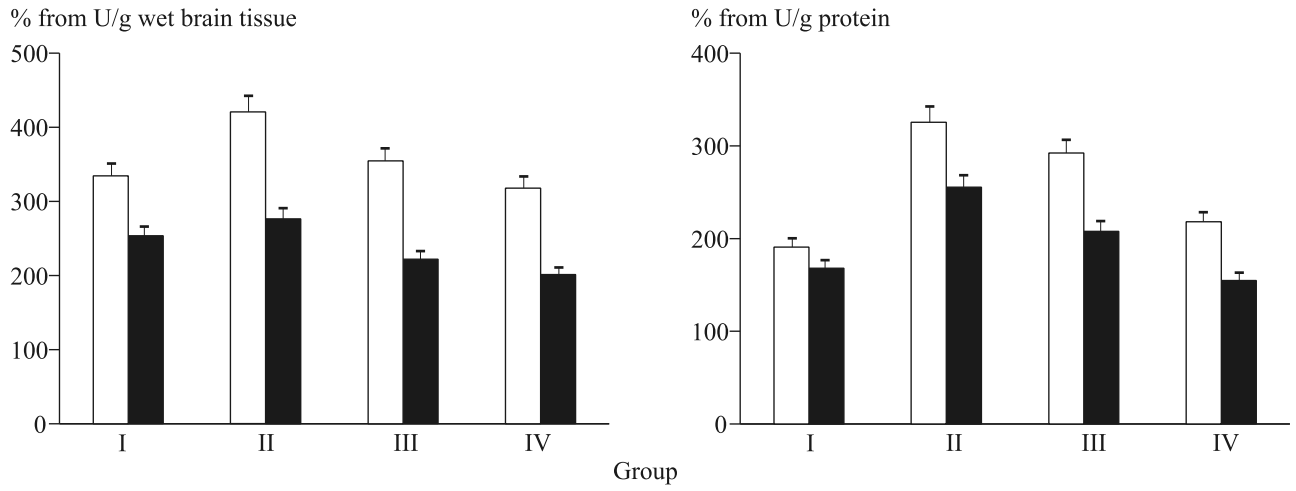

Fig. 2. Activities of GP (I), GR (II), G-6-PDH (III), and NADP-IDH (IV) in rat brain. 100%: enzyme activity in the control (for GP: 0.073 U/g wet tissue and 0.011 U/mg protein; for GR: 0.034 U/g wet tissue and 0.0047 U/mg protein; for G-6-PDH: 0.11 U/g wet tissue and 0.013 U/mg protein; for NADP-IDH: 0.085 U/g wet tissue and 0.011 U/mg protein).

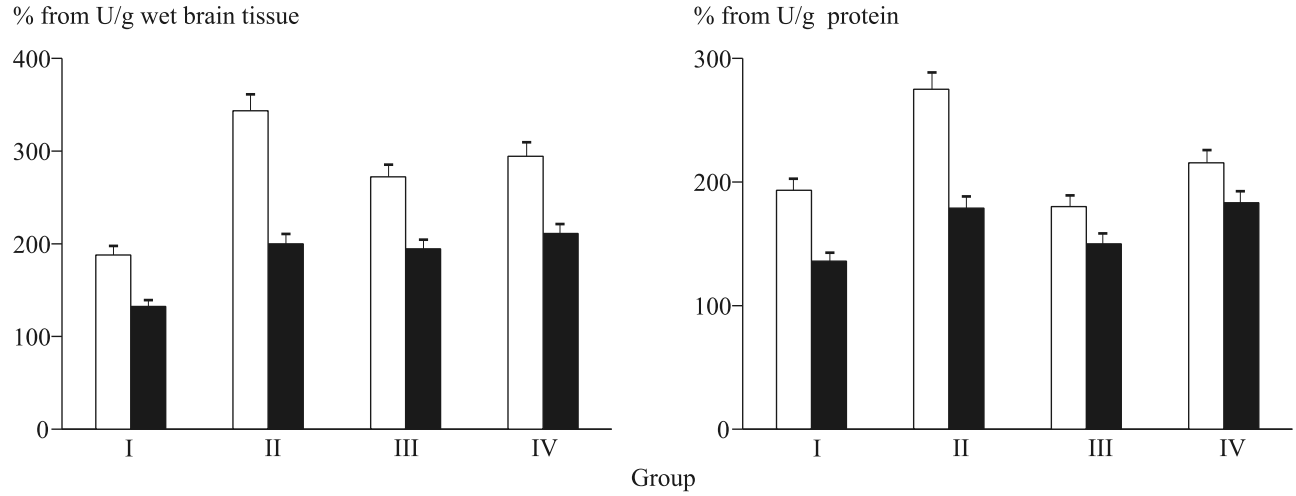

Fig. 3. Activities of GP (I), GR (II), G-6-PDH (III) and NADP-IDH (IV) in rat serum. 100%: enzyme activity in the control (for GP: 0.059 U/ml and 0.0015 U/mg protein; for GR: 0.023 U/ml and 0.0008 U/mg protein; for G-6-PDH: 0.018 U/ml and 0.001 U/mg protein; for NADP-IDH: 0.036 U/ml and 0.0013 U/mg protein).

and serum, respectively, in comparison with the corresponding values obtained during ischemia–reperfusion (Figs. 2 and 3). In addition, GP and GR activities in terms of U/g wet brain decreased by 1.3 and 1.5 times (Fig. 2) and in terms of U/ml serum by 1.4 and 1.7 times (Fig. 3), respectively.

Thus, treatment with this guanidine derivative reduced activity of GP, which is addressed toward deactivation of organic and inorganic peroxides, in comparison with the values observed under pathological conditions. Alongside with reduction in reduced glutathione requirements, activity of GR involved in redox-cycle of this compound was also reduced. It can be hypothesized that 2,4-dimethoxyphenyl biguanide reduced the strain of the glutathione system during oxidative stress induced by ischemia–reperfusion via inhibition of free-radical reaction [7].

Since oxidative stress is associated with intensified functioning of the glutathione system, which requires permanent NADPH delivery, previously revealed increased G-6-PDH and NADP-IDH activities in tissues of rats with postischemic reperfusion of the brain can be explained by the necessity for delivery of reduced equivalents for functioning of this element of antiradical protection under conditions of oxidative stress. So, activity of these enzymes in terms of U/g wet brain increased by 3.5 and 3.2 times (Fig. 2) and in terms of U/ml serum by 2.7 and 2.9 times, respectively, in comparison with the control values (Fig. 3). In addition, a significant increase in specific activity of these enzymes was noted (Figs. 2 and 3) [6].

Administration of 2,4-dimethoxyphenyl biguanide to animals with ischemia–reperfusion of the brain was associated with reduction of G-6-PDH and NADP-

IDH activity in the brain in terms of U/g wet tissue by 1.6 times and specific activity by 1.4-fold in comparison with the corresponding values under pathological conditions (Fig. 2). Moreover, serum activity of this enzyme in terms of U/ml was reduced by 1.4 times and specific activity by 1.2 times (Fig. 3).

Analysis of the effects of the studied biguanide derivative suggests that administration of this compound results in inhibition of free-radical oxidation, associated with less pronounced mobilization of the GR/GP system; in addition, activity of NADPH-regenerating enzymes also decreased in comparison with the values observed under pathological conditions. Thus, the results of our experiments explain antioxidant and protective effects of 2,4-dimethoxyphenyl biguanide, which is of substantial interest in the context of pharmacological correction of metabolic changes under pathological brain conditions associated with oxidative stress.

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