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INFLUENCE OF GLYCYL-PROLINE ON THE CHANGES OF NEUROACTIVE AMINO ACID METABOLISM AND OXIDATIVE STRESS PARAMETERS IN THE RAT BRAIN IN EXPERIMENTAL PARKINSON'S MODEL

Abstract. *We studied influence of the dipeptide glycyl-proline on glutamate and GABA metabolism, Krebs cycle enzyme activities, and parameters of oxidative stress activity in the rat brain in experimental model of parkinsonism, as well as morphological structure of the pyramidal neurons in the rat forebrain. Experimental parkinsonism was modelled by injections of rotenone (2 mg/kg, i.p.) on the days 1, 2, 5 and 6 of the experiment. Biochemical and histological examinations were carried out after 7 days since the beginning of the experiment. We showed that after rotenone injections most of the animals had some deviations in the pyramidal neurons in the fifth layer of the cortex. They were characterized by neuron cytoplasm hyperchromatosis, appearance of shadow cells and mass death of the pyramidal neurons in the cortex ganglionic layer. When the rotenone was injected simultaneously with the gly-pro, morphological deviations were observed only in some pyramidal neurons in the cortex ganglionic layer. They had hyperchromatosis and corrugation in pyramidal neurons and total absence of dendrites. Administration of rotenone is accompanied by significant disturbances of energetic metabolism processes and metabolism of the glutamate and GABA, with lesser effect on lipid peroxidation. Intranasal administration of the dipeptide gly-pro leads to a significant reduction in energetic metabolism disorders and morphological changes in the rat forebrain. We suppose that the dipeptide gly-pro may be used as an effective neuroprotective substance for easing the neurodegenerative processes in the brain.*

Key words: *forebrain, rotenone, GABA, glutamate, glutathione.*

Introduction. For neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, circulatory disorders in the brain), a characteristic number is pathochemical processes are inextricably linked: the deposition of amyloid plaques, the formation of neurofibrillary tangles, cholinergic neurotransmitters deficits in forebrain structures, excitotoxic expression products, loss of neuronal and synaptic structures in the hippocampus and the cortex, and the development of oxidative stress and apoptosis [30]. The brain's ability to withstand shifts in redox potential declines compared to other organs and tissues, due to the fact that the activity of antioxidant enzymes in the brain is decreased, while the content of unsaturated fatty acids increases. This is

accompanied by a lighter form of reactive oxygen species and lipid peroxidation products [10, 20]. At present, more than 100 known pathological processes are accompanied by the activation of free radical reactions [6, 13, 18, 26, 34]. This is particularly dangerous in the brain, where the violation of the membrane integrity of the neuron activation processes by peroxide leads to increased release of excitotoxic neurotransmitter glutamate. Neuronal cells affected by neurodegenerative diseases, for example, in Parkinson's disease, amyotrophic lateral sclerosis etc., present alterations in the oxidative stress parameters and the main processes of energetic formation [23, 24]. Brain tissue requires a continuous supply of energy substrates in the form of the limited sources of energy formation in

the brain, a high metabolic rate and a constant supply of oxygen [5, 9, 23]. One of the most popular experimental models of Parkinson's disease is a model using rotenone [1, 2, 7]. Rotenone is a high-affinity specific inhibitor of respiratory chain complex I of the mitochondria (NADH dehydrogenase). Due to the poor absorption by the stomach and the intestines, toxic effects of rotenone are relatively low when administered orally. However, 15 minutes after its intravenous administration it reaches the highest concentration in the CNS. Toxic effect of the drug leads to the appearance of characteristic pathological symptoms: rats appear to have dose-dependent catalepsy, decreased locomotor activity, inability to maintain posture, and a significant destruction of dopaminergic neurons in the striatum and substantia nigra [1, 2].

Over the last years, research opened a lot of new components of the chemical regulation of the pathophysiological processes, with the, so called, regulatory peptides being the most popular ones at the moment. Their key role is homeostasis maintenance, as they primarily define the basic parameters of the formation of compensatory-adaptive reactions to stress impact and disturbance of the homeostatic balance [8, 28, 32]. Neuropeptides have been successfully used for treating encephalopathy, and ischemic brain states, as well as for increasing the resistance to stress and minimizing the effects of poisoning. and improve efficiency of specific therapy. The distinctive feature of exogenous neuropeptide is that they exhibit neuroprotective activity when introduced into the body in femtomolar concentrations [3].

In this study, we investigated the influence of the dipeptide "glycyl-proline" on changes of glutamate and GABA metabolism, Krebs cycle enzyme activities, and parameters of oxidative stress activity in the rat brain in experimental model of parkinsonism, as well as morphological structure of the pyramidal neurons in the rat forebrain.

Objective: To establish the influence of glycyl-proline on the changes of neuroactive amino acid metabolism and oxidative stress parameters in the rat brain in experimental parkinson's model

Materials and methods. The experiments were carried out on male rats Wistar CRL: (WI)WUBR

with the body mass 180-220 g. Experimental parkinsonism was modelled by injections of rotenone (2 mg/kg, i.p.) on the 1, 2, 5 and 6 day of the experiment. Biochemical and histological examinations were carried out 7 days since the beginning of the experiment.

Biochemical parameters were measured in the rat forebrain. We studied such parameters of the oxidative stress activity as an initial level of TBA-reactive substances (TBARS) and their formation in the presence of Fe-ascorbate according to the method [25]. We estimated activity of the main antioxidant system in the brain — glutathione system, too. Determination of GSH was made with 5, 51-dithiobis-(2-nitrobenzoic acid by method [14], activity of glutathione peroxidase (GPx) was measured by [27], activity of glutathione reductase (GR) by method [22].

Activity of glutamate decarboxylase (GAD) was measured fluorometrically [17], GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSA-DH) were studied fluorometrically, too [12]. Activities of succinate dehydrogenase (SDH) and 2-oxoglutarate dehydrogenase (2-OGDH) were measured by spectrophotometric methods [16, 21]. Activity of glutamate dehydrogenase (GDH) was studied spectrophotometrically, too with using of 2-oxoglutarate as a substrate [11]. Белок измеряли по методу Hartree [19].

Forebrain for morphological studies was dissected with a transection at the level of the two colliculi and meninges were carefully removed. The sections were coloured by Nissl's technique [15, 29, 35], and pyramidal neurons of ganglionic layer of the rat forebrain sensomotoric part were studied [15, 34].

We studied influence of dipeptide glycyl proline (gly-pro) which was synthesized in the Laboratory of Applied Biochemistry of the Institute of Bioorganic Chemistry of the National Academy of Science of Belarus (head — Dr. Golubovich V.P.) [4]. The gly-pro was giving intranasally during 6 days of the experiment. Comparative analysis of various administration of the gly-pro (intramuscularly, intraperitoneally, intravenously, intranasally, subconjunctivally) into the model organism showed that the intranasal administration of the dipeptide provides the most effective neuropeptide actions [31].

Results and discussion. Morphological data showed that intact animals had normal structure of pyramidal neurons in the forebrain (Fig. 1) neurons had pyramidal form, nucleus is in the center, cytoplasm and nucleus had pronounced border.

After rotenone injections the most animals had some deviations in the pyramidal neurons of the fifth layer of the cortex (Fig. 2). They were characterized by neuron cytoplasm hyperchromatosis, appearance of shadow cells and mass death of the pyramidal neurons in the cortex ganglionic layer. Almost all the neurons exhibited hyperchromatosis and twisted apical dendrites.

When the rotenone was injected simultaneously with the gly-pro, morphological deviations were observed only in some pyramidal neurons in the cortex ganglionic layer (Fig. 3).

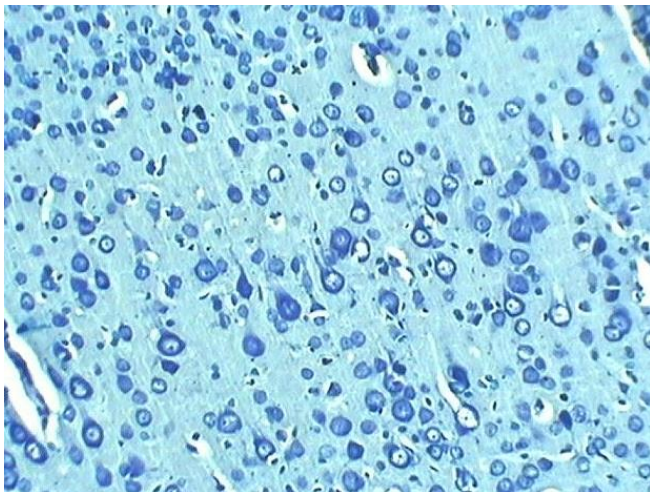


Fig. 1 – Structure of the pyramidal neurons in the ganglionic layer of sensomotoric cortex of intact rats. Colouring according to Nissl's technique. Multiplication 100

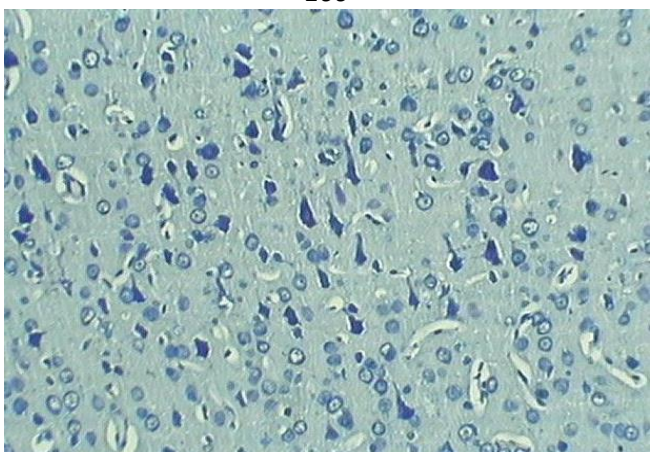


Fig. 2 – Structure of the pyramidal neurons in the ganglionic layer of sensomotoric cortex of intact rats after injections of rotenone (2 mg/kg, i.p.). Colouring according to Nissl's technique. Multiplication 100

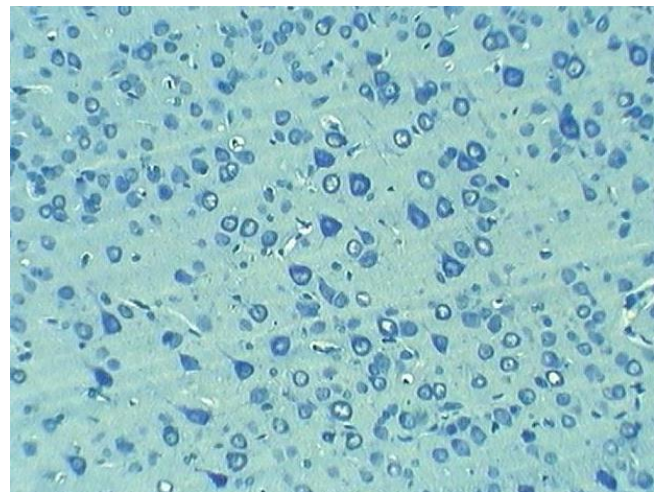


Fig. 3 – Structure of the pyramidal neurons in the ganglionic layer of sensomotoric cortex of intact rats after injections of rotenone (2 mg/kg, i.p.) and gly-pro (0,2 mg/kg, intranasally). Colouring according to Nissl's technique. Multiplication 100

They had hyperchromatosis and corrugation in pyramidal neurons and total absence of dendrites. Studying of biochemical parameters showed that the rotenone caused decrease of the TBARS formation in the presence of Fe-ascorbate to 83% compared to the control value in the forebrain ($p < 0.05$). Gly-pro returned this parameter to the control value.

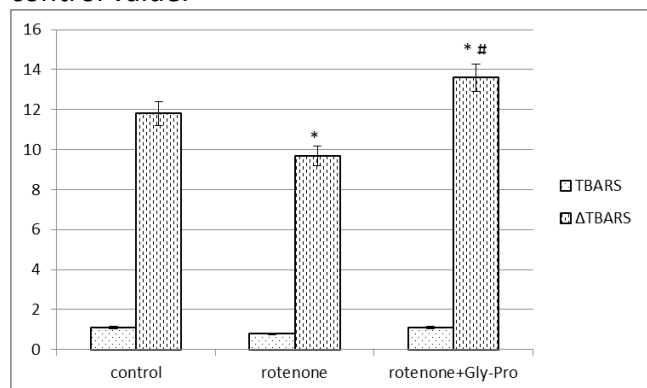


Fig. 4 – Influence of gly-pro (0,2 mg/kg, intranasally) and rotenone (2 mg/kg, i.p.) on the TBARS level and its Fe-ascorbate-induced formation in the rat forebrain. * – $p < 0,05$ concerning intact control; # – $p < 0,05$ concerning rotenone

After rotenone administration we did not detect any changes of the level of reduced form of glutathione and activity of the GPx in the forebrain (tab. 1). Activity of the GR was only increased after the rotenone administration and its activity showed a further increase in the presence of the dipeptide.

Studying of the parameters of the Krebs cycle enzymes and enzymes of glutamate and GABA

Table 1

Influence of gly-pro (0,2 mg/kg, intranasally) and rotenone (2 mg/kg, i.p.) on the parameters of glutathione system in the rat forebrain

Groups	GSH, mcmol/g tissue	GPx, nmol /mg protein*min	GR, nmol /mg protein*min
Control	1,28 ± 0,069	34,00 ± 2,18	21,83 ± 4,26
Rotenone	1,42 ± 0,043	32,88 ± 4,64	33,79 ± 5,44*
Rotenone+gly-pro	1,40 ± 0,05	31,57 ± 5,9	39,68 ± 4,48*#

* – p•0,05 concerning intact control; # – p•0,05 concerning rotenone

metabolism showed that the rotenone administration caused decrease of the GDH and OGDH activities and increase of the SDH activity in the forebrain hemispheres (Tab. 2). Gly-pro did

not influence the disturbance of the GDH but returned OGDH activity to the normal value. Activity of SDH increased in these conditions even more.

Table 2

Influence of gly-pro (0,2 mg/kg, intranasally) and rotenone (2 mg/kg, i.p.) on the activities of glutamate and GABA metabolism enzymes in the rat forebrain

Groups	GDH	SDH	OGDH	GAD
Control	124,80±6,21	10,61±0,17	2,51±0,59	1,98±0,12
Rotenone	92,45±2,84*	14,76±0,46*	1,58±0,5*	2,63±0,24*
Rotenone+gly-pro	90,92±5,79*	24,1±1,89*#	2,96±0,53#	1,68±0,13#

* – p•0,05 concerning intact control; # – p•0,05 concerning rotenone

In the presence of rotenone we observed significant increase of the GAD activity and decrease of the activities of GABA-T (to 75%) and SSA-DH (to 80%) (Fig. 5). Administration of gly-pro returned activities of the enzymes to control values.

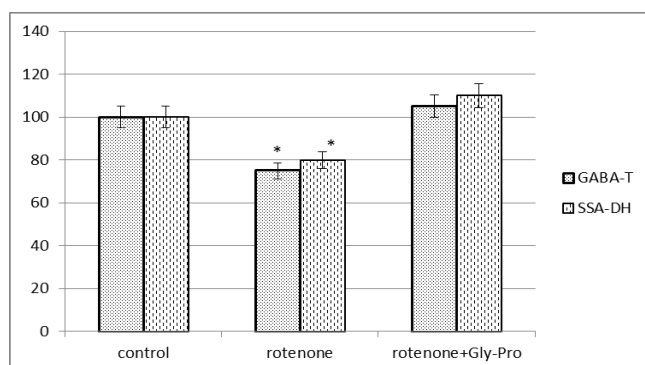


Fig. 5 – Influence of gly-pro (0,2 mg/kg, intranasally) and rotenone (2 mg/kg, i.p.) on the activities of GABA metabolism (GABA-T and SSA-DH) in the rat forebrain

Conclusions. Administration of rotenone (2 mg/kg) is accompanied with significant alterations in energetic metabolism processes and metabolism of the important amino acids in these processes — glutamate and GABA whereas changes of the parameters of oxidative stress is decreased in the forebrain. These changes may

play important role in the development of histomorphological alterations in the brain tissue caused by the rotenone injections. Intranasal administration of the dipeptide gly-pro leads to a significant reduction in energy metabolism disorders and morphological changes in the rat forebrain. We suppose that the dipeptide gly-pro may be used as an effective neuroprotective substance for easing of neurodegenerative processes in the brain.

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