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Selank and Short Peptides of the Tuftsin Family in the Regulation of Adaptive Behavior in Stress

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White laboratory male rats, inbred male C57BL/6 and Balb/c mice, and male Wistar rats, all previously divided on the basis of the type of emotional reactivity, were used to compare the effects of ten peptide compounds of the tuftsin family and Selank on the behavioral manifestations of emotional stress created by a conflict situation. Peptides were shown to have positive emotional effects and antistress actions. Individual physiologically significant effects were seen, due to the molecular structures of the study peptides and/or their degradation fragments. The results demonstrate the potential for the synthesis of peptide compounds with predictable directions of pharmacological actions and safe for wide use.

KEY WORDS: emotional stress, adaptation, conflict situations, peptides, tuftsin, Selank.

Functional systems theory, proposed by P. K. Anokhin and developed to its current stage by K. V. Sudakov [1, 16, 17] and a large cohort of his colleagues and students, determines current approaches to the analysis of the psychophysiological mechanisms of impairments of adaptive behavior induced by stress [14, 18, 19]. From these points of view, the conflict situation is an important factor in the genesis of emotional stress, which produces negative emotional tension, fear, and anxiety [21]. Models provoking emotional stress by creating conflict situations are particularly important among the many stresses and models of stress reactions from the current point of view in experimental psychophysiology and neuropharmacology. These situations have disorganizing effects on such important components of functional behavioral systems as the programming of purposive activity, correction of errors, stability of the dominant motivation, and decision-taking processes. The role of the immunomodulating system and its tightly associated peptidergic system in making the connection between different types of emotional stress and genetically determined properties of neurotransmitter systems have been studied intensely [2, 4, 8, 13]. The hypothesis that the resistance of biological objects to emotional stress should be determined by endogenous peptide substances was proposed as long ago as the 1960s by K. V. Sudakov [20]. Nonetheless, the role of neuropeptides in forming emotional stress and in the possibility of peptidergic correction of impairments of adaptive behavior in stress situations has received insufficient study.

Our studies over recent years have been directed towards investigation of the psychotropic and stress-protective properties of peptide compounds, which might be combined in the tuftsin family and the heptapeptide Selank [3, 11]. Tuftsin is fragment 289–292 of endogenous immunoglobulin G (Thr-Lys-Pro-Arg). Selank is a synthetic heptapeptide of original structure, including the tuftsin tetrapeptide Thr-Lys-Pro-Arg and three natural L-amino acids Pro-Gly-Pro. The other peptide compounds used here were prepared by removing individual amino acids from the C and N terminals of the heptapeptide Selank molecule. Peptides were synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences and pharmacological studies were performed at the Institute of Pharmacology, Russian Academy of Medical Sciences. Previous studies [2, 5] have demonstrated that the immunotropic peptide tuftsin had stress-protective proper-

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ties, preventing adrenal weight loss in rats subjected to immobilization stress, along with hepato- and leukotropic effects, and having generally activatory effects on the hypothalamic-hypophyseal-adrenal system [4, 5]. However, this compound had transient and unstable actions, with a biphasic effect, and was sensitive to the actions of degrading factors.

Modification of the structure of the tuftsin molecule (attachment of the amino acid sequence Pro-Gly-Pro) led to the creation of a new synthetic original tuftsin derivative, the heptapeptide Selank, which is resistant to the degrading actions of external and internal factors. Pharmacological studies of the heptapeptide Selank allowed it to be characterized as a peptide compound with anxioselective and nootropic actions with potential for clinical application [9, 12, 15, 23]. Considering the important neuropsychotropic action of the family-forming peptides tuftsin and Selank, it is important to study the effects of new peptide compounds of this group on the adaptive behavior of animals in conflict stress-provoking situations.

The aim of the present work was to conduct a comparative study of the actions of new peptide compounds of the tuftsin and Selank families and their various degradation fragments on the behavioral manifestations of emotional stress in mice and rats.

METHODS

Experiments were performed on small laboratory animals (male mice weighing 20–22 g; male rats weighing 230–240 g). Animals were kept in standard animal-house conditions at the Science Research Institute of Pharmacology, Russian Academy of Medical Sciences, with a natural light regime and a standard diet. Experiments were started at least two months after animals arrived from the supplier. Experiments were performed in the autumn-winter period, from 10:00 to 14:00. Use was made of a complex of behavioral psychophysiological models of emotional stress formed in conflict situations without any kind of injurious treatments. The complex behavioral methods and the actions of peptide compounds were assessed in terms of the current concept of the systems organization of emotional behavior [13, 19]. The anxioselective actions of compounds were identified using a pharmacological experimental approach developed and validated at the Science Research Institute of Pharmacology, Russian Academy of Medical Sciences, using two lines of mice with genetically determined opposite types of emotional stress responses (ESR) on testing in the open field: these were Balb/c mice, with "passive" ESR, and C57BL/6 mice, with "active" ESR. Given the needs of the two behavioral methods employed here, both white laboratory mice and Wistar rats were used.

An open field method was used to reproduce emotional stress in mice with genetically determined types of ESR: the open field was a round arena 1 m in diameter and was divided by four concentric lines into peripheral, intermediate and central zones and by radii into 48 curved fields ("squares") with sides of 50 cm; the area was illuminated without shadows (four 75-W lamps at a height of 1 m above the floor). The observation time was 2 min; movement activity (square crossings and vertical rearings) was measured in relation to area zones and in total. The Porsholt forced swimming method used white laboratory mice. An unavoidable conflict situation was created by placing animals in a cylinder containing water. The diameter of the cylinder was 10 cm and its height was 25 cm; the water temperature was 21°C and the observation period lasted 15 min. The latent period of refusing to swim actively (sec) was measured, along with the total duration of "immobilization." The Henderson extrapolatory escape method was used with Wistar rats. Rats were placed tail down in a bottomless cylinder inserted into a basin of water. The walls of the cylinder prevented the animal from swimming, thus hindering escape from the acute stress situation (diving). The cylinder diameter was 12 cm, its height was 22 cm, and the depth of the basin was 50 cm; the cylinder was inserted 2.5 cm into the water. The observation period was 2 min. The latent period of the first escape-directed movement response (sec) was measured, along with the latent period of escape (sec), the number or errors, and the level of effective responses. Assessment of orientational-investigative responses (OIR) in terms of a general measure of the adaptability of the animals (rats) to new situations was performed using the Éra-2 apparatus, developed and validated at the Science Research Institute of Pharmacology, Russian Academy of Medical Sciences – this is an open field without the stress imposed by bright light, in conditions of sound insulation as required for experiments on rats. The rats' behavior was recorded automatically using a light diode minute by minute. The observation periods were 3 and 5 min, as well as 4–5 h when animals were placed into a new experimental situation.

Ten original peptide compounds (names and structures are shown in the Tables) were studied. All peptides were given i.p. at doses of 300 μg/kg, which is optimal for the neurotropic actions of the widely studied heptapeptide Selank, allowing comparison of the effects of synthetic peptides as individual compounds. Peptides were given in a volume of 0.2 ml for mice and 0.4 ml for rats. Controls received the same volume of physiological saline. Tests were performed at 30 min.

Data were analyzed statistically using Student's t test and the non-parametric Wilcoxon–Mann–Whitney U test for independent sets. Differences were assessed as significant at $p < 0.01$ and $p < 0.05$.

RESULTS AND DISCUSSION

There are good grounds for regarding the behavioral response to the conflict situation and aversive treatments as

TABLE 1. The Effects of Peptide Compounds Based on Tuftsin and Selank on the Behavior of High-Anxiety Mice of Line Balb/c with the "Passive" Type of Emotional Stress Response in the Open Field

No.	Formula and name of peptide	Movement activity in open field			
		peripheral	central	vertical	total
0	Control (physiological saline)	29.4 ± 13.0	0.35 ± 0.10	0.54 ± 0.15	31.79 ± 12.66
1	Thy-Lys-Pro-Arg, tetrapeptide, Tuftsin	35.44 ± 5.7*	1.5 ± 0.8	0.3 ± 0.7	37.18 ± 4.80
2	Thr-Lys-Pro-D-Arg, D-Arg^4-Tuftsin	20.00 ± 3.34	1.05 ± 0.10	0.14 ± 0.05	21.64 ± 3.40
3	Thr-Lys-Pro-Arg-Pro-Gly-Pro, heptapeptide, Selank	50.13 ± 7.25*	2.33 ± 1.03**	0.46 ± 0.16	52.92 ± 8.33*
4	Thr-Lys-Pro-Arg-Phe-Pro-Gly-Pro, octapeptide	84.42 ± 2.87*	5.71 ± 1.40*	0.85 ± 0.60	93.62 ± 8.00*
5	Thr-Lys-Pro-Arg-Pro-Gly, hexapeptide	27.7 ± 6.5	1.50 ± 0.51	3.50 ± 0.04*	32.70 ± 2.35
6	Lys-Pro-Arg-Pro-Gly-Pro, hexapeptide	55.00 ± 0.85*	0.8 ± 0.4	1.30 ± 0.12	55.82 ± 3.0*
7	Glu-His-Phe-Pro-Gly-Pro, hexapeptide	25.57 ± 19.30	1.71 ± 0.15	0.14 ± 0.09	26.85 ± 13.30
8	Pro-Arg-Pro-Gly-Pro, pentapeptide	24.8 ± 13.4	1.08 ± 0.90	2.02 ± 0.60	27.9 ± 5.8
9	Arg-Pro-Gly-Pro, tetrapeptide	25.44 ± 5.50	0.0	0.33 ± 0.09	25.77 ± 3.90
10	Thr-Lys, dipeptide	81.18 ± 3.34**	6.4 ± 1.7*	2.0 ± 0.8	87.98 ± 3.80**

Notes. Here and in Tables 2 and 3: numbers in columns show mean values for the group; the number of animals in each group was $n = 10$; control values are the mean for all groups. *$p < 0.05$; **$p < 0.01$.

TABLE 2. The Effects of Peptide Compounds Based on Tuftsin and Selank on the Behavior of Mice of Line C57BL/6 with the "Active" Type of Emotional Stress Response in the Open Field

No.	Formula and name of peptide	Movement activity in open field			
		peripheral	central	vertical	total
0	Control (physiological saline)	86.5 ± 5.5	30.5 ± 8.4	24.4 ± 6.1	157.98 ± 22.20
1	Thy-Lys-Pro-Arg, tetrapeptide, Tuftsin	71.8 ± 5.0	38.5 ± 3.1	20.8 ± 9.4	131.15 ± 11.50
2	Thr-Lys-Pro-D-Arg, D-Arg^4-Tuftsin	68.5 ± 2.5	18.50 ± 0.49	18.8 ± 5.0	105.80 ± 7.09*
3	Thr-Lys-Pro-Arg-Pro-Gly-Pro, heptapeptide, Selank	88.4 ± 15.0	29.15 ± 9.00	28.4 ± 8.0	145.95 ± 9.60
4	Thr-Lys-Pro-Arg-Phe-Pro-Gly-Pro, octapeptide	88.7 ± 9.4	28.6 ± 1.8	24.8 ± 5.0	142.1 ± 16.2
5	Thr-Lys-Pro-Arg-Pro-Gly, hexapeptide	78.6 ± 5.6	25.4 ± 2.3	18.3 ± 9.5	122.3 ± 17.4
6	Lys-Pro-Arg-Pro-Gly-Pro, hexapeptide	98.3 ± 5.9	28.3 ± 13.0	18.8 ± 8.0	145.4 ± 26.9
7	Glu-His-Phe-Pro-Gly-Pro, hexapeptide	78.3 ± 9.4	24.3 ± 3.3	15.6 ± 9.1	118.2 ± 21.8
8	Pro-Arg-Pro-Gly-Pro, pentapeptide	69.7 ± 10.0	24.8 ± 8.0	23.8 ± 5.3	118.3 ± 23.3
9	Arg-Pro-Gly-Pro, tetrapeptide	78.40 ± 7.15	20.0 ± 6.8	20.4 ± 6.9	118.80 ± 20.85
10	Thr-Lys, dipeptide	87.45 ± 6.1	31.8 ± 6.0	20.8 ± 1.7	146.05 ± 14.80*

part of the overall stress response of the body, formed on the basis of an emotionally negative state, clear indications of which are fear and anxiety. The behavioral ESR of the animal to a stress-inducing situation is to a considerable extent determined genetically and is associated with the individual typological characteristics [4, 7, 15, 21]. The open field is a widely used model reproducing emotional stress in studies with small laboratory animals (mice, rats). In this experimental situation, the ESR in rodents is formed on the basis of a conflict between the innate need to investigate territory and the tendency to avoid the danger of the "frightening" brightly illuminated open unfamiliar space. Our studies were performed on mice with inheritance-dependent forms of ESR: the "passive," associated with a series of behavioral and biochemical measures with high levels of anxiety and fear (Balb/c mice), and "active" (C57BL/6 mice) forms of ESR in the open field. The overall results of these experiments are presented in Tables 1 and 2. These experiments demonstrated that the study peptide compounds including the N-terminal fragment Thr-Lys had different levels of ability to block the open field parameters typical of Balb/c mice: the freezing reaction did not appear, total movement activity generally increased, and there were increases in the numbers of excursions to the most "dangerous" central

TABLE 3. The Effects of Peptide Compounds Based on Tuftsin and Selank on the Behavior of Mice in the Porsholt Forced Swimming Test

Control, formula, and name of peptide	Porsholt forced swimming measures		
	duration of immobility, sec	episodes of immobility	latent period of first episode of immobility, sec
Physiological saline,	489 ± 36	14.8 ± 1.7	99.3 ± 7.4
Thy-Lys-Pro-Arg (tuftsin)	406 ± 20*↓	14.1 ± 2.4	120.0 ± 9.5↑
Physiological saline,	342.0 ± 26.0	11.14 ± 3.5	77.5 ± 4.9
Thy-Lys-Pro-D-Arg (D-Arg⁴-tuftsin)	391.1 ± 40.4*↑	10.25 ± 2.00	72.77 ± 8.4↓
Physiological saline,	514.1 ± 9.8	17.9 ± 1.6	85.3 ± 10.3
Thr-Lys-Pro-Arg-Pro-Gly-Pro (Selank)	382.1 ± 33.0*↓	13.1 ± 2.1*↓	100.3 ± 9.0*↑
Physiological saline,	436.0 ± 42.1	20.1 ± 2.2	89.3 ± 10.3
Lys-Pro-Arg-Pro-Gly-Pro (hexapeptide)	376.90 ± 27.08*↓	17.7 ± 2.9↓	117.3 ± 9.9*↑
Physiological saline,	482.30 ± 35.09	13.3 ± 2.9	103.2 ± 17.1
Pro-Arg-Pro-Gly-Pro (pentapeptide)	380.0 ± 20.7*↓	14.0 ± 2.7	134.1 ± 15.0*↑
Physiological saline,	444.0 ± 45.0	18.1 ± 1.8	105.3 ± 11.2
Arg-Pro-Gly-Pro (tetrapeptide)	363.0 ± 34.0*↓	16.3 ± 0.9↓	198.2 ± 9.3*↑
Physiological saline,	445.5 ± 38.1	23.5 ± 3.7	95.2 ± 10.5
Pro-Gly-Pro (tripeptide)	416.00 ± 49.08	19.2 ± 2.0	173.1 ± 13.8*↑
Physiological saline,	412.2 ± 42.0	14.2 ± 2.1	118.4 ± 9.9
Pro-Arg (dipeptide)	395.5 ± 15.0*↓	14.4 ± 1.9	114.3 ± 12.1↓
Physiological saline,	440.0 ± 20.8	16.3 ± 3.8	105.0 ± 10.9
Pro-Arg-Gly-Pro (tetrapeptide)	306.3 ± 34.1*↓	14.3 ± 0.9↓	98.1 ± 9.3↓

zones of the open field and the numbers of vertical rearings (Table 1).

The behaviorally "passive" type of ESR was replaced by orientational-investigative activity directed to seeking active release from the experimental stress situation. The same compounds had no significant effect on the behavior of mice with the initially "active" type of ESR in the open field (Table 2). Previously obtained data have demonstrated that the magnitudes of the anti-anxiety effects of peptides were dose-dependent, without the appearance of sedation and inhibition [10, 15]. Removal of the threonine (Thr) residue from the N terminus of the molecule, keeping the lysine (Lys) residue, gave hexapeptide Lyz-Pro-Arg-Pro-Gly-Pro, in which the magnitude of the anti-anxiety action on the behavior of Balb/c mice was decreased without any significant reduction in the activating effect on total movement activity.

At high levels of peripheral activity, the numbers of excursions to the central "dangerous" zones and vertical rearings both decreased. Rapid movements of the rodents in the periphery of the open field combined with low numbers of excursions to the central zones and vertical rearings are characteristic for the active form of anxiety in the stress situation. In this regard, the effects observed here may be associated with decreases in the magnitude of the anti-anxiety action without loss of the overall activating effect.

Introduction of phenylalanine in position 5 (Thr-Lys-Pro-Arg-Phe-Pro-Gly-Pro) was accompanied by activation of behavior with no increase in the number of vertical rearings (values were 2.9, 16.3, and 1.6) (Table 1). Attention is drawn to the fact that administration of the shortest peptide (Thr-Lys) to mice, this peptide being present in all the other compounds with significant activating and various levels of anti-anxiety activities listed above, was also accompanied by an anti-anxiety effect. However, the dipeptide, lacking the stabilizing Pro-Gly-Pro group, was unstable to degradation. The absence of the Thr-Lys fragment from study peptides led to reduction or loss of anti-anxiety activity. These data appear to be of great importance, because they open the pathway to creating peptide compounds with a predominance of anti-anxiety or, conversely, stimulating actions on behavior in a stress situation.

The Porsholt forced swimming test (unavoidable stress) required the use of white laboratory mice [24]. This test has been proposed by a series of authors as a model of an anxiety state and/or depression ("despair behavior") arising as a result of the impossibility of realizing the motivation to escape a situation threatening the animal's life. In accordance with the method, the ratio of the active swimming time in the closed space and the immobility time (immobilization, withdrawal from activity) is regarded as reflecting the ratio of excitation to anxiety [14, 22, 25]. The results obtained from thee experiments are shown in Table 3. Most of the study peptides had some degree of activating effect on mouse behavior in the swimming test. Attention is drawn to

TABLE 4. Comparative Effects of Peptides of the Tuftsin Family and Selank on the Escape Behavior of Rats from an Acute Stress Situation (the "inactive" group)

Structure and name of peptide	LP of movement response, sec	LP of escape from the acute stress situation, sec	Number of erroneous escape attempts	Level of affective responses, units
Control – physiological saline	2.5 ± 1.3	None	24.1 ± 4.2	9.97 ± 2.9
Thr-Lys-Pro-Arg, tuftsin	3.9 ± 1.6	29.7 ± 5.5*↓	10.5 ± 2.9*↓	3.1 ± 1.1*↓
Thr-Lys-Pro-Arg-Pro-Gly-Pro, heptapeptide	3.8 ± 1.0*	21.2 ± 5.3*↓	6.1 ± 1.4*↓	4.2 ± 2.1*↓
Lys-Pro-Arg-Pro-Gly-Pro, hexapeptide	2.2 ± 0.9	None	29.9 ± 5.8	14.2 ± 3.9
Pro-Arg-Pro-Gly-Pro, pentapeptide	4.2 ± 2.2	28.7 ± 7.4*↓	5.9 ± 1.7*↓	3.3 ± 1.5*↓
Arg-Pro-Gly-Pro, tetrapeptide	3.8 ± 1.5	17.3 ± 4.6*↓	9.8 ± 3.1*↓	2.7 ± 0.9*↓
Pro-Gly-Pro, tripeptide	1.9 ± 0.5	None	36.6 ± 6.8	18.5 ± 4.5
Pro-Arg, dipeptide	5.9 ± 3.3	None	10.8 ± 2.2*↓	2.1 ± 1.8*↓

Notes (here and Table 5). LP = latent period; the number of animals in each group was $n = 10$; control values are means for all groups. None = no performances during the 5-min observation period. *$p < 0.05$.

the fact that peptide compounds whose central or N-terminal regions contained the Pro-Arg fragment, which is very stable to the actions of tissue peptidases (proline peptidases), as well as the Pro-Arg fragment itself, significantly ($p < 0.05$) decreased the emotionally negative effects of this stress situation on the behavior of the mice. These compounds included tufstin, the heptapeptide, the hexapeptide, and the pentapeptide, as well as the tetrapeptide and the dipeptide Pro-Arg itself. Administration of these peptides increased the duration of active swimming and decreased the numbers of cessation of (episodes of immobility) and withdrawals from activity. Compound D-Arg^4-tuftsin had no statistically significant effect on mouse behavior in this test, which may partly be associated with the initially high level of activity in controls (which, however, was not beyond the significant range for controls). It should be noted that the increase in the time to the first episode of immobility (withdrawal from activity) was significantly greater after administration of peptides containing the stabilizing C-terminal Pro-Gly-Pro group along with the Pro-Arg group. The time to the first withdrawal from activity increased significantly with these peptides, by an average factor of 1.2–1.3. The most characteristic features in this test were the behavior of mice given the hexapeptide Lys-Pro-Arg-Pro-Gly-Pro. The decrease in the total duration of immobility and the increase in the duration of active swimming ($p < 0.05$) were accompanied by a sharp increase in general movement activity during the first few minutes of the experiment. The swimming period was also accompanied by jumps and vocalizations. The only property of tripeptide Pro-Gly-Pro was to increase the latent period of the first episode of immobility; it had no effects on any other parameter. Conversely, dipeptide Pro-Arg decreased the duration of immobility without affecting the latent period of the first episode of immobility.

Both antidepressants and tranquillizer-anxiolytics are known to have similar actions on the behavior of mice in the Porsholt test [14, 22]. The results presented above can thus be regarded as evidence not only for the appearance of anti-stress properties for the study peptides, with increases in the motivation to avoid the aversive situation, but also as evidence that they have insignificant levels of antidepressant activity.

The effects of peptides on adaptive behavior, associated with the possibility of performing active escape from the stress situation, were studied using the widely known Henderson diving test. This test requires rats (Wistar rats) and yields data characterizing the effects of peptides on extrapolatory escape behavior based on the simplest activity and allows these data to be compared with results obtained by other authors using the same method.

Physiological, clinical, and pharmacological profiling has constantly emphasized that the emotional stress response of a subject (as in animal experiments) in a uniform conflict situation is to a large extent determined by the type of emotional-behavioral reactivity [7, 13, 16]. Studies were performed in rats previously divided into two groups on the basis of their behavior in the open field situation. One group consisted of animals characterized by high levels of movement and investigative activity in the open field (total square crossings per 4 min amounted to 80.2 ± 10.8). The other group consisted of animals with freezing responses and low levels of movement activity in the open field (total square crossings 48.8 ± 5.1). The effects of study peptides on measures of adaptive behavior were compared in these two groups in terms of their ability to escape the aversive stimulus by diving and solving the extrapolatory task on the basis of the simplest assessed activity.

Rats with low levels of activity in the open field, when placed in this stress situation, responded with excitation and vocalization and performed numerous erroneous actions in attempting to escape (this was the "inactive" group, with a poor ability to resolve the task). Over an observation period of 2 min, these animals were unable to evaluate the parameters of the experimental situation appropriately and cor-

TABLE 5. Comparative Effects of Peptides of the Tuftsin Family and Selank on the Escape Behavior of Rats from an Acute Stress Situation (the "active" group)

Structure and name of peptide	LP of movement response, sec	LP of escape from the acute stress situation, sec	Number of erroneous escape attempts	Level of affective responses, units
Control – physiological saline	5.80 ± 2.08	22.57 ± 5.10	3.68 ± 1.60	None
Thr-Lys-Pro-Arg, tuftsin	3.1 ± 1.4↓	10.6 ± 3.1↓	None	None
Thr-Lys-Pro-Arg-Pro-Gly-Pro, heptapeptide	5.0 ± 1.2	9.7 ± 2.6*↓	None	None
Lys-Pro-Arg-Pro-Gly-Pro, hexapeptide	4.8 ± 1.5	16.2 ± 5.0	7.5 ± 0.8*↑	None
Pro-Arg-Pro-Gly-Pro, pentapeptide	6.1 ± 0.9	14.2 ± 4.0*↓	None	None
Arg-Pro-Gly-Pro, tetrapeptide	5.9 ± 2.1	9.1 ± 2.3*↓	None	None
Pro-Gly-Pro, tripeptide	4.1 ± 1.3	30.5 ± 7.4	9.4 ± 1.5*↑	3.4 ± 1.2*↑
Pro-Arg, dipeptide	12.8 ± 5.7	40.1 ± 9.4*↑	None	None

rectly or to extrapolate a route for escape (Table 4). In this group, all peptide compounds used in this study (except hexapeptide Lys-Pro-Arg-Pro-Gly-Pro and tripeptide Pro-Gly-Pro) decreased the level of affective features, decreased the number of correct attempts to escape, and, after a short series of attempts to jump out of the cylinder, animals made successful attempts to escape by entering the water and diving under the bottom edge of the cylinder. The most effective compounds in this test were heptapeptide Thr-Lys-Pro-Arg-Pro-Gly-Pro and pentapeptide Pro-Arg-Pro-Gly-Pro. Administration of these compounds was followed by a six-fold decrease in the number of erroneous actions ($p < 0.05$) and an average three-fold reduction in the level of affective changes ($p < 0.05$). The hexa- and tripeptides identified above increased emotional reactivity, which prevented the animals from detecting a route of escape.

Rats with high levels of movement activity in the open field in this same experimental situation responded with clear orientational responses directed to seeking an escape route, after which the rats successfully found the route to escape from the stress situation, diving beneath the wall of the cylinder and reaching the edge of the basin ("active" group, task-solving). In this group of animals, all peptides except hexapeptide Lys-Pro-Arg-Pro-Gly-Pro and tripeptide Pro-Gly-Pro completely blocked the observed errors in behavior and affective responses, decreasing the latent period of the directed escape response (Table 5). Administration of the hexapeptide containing the N-terminal residue not protected by lysine increased the number of unsuccessful attempts and movement responses in the cylinder. However, this activation did not led to optimization of escape, which appeared to be extreme.

In general, the test compounds can be divided into two groups depending on the direction of their effects on the animals' behavior in this test. The first group included hexapeptide Lys-Pro-Arg-Pro-Gly-Pro, tripeptide Pro-Gly-Pro, and dipeptide Pro-Arg, which had no optimizing action on the ability of the animals to escape from the stress situation. The second group included heptapeptide Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), pentapeptide Pro-Arg-Pro-Gly-Pro, tetrapeptide Arg-Pro-Gly-Pro, and tuftsin (Thr-Lys-Pro-Arg), which had significant and marked optimizing actions on the ability of the animals to identify an escape route from the stress situation. Far from every change in emotional reactivity produced an optimizing effect on the ability to extrapolate. Thus, dipeptide Pro-Arg, which significantly decreased the affective response to stress, did not facilitate performance of the escape behavior. Addition of the amino acid sequence Pro-Gly-Pro to the C terminal of tuftsin (to make the heptapeptide Selank) led to an increase in the activity of the new peptide as compared with tuftsin, while tripeptide Pro-Gly-Pro itself had no positive effect in this test, though it did increase emotional reactivity, which hindered solution of the extrapolatory task.

Thus, the data obtained here can be interpreted not only in terms of the peptides having emotiotropic effects with positive effects on the behavior of animals in a model stress situation, but also in terms of the properties of individual peptides to optimize extrapolatory activity, at least in the experimental situation described above using this population of rats.

A further series of experiments was performed to study the effects of peptides on orientational-investigative responses (OIR), which to a large extent determine subsequent behavioral activity [1, 7]. Studies were performed using the Éra-1 automated apparatus developed for assessment of orientational-investigative reactions. Overall data are presented in Table 6. Heptapeptide Selank, the tetrapeptide, and the hexapeptide had the most marked and significant activatory effects on orientational-investigative responses in rats. Animals given the heptapeptide demonstrated the largest increases in all measures of movement (horizontal, vertical) and investigative (hole investigations) activities. The number of vertical rearings increased two-fold and the number of hole investigations increased three-fold compared with the control group ($p < 0.05$). The other peptides had less marked effects. The tetrapeptides Pro-Arg-Pro-Gly and Thy-Lys-Pro-D-Arg (D-Arg-tuftsin) led to decreases in all measures.

TABLE 6. The Effects of Peptide Fragments in the Molecule of the Heptapeptide Selank on Measures of Orientational-Investigative Responses of Rats in an Unfamiliar Situation with One Placing in the Éra-1 Apparatus

Amino acid sequence	Peripheral activity	Vertical activity	Number of opening investigations	Quantity of grooming
Thy-Lys-Pro-Arg, tuftsin	+62.1↑	+14.7*↑	+19.8*↑	–0.3≈
Thr-Lys-Pro-D-Arg, D-Arg^4-tuftsin	–44.1↓	–2.1*↓	–3.2↓	–3.0↓
Thr-Lys-Pro-Arg-Pro-Gly-Pro, heptapeptide, Selank	+63.4*↑	+10.2*↑	+23.0*↑	+0.9↑
Lys-Pro-Arg-Pro-Gly-Pro, hexapeptide	+192.0*↑↑	+19.2*↑	+34.0*↑↑	+1.4*↑↑
Pro-Arg-Pro-Gly-Pro, pentapeptide	+25.8≈	+14.1≈	+23.2*↑	+0.3≈
Pro-Arg-Pro-Gly, tetrapeptide	–114.3*↓↓	–2.4↓	+3.7≈	–2.0*↓
Pro-Gly-Pro, tripeptide	+30.9≈	–2.3↓	+7.7≈	–1.1↓
Pro-Arg, dipeptide	+86.4≈	+15.4≈	+24.9≈	–3.0↓

Notes. In columns, "+" indicates increases and "–" indicates decreases in parameters compared with controls; arrows show the overall direction of the effect, while the number of arrows shows the extent of effects; ≈ indicates no clear effect with a large spread of data points; the number of animals in each group was $n = 11$. *$p < 0.05$; the heptapeptide Selank was used at a standard dose of 300 μg/kg.

Repeated placing of animals in the same situation (4-h observation) after treatment with the hexapeptide and the heptapeptide (but not tuftsin) showed significantly greater extinction of orientational-investigative responses than in control rats as determined by behavioral measures.

Thus, use of quite simple models suitable for reproducing stress situations excluding any harmful influences from other factors allowed us to compare the actions of a large group of peptides of the tuftsin family and Selank (their derivatives and/or fragments, consisting of peptides produced synthetically by cleavage of individual amino acids from the N or C terminals of the heptapeptide Selank). The results reflect the directions of the isolated actions of these peptides as independent structures on the behavior of animals in these tests, allowing their effects to be compared after administration of doses of 300 μg/kg without consideration of the molar doses needed for analysis of the contributions of individual fragments to the effects of the whole Selank molecule.

Individual peptides were found to produce differently directed changes and sets of changes in the behavioral emotional stress responses and in the optimizing effects on the appearance of adaptive behavior in the animals directed to achieving a useful result – escape from the stress situation. The results show that not every decrease (or increase) in emotional reactivity in stress-inducing conditions optimized solution of the extrapolatory task (escape) even in animals which were initially able to solve the task (Tables 4 and 5).

The most universal positive effects in model situations were seen with the heptapeptide Selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro) and the pentapeptide Pro-Arg-Pro-Gly-Pro, which had the most marked anti-anxiety and activating actions on the behavior of animals in the stress situation (Table 1). These peptides also increased measures of orientational-investigative responses, reflecting overall readiness for active behavior (Table 6).

Peptides with the most marked depriming (sedative) actions (dipeptide Pro-Arg, tetrapeptide D-Arg^4-tuftsin) decreased measures of orientational-investigative responses but did not facilitate the achievement of a useful result of an action in complicated emotionally stressful conditions (extrapolatory escape), though they had anti-stress effects as identified from decreases in emotional reactivity.

Comparison of the "structure-activity" measure among the study peptides led to the suggestion that the activating influences on the behavior of animals with the "passive" form of ESR and on measures of orientational-investigative responses may be associated with the present of the lysine (Lys) residue on the N terminal of the molecule and the Pro-Gly-Pro group on the C terminal. Peptides containing the Thr-Lys group at the N terminal and the Pro-Arg fragment within their structures had opposite actions. The group in which it was most difficult to follow "structure-activity" relationships consisted of peptides with positive effects on behavioral measures associated with extrapolatory activity in the stress situation.

Comparison of "structure-activity" relationships has potential for improving our understanding of the mechanisms of endogenous self-regulation and resistance to emotional stress and for identifying the most appropriate exogenous influences on the mechanisms of adaptation when these are weakened in the situation of extreme emotional stress.

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