http://dx.doi.org/ 10.35630/2199-885X/2021/11/4.16

EFFECT OF MELANOCORTINS AND EXPERIMENTAL SOCIAL STRESS ON THE LEVEL OF CASPASE-3 AND CASPASE-8

Received 01 September 2021;
Received in revised form 18 September 2021;
Accepted 20 September 2021

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ABSTRACT — At present the study of stress-generating effects and key mechanisms of regulation of apoptosis remain in the focus of attention of researchers. Stress causes dysfunctions of many body systems which lead to disruption of homeostasis and molecular cell mechanisms of programmed cell death. In this context, the problem of pharmacological regulation and correction of these disorders poses an urgent problem. Neuropeptide compounds such as melanocortins are interesting in this direction as regulators and correctors. Under conditions of experimental social stress activation of apoptotic processes was established due to an increase in the level of caspase-3 and caspase-8. The studied neuropeptides Semax (ACTH (4-7)-Pro-Gly-Pro) and ACTH (6-9)-Pro-Gly-Pro exhibit antiapoptotic effects under conditions of induced social stress in rats. This action of neuropeptides is manifested by a decrease in the activity of the initiating and effector caspases in the blood serum of experimental animals.

KEYWORDS — experimental social stress, neuropeptides, melanocortins, Semax, ACTH(4-7)-Pro-Gly-Pro, ACTH(6-9)-Pro-Gly-Pro, apoptosis, caspases.

INTRODUCTION

Nowadays the study of the impact of stress, including social stress, on various pathological processes in the body is an actual problem. It has been proven that stress causes dysfunction of many body systems [5, 11]. Numerous studies have been carried out confirming the fact that the influence of stress factors can lead to the development of endocrine, immune, antioxidant, metabolic and other types of disorders leading to disruption of homeostasis, which in turn in its turn can damage molecular-cell apoptotic mechanisms [3, 4, 8]. In recent years the assessment of apoptotic processes is often carried out by determining the activity of caspases, both initiator and effector. Caspases are able to activate each other, forming a caspase cascade. In the works of modern researchers much attention is paid to the study of caspase-3 and caspase-8, which makes it possible to determine the receptor pathway for the initiation of programmed cell death [1, 2]. It has been established that the process of initiation of effector caspase-3 by caspase-8 is characteristic of the development of apoptosis in lymphoid and endothelial cells, which in turn contributes to the development of immune dysfunction, as well as pathology of the cardiovascular, urinary and other systems. It should be noted that when the body is exposed to stress factors, activation of apoptotic processes is observed, including caspase-3-dependent apoptosis [6]. A group of scientists have proven that, along with caspase-3, caspase-8 plays one of the main roles in the realization of pathological conditions observed under the influence of chronic stress [1, 7]. In this connection, the problem of pharmacological regulation and correction of these changes is becomes ever more urgent. Neuropeptide compounds that play the role of regulatory molecules that are produced by almost all body systems, which determines their broad pharmacological activity, are of great interest in this direction. It has been established that neuropeptides are capable of exerting metabolic and antioxidant activity, as well as participating in the processes of induction of various trophic factors and cytokines, regulation of apoptotic processes [8, 10, 11].

Melanocortins are of particular interest as promising remedies for the correction of disorders in the mechanisms of apoptosis: Semax (ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro, synthesized at the Institute of Molecular Genetics of the National Research Center Kurchatov Institute.

The aim of research:
to study the effect of melanocortins on the level of initiating and effector caspases in the blood serum of white rats under conditions of experimental social stress.

MATERIAL AND METHODS

The study was carried out on 70 male white rats aged six months. All manipulations with laboratory animals were performed in accordance with the requirements of international and national regulations on the protection of animals used for scientific purposes.

When modeling *social* stress, we adhered to the conditions when animals lived in conditions of sensory contact with the subsequent formation of an aggressive and submissive type of behavior. In order to form *social* stress, the animals were separated in pairs by a transparent partition in the cage, which provided sensory contact in the absence of physical contact. The partition was removed for 10 minutes every day and the inter-male confrontations were observed. As a result, groups of rats with aggressive (aggressor) and submissive (victim) types of behavior were formed. Aggressiveness of rats was assessed by the presence of attack and vertical and lateral stands, and submissiveness by the presence of immobility, sniffing, autogrooming, and vertical *protective* stands.

After stressing the rats were divided into groups: intact males (control); animals exposed to *social* stress for 20 days (stress); and experimental groups which received intraperitoneally Semax and ACTH(6-9)-Pro-Gly-Pro at a dose of 100 μg/kg/day for 20 days starting from the 1st day of stress exposure.

The effect of neuropeptides on the activity of apoptosis processes was assessed by determining the level of initiating and effector caspases (caspase-8 and caspase-3) (ELISA Kit for Caspase-8 and ELISA Kit for Caspase-3; USA) in the blood serum of white rats by enzyme immunoassay.

The experiment results were statistically processed by 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 difference was considered at p.0.05.

RESULTS

The results reflecting the effect of melanocortins on the activity of apoptosis processes were assessed by determining the level of caspase-3 and caspase-8 in the blood serum of white rats under conditions of experimental social stress, are presented in the Table 1.

In groups of experimental animals with an aggressive type of behavior, the following changes were noted: the level of caspase-3 in the stress group increased 1.8 (p1<0.05) times in comparison with the control group; compounds ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro contributed to a decrease in the level of the studied marker in 1.4 (p_2<0.05) and 1.2 (p_2<0,05) times, respectively, in comparison with the *social* stress group. A change in the level of caspase-8 was noted: in the group of *social* stress, an increase of 2.6 (p_1<0.05) times was noted in relation to the control group; against the background of the introduction of ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)-Pro-Gly-Pro, a decrease in the level of the indicator in 2 (p_2<0.05) and 1.1 (p_2>0,05) times in comparison with the group of stressed animals respectively.

In groups of animals with a submissive type of behavior when determining the level of caspase-3, the following changes were noted: in rats of the *social* stress group, this indicator increased 1.6 (p_1<0.05) times in relation to the control group; under the conditions of administration of the test compounds a tendency towards a decrease in this marker was observed; the level of caspase-8 increased 2.4 (p_1<0.05) times in relation to intact animals; the administration of the compounds ACTH(4-7)-Pro-Gly-Pro and ACTH(6-9)Pro-Gly-Pro contributed to a decrease in the level of the studied marker by an average of 1.3 times (p_2>0.05) compared to stressed rats.

According to the results of the study, it was found that under conditions of experimentally social stress, an increase in apoptotic processes was observed accompanied by an increase in the level of caspase-3 and caspase-8 in the blood serum of white rats. The administration of Semax (ACTH (4-7)-Pro-Gly-Pro) and ACTH (6-9)-Pro-Gly-Pro against the background of stress contributed to a decrease in the level of the studied parameters, which is most likely associated with the presence of antiapoptotic action in melanocortins due to inhibition of the caspase-dependent cascade of apoptosis reactions.

This pathway, along with caspases, is realized with the participation of cell death receptors, which include tumor necrosis factor. Previously, the presence of antioxidant action and the ability of melanocortins to influence the level of pro- and anti-inflammatory cytokines have been proven. It has been established that Semax (ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro under conditions of *social* stress cause an inhibition of free radical oxidation processes and reduce the concentration of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [9, 10].

CONCLUSION

Thus, effector caspase-3 and initiating caspase-8 are actively studied as targets for various pathological conditions, in which the processes of programmed cell death are triggered. Expression of caspases-3 and -8 is an indicator of the cytotoxicity of an apoptotic stimulus, which makes these markers an important part of research on apoptotic processes. These molecules-markers of the external and internal pathways of apoptosis can serve as targets for the action of stress-protective drugs of neuropeptide structure. The study established the presence of Semax

Table 1. *The level of caspase-3 and caspase-8 in blood serum of white rats under conditions of experimental social stress and administration of melanocortins*

Groups of experimental animals	Markers of apoptosis	
	Caspase-3 (pg / ml)	Caspase-8 (pg / ml)
Control	17,41±1,22	2,33±0,91
Animals with an aggressive type of behavior		
Experimental social stress	30,62±4,23*	6,14±1,21*
Experimental social stress + ACTH(4-7)-Pro-Gly-Pro	21,49±1,23#	3,08±0,91#
Experimental social stress + ACTH(6-9)-Pro-Gly-Pro	24,84±2,11	5,15±1,20
Animals with submissive type of behavior		
Experimental social stress	27,83±3,81*	5,64±0,87*
Experimental social stress + ACTH(4-7)-Pro-Gly-Pro	23,74±1,42	4,26±1,22
Experimental social stress + ACTH(6-9)-Pro-Gly-Pro	24,30±2,13	4,58±0,98

Note: ** — p1 <0.05; ** — p1 <0.01; *** — p1 <0.001 — relative to the control; # — p2 <0.05; ## — p2 <0.01; ### — p2 <0.001 — relative to stressed animals (Student's t-test)*

(ACTH(4-7)-Pro-Gly-Pro) and ACTH(6-9)-Pro-Gly-Pro under stressful effects of anti-apoptotic action due to inhibition of the caspase-dependent cascade of apoptosis reactions.

Acknowledgments

The reported study was funded by Russian Foundation for Basic Research (RFBR) according to the research project Nr. 19-04-00461.

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