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ANIMAL AND HUMAN PHYSIOLOGY

Physiological Effects of Selank and Its Fragments

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Abstract—The development of Selank based on the natural regulatory peptide tuftsin allowed combining the native bioactivity of the molecule with the design engineering of certain physiological functions. Based on a summary of the experimental and clinical data, a comparative analysis of the structural and functional regularities of the effects of Selank and its fragments was performed. The qualitative and quantitative characteristics of correctional processes (anxiolytic, cognitive, immunostimulatory, anti-ulcer, etc.), triggered by the peptide drug and its analogues, are summarized into a single scheme. It is assumed that the Selank effect on the network of cascade processes of peptides and mediators is carried out through enkephalin, the key player of this system.

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MOLECULAR ASPECTS

Regulatory peptides (RPs) and their fragments are effective programs for control of various physiological processes of organisms. All of them form the top floor of the hierarchy of regulatory elements and serve as the basis for intercellular interactions of various modalities. On the one hand, each peptide is characterized by a unique combination of physiological effects, and on the other hand, it has a spectrum of bioactivities overlapping with other RPs. Cumulatively, the totality of RPs form an effective functional continuum. The use of the objects of this system for therapeutic purposes allows one to develop drugs, the action of which is as close as possible to the action of natural control mechanisms with minimal side effects. However, the pronounced polyfunctionality of oligopeptides can cause a number of problems when used as drugs. That is why it is especially important to determine the minimum amino acid sequence of a RP responsible for certain physiological functions (pharmacophore). Such mapping of peptide chains is extremely necessary for the development of new peptides with a specific physiological profile. In addition, it is known that intense proteolysis, to which peptides are susceptible, sharply limits the time interval for the drug to function. Therefore, the task of prolonging the action of RPs is very relevant. There is a need to protect important functional sites of the oligopeptide with specific amino acid sequences without introduction of additional sites for endopeptidases to the molecule.

One of the starting points and reference models of peptide engineering is Thr–Lys–Pro–Arg tetrapeptide, tuftsin. It is a peripheral regulator of immunity, which represents a short fragment of the human immunoglobulin G heavy chain. The peptide drug Selank was developed on the basis of this endogenous regulator. The Pro–Gly–Pro combination was chosen as a protective sequence from the tuftsin C-terminal. On the one hand, this tripeptide is a protective group against proteases, which allows prolonging the action of the peptide several times. On the other hand, such a tripeptide itself performs a number of additional positive functions. Moreover, it is known that such endogenous regulators in the form of glyprolines, products of collagen and elastin metabolism, are continuously formed in the body. All this allows us to enhance the Selank nativeness. Thus, the Selank sequence represents the Thr–Lys–Pro–Arg–Pro–Gly–Pro heptapeptide, which has a much more prolonged effect compared with its natural analog.

The aim of this work is to study all currently known pharmacophores in the molecules of tuftsin and Selank, which are responsible for the multiple central and peripheral effects of the peptides. All these functional activities of the drugs were tested in various laboratory and clinical studies.

EFFECTS ON THE NERVOUS SYSTEM

Regulation of anxiety. In the 21st century, a century of social problems and overloading of information channels, the problem of regulating anxiety comes out on top. It has been shown that anxiety states are under multilevel control of various systems of the body regulation. Many neurotransmitters, as well as various neurohumoral processes, form a complex branching network of trigger mechanisms and their counterbalances. The use of drugs of a synthetic profile causes a

harsh disturbance of the entire fine-tuning of molecular mechanisms. Benzodiazepine tranquilizers are most often used in medical practice. Along with the achievement of the target function, they cause a number of undesirable side effects, such as muscle relaxation, hypnosedative effect, addiction, withdrawal syndrome, and amnesia. Antidepressants and drugs of other groups, prescribed for relief of anxiety in combination with depression, trigger side processes caused by cholinergic, adrenergic, and serotonergic mechanisms.

The search for and development of drugs, on the one hand, that are as close as possible to natural molecular agents, and on the other hand, that have a rather narrow and selective anxiolytic profile simultaneously with various targets of action, is one of the most important tasks in pharmacology. The obvious advantages of pharmacological drugs of peptide nature include the following: firstly, their low toxicity, since they are metabolized to amino acids; secondly, the fact that they carry a replica from endogenous oligopeptide regulators already created by nature and optimized for a complex branching regulation network of the central nervous system status. Thus, the negative effect of a harsh penetration into the endogenous sphere of homeostasis disappears. The discovery of the anxiety reduction effect in tuftsin determined the development of a direction associated with the development of Selank as an anxiolytic drug, its testing, and testing of its analog fragments in preclinical and clinical trials.

Patients with various forms of anxiety-phobic disorders of the neurotic level were examined (Zozulya et al., 2001). The authors of the study showed that Selank reduces behavioral manifestations of anxiety and does not cause side effects typical for most common anxiolytics. As a result of a preliminary analysis of patients (prior to the use of Selank), a pronounced decrease in the half-life of enkephalins and in the total enkephalinase activity in the blood plasma of patients was found. However, this effect was observed in patients with generalized anxiety disorders (GAD), but was absent in patients with panic disorders. It was suggested that this was due to the relatively low concentration of endogenous inhibitors of enkephalin-degrading enzymes in the blood of patients with GAD.

The high activity of enzymes degrading endogenous opioid peptides determines the rapid breakdown of the latter (for enkephalins, the half-life in the blood is 1–2 min). Serum enkephalin-degrading enzymes include aminopeptidases, which provide 70% of the total enkephalinase activity, and diamino- and dicarboxypeptidases, such as endopeptidase 24-11 (enkephalinase B) and angiotensin-converting enzyme, or enkephalinase A, more specific to enkephalins.

At the same time, it was found that Selank dose-dependently inhibits the rate of enzymatic hydrolysis of enkephalin in the blood serum. The concentration of the peptide at which a 50% inhibition of the enzymes is observed was 15 μM. In addition, the effectiveness of Selank in inhibiting enkephalinases was significantly more pronounced than for the known peptidase inhibitors, bacitracin and puromycin. In addition to the Selank heptapeptide itself, its pentapeptide fragments also possessed inhibitory ability (Kost et al., 2001). However, tri-, tetra-, and hexapeptide fragments did not cause this effect.

Analyzing the mechanisms of enkephalinase inhibition, it can be assumed that Selank interacts with serum enkephalin-degrading enzymes, serving like enkephalins as their substrate. Indirect evidence of this is the data that the half-life of Selank in the blood is only a few minutes, like that of enkephalins.

Clinical comparative studies were conducted in patients with GAD and neurasthenia, where medazepam was used as a reference drug (Zozulya et al., 2008). As a result of the investigation, it turned out that the anxiolytic effect of Selank is comparable to the action of medazepam. In addition, it is worth noting the important and beneficial side effects of this heptapeptide. Thus, as a result of treatment with Selank, antiasthenic and psychostimulatory effects on the body were detected. In these studies, clinical and biological analysis of the level of enkephalins in the blood serum was also carried out. It was found that in patients with GAD and neurasthenia there is a reduced content of leu-enkephalin, which correlates with the duration of the disease and the severity of a number of symptoms that reflect manifestations of anxiety, asthenia, and vegetative disorders. The clinical efficiency of Selank was compared with the characteristics of its effect on the rate of leu-enkephalin hydrolysis in the blood serum of patients. The studies showed that the enkephalin content increased under treatment with Selank (mainly in patients with GAD). Based on the data obtained, it can be assumed that the effect of Selank is associated with activation of the opioid system and enhancement of its protective role under an increased level of anxiety. No similar dependence was found from treatment with medazepam.

GABA-ergic system. The normal functioning of the central nervous system is based on the optimal balance of the excitatory and inhibitory systems. The disturbance of this balance leads to the development of pathological conditions. The regulation and therapy of such diseases necessitate detailed study of both systems.

The pronounced anxiolytic effect of Selank prompted us to perform studies on the interaction of the drug with the inhibitory system, the main molecular agent of which is gamma-aminobutyric acid (GABA). It is known that GABA causes a calming effect, reduces excitation, reduces the heart rate, has mild hypotensive as well as antihypoxic and anticonvulsant effects, increases the productivity of thinking, has a positive effect on the recovery of movements and speech, and improves memory. Currently, there are many allosteric modulators of $GABA_A$ receptors.

However, the molecular mechanisms underlying such interactions are not yet fully understood. The most studied allosteric modulators of $GABA_A$ receptors include benzodiazepines, which are widely used in medical practice, but have a number of undesirable side effects. Thus, the need to test the usefulness and effectiveness of peptide drugs with minimal side effects becomes obvious.

Selank and its Arg–Pro–Gly–Pro fragment were studied to determine the formation of direct and indirect changes in the main parameters of specific GABA ligand-receptor interactions on the plasma membranes of brain nerve cells (V'yunova et al., 2014). The presence of these peptides was found to alter the amount of the specifically bound [^{3}H]GABA ligand. Preliminary intranasal introduction of Selank also caused changes in the number of specific [^{3}H]GABA binding sites, but did not affect the receptor affinity. Preliminary intranasal administration of the Arg–Pro–Gly–Pro fragment caused more complex delayed effects. Thus, the effects of peptides on cellular regulatory mechanisms unrelated to the ligand-receptor interaction were shown.

Dopaminergic system. In previous studies, it was found that Selank, which has a pronounced anxiolytic effect, does not have negative side effects (sedative and muscle relaxant) that are typical of benzodiazepine tranquilizers. Moreover, it was shown that the tranquilo-activating effect is typical for Selank, mechanisms of which are still little studied. To clarify the situation on the ability of Selank to change the state of the dopaminergic system, a corresponding study was conducted (Meshavkin et al., 2006). In this work, the effect of the peptide on behavioral manifestations of the hyperactive dopaminergic system induced by the administration of apomorphine was studied. As a result of the analysis, it was revealed that intraperitoneal Selank (0.01, 0.1, 1, and 10 mg/kg) reduced apomorphine-induced behavioral manifestations of the dopamine system hyperfunction in the verticalization test in mice. The reference drug in these studies was the antipsychotic olanzapine, administered in doses comparable to therapeutic (0.1 and 1 mg/kg intraperitoneal). It turned out that the effect of Selank is comparable to the effect of olanzapine. Moreover, it was shown that the effect of Selank is blocked by the nonselective opioid receptor antagonist naloxone. Radioreceptor analysis did not detect in Selank the ability to displace the nonselective antagonist of D_2-dopamine receptors, ^{3}H-spiperone, and the ligand of δ- and μ-opioid receptors, ^{3}H–DADLE, from specific binding sites on membranes of the rat brain. Thus, on the basis of the data obtained, it was assumed that the detected behavioral effect of Selank is mediated by its modulating effect on the endogenous opioid system.

Processes of learning and memory formation. The assessment of the peptide effect on cognitive processes is of particular interest. Thus, a comparative study of the original peptide, tuftsin, Selank and their fragments, formed during degradation, on processes of learning and memory was carried out (Kozlovskaya et al., 2001, 2002). It is known that tuftsin undergoes degradation under the influence of tissue peptidases, aminopeptidase N, dipeptidyl aminopeptidase IV, prolyl peptidase, or prolyl endopeptidase, which enters the bloodstream as a result of partial hemolysis of erythrocytes under stressing effects. In addition, it was shown that the primary degradation of Selank can begin at the C-terminal region of the molecule by elimination of the Pro–Gly–Pro group, undergoing further hydrolysis with the formation of short fragments. Thus, during degradation of the molecule, a sufficiently large spectrum of peptide fragments of various lengths can be detected. The identification of pharmacophores responsible for mnestic functions is of particular interest for the design of cognitive peptide drugs. To assess the influence of molecules on the processes of learning and memory, the method of developing conditional passive avoidance response (CPAR) was used in the study.

It was found that tuftsin showed only a tendency to increase the ability of animals to reproduce the recorded learning indicators. At the same time, Selank itself had a pronounced, stable, and prolonged positive influence on the same indicators. All these data indicate improved memorization of the experimental situation, as well as preservation of the developed reaction of electrical irritation avoidance.

Evaluation of the effects of peptide fragments resulting from the elimination of individual amino acids from the C- and N-terminus of their molecules, allowed us to build a certain map of the correspondence between the position of the residue of a certain amino acid and the effectiveness of the effect on the CPAR indicators.

Thus, a relative increase in the amount of proline in the peptide molecules led to a positive effect of the compound effect on CPAR characteristics. The Pro–Arg–Pro–Gly–Pro pentapeptide, the molecule of which contains a relatively large number of proline fragments as compared to the other peptides studied, as well as the Pro–Gly–Pro tripeptide, had a pronounced optimizing effect on the recorded parameters.

The content of arginine in the peptide molecules did not significantly affect the indicators of the CPAR development, if arginine was between two proline residues. However, the presence of arginine in the peptide molecule, not covered by proline, caused the deterioration of all learning indicators.

The presence of threonine in the molecule of the peptides studied did not significantly affect the rates of development and preservation of CPAR. Nevertheless, the absence of threonine at the N-terminal of the Lys–Pro–Arg–Pro–Gly–Pro hexapeptide molecule, despite the presence of three proline residues, worsened the effect of this peptide on learning when com-

paring its effect with the action of Selank containing threonine.

Thus, the pentapeptide and heptapeptide Selank had an optimal effect on the model of CPAR in a series of peptide sequences studied.

IMMUNE MODULATING EFFECT

The development of effective antiviral drugs, devoid of adverse side effects, is now a very important task. Endogenous peptides with antiviral activity are a unique model for the development of effective drugs. Since tuftsin has antiviral effects, close attention was paid to study of the antiviral drug potential of Selank and its multiple analog fragments.

The activity of Selank against the influenza virus A/Aichi2/68 (H_3N_2 strain) was studied (Ershov et al., 2009). The experiments were carried out in systems in vitro and in vivo. Recombinant interferon-α (IFN-α) at a concentration of 10^4 IU/mL was used as a reference drug. In both cases, a pronounced antiviral effect of the drug was found. Evaluation of the highest efficiency of the peptide was carried out in two modes: according to the prophylactic regimen, when the drug was added 24 h before the infection, and according to the treatment scheme, when the drug was used after the infection. Selank at a concentration of 10^{-6} M, added to the cell culture according to the prophylactic scheme, showed the greatest efficiency. Reproduction of the virus was completely suppressed. Moreover, under this scheme of administration, the antiviral activity of Selank significantly exceeded the efficiency of recombinant IFN-α.

Studies conducted in a system in vivo also showed that the highest survival rate of laboratory animals was observed with the introduction of the drug according to the prophylactic scheme. In the case of using the therapeutic scheme of Selank administration, it turned out that a concentration of the drug of 100 μg/kg was more effective than a concentration of 200 μg/kg. The cellular and molecular parameters of the immunomodulatory activity were evaluated. Thus, the use of Selank in vivo induced expression of the IFN-α gene and interleukin-12 (IL-12) (p35 and p40 subunits), without affecting expression of the genes IL-4, IL-10, and tumor necrosis factor α (TNFα).

Thus, the mechanism of antiviral action of Selank is related to its ability to modulate the Tx1/Tx2/T*reg*-cytokine balance. Previously it was shown that tuftsin realizes its immunomodulatory effect through specific receptors localized on the membrane of polymorphonuclear leukocytes, monocytes, and peripheral macrophages. The authors of that study suggest that Selank may also manifest its effect by interacting with similar receptors. In addition, the peptide effect may be mediated by its effect on the state of the CNS. Summarizing the data, one can conclude that the use of Selank enhances expression of cytokine genes that regulate the development of cell-mediated immune responses (Tx1-cytokines) and stimulate antiviral immunity (IFN-α and IFN-γ).

Analyzing the relationship between the regulatory role of cytokines and the development of mental pathology, it is worth noting that abnormalities in the cytokine balance were found in many neurological and mental diseases. For example, an increased content of the IFN-γ, IL-6, IL-1 receptor has been detected in patients suffering from depression. An elevated level of IL-10 was found in the plasma of schizophrenic patients. In addition, the relationship between mental state and susceptibility to infectious diseases is well known. Many studies have shown that depression and other mental disorders lead to the suppression of natural defense mechanisms. Moreover, even under exposure to short-term severe psychological stressors, changes in cytokine production and a decrease in the immunoreactivity resulting in the reactivation of latent viral infections are observed. It was shown that drugs used to treat mental diseases have immunoregulatory properties. For example, the introduction of clomipramine and sertraline into the culture medium affected the mitogen-stimulated secretion of IFN-γ and IL-10 significantly and in different directions. Thus, these antidepressants, increasing the secretion of IL-10 in the cell culture, affect the cytokine balance by reducing the IFN-γ/IL-10 ratio.

A study on the relationship between the immunoregulatory properties of Selank and mental diseases was conducted (Uchakina et al., 2008). In vitro use of Selank (10^{-7} M) in the culture medium completely suppressed expression of the IL-6 gene by peripheral blood leukocytes of patients with depressive disorders, but did not affect this gene expression in the blood cells of healthy donors. At the same time, a significant increase in the concentration of IL-6 itself, which was observed in the culture medium of the peripheral blood of patients cultured with Selank, was noted. Based on these data, it can be assumed that Selank triggers certain signaling mechanisms that affect the production of the IL-6 protein in the form of feedback, stimulating its excretion and at the same time blocking expression of its gene. Since elevated levels of IL-6 are usually associated with depression, blocking of IL-6 gene translation should be considered as a pronounced protective role of Selank. At the same time, IL-6 is an activator of the secretion of the anti-inflammatory cytokine IL-10, which has neuroregenerating properties, which are manifested in activation of growth factor secretion of neurons.

Studies on the relationship of the immunotropic properties of Selank and mental diseases in vivo were also conducted. Changes in the Tx1/Tx2-cytokine balance were noted in the blood serum of patients with GAD and neurasthenia treated with Selank for 14 days. The dynamics of these changes had a reliable inverse correlation. Thus, the detected cytokine-regu-

lating properties of Selank allow one to consider it an immunomodulator for use in people with anxiety-asthenic diseases. It is also important to take into account the adaptogenic properties of this peptide in the case of its use under stress-inducing environmental factors for the prevention of infectious diseases.

In the study of various functional profiles of the peptide, it is always important to identify the pharmacophore. For a detailed mapping of the peptide structures of Selank and its analogues with respect to their antiviral activities, 17 new peptides were synthesized (Andreeva et al., 2010). The structure of the Selank fragments was designed by sequential truncation of the N- and C-terminal amino acids. In addition, various sequences that are homologous to tuftsin and Pro–Gly–Pro were developed. Studies of the antiviral effect of drugs (at a concentration of 10^{-6} M) were performed both in vitro and in vivo. Three regimens for treatment with Selank and its analogues were developed: firstly, the use of the peptides before infection with the virus as a prophylactic agent; secondly, the use of the peptides simultaneously with the virus as a means of emergency prophylaxis; thirdly, the use of the peptides after the infection with the virus as a therapeutic drug. The excess of the antiviral activity of the peptides compared with the properties of the known antiviral drugs was the comparison criterion. It was found that almost all the peptides studied had pronounced antiviral activity, exceeding that of the comparison drugs. This effect was repeated at various concentrations under different times of drug administration. In addition, it was determined that the presence of proline and glycine residues at the C-terminus of the molecule provided a pronounced antiviral effect. Thus, the minimum pharmacophore fragment responsible for the pronounced antiviral effect, which is comparable with that of the comparison drugs, is the Gly–Pro dipeptide.

The study of the transcriptome response of the body to the introduction of Selank and its analogs involved in the regulation of inflammatory processes is of interest. The assessment of gene expression changes in the hippocampus and spleen in response to a single administration and a full course of Selank was performed (Kolomin et al., 2010). As a result of the study, it was found that expression of five genes changed more than two times in both modes of drug administration. Moreover, a quantitative assessment showed that, in the rat spleen, the effect of Selank on the expression of the five selected genes was more pronounced than in the hippocampus. In addition, differences in the effectiveness of two modes of drug administration were found as a result of the study. So, under a single peptide administration, the strongest increase in gene expression was observed (more than 4.5 times). During the course administration of Selank, the effect on expression was less pronounced (no more than two times). Later, the effects of Selank and two of its fragments on expression of a larger number of genes involved in inflammation processes were studied (Kolomin et al., 2011). Thus, 84 mouse spleen genes associated with molecular agents of inflammatory processes were analyzed. As a result of the study, it was revealed that 34 genes (*Bcl6* and others) involved in inflammatory processes significantly changed under intraperitoneal injection of the peptide drugs (100 μg/kg).

For a more detailed investigation of the immunomodulatory activity of Selank, a study of changes in the gene expression of chemokines and cytokines in the mouse spleen under the influence of both the whole Selank peptide and its fragments Gly–Pro, Arg–Pro–Gly–Pro, and tuftsin 6 and 24 h after a single peptide administration was performed (Kolomin et al., 2011). It was shown that all drugs can cause significant changes in gene expression of chemokines, cytokines, and their receptors in the mouse spleen in all regimens of peptide administration. Moreover, the previously found Gly–Pro pharmacophore, which has a pronounced antiviral activity, caused a change in the mRNA level of most of the genes examined.

Thus, one can conclude that Selank has pronounced immunotropic activity. The ability of this peptide to induce the secretion of interferons that protect cell cultures from the cytopathic effect of viruses allows us to hope for its effective use as an antiviral drug, as well as for aborting changes in the psychoimmune status under stress-inducing factors and for the development of adaptogenic properties of the body.

EFFECTS ON THE GASTROINTESTINAL TRACT

It is known that damage to the gastrointestinal tract occurs as a result of an imbalance of central mechanisms (stress models) and in the case of peripheral "direct" damage to the mucous membrane. The targeted search for anti-ulcer drugs that act in an integrated manner at different links of the pathological process is very relevant. In this case, the development of peptide drugs, especially proline-containing molecules, plays an important role. The effect of Selank and its three fragments, obtained by sequential cleavage of amino acid residues from the N-terminus of the molecule, was studied in various models of ulcer formation (Pavlov et al., 2004). The ability and effectiveness of ulcer healing in disorders of the gastric mucosa homeostasis were studied. In this work, three experimental models of ulceration were used: ethanol (emphasis on peripheral mechanisms of ulcer formation), water-immersion immobilization (stressing, emphasis on central mechanisms), and acetate (study of the ability to form and heal ulcers). The study of the anti-ulcer effect of Selank, as well as its three fragments, showed that they all effectively act in two directions determining the protective effect in the form of increasing the resistance of the gastric mucosa to the action of various ulcerogenic factors and the therapeutic effect in the form of accelerated healing of ulcers.

To assess the effect of glyprolines on the expression of cytokine genes, a study using stress and acetate models of ulcer formation was conducted (Sangadzhieva et al., 2014). Pro–Gly–Pro under intranasal administration (3.7 μmol/kg) had a pronounced protective effect on models of stress (59.4%) and acetate (78.5%) ulceration in rats. It was shown that, in control animals in models of stress damage to the gastric membrane, the destruction of the mucous membrane in several cases was accompanied by an increase in TNFα transcription and inhibition of interleukin IL-4 transcription. In models of acetate ulcers, the development of damage correlated with a decrease in gene expression of a number of cytokines: IFN-α, IFN-γ, IL-2, IL-4, IL-6, IL-12, and TNFα. At the same time, the protective anti-ulcer effect of Pro–Gly–Pro was observed, which was accompanied by an increase in IL-6 transcription.

N-Acetyl-Pro–Gly–Pro, unlike Pro–Gly–Pro, did not reduce the area of stress damage, but was characterized by a tendency to prevent the development of acetate ulcers. In addition, when using *N*-Acetyl-Pro–Gly–Pro, there was an increase in the expression of cytokine genes of the Tx1 and Tx2 type: IFN-α, IFN-γ, IL-4.

FIBRINOLYTIC AND HYPOGLYCEMIC EFFECTS

Glyprolines Pro–Gly and Pro–Gly–Pro, which were chosen for the formation of the C-terminus of the Selank molecule, are constantly formed in the body during the synthesis and degradation of collagen and are actively involved in the regulation of the hemostasis system. One of the diseases with pronounced systemic disorders of hemostatic parameters is diabetes, in which there is a suppression of the anticoagulation system function, an increase in the degree of platelet aggregation, and a decrease in the activity of anticoagulant and fibrinolytic systems. In addition, it was shown that the fragment of bradykinin, which includes the Pro–Pro–Gly–Phe peptide, inhibits the coagulation activity of thrombin and platelet aggregation induced by thrombin. Several studies using the diabetogenic metabolite alloxan have been conducted (Ul'yanov et al., 2009; Lyapina et al., 2010; Andreeva et al., 2013), which allowed determination of the role of glyprolines and their analogues in the system of blood coagulation and thrombosis. As a result of the experiments, it was found that proline-containing peptides protect the organism of animals from the threat of thrombus formation and have antitoxic and protective antidiabetogenic effects. Repeated (for 7 days) intranasal administration of the Pro–Gly–Pro–Leu peptide to animals (1 mg/kg) prior to the injection of alloxan provided effective protection of the body against the development of insulin-dependent diabetes mellitus and prevented the development of hypercoagulatory changes in the hemostasis system. At the same time, against the background of preliminary alloxan injection, the same peptide under the same experimental conditions slowed down the development of symptoms of diabetes. Repeated (7 days, 1 mg/kg) intranasal administration of the Pro–Gly–Arg tripeptide prior to the administration of a diabetogenic dose of alloxan also ensured effective protection of the body against the development of insulin-dependent diabetes mellitus. The Pro–Gly–Pro–Arg tetrapeptide did not cause a hypoglycemic effect during the provocation of diabetes mellitus.

Summarizing research was conducted to form the integration concept of the regulatory role of glyprolines (Lyapina et al., 2013). Animals with insulin-dependent alloxan diabetes (of the first type) and persistent hyperglycemia, similar to the development of insulin-independent diabetes (of the second type), in humans were chosen as the two reference models. It was shown that repeated intranasal administration of di-, tri-, and tetrapeptides of the glyproline series with additional inclusion of arginine and leucine in different positions caused both therapeutic and prophylactic effects, namely combined normoglycemic and antithrombotic changes in the parameters of the blood.

CASCADE PROCESSES

As noted above, an increase in the enkephalin content occurs under the introduction of Selank into the body. Such activation of the opioid system may underlie a wide range of effects of the heptapeptide on various cascade processes occurring in the body.

Previously, we analyzed in detail the main volume (over the past 40 years) of literature sources to identify possible inducing and inhibiting effects of various RPs and a number of mediators (Koroleva and Mjasoedov, 2012; Koroleva et al., 2012). To determine the role of each peptide in the control of physiological functions, a database of representatives of the main RP families and mediators was developed; it included consideration of the direction of the effect, various doses and methods of introducing the molecules, types of organisms, receptor mechanisms, etc. During complex analysis of all extensive information, numerous regularities of peptide control of processes in the body were revealed. An important role of system-organizing factors is played by opioids, in particular leu-enkephalin. This peptide modulates the release of many other RPs, as well as important mediators such as dopamine, serotonin, norepinephrine, acetylcholine, and others. The width of the spectrum of the main behavioral and physiological processes allows one to attribute this peptide to the top management of endogenous regulators. In addition, leu-enkephalin is among the factors of the reward system (FRS). This system, which determines the level of positive emotions, is a multicomponent system with a complex network of vectors of cascade effects, which allow the formation of logic

schemes for the suppression of pathophysiological processes.

By regulating the content of enkephalin, Selank and its fragments have the ability to expand the zone of influence and access to a wide range of extremely important mediators and RPs, as well as to a significant number of downstream controlled physiological processes. All this allows the formation of long-lasting stable induction cascades and an increase in the therapeutic range of the effects. Thus, it is possible to come close to the correction of biological processes as effectively and as naturally as possible (a minimum of side effects, since this system has already been tested by nature itself).

CONCLUSIONS

Fundamental research in the development of various pharmacological drugs should be as close as possible to the search for target objects among the internal reserve molecular programs. This approach allows one to identify the most physiological molecular schemes for the regulation of bioprocesses. The main directions of further development of drug design can be both the enhancement of existing physiological effects and the introduction of new properties, the elimination or reduction of undesirable effects, and the development of stabilized structures more resistant to metabolic degradation (proteolysis). All these objectives were successfully solved when developing the drug Selank. This peptide has pronounced anxiolytic, antidepressant, stress-protective, cognitive, immunomodulatory, antiviral, anti-ulcer, fibrinolytic, and hypoglycemic effects. Along with such a wide range of therapeutic effects, Selank is characterized by a high level of safety, since it is metabolized to natural amino acids. Moreover, Selank, having a biochemical profile most similar to two natural regulators, tuftsin and collagen derivatives, "finely" fits into the existing system of regulating physiological functions without causing a harsh sharp change in the bioparameters typical for the effects of many common drugs. The results of study of the relationship between the structure and physiological activity in the series of tuftsin, Selank, and their numerous analogues, which allowed mapping of the amino acid sequences according to the direction of pharmacological effects, have a special scientific and applied value.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

Andreeva, L.A., Nagaev, I.Yu., Mezentseva, M.V., Shapoval, I.M., Podchernyaeva, R.Ya., Shcherbenko, V.E., Potapova, L.A., Russu, L.I., Ershov, F.I., and Myasoedov, N.F., Antiviral properties of structural fragments of the peptide Selank, *Dokl. Biol. Sci.*, 2010, vol. 431, pp. 79–82.

Andreeva, L.A., Myasoedov, N.F., Lyapina, L.A., Grigor'eva, M.E., Obergan, T.Yu., and Shubina, T.A., Effect of the Pro–Gly–Pro peptide on hemostasis and lipid metabolism in rats with hypercholesterolemia, *Dokl. Biol. Sci.*, 2013, vol. 453, pp. 333–335.

Ershov, F.I., Uchakin, P.N., Uchakina, O.N., Mezentseva, M.V., Alekseeva, L.A., and Myasoedov, N.F., Activity of the immunomodulator Selank in experimental influenza infection, *Vopr. Virusol.*, 2009, vol. 5, pp. 19–24.

Kolomin, T.A., Shadrina, M.I., Agniullin, Ya.V., Shram, S.I., Slominskii, P.A., Limborskaya, S.A., and Myasoedov, N.F., Transcriptomic response of rat hippocampus and spleen cells to single and chronic administration of the peptide Selank, *Dokl. Biochem. Biophys.*, 2010, vol. 430, pp. 5–6.

Kolomin, T.A., Shadrina, M.I., Slominsky, P.A., Limborska, S.A., and Myasoedov, N.F., Changes in expression of the genes for chemokines, cytokines, and their receptors in response to Selank and its fragments, *Russ. J. Genet.*, 2011a, vol. 47, no. 5, pp. 629–631.

Kolomin, T., Shadrina, M., Andreeva, L., Slominsky, P., Limborska, S., and Myasoedov, N., Expression of inflammation-related genes in mouse spleen under tuftsin analog Selank, *Regul. Peptides*, 2011b, vol. 170, pp. 18–23.

Koroleva, S.V. and Myasoedov, N.F., Dynamic hierarchy of regulatory peptides. The structure of the inductive relationships of regulators as a target for therapeutic interventions, *Usp. Fiziol. Nauk*, 2012, vol. 43, no. 3, pp. 38–47.

Koroleva, S.V., Nikolaeva, A.A., and Ashmarin, I.P., Types of bioinformatic programs in the continuum of regulatory peptides and non-peptide mediators. Traits of interaction of dopamine and serotonin systems, *Neurochem. J.*, 2012, vol. 6, no. 2, pp. 132–143.

Kost, N.V., Sokolov, O.Yu., Gabaeva, N.V., Grivennikov, I.A., Andreeva, L.A, Myasoedov, N.F., and Zozulya, A.A., Semax and Selank inhibit the enkephalin-degrading enzymes of human serum, *Russ. J. Bioorg. Chem.*, 2001, vol. 27, no. 3, pp. 156–159.

Kozlovskaya, M.M., Seredenin, S.B., Kozlovskii, I.I., Val'dman, E.A., Andreeva, L.A., and Myasoedov, N.F., Comparative study of the effect of tuftsin fragments on the indices of passive avoidance response, *Khim.-Farm. Zh.*, 2001, vol. 35, no. 3, pp. 3–5.

Kozlovskaya, M.M., Seredenin, S.B., Kozlovskii, I.I., Val'dman, E.A., Andreeva, L.A., and Myasoedov, N.F., Comparative analysis of the structural and functional characteristics of the peptide Selank, *Psikhofarmakol. Biol. Narkol.*, 2002, vol. 2, nos. 1–2, pp. 203–210.

Lyapina, L.A., Myasoedov, N.F., Andreeva, L.A., Ul'yanov, A.M., Obergan, T.Yu., Shubina, T.A., and Pastorova, V.E., Fibrinolytic and hypoglycemic effects of the Pro–Gly–Pro–Leu peptide during development of insulin-dependent diabetes in rats, *Biol. Bull.* (Moscow), 2010, vol. 37, no. 3, pp. 316–320.

Lyapina, L.A., Myasoedov, N.F., Grigor'eva, M.E., Shubina, T.A., and Andreeva, L.A., The modern concept of the regulatory role of peptides of the glyproline family in the correction of hemostasis system function during development of diabetes mellitus, *Biol. Bull.* (Moscow), 2013, vol. 40, no. 4, pp. 386–393.

Meshavkin, V.K., Kost, N.V., Sokolov, O.Yu., Zolotarev, Yu.A., Myasoedov, N.F., and Zozulya, A.A., Naloxone-blocked depriming effect of anxiolytic Selank on apomorphine-induced behavioral manifestations of hyperfunction of dopamine system, *Bull. Exp. Biol. Med.*, 2006, vol. 142, no. 5, pp. 598–600.

Pavlov, T.S., Samonina, G.E., Andreeva, L.A., Myasoedov, N.F., and Ashmarin, I.P., A new property of the synthetic anxiolytic Selank and its derivatives, *Dokl. Biol. Sci.*, 2004, vol. 397, pp. 281–283.

Sangadzhieva, A.D., Bakaeva, Z.V., Samonina, G.E., Mezentseva, M.V., Andreeva, L.A., and Myasoedov, N.F., Effect of glyprolines PGP and *N*-acetyl-PGP on the expression of cytokine genes during stress- and acetate-induced ulceration, *Moscow Univ. Biol. Sci. Bull.*, 2014, vol. 69, no. 4, pp. 141–146.

Uchakina, O.N., Uchakin, P.N., Myasoedov, N.F., Andreeva, L.A., Shcherbenko, V.E., Mezentseva, M.V., Gabaeva, M.V., Sokolov, O.Yu., Zozulya, A.A., and Ershov, F.I., Immunomodulatory effects of Selank in patients with anxiety-asthenic disorders, *Zh. Nevrol. Psikhiatr.*, 2008, vol. 108, no. 5, pp. 71–75.

Ul'yanov, A.M., Lyapina, L.A., Obergan, T.Yu., Shubina, T.A., Pastorova, V.E., Andreeva, L.A., Myasoedov, N.F., and Ashmarin, I.P., Antidiabetogenic action of Pro–Gly–Arg and Pro–Gly–Pro–Arg peptides after their intranasal chronic administration to animals, *Biol. Bull.* (Moscow), 2009, vol. 36, no. 6, pp. 630–633.

V'yunova, T.V., Andreeva, L.A., Shevchenko, K.V., Shevchenko, V.P., and Myasoedov, N.F., Peptide regulation of specific ligand–receptor interactions of GABA with the plasma membranes of nerve cells, *Neurochem. J.*, 2014, vol. 8, no. 4, pp. 259–264.

Zozulya, A.A., Kost, N.V., Sokolov, O.Yu., Gabaeva, N.V., Grivennikov, I.A., Andreeva, L.A., Zolotarev, Yu.A., Ivanov, S.V., Andryushchenko, L.N., Myasoedov, N.F., and Smulevich, A.B., The inhibitory effect of Selank on enkephalin-degrading enzymes as a possible mechanism of its anxiolytic activity, *Bull. Exp. Biol. Med.*, 2001, vol. 131, no. 4, pp. 315–317.

Zozulya, A.A., Neznamov, G.G., Syunyakov, T.S., Kost, N.V., Gabaeva, M.V., Sokolov, O.Yu., Serebryakova, E.V., Sirantchieva, O.A., Andryushchenko, A.V., Telesheva, E.S., Syunyakov, S.A., Smulevich, A.V., Myasoedov, N.F., and Seredenin, S.B., The efficacy and possible mechanisms of action of the new peptide anxiolytic Selank in the treatment of generalized anxiety disorder and neurasthenia, *Zh. Nevrol. Psikhiatr.*, 2008, vol. 4, pp. 38–48.

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