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INFLUENCE OF NEUROPEPTIDES ACTH(4-7)-PRO-GLY-PRO AND ACTH(6-9)-PRO-GLY-PRO ON THE INTENSITY OF REDOX REACTIONS UNDER EXPERIMENTAL DEPRESSION

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ABSTRACT — The experiment is devoted to the study of the antioxidant properties of neuropeptides from melanocortins ACTH(4-7)-Pro-Gly-Pro (Semax) and ACTH(6-9)-Pro-Gly-Pro under conditions of experimental depression. The study was carried out on white outbred male rats. In the process of modeling experimental depression (social stress) inter-male confrontations were observed as a result of which groups of animals with aggressive and submissive behaviors were formed. The free radical oxidation processes were assessed by determining the activity of catalase, the initial content of malondialdehyde (MDA), the rate of spontaneous and ascorbate-dependent lipid peroxidation (LPO) in the hypothalamic and prefrontal regions of the brain by spectrophotometric method. It was found that under the influence of melanocortins, there is a pronounced suppression of the processes of free radical oxidation in the hypothalamic and prefrontal regions of the brain, which arose against the background of a stressful load which is manifested by a decrease in the indicators of the oxidative process.

KEYWORDS — experimental depression, experimental social stress, antioxidant activity, lipid peroxidation, neuropeptides, melanocortins, Semax.

The problem of studying the processes of free radical oxidation is of particular interest from scientists. Numerous studies have shown that oxidative processes occur in a living organism at a constant rate and its change is one of the leading mechanisms of damage to biological membranes [1, 2, 3]. It has been established that an imbalance between prooxidant and antioxidant processes is a pathogenetic link of different diseases, which is most pronounced in the development of autoimmune neurodegenerative, oncological and many other pathological conditions [4, 5, 6]. It has been proven that the development of free radical processes in the body is directly associated with the impact of various stress factors. Thus, scientific research has shown that any stress response is accompanied by an increase in the level of reactive oxygen species and the development of oxidative stress [7, 8].

Thus the need to create drugs with antioxidant activity is an urgent task of modern medicine. Neuropeptides are of particular interest. Being in fact regulatory peptides and synthesized in almost all tissues of the body they have undeniable advantages, such as high efficiency, lack of toxicity and safety. It has been established that this type of biologically active compounds is involved in such processes as the regulation of metabolism, maintenance of homeostasis, impact on immune processes and functions of the higher nervous system (memory, learning, sleep) and much more [9, 10].

ACTH(4-7)-Pro-Gly-Pro is one of representative of the neuropeptides of the melanocortin family. It is the registered drug Semax, which is an analogue of the ACTH4-10 fragment completely devoid of hormonal activity and with proven neurometabolic, neuroprotective, psychostimulating, immunotropic and other pharmacological effects. Along with the already registered drug Semax, a new synthesized compound from the melanocortin family ACTH(6-9)-Pro-Gly-Pro is being actively studied [11].

Aim

In this connection the aim of this work was to assess the antioxidant activity of neuropeptides of the melanocortin family ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro under conditions of experimental social stress.

MATERIAL AND METHODS

The study was carried out on white outbred male rats. Animals were divided into groups (n = 10): 1st group — intact control; 2nd group — rats exposed to experimental depression for 20 days, 3rd group — experimental animals exposed to experimental depression and receiving ACTH(4-7)-Pro-Gly-Pro at a dose of 100 μg/kg per day intraperitoneally for 20 days; 4th group — rats exposed to experimental depression

and receiving ACTH(6-9)-Pro-Gly-Pro at a dose of 100 μg/kg per day intraperitoneally for 20 days. For modeling experimental depression (social stress) animals were placed in pairs in cages separated by a transparent partition with holes which allowed rats to see, hear and perceive each other's smells but at the same time prevented physical interaction. The septum was removed for 10 minutes every day resulting in inter-male confrontation. As a result groups of animals with aggressive and submissive behaviors were formed.

The intensity of free radical oxidation processes was assessed by determining the activity of catalase, the initial content of malondialdehyde (MDA), the rate of spontaneous and ascorbate-dependent lipid peroxidation in the hypothalamic and prefrontal regions of the brain by the spectrophotometric method.

The experiment results were statistically processed using the following programs: Microsoft Office Excel 2007 (Microsoft, USA), BIOSTAT 2008 Professional 5.1.3.1. To process the obtained results, a parametric method was used with the Student t-test with the Bonferroni correction. Statistically significant differences were considered at $p<0.05$.

RESULTS AND DISCUSSION

The results of a study to determine the intensity of indicators of peroxidation and catalase activity in the hypothalamic region of the brain under the influence of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro under conditions of social stress are presented in the table 1.

Modeling of experimental depression in the form of long-term exposure to social stress led to a statistically significant increase in the content of peroxidation products in the hypothalamic region of the rat brain both in the group with aggressive and submissive behaviors as compared with the control group of animals: the initial MDA level increased by 55% ($p<0.001$) and 63% ($p<0.001$), respectively; the rate of spontaneous LPO — in both groups more than 2 times ($p<0.001$); the rate of ascorbate-dependent LPO by 47% ($p<0.01$) and 68% ($p<0.001$), respectively, and the catalase activity increased by 53% ($p<0.01$) and 49% ($p<0.01$), respectively.

In the group of animals with an aggressive type of behavior upon administration of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro there was a decrease in the baseline MDA level by 32% ($p<0.05$) and 25% ($p<0.05$), respectively. The rate of spontaneous and ascorbate-dependent LPO also decreased in comparison with the group of animals with experimental social stress: by 50% ($p<0.01$), 30% ($p<0.01$) and 42% ($p<0.01$), 28 % ($p<0.05$) respectively. When assessing the activity of catalase a decrease in this indicator was observed on average by 30% ($p<0.05$) in comparison with the group of stressed animals.

In the group of animals with a submissive type of behavior identical statistically significant changes were observed. Thus the initial level of TBA-reactive products decreased under the influence of melanocortins by an average of 30% ($p<0.05$) relative to the stress group. The rate of spontaneous and ascorbate-dependent LPO during exposure to ACTH(4-7)-Pro-Gly-Pro decreased by more than 40% ($p<0.01$); under the influence of ACTH(6-9)-Pro-Gly-Pro these indicators decreased by an average of 35% ($p<0.05$). The introduction of the studied neuropeptide compounds led to a statistically significant decrease in catalase activity by almost 30% ($p<0.05$) in comparison with stressed animals.

The results of the study of lipid peroxidation and catalase activity in the prefrontal zone of the brain under the influence of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro under conditions of experimental depression are presented in Table 2.

When studying the processes of free radical oxidation in the prefrontal zone of the brain of rats under conditions of experimental social stress an increase in the products of peroxidation was noted in comparison with the control in groups with aggressive and submissive behaviors. It was found that the baseline MDA level increased by 76% ($p<0.001$) and 50% ($p<0.01$); the rate of spontaneous LPO — 1.7 and 1.9 times ($p<0.001$); the rate of ascorbate-dependent LPO by an average of 65% ($p<0.01$) and the catalase activity increased by 59% ($p<0.05$) and 72% ($p<0.01$), respectively.

In the group of animals with an aggressive type of behavior under the influence of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro there was a change in all indicators of free radical oxidation in comparison with the group of stressed animals: the initial level of malonic dialdehyde decreased by almost 40% ($p<0.01$); the rate of spontaneous and ascorbate-dependent lipid peroxidation decreased by almost 40% ($p<0.05$); catalase activity decreased by 34% ($p<0.05$) and 28% ($p<0.05$) respectively.

A similar trend in changes in the indices of the free radical oxidation process was observed in the group of animals with a submissive type of behavior. Against the background of the introduction of ACTH(4-7)-Pro-Gly-Pro the initial level of MDA decreased by 21% ($p<0.05$), the rate of spontaneous and ascorbate-dependent LPO — by 40% ($p<0.01$) and 25% ($p2<0.05$) respectively, catalase activity — by 40% ($p<0.01$) in comparison with the stress group. The neuropeptide compound ACTH(6-9)-Pro-Gly-Pro reduced the listed parameters by an average of 30% ($p<0.05$).

Table 1. *Influence of melanocortins on lipid peroxidation indicators and catalase activity in the hypothalamic area of the brain under experimental social stress*

Experimental groups (n = 10)	Lipid peroxidation indicators			Catalase activity, %
	The initial level of MDA, M±m, nmol/g tissue	The rate of spontaneous lipid peroxidation, M ± m, nmol/g · h	The rate of ascorbate-dependent lipid peroxida-tion, M ± m, nmol/g · h	
Control	26,5±2,1	2,2±0,3	14,6±1,0	5,5±0,5
Animals with an aggressive type of behavior				
Social stress	41,1±3,1***	5,0±0,6***	21,5±1,5**	8,4±0,8**
Social stress + ACTH(4-7)-Pro-Gly-Pro	28,1±3,1#	2,5±0,3##	15,1±1,2##	5,9±0,6#
Social stress + ACTH(6-9)-Pro-Gly-Pro	30,8±2,9#	2,9±0,3##	15,4±1,3#	6,1±0,6#
Animals with a submissive type of behavior				
Social stress	42,3±3,6***	4,7±0,6***	24,5±2,1***	8,2±0,9**
Social stress + ACTH(4-7)-Pro-Gly-Pro	30,1±3,2#	2,6±0,2##	14,2±0,8###	5,9±0,8#
Social stress + ACTH(6-9)-Pro-Gly-Pro	29,7±3,1#	3,0±0,3#	16,2±0,7##	6,1±0,5##

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### — p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons).*

Table 2. *Influence of melanocortins on lipid peroxidation indicators and catalase activity in the prefrontal zone of the brain under experimental social stress*

Experimental groups (n = 10)	Lipid peroxidation indicators			Catalase activity, %
	The initial level of MDA, M±m, nmol/g tissue	The rate of spontaneous lipid peroxidation, M ± m, nmol/g · h	The rate of ascorbate-dependent lipid peroxida-tion, M ± m, nmol/g · h	
Control	18,2±1,5	4,5±0,4	14,3±1,3	6,5±0,7
Animals with an aggressive type of behavior				
Social stress	32,1±3,2***	7,8±0,8***	24,2±2,1***	10,3±1,1*
Social stress + ACTH(4-7)-Pro-Gly-Pro	20,3±2,1#	4,9±0,6#	14,7±1,5##	6,8±0,9#
Social stress + ACTH(6-9)-Pro-Gly-Pro	19,7±2,7##	5,5±0,8#	15,3±1,7##	7,4±0,8#
Animals with a submissive type of behavior				
Social stress	27,3±2,1**	8,7±0,8***	23,1±1,8**	11,2±1,2**
Social stress + ACTH(4-7)-Pro-Gly-Pro	20,7±1,7#	5,2±0,5##	17,3±1,2#	6,7±0,8##
Social stress + ACTH(6-9)-Pro-Gly-Pro	19,0±1,7##	5,8±0,7#	15,2±1,3#	8,1±0,7#

Note: ** — p<0,05; ** — p<0,01;*** — p<0,001 — comparing with control; # — p<0,05; ## — p<0,01; ### – p<0,001 — comparing with stress (Student's t-test with Bonferroni amendment for multiple comparisons).*

CONCLUSION

Taking into account the results obtained it can be concluded that pronounced inhibition of free radical oxidation processes in the hypothalamic and prefrontal regions of the brain that arose against the background of experimental depression which is manifested by a decrease in the indicators of the oxidative process, is observed under the influence of neuropeptide drugs of the melanocortin family ACTH (4-7)-Pro-Gly-Pro and ACTH(6- 9)-Pro-Gly-Pro.

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