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Innovative Drugs: From Basic Research to Production

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Abstract—The results of studies of the molecular action mechanism of the peptides Semax and Selank—simple glyprolines that are used to prepare peptide-based drugs—are presented. A concept of creating an amino acid sequence of peptides is given; their interaction with receptor systems of the cell and their influence on the cell's transcriptome, which forms the cell-mediated response, are considered. On the basis of the findings, pharmaceutical compositions of peptide drugs have been developed and their production and introduction into clinical practice have been organized.

Keywords: peptides, medical preparations, ligands, receptors, transcriptome, proteome, Semax, Selank, glyprolines.

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The problem of the specificity and safety of drugs is the main problem of pharmacology; from this point of view, peptides as a class of physiologically active substances that exert a regulatory and neuromodulatory action on physiological processes of the organism are very interesting. Despite the efforts of pharmacologists throughout the world, peptide-based drugs are few. This is due to the polyfunctionality and rapid degradation of peptides under the action of various proteases and the complexity and ambiguity of the mechanism of their activity.

We have proposed a concept of the targeted design of peptides with definite physiological properties; it is based on the use of the structure of natural peptides with a known physiological action. Structural–functional studies have helped identify the minimal amino acid sequence with a certain physiological action, which can be prolonged by attaching definite amino acid residues to the C- and N-ends of the peptide. Thanks to this approach, we managed to identify groups of neurotropic [1], analgesic [2], neuroleptic [3], and antiviral [4] peptides and to create a number of substances for medical preparations and medical preparations themselves [5]. We have covered the difficult road from a scientific idea to obtaining a medical preparation, organizing its production, and introducing it into clinical practice.

* RAS Academician Nikolai Fedorovich Myasoedov is a deputy director of the RAS Institute of Molecular Genetics.

FUNDAMENTAL PROBLEMS OF THE CREATION OF PEPTIDE-BASED DRUGS

Figure 1a shows a diagram of the possible mechanism of interaction between peptides and the cell, including the interaction of peptides with the cell membrane, signal transmission to the cell nucleus, the activation of early transcription factors, and changes in the cell's transcriptome and proteome. The influence of peptides on the cell's proteome is exemplified by the Semax-induced activation of the genes of trophic factors [6] and their protein products [7]. The complexity of intracellular regulatory processes is demonstrated in Fig. 1b, which depicts regulatory circuits of an early c-Myc transcription factor.

Semax and its tritium-tagged fragments specifically bind to rat brain membranes [8, 9]. However, competitive binding in the presence of agonists and antagonists of the main neuroreceptor systems did not allow us to identify concrete receptor systems that are affected by Semax.

Semax and its fragments act on all receptor systems studied. Our recent studies have shown that the picture of interaction between peptides and various receptor systems is even more complex because low concentrations of Semax and its fragments reveal new specific binding sites. Experimental data on the specific binding of peptides make it possible to suggest that peptides act allosterically on various receptor systems, which leads to changes in the effect of endogenous orthosteric ligands and to specific responses of cells. This

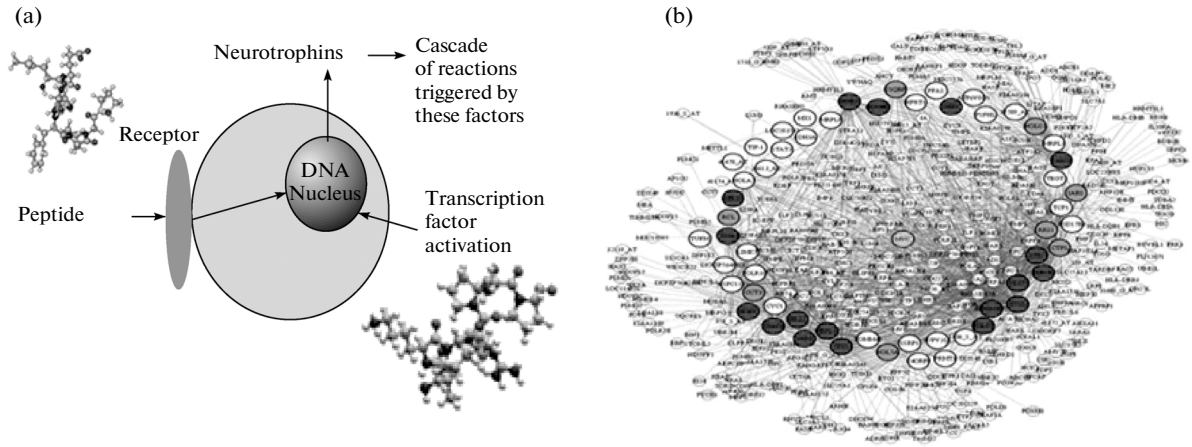

Fig. 1. Diagram of interaction between peptides and the cell. (a) Possible mechanisms of peptides' action; (b) regulatory circuits of an early transcription factor.

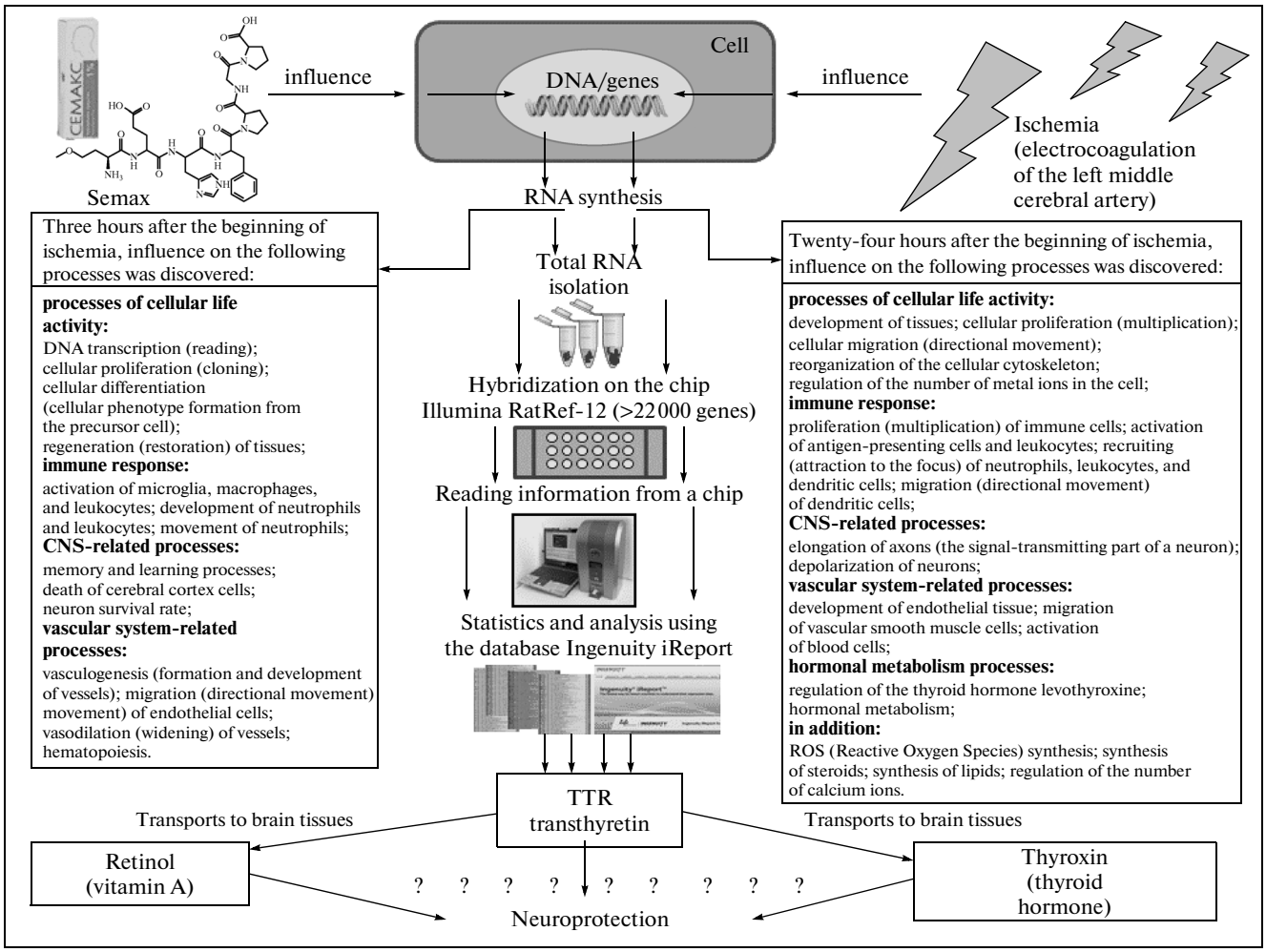

Fig. 2. Influence of the peptide Semax on intracellular processes under ischemia in rats.

suggestion explains the polyfunctionality of peptides, as well as the specific and "mild" character of their action.

Let us consider the influence of the Semax peptide on intracellular processes on a model of focal cerebral ischemia in rats, caused by the electrocoagulation of

Table 1. Degree of functional recovery (by the Barthel index) by the 30th day of carotid ischemic stroke

Outcome of functional recovery	Standard therapy ($n = 40$)	Semax, dose		
		6 mg/day ($n = 40$)	12 mg/day; ($n = 40$)	18 mg/day ($n = 40$)
Group as a whole	7/40(17.5%)	3/40(7.5%)	2/40(5%)#	1/40(2.5%)#
Severe disability	9/40(22.55)	4/40(10%)	2/40(5%)#	2/40(5%)#
Moderate disability	16/40(40%)	18/40(45%)	6/40(15%)##**	7/40(17.5%)#*
Good recovery	8/40(20%)	15/40(37.5%)	30/40(75%)###**	30/40(75%)###*

Source: *The Use of the Peptide Neuroprotector Semax 1% in the First Hours of Cerebral Stroke (Brain Ischemia): Methodological Recommendations*, Ed. by V. I. Skvortsova (Moscow, 2011).

Table 2. Course and outcomes of dyscirculatory encephalopathy (DE) under the use of Semax in different doses compared to reference groups, % of observations

Study groups	Variant of DE course		Outcome	
	stable	progressive	TIA	stroke
DEI (n=32)				
6 mg/day	75.0*	25.0*	– **	–
9 mg/day	81.2**	18.7**	– **	–
Reference I	42.9	57.1	14.3	–
DEII (n=65)				
6 mg/day	705.0*	30.0*	10.0	5.0*
9 mg/day	72.7*	27.3*	9.1	9.1
12 mg/day	73.9*	26.1*	8.7	– *
Reference I	41.0	59.0	7.7	12.8
DEIII (n=90)				
6 mg/day	53.9	46.1	7.6	7.6
9 mg/day	51.6	48.4	3.2*	6.4*
12 mg/day	54.6*	45.4*	9.1	6.1
Reference I	36.2	64.6	13.7	15.5*

Source: E. I. Gusev, V. I. Skvortsova, and E. I. Chukanova, "Semax in preventing the progression and development of acute conditions in patients with dyscirculatory encephalopathy," Zh. Nevrol. Psikhiatr. im. S.S. Korsakova, No. 2 (2005).

the region of the middle cerebral artery (the closest to a human stroke). Figure 2 shows the diagram of the experiments [10]. Ischemia was induced in rats; half of them received Semax. After 3 and 24 hours, they were withdrawn from the experiment, and the RNAs of the brain cells of the animals under ischemia or the ischemia + Semax action were analyzed on Illumina chips, which contained 22000 rat genes. The chips are plates covered with oligonucleotides taken from a sequence of genes of a definite structure. It was discovered that ischemia had changed the expression of several hundred genes; the ischemia + Semax action changed the expression of another several dozen. Figure 2 shows the main cellular processes that correspond to the genes that responded to the ischemia + Semax action compared to the ischemic tissue after 3 and 24 hours. The share of genes related to the immune response was 9% after 3 hours and 53% after 24 hours. A strong activation of the gene transthyretin was also found; transthyretin codes the protein carrier of thyronine and retinol [11].

If the results are confirmed at the protein level, we will be able to show what underlies the neuroprotective properties of Semax (the question mark in Fig. 2).

These studies agree with the clinical results in works supervised by RAS Corresponding Member V.I. Skvortsova and RAS Academician E.A. Gusev in curing stroke and treating dyscirculatory encephalopathy and other neurological diseases (Tables 1, 2), as well as with those by associates of the RAS Institute of Eye Diseases in treating the central optic nerve (Fig. 3) (let me mention N.L. Sheremet, Dr. Sci. (Med.), first of all) (Figs. 3c, 3d). The positive influence of Semax on cognitive processes and its neuroprotective activity made it possible to use drugs based on this peptide in the therapy of Parkinson's disease, Huntington's disease (RAS Scientific Center of Neurology) [12], and early stages of Alzheimer's disease (RAS Mental Health Research Center) [13]. A positive effect was reached in all cases.

Numerous studies have shown the important role of the prenatal and early neonatal periods of life in the development and formation of neurophysiological mechanisms and mental functions. For example, in rats that were affected pharmacologically (neonatal induction of the antidepressant fluvoxamine) in the early period of development, as well as in the model of neonatal stress and acute neonatal hypoxia, the anxiety level is higher, the learning ability is impaired, and neurochemical indicators are different. The rats that received Semax in these conditions demonstrated a decrease in the anxiety level, better learning ability, and normalization of the neurochemical indicators. These data open new possibilities in the use of Semax to correct the negative effects of centrally acting medical preparations, especially in children.

In 2012, the RAS Institute of Molecular Genetics jointly with the RAS Research Institute of Pharmacology completed long-standing studies on the construction, examination, and production of a new medical preparation, Selank [14]. Selank (Thr–Lys-Pro–Àrg–Pro–Gly–Pro) is a stimulatory anxiolytic without side effects. It was obtained through the condensation of the peptides tuftsin (Thr–Lys-Pro–Arg) and glyproline (Pro–Gly–Pro). Today Selank is used to cure patients with anxiety syndromes and those with organic emotionally labile disorder. New indications for the use of this preparation in neurology and pediatrics, as well as under viral diseases and hemorrhagic stroke, have been identified.

Figure 4 shows the structure and physiological properties of this clinically used preparation. Figure 4a presents the structure of the $GABA_A$ receptor—the major inhibitory receptor, which transmits Cl^- ions into the cell and thus affects the potential of the neuron's action. The ligand of this receptor is γ-aminobutyric acid (GABA). Allosteric binding sites of this receptor are also shown here. Figure 4b shows the specific binding of [^{3}H]GABA with receptors of the cell membranes of the rat cerebral cortex, as well as the influence of the pretreatment intranasal introduction

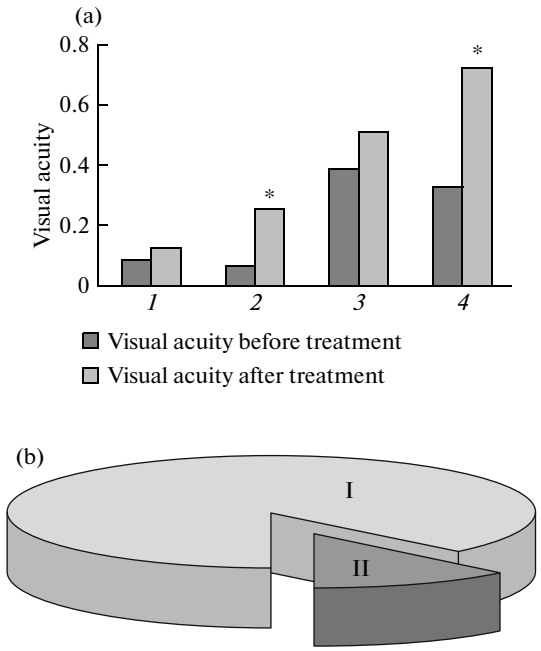

Fig. 3. Clinical data from the therapy of central optic nerve diseases by the Semax preparation. (a) Influence of Semax on visual acuity in patients with optic nerve diseases ((*1*) the reference group with an initial visual acuity of 0.1 and lower, (*2*) the group of patients with an initial visual acuity of 0.1 and lower who took Semax, (*3*) the reference group with an initial visual acuity of 0.2 and higher, (*4*) the group of patients with an initial visual acuity of 0.2 and higher who received Semax). * Significant differences from the initial values. (b) Visual acuity three months after complex treatment with the Semax preparation (I—87.5%—visual acuity is stable, II—12.5%—visual acuity has partially decreased).

G. S. Polunin, S. M. Nurieva, D. L. Bayaldin, et al., "Determining the therapeutic efficacy of the new domestic preparation Semax under optic nerve diseases," Vestn. Oftal'mol. **116** (1), 15 (2000). N. L. Sheremet, G. S. Polunin, A. N. Ovchinikov, et al., "Experimental substantiation of the use of the neuroprotector Semax in the treatment of optic nerve diseases," Vestn. Oftal'mol. **120** (6), 25 (2004).

of Selank or GABA on this binding. We can see that pretreatment with GABA introduction increases binding by 40%. A similar picture is observable under the intranasal introduction of Selank. Figure 4c depicts the specific binding of [^{3}H]GABA on cell membranes of the rat cerebral cortex in the presence of different concentrations of Selank. In Fig. 4c, we can see the influence of Selank in a wide range of concentrations on the binding of the orthosteric ligand [^{3}H]GABA, as well as the region of the therapeutically significant effect of this peptide. In the case of Selank, we managed to identify the sites of the specific binding of this peptide as well. Figure 4d shows the competitive influence of Selank on the binding of tritium-tagged diazepam with a bendiazepin binding site. Selank

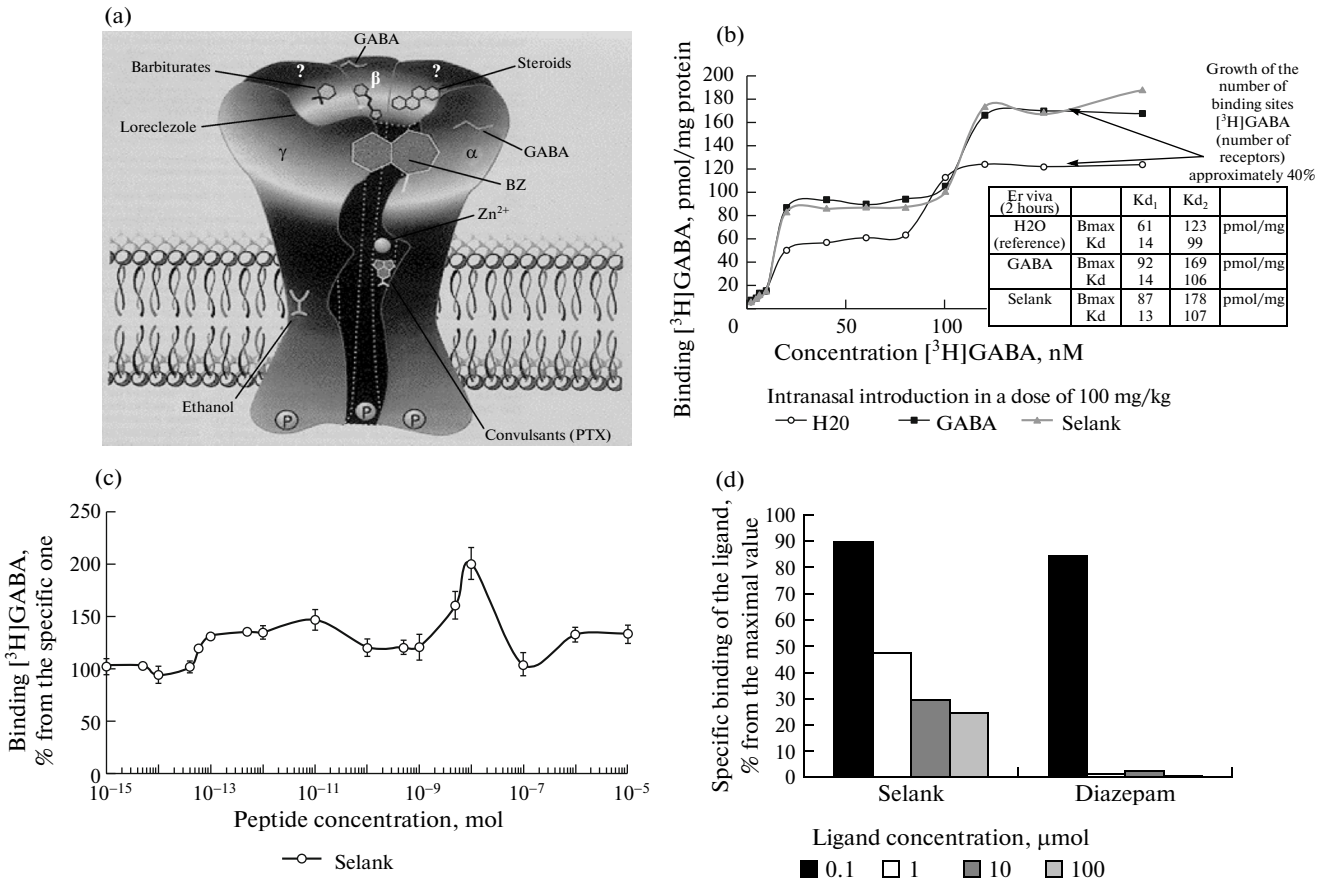

Fig. 4. Structure of the $GABA_A$ receptor and its interaction with the peptide Selank. (a) Structure of the $GABA_A$ receptor; (b) specific binding of [^{3}H]GABA on plasmatic membranes of the rat cerebral cortex two hours after the introduction of Selank or GABA; (c) specific binding of [^{3}H]GABA (20 nM) on plasmatic membranes of the rat cerebral cortex in the presence of peptides; (d) specific binding of [^{3}H]Diazepam (30 nM).

competes with diazepam, which is itself an allosteric regulator.

The results obtained constituted the basis for a new therapeutical scheme to treat anxiety disorders, which was developed by the RAS Mental Health Research Center and envisaged the concomitant use of bendiazepins with Selank; this made it possible to decrease significantly the negative effects of bendiazepins, preserving at the same time the specific character of their action.

The influence of Selank on intracellular regulation has been studied. The genes involved in the neuroreception and functioning of the GABAergic system, the change in the transcription of which under the action of Selank had been studied, were investigated. Figure 5 shows how gene expression in the rat brain changes three hours after the introduction of GABA or Selank. The influence of both preparations on gene transcription is the same; however, Selank specifically activates (by 100 times) the *Hcrt (hypocretin)* gene, important for the functioning of the cell, which is reflected in the same figure. The change in the transcriptome of neurons under the action of peptides has been studied. Semax and the genes involved in neuroprotection change in the expression of 27 out of the 84 genes. Selank and the genes involved in the anti-inflammatory effect change in the expression of 34 out of the 84 genes. The change in the transcriptome under the action of the peptides Thr–Lys-Pro–Àrg, Pro–Gly–Pro, Gly–Pro, and Arg–Pro–Gly–Pro has been studied.

A positive influence of glyprolines (short proline-containing peptides) on various disorders under metabolic syndrome has been found in rats in recent years [15]. Metabolic syndrome includes several risk factors, each of which favors the development of cardiovascular diseases: a high level of blood glucose, triglycerides, total cholesterol, and low-density lipoproteins; obesity; hypertension; and thrombocyte adhesion. Peptides normalize these indicators, affecting hemostasis parameters, lipid pattern indicators, the level of glucose, and changes in the body mass.

On the basis of these studies, the tetrapeptide Pro–Gly–Pro–Leu (glypropol) was chosen as a candidate

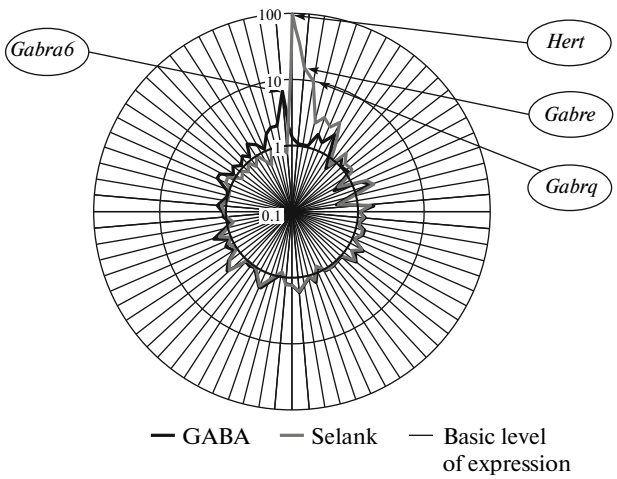

Fig. 5. Change in gene expression in the rat brain three hours after the introduction of GABA and Selank.

for the creation of the drug. Preclinical studies and the first phase of clinical studies have been completed.

The action of peptides, which leads to positive therapeutical effects, can be targeted both at receptor systems (mainly thanks to allosteric binding sites) and at a directional change in the transcriptome (at the expense of changes in the activity of definite genes). While the effect on certain receptor systems has been a task of pharmacology for a long time, the activation of definite genes, which causes a targeted change in the proteome and, as a consequence, definite physiological responses, is a new area of pharmacology, a new target of pharmacological influence. Physiologically active peptides are the most appropriate instrument of this influence.

THE PRODUCTION OF PEPTIDE-BASED DRUGS

Peptide-based medical preparations are science-intensive products. The therapeutic effect for the peptide Semax is reached with a dose of ~0.5 mg/day; that of Selank, at ~0.75 mg/day. The total demand of the country and its exports for peptide (for example, Semax-based drugs) is ~3.5 kg per year. In organizing the production of medical preparations based on Semax, Selank, and other peptides, everything had to be done for the first time, including the development of regulatory documentation. At present, its pharmaceutical production by the Peptogen Closed Joint-Stock Company Innovative Research-and-Production Center has been GMP licensed and already participates in solving technological issues related to the creation of new preparations.

Among the organizations with which we collaborated and without which this work would have been impossible are the Mental Health Research Center, Russian Academy of Medical Sciences; the Faculty of Biology and the Faculty of Fundamental Medicine, Moscow State University; Pirogov Russian National Research Medical University; the Institute of Molecular Genetics, Russian Academy of Sciences; the Scientific Center of Children's Health, Russian Academy of Medical Sciences; the Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences; and the Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences.

Since the development of a new chemical structure and drugs, the organization of production, and marketing take at least 10–15 years, require great material costs, and can at any moment yield a negative result, both basic research and technology need state support. Basic research that yields new targets, new structures, and their action mechanisms can somehow be performed on a long-term grant basis, while all the rest, starting from preclinical studies, is possible only within long-term state programs.

In conclusion, we would like to recall the late participants in this work who contributed much to it: Academician I.P. Ashmarin; V.N. Nezavibat'ko, Cand. Sci. (Chem.); and M.A. Ponomareva-Stepnaya, Cand. Sci. (Chem.).

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