REPRESENTATIVES OF NEUROPEPTIDES — SELANK AND PRO-GLY-PRO-LEU AS MODULATORS OF IMMUNOREACTIVITY IN CONDITIONS OF "SOCIAL" STRESS

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ABSTRACT — This study is devoted to the experimental study of the immunocorrecting action of neuropeptides Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) and Pro-Gly-Pro-Leu on the model of "social" stress. Functional activity of the immune system was assessed on the basis of standard immunopharmacological tests: delayed-type hypersensitivity reaction (DTH), direct agglutination test (DAT), latex test for studying the phagocytic activity of peripheral blood neutrophils, evaluation of changes in the leukocyte formula. As a result of the experiment, it was found that, under the influence of "social" stress, changes in the immune response are multidirectional, which confirms the theory of "immune disbalance" under the action of stressors. As a result of studying the effect of neuropeptides under the conditions of "social" stress, it was found that Selank and Pro-Gly-Pro-Leu proved to be an effective correctors, restoring the cellular and humoral immunogenesis reactions, the phagocytic activity of neutrophils and indicators of leukocyte formula.

INTRODUCTION

Studies concerning the mechanisms of the dysfunctional changes in the immune system under stress, as well as the development of methods for their correction, are one of the main directions of development of modern immunology and pharmacology. The greatest achievement of molecular biology and medicine was the possibility of synthesizing bioregulators, in particular of neuropeptides, and the creation on their basis of new highly effective drugs, exhibiting, among other things, stress protective properties [7, 10]. It is important to emphasize that molecules in the form of short fragments of peptide compounds are characterized by a high degree of safety due to their complete proteolytic degradation [1]. It should be noted that at the moment a representative of neuropeptides is actively used in clinical medicine — Selank synthesized at the Institute of Molecular Genetics of the Russian Academy of Sciences. This drug was created by attaching a tripeptide Pro-Gly-Pro to the C-termini of an unstable regulatory peptide taftsin which solved the problem of in vivo stabilization and supplemented it with the effects of Pro-Gly-Pro itself [6]. Along with the already registered drug Selank, glyproline Pro-Gly-Pro-Leu is of considerable interest from the standpoint of the promise of practical implementation in clinical pharmacology [3, 5].

The aim of research

To study of the immunomodulatory effects of Selank and Pro-Gly-Pro-Leu on the model of "social" stress.

MATERIAL AND METHODS

White non-linear rats (males, 6–8 months old) were used as experimental animals. In order to create a "social" stress in the experiment a model of intermale 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) [2, 8, 9]. Groups of animals with alternative types of behavior were formed: aggressive type — in case of repeated victories experience (winner, aggressor) and submissive type - in case of defeats (victim). Laboratory animals were divided into 5 groups (n = 10): a group of intact males; a group of animals that were exposed to stress for 20 days (sensory contact); a group of individuals treated intraperitoneally with Selank at a dose of 100 μg/kg/day under conditions of 20-day stress exposure (sensory contact) in a course of 20 days; a group of rats treated intraperitoneally with Pro-Gly-Pro-Leu at a dose of 100 μg/kg/day under conditions of 20 days of stress exposure (sensory contact) in a course of 20 days. Functional activity of the immune system of laboratory animals was assessed on the basis of standard immunopharmacological tests: delayed-type hypersensitivity reaction (DTH), direct agglutination test (DAT), latex test for studying the phagocytic activity of peripheral blood neutrophils, evaluation of changes

in the leukocyte formula. Particle T-dependent antigen (the erythrocytes of the ram) was used as an antigenic stimulus in DTH and DAT [4].

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 ITS DISCUSSION

In the course of the experiments, it was found that long-term inter-male confrontations caused the suppression of DTH and DAT in animals with both aggressive and submissive types of behavior compared to control animals. The delayed-type hypersensitivity reaction index in aggressors decreased by more than 45% (p<0,01), in victims — by more than 30% (p<0,05). In relation to the humoral immunity in animal aggressors, more pronounced changes in indicators were observed: a decrease in antibody titer in the aggressors — by more than 80% (p<0,001), in victims — by more than 50% (p<0,001) compared with the control indicators (Table 1). As can be seen from the results presented in Table 1, the neuropeptides contributed to the restoration of the indices of both immunity units. The index DTH in aggressors increased in conditions of introduction of Selank by 80% (p<0,01), Pro-Gly-Pro-Leu — by 60% (p<0,05), in victims — by an average of 30%, but statistically significant indicators were only in the group of animals that were administered Selank (p<0,05). With regard to the formation of anti-erythrocyte antibodies in DAT, the hemagglutinin titer indices increased in animals with aggressive type of behavior on average more than 5 times (p<0,001), in animals with submissive type of behavior — on average almost 2 times (p<0,01) (Table 1).

When studying the indicators of phagocytic activity of peripheral blood neutrophils in animals exposed to "social" stress, an increase in the phagocytic index (PhI) and phagocytic number (PhN) was found in rats with both aggressive and submissive behaviors. There was an increase in the phagocytic index by 20% in aggressors (p>0,05) and almost 30% in victims (p<0,05), phagocytic number — by 40% in aggressors and by 20% in victims (p>0,05), which indicates a hyperreactivity of the nonspecific element of the immune system (Table 2). When assessing phagocytosis in the group of animals treated with neuropeptides on the background of the impact of "social" stress, it was found that the introduction of these compounds leads to the restoration of the parameters of nonspecific immunoreactivity. The phagocytic number decreased in aggressors and victims by an average of 30% (p<0,05) under the conditions of administration of Pro-Gly-Pro-Leu, in the group of animals treated with Selank — by 20% (p>0,05). With respect to the phagocytic index, there was a tendency to a decrease in this indicator in animals with aggressive and submissive types of behavior (Table 2).

An important stage of our work was to determine the total number of leukocytes, as well as the study of indicators of leukocyte formula. Under the conditions of "social" stress, there was a decrease in the total number of leukocytes by an average of 30% (p<0,05) in both the aggressors and the victims, relative to the control group. The leukocyte formula in stressed animals showed a decrease in the percentage of eosinophils by 30% (p<0,05) in aggressors and more than 40% (p<0,01) in victims. It should also be noted a statistically significant increase in segmented neutrophils by an average of 2 times (p<0,001), band staple — by more than 50% (p<0,01) in aggressors and almost 2 times in victims (p<0,01) (Table 3). It was established that neuropeptides on the background of stress contributed to an increase in the total number of leukocytes: in the group of aggressors the introduction of Selank almost 2 times (p<0,001), Pro-Gly-Pro-Leu — 1,5 times (p<0,01); in the group of victims the introduction of Selank — by more than 1,5 times (p<0,01), with the introduction of Pro-Gly-Pro-Leu there was only a tendency to increase (p>0,05). When assessing the number of eosinophils in aggressors under the influence of Selank and Pro-Gly-Pro-Leu, there was only a tendency to increase this indicator. In leukocyte formula in rats with a submissive type of behavior, the increase in the percentage of eosinophils was determined, which averaged 30% (p<0,05). In addition, in individuals with an aggressive type of behavior, the introduction of Selank and Pro-Gly-Pro-Leu led to a decrease in the band neutrophils by an average of 40% (p<0,05 and p<0,01 respectively). With the introduction of Selank and Pro-Gly-Pro-Leu in animals with a submissive type of behavior, the number of band neutrophils significantly decreased on average by 50% (p<0,01 and p<0,001 respectively). The percentage of segmented forms of neutrophils decreased in all groups by more than 40% relative to stressed animals (Table 3).

CONCLUSION

As a result of the experiment, it was found that under the influence of "social" stress, changes in immunoreactivity are multidirectional, which indicates the formation of an immune imbalance, manifested by activation of some and suppression of other parts of the immune system. As a result of studying the effect

Table 1. *The effect of neuropeptides on the formation of DTH and DAT under the conditions of "social" stress*

(M ± m) Experimental groups (n = 10) / Indicators	Index DTH, %	Titer of antibodies in DAT, log2
Animals with an aggressive type of behavior		
Control	30,83 ± 3,52	224,77 ± 23,27
"Social" stress	16,57 ± 1,75**	40,46 ± 5,81***
"Social" stress + Selank (100 mcg /kg/day)	30,38 ± 3,48##	210,56 ± 22,54###
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	26,47 ± 3,61#	181,65 ± 20,37###
Animals with a submissive type of behavior		
Control	30,83 ± 3,52	224,77 ± 23,27
"Social" stress	20,78 ± 2,54*	103,55 ± 11,64***
"Social" stress + Selank (100 mcg /kg/day)	28,26 ± 2,66#	231,19 ± 34,91##
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	26,40 ± 2,86	205,51 ± 23,27##

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. *The effect of neuropeptides on the phagocytic activity of neutrophils under conditions of "social" stress*

(M ± m) Experimental groups (n = 10) / Indicators	Phagocytic index	Phagocytic number, %
Animals with an aggressive type of behavior		
Control	17,7 ± 1,68	53,3 ± 3,66
"Social" stress	21,0 ± 1,85	74,3 ± 7,37*
"Social" stress + Selank (100 mcg /kg/day)	16,3 ± 1,87	57,6 ± 4,23
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	17,4 ± 1,68	49,2 ± 4,04#
Animals with a submissive type of behavior		
Control	17,7 ± 1,68	53,3 ± 3,66
"Social" stress	22,9 ± 1,61*	63,7 ± 4,73
"Social" stress + Selank (100 mcg /kg/day)	18,4 ± 1,58	50,5 ± 4,65
"Social" stress + Pro-Gly-Pro-Leu (100 mcg /kg/day)	19,2 ± 0,96	45,8 ± 5,15#

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)*

of neuropeptides under the conditions of "social" stress, it was found that Selank and Pro-Gly-Pro-Leu proved to be an effective correctors, restoring the cellular and humoral immunogenesis reactions, the phagocytic activity of neutrophils and indicators of leukocyte formula.

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Table 3. *The effect of neuropeptides on the leukocyte formula of animals under "social" stress*

(M ± m) Indicators / Experimental groups (n = 10)	Total number of leukocytes, x10⁹/l	Eosinophils, %	Band neutrophils, %	Segmented neutrophils, %	Lymphocytes,%	Monocytes, %
Animals with an aggressive type of behavior						
Control	11,7 ± 0,93	2,8 ± 0,33	2,2 ± 0,23	12,7 ± 1,59	81,5 ± 5,95	0,83 ± 0,15
"Social" stress	8,3 ± 0,82*	2,0 ± 0,21*	3,4 ± 0,25**	26,7 ± 1,81***	67,1 ± 4,27	0,71 ± 0,10
"Social" stress + Selank (100 mcg /kg/day)	15,7 ± 1,24###	2,4 ± 0,20	2,1 ± 0,36#	16,0 ± 2,10##	78,7 ± 4,87	0,86 ± 0,11
"Social" stress + Pro-Gly-Pro-Leu (100 mcg/kg/day)	12,0 ± 0,76##	2,3 ± 0,40	1,9 ± 0,42##	14,6 ± 1,36###	79,9 ± 4,11	1,0 ± 0,10#
Animals with a submissive type of behavior						
Control	11,7 ± 0,93	2,8 ± 0,33	2,2 ± 0,23	12,7 ± 1,59	81,5 ± 5,95	0,83 ± 0,15
"Social" stress	8,4 ± 0,77*	1,6 ± 0,11**	4,1 ± 0,40**	27,1 ± 2,11***	66,4 ± 4,77	0,71 ± 0,10
"Social" stress + Selank (100 mcg /kg/day)	13,1 ± 0,58###	2,1 ± 0,22#	2,1 ± 0,37##	16,1 ± 2,57##	78,9 ± 4,87	0,86 ± 0,11
"Social" stress + Pro-Gly-Pro-Leu (100 mcg/kg/day)	9,6 ± 0,77	2,1 ± 0,18#	1,6 ± 0,30###	13,9 ± 1,51###	81,6 ± 6,04	0,86 ± 0,11

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)*

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