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# THE INFLUENCE OF PRO-GLY-PRO-LEU ON THE LEVEL OF CYTOKINES UNDER THE CONDITIONS OF "SOCIAL" STRESS

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**ABSTRACT** — The experiment investigated the effect of Pro-Gly-Pro-Leu on the level of cytokines under the conditions of "social" stress. The level of cytokines under study was determined by enzyme-linked immunosorbent assay. The stress-induced changes observed in the experiment consist in increasing the production of IL-1 $\beta$ , IL-6, TNF- $\alpha$  and TGF- $\beta$ , which allows us to consider stress exposure as an inducer of the production of pro-inflammatory interleukins and various growth factors. It was found that the introduction of Pro-Gly-Pro-Leu is accompanied by the restoration of increased values of the studied cytokines in stressed animals almost to the level of indicators of the control group. The obtained results indicate the pronounced stress-protective properties of this compound, which are manifested in the restoration of the level of the studied cytokines to the level of control values.

**KEYWORDS** — "social" stress, peptide, glyprolins, cytokines, interleukin-1 $\beta$ , interleukin-4, interleukin-6, tumor necrosis factor- $\alpha$ , transforming growth factor  $\beta$ 1.

## INTRODUCTION

Currently, special attention is paid to the creation of drugs with stress-protective activity, which is directly related to modern human conditions, constantly accompanied by various stressful factors, which often entail the development of a complex of reactions, both compensatory and pathological, and, ultimately, they can lead to disruption of the immune mechanisms of homeostasis [1, 2, 3, 4, 5].

Modern studies indicate that in the development of immune disorders caused by stress exposure, the leading place is occupied by the production of cytokines, such as interleukins, neurotransmitters, hormones, neuropeptides, etc., whose main role is to regulate intercellular and intersystem interactions and to act functions of stress-limiting and immune systems of the body [6, 7, 8, 9, 10].

Of particular interest today are drugs from the group of peptide analogues with proven regulatory

function relative to homeostasis and the ability to participate in the formation of adaptive changes under the influence of stress factors [11]. It was established that peptide compounds related to glyprolins, along with the above properties, have neuroprotective, neurotrophic, anxiolytic and other types of activity, however, information on the immunotropic effect of these substances is insufficient [12, 13].

### *The aim of research:*

study the effect of Pro-Gly-Pro-Leu on the level of cytokines (IL-1 $\beta$ , IL-4, IL-6, TNF- $\alpha$  and TGF- $\beta$ 1) under the conditions of "social" stress.

## MATERIAL AND METHODS

White non-linear rats (males, 6–8 months old) were used as experimental animals. The effect of the peptide compound Pro-Gly-Pro-Leu on the plasma concentration of cytokines was carried out on the model of social stress — "sensory contact". Laboratory animals were divided into 3 groups ( $n = 10$ ): a group of intact males; a group of animals that were subjected to stress for 20 days (sensory contact); a group of animals that received intraperitoneally Pro-Gly-Pro-Leu at a dose of 100 mcg / kg / day under conditions of 20-day stress (sensory contact) course of 20 days.

In order to create a "social" stress in the experiment a model of inter-male confrontations was chosen. Animals were placed in pairs in experimental cells separated by a septum which prevents physical contact but has openings that provide sensory contact. Every day the partition was removed for 10 minutes which overwhelmingly led to agonistic collisions (confrontations) [14, 15]. Then groups of animals with an aggressive and submissive type of behavior were formed.

The study of the cytokine level under the influence of Pro-Gly-Pro-Leu on the model of "social" stress was carried out by the method of enzyme-linked immunosorbent assay (ELISA) using test systems based on monoclonal antibodies manufactured by BenderMedSystems (Austria) according to the guidelines attached to the sets.

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 Bon-

ferroni correction. Statistically significant differences were considered at  $p < 0.05$ .

## RESULTS

The results of the determination of serum cytokines IL-1 $\beta$ , IL-4 and IL-6 are presented in Table 1.

of stressed animals however, compared with the control group, the changes did not have statistical significance; the level of IL-6 decreased by 1.2 times ( $p > 0.05$ ) and almost reached the level of the group of intact animals. In the group of animals with a submissive type of behavior, a decrease in the levels of IL-1 $\beta$  and IL-6 was

**Table 1.** The concentration of interleukins under the influence of Pro-Gly-Pro-Leu in conditions of «social» stress

Experimental groups (n=8)	IL-1 $\beta$ , M $\pm$ m, pg / ml	IL-4, M $\pm$ m, pg / ml	IL-6, M $\pm$ m, pg / ml
Control	107,80 $\pm$ 10,76	3,97 $\pm$ 0,18	79,60 $\pm$ 5,40
Animals with an aggressive type of behavior			
«Social» Stress	152,80 $\pm$ 15,36 *	3,38 $\pm$ 0,27	100,60 $\pm$ 8,20*
«Social» stress + Pro-Gly-Pro-Leu (100 mg / kg / day)	95,60 $\pm$ 11,26##	3,96 $\pm$ 0,12	82,20 $\pm$ 5,40
Animals with a submissive type of behavior			
«Social» Stress	171,60 $\pm$ 12,94**	3,48 $\pm$ 0,22	104,00 $\pm$ 7,20*
«Social» stress + Pro-Gly-Pro-Leu (100 mg / kg / day)	97,80 $\pm$ 14,46##	3,84 $\pm$ 0,11	82,20 $\pm$ 8,3

**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)

Compared with the control group of animals, an increase in the concentration of IL-1 $\beta$  and IL-6 was observed in animals with experimental “social” stress and aggressive behavior by 42% ( $p < 0.05$ ) and 26% ( $p < 0.05$ ), respectively. whereas in animals with submissive behavior, 60% ( $p < 0.01$ ) and 30% ( $p < 0.05$ ), respectively; a decrease in the level of IL-4 was also observed in groups of animals with simulated stress, however, these changes were not significant.

When the peptide compound was intraperitoneally administered under the code Pro-Gly-Pro-Leu at a dose of 100 mg / kg, a 1.6-fold decrease in the concentration of IL-1 $\beta$  was observed in animals with aggressive behavior ( $p < 0.05$ ) compared with the group

observed in comparison with the stressed group by 1.8 ( $p < 0.01$ ) and 1.3 ( $p > 0.05$ ) times, respectively. In relation to the intact control, the concentration of IL-1 $\beta$  decreased by 1.1 ( $p > 0.05$ ). Against the background of the introduction of the peptide compound, an increase in the concentration of IL-4 in blood serum was noted as in both groups of animals, however, these changes were not significant.

The results of the determination of TNF- $\alpha$  and TGF- $\beta$ 1 in blood serum are presented in Table 2. A statistically significant increase in the level of TGF- $\beta$ 1 by more than 2 times ( $p < 0.001$ ) was found in the social stress group in animals with an aggressive type of behavior, and in the group with a submissive type

**Table 2.** The concentration of TNF- $\alpha$  and TGF- $\beta$ 1 under the influence of Pro-Gly-Pro-Leu in conditions of «social» stress

Experimental groups (n=8)	TGF- $\beta$ 1, M $\pm$ m, pg / ml	TNF- $\alpha$ , M $\pm$ m, pg / ml
Control	138,8 $\pm$ 17,6	81,8 $\pm$ 7,8
Animals with an aggressive type of behavior		
«Social» stress	292,4 $\pm$ 25,8***	93,4 $\pm$ 5,8
«Social» stress + Pro-Gly-Pro-Leu (100 mg / kg / day)	207,8 $\pm$ 16,2#	82,2 $\pm$ 6,2
Animals with a submissive type of behavior		
«Social» stress	230,0 $\pm$ 24,6**	91,4 $\pm$ 7,8
«Social» stress + Pro-Gly-Pro-Leu (100 mg / kg / day)	165,8 $\pm$ 18,4#	78,8 $\pm$ 7,2

**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)

of behavior — 1.7 times ( $p < 0.05$ ) in comparison with the control group; under the same conditions, there was a statistically significant decrease in TGF- $\beta$ 1 in 1.4 ( $p < 0.05$ ) in two groups compared with stressed animals. When assessing the level of TNF- $\alpha$  in conditions of "social" stress in animals with various types of behavior, there was a tendency to increase this indicator; with the introduction of Pro-Gly-Pro-Leu, the level of the studied cytokine almost reached the control values, but these changes were not statistically significant.

Thus, when studying the effect of the peptide compound of the glyproline group Pro-Gly-Pro-Leu on the level of cytokines under conditions of "social" stress, it was found that this peptide is able to reduce the concentrations of IL-1 $\beta$ , IL-6 and TNF- $\alpha$  and TGF- $\alpha$   $\beta$ 1 almost to the level of control values.

## DISCUSSION

Currently, one of the priority areas is a more detailed study of the mechanisms of cytokine regulation, as one of the leading links that determine the intercellular interaction both in normal and in pathological conditions. It is proved that the level of cytokines changes in any pathological process, including with stress-mediated etiopathogenetic cause. In this study, "social" stress was accompanied by an increase in the concentration of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and TGF- $\beta$ 1 in the blood serum, confirming the fact that the stressful effect on the body leads to the induction of the synthesis of pro-inflammatory cytokines and growth factors that have a significant effect on the body as a whole, which provokes a change in the functional activity of the stress-limiting and immune systems of the body. The results obtained when studying the effect of the peptide compound of the glyproline group Pro-Gly-Pro-Leu on the level of cytokines under conditions of "social" stress indicate the presence of stress-protective activity of the peptide compound of the glyproline series under the code Pro-Gly-Pro-Leu.

## CONCLUSION

Thus, the results of a study of the effect of a peptide compound of glyprolin nature Pro-Gly-Pro-Leu on the plasma concentration of cytokines such as IL-1 $\beta$ , IL-4, IL-6, TNF- $\alpha$ , and TGF- $\beta$ 1, under conditions "social" stress indicates the need for a detailed analysis of the role of cytokine and growth factors in the development of stress-induced changes in order to find optimal correction tools.

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