# Role of Aqueous Medium in Mechanisms Underlying the Influence of Immunoactive Peptides in Ultralow Doses

**E. I. Grigor'ev, V. Kh. Khavinson, V. V. Malinin, A. E. Grigor'ev, and T. A. Kudryavtseva**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 8, pp. 173-178, August, 2003
Original article submitted August 26, 2002

The mechanism underlying the influence of peptides in ultralow doses is based on distant signal transduction from the ligand molecules to target cells by means of water solitons. Biological activity of synthetic immunoactive peptides in concentrations below $10^{-18}$ M depends on their structural and conformational characteristics. The data on temperature dependencies of the near-infrared spectrum for solutions of these peptides are presented.

**Key Words:** *ultralow doses; peptides; signal transduction; solitons; infrared spectroscopy*

Little is known about the mechanism of ligand-receptor interaction, role of water in signal transduction, effect of ultralow doses (ULD), and formation of complexes between structurally different substances and similar receptors. The relationship between biological activity and structure of compounds casts some doubt on the necessity of forming complementary ligand-receptor complexes during signal transduction [5]. This is of particular importance in treatment with ULD of ligands, when the possibility for formation of ligand-receptor complexes sharply decreases ($10^{-12}$-$10^{-13}$ M) and reaches a minimum ($10^{-18}$ M and lower). There is no question that substances in specified concentrations produce biological effects [2,3,10]. The dose-activity dependence is of considerable interest with this respect. Previously we studied the initial stages of signal formation and transduction to target cells. Attention was given to distant transduction of signals in the aqueous medium to receptors, which is realized by the soliton mechanism [5]. The soliton model for the structure of water completely explains its unique properties. There are estimated and experimental data on the concentration of solitons. The effects of temperature and magnetic fields are discussed. Recent studies proposed three-dimensional models of the bound structure along which solitons spread [9]. Published data suggest that the aqueous medium is a universal receptor system for weak electromagnetic fields of different ranges [12]. Here we evaluated the role of water in signal formation and transduction to target cells. This study would extend our knowledge on mechanisms underlying the action of substances in ULD.

## MATERIALS AND METHODS

We studied synthetic peptides Vilon (Lys-Glu) [8] and Epithalon (Ala-Glu-Asp-Gly) synthesized at the St. Petersburg Institute of Bioregulation and Gerontology [14]. Peptides were subjected to preparative high-performance liquid chromatography (Beckman, 168 Diod Array Detector Module, 126 Solvent Module). The purity of preparations was >99%. Conformation was assayed in field MM2 (aqueous medium, CSChem3DPro). The effects of Vilon and Epithalon on proliferative activity of mouse thymocytes, experimental data, and results of statistical treatment were described previously [11]. The suspension of cells was incubated with preparations in various concentrations to evaluate the direct mitogenic effect of peptides. Incubation was performed in the presence of concanavalin A (Con A) to determine comitogenic activity of the peptides. Con A and interleukin-1 (IL-1) were added to reveal the

St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences. Address for correspondence: ibg@mail.wplus.ru. Grigor'ev E. I.

0007-4888/03/1362-0150$25.00 

modulatory effect of peptides on comitogenic activity of IL-1. Peptides were added to the incubation medium in concentrations of $10^{-7}$-$10^{-18}$ M. Temperature dependencies of the near-infrared spectrum (5180 $cm^{-1}$) were determined for aqueous solutions of preparations at 5-45°C. We used deionized water (specific resistance >17 MΩ/cm, Labconco Water Pro. PS).

## RESULTS

Neither Vilon, nor Epithalon in various concentrations produce direct mitogenic effects. They did not induce thymocyte blast transformation in the absence of lectin. However, the addition of peptides in similar concentrations to the incubation medium containing thymocyte suspension and Con A promoted incorporation of $^3$H-thymidine into DNA of proliferating cells. Therefore, under these conditions the test peptides produced a comitogenic effect on thymocyte proliferation. Vilon was most potent and stimulated blast transformation by 1.4-2.8 times. Epithalon was less active than vilon (Fig. 1, *a*). Vilon most significantly potentiated the comitogenic effect of IL-1 (by 1.6 times, Fig. 1, *c*). It should be emphasized that the dose-response relationship was described by a bimodal curve with 2 maxima at $10^{-9}$-$10^{-10}$ and $10^{-15}$-$10^{-16}$ M (ultralow concentrations). The curve with this shape most often describes the dose-response relationship for ultralow concentrations [2,3,10]. The mechanism of this phenomenon remains unclear. Modern hypotheses cannot explain the effects of ULD [1,10]. It remains unclear why the amount of molecules of biologically active substances (BAS) is very low near the target cell entering the composition of ligand-receptor complexes. Moreover, the number of these molecules approaches zero at concentrations of $10^{-18}$-$10^{-19}$ M. It should be noted that the concentration of ligands is 4-6 orders of magnitude lower than the minimum dissociation constants for the

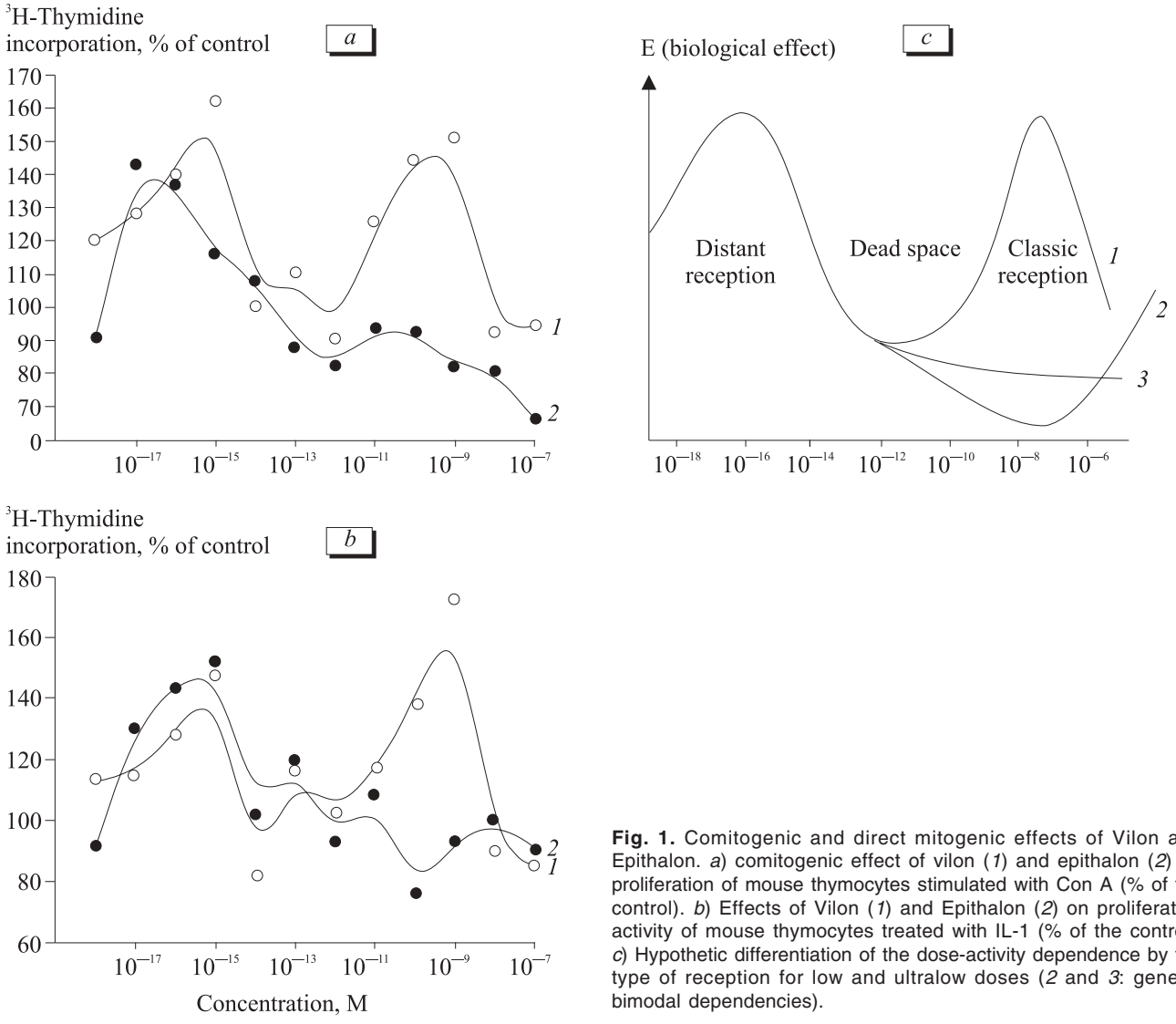


**Fig. 1.** Comitogenic and direct mitogenic effects of Vilon and Epithalon. *a*) comitogenic effect of vilon (*1*) and epithalon (*2*) on proliferation of mouse thymocytes stimulated with Con A (% of the control). *b*) Effects of Vilon (*1*) and Epithalon (*2*) on proliferative activity of mouse thymocytes treated with IL-1 (% of the control). *c*) Hypothetic differentiation of the dose-activity dependence by the type of reception for low and ultralow doses (*2* and *3*: general bimodal dependencies).

ligand-receptor complexes ($10^{-10}$-$10^{-11}$ M) [4]. That is why the extremely improbable formation of complexes with these receptors becomes practically impossible for substances in specified concentrations. It is related to the half-life of peptide preparations, transport to target tissues, and diffusion through a gel structure of the intercellular matrix to the target cells [13]. Proteases located on the surface of endoplasmic membranes serve as a final barrier for peptides [15]. The biological effect observed in the presence of endogenous ligands at concentrations that surpass the dose of preparations by an order of magnitude [7] cannot be explained by the classical theory of ligand-receptor complexes. Apart from the above-mentioned reasons, this theory is not consistent with the law of mass action. We believe that it is important to follow the real experimental conditions, take into account a possibility for the absence of ligand molecules for preparations in ULD near target cells and, therefore, consider the probability of signal transduction to target cells without the formation of ligand-receptor complexes.

We first proposed a possible distant mechanism of signal formation and transduction form the ligand molecules to target cells in aqueous medium, which is mediated by solitons. The possibility of these processes was confirmed experimentally [5]. With respect to the bimodal dose-response relationship, the first peak for relatively high concentrations ($10^{-10}$-$10^{-7}$ M)

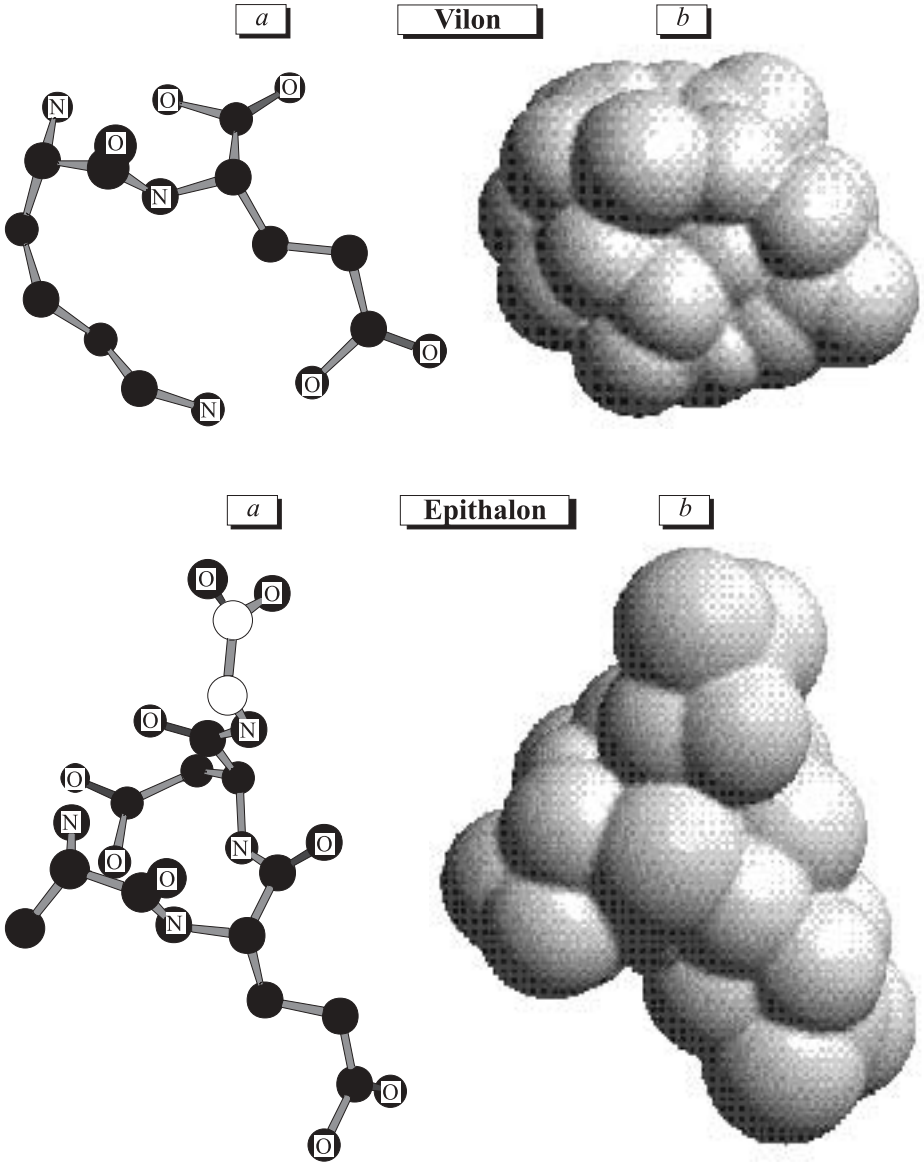


**Fig. 2.** Minimized conformations (*a*) and solvent-available surfaces (*b*, MM2, aqueous medium, CSChem3DPro) for ionized molecules of Vilon (H-Lys-Glu-OH) and Epithalon (H-Ala-Glu-Asp-Gly-OH). Vilon: molecular weight, 275.31; formal charge, 0; solvent-available surface, 437 Å$^2$; volume without solvent, 229 Å$^3$; number of ionogenic functional groups, $NH_3^+$ (2), $COO^-$ (2). Epithalon: molecular weight, 388.34; formal charge, 2; solvent-available surface, 566 Å$^2$; volume without solvent, 308 Å$^3$; number of ionogenic functional groups, $NH_3^+$ (1), $COO^-$ (3).

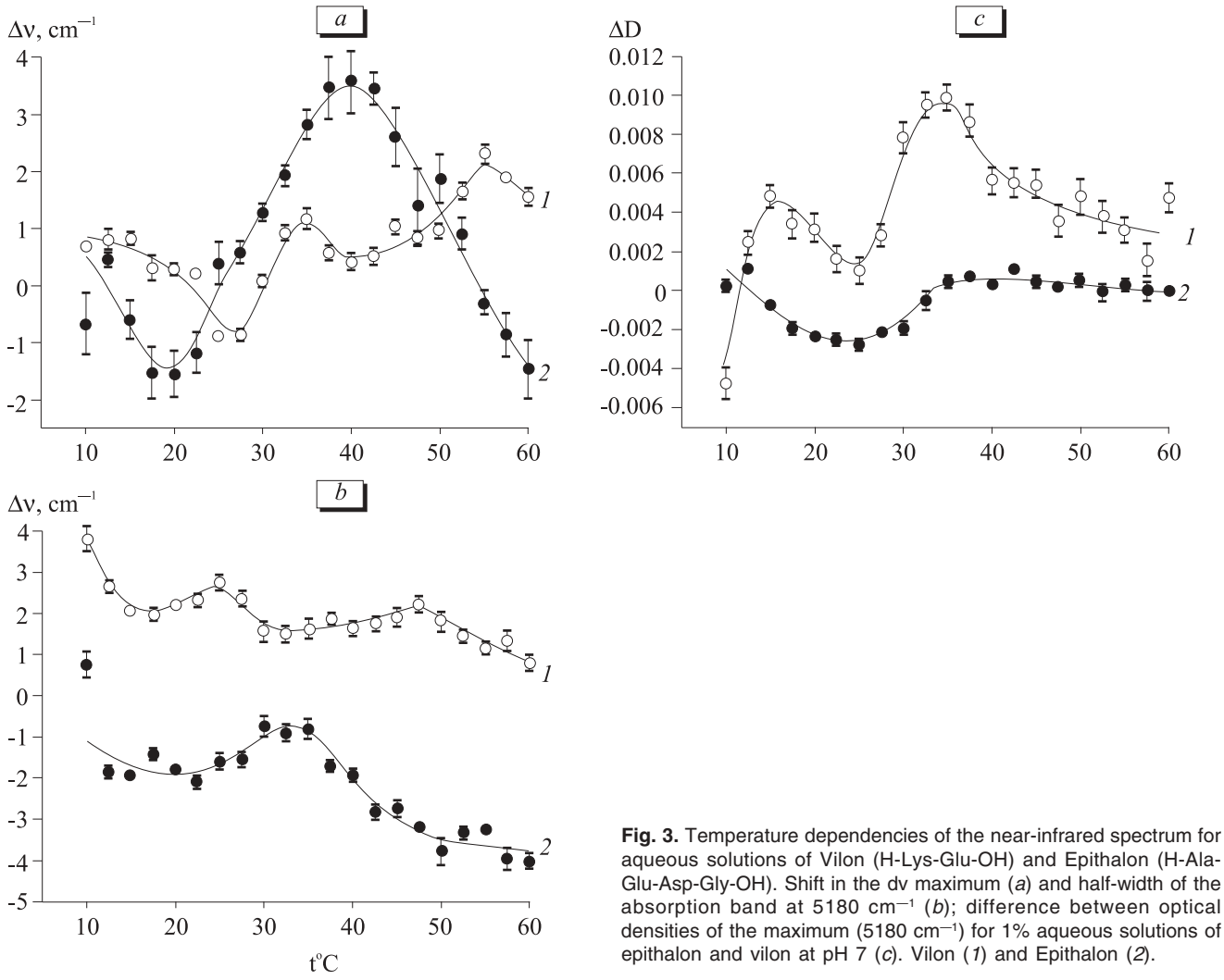


**Fig. 3.** Temperature dependencies of the near-infrared spectrum for aqueous solutions of Vilon (H-Lys-Glu-OH) and Epithalon (H-Ala-Glu-Asp-Gly-OH). Shift in the dv maximum (*a*) and half-width of the absorption band at 5180 $cm^{-1}$ (*b*); difference between optical densities of the maximum (5180 $cm^{-1}$) for 1% aqueous solutions of epithalon and vilon at pH 7 (*c*). Vilon (*1*) and Epithalon (*2*).

corresponds to the classic mechanism of ligand-receptor complexes (contact reception). Minimum dissociation constants for ligand-receptor complexes containing various compounds are $10^{-10}$-$10^{-11}$ M [4,10]. These characteristics for Vilon and Epithalon were not evaluated. The second peak for ultralow concentrations ($10^{-14}$ M and lower) corresponds to the non-contact mechanism of reception (extremely low amount or absence of ligand molecules) that can be realized distantly with water solitons. Modulated water solitons are also formed in the presence of ligands at higher concentrations (zone of classic reception). In that instance the intensity of signals surpasses the possible sensitivity limit of membrane receptors by several orders of magnitude. Reception of signals by the mechanism of resonance is impossible under these conditions. The dead space between these peaks does not realize or realizes insignificantly both types of reception. On the one hand, ligand concentration in this dead space is below the limit necessary for complex formation with receptors. On the other hand, the amount of ligands is too much for wave (soliton) transduction. The amplitude of signals is very high and, therefore, their reception by membrane structures resonating with weaker signals is impossible. The range of concentrations and shape of curves are specific for various BAS, biological activities, and experimental conditions. In our experiments with Vilon and Epithalon, 2 maxima of biological activity corresponded to the specified ranges of concentrations. Vilon and Epithalon had a comparable biological activity in 2 similar biological tests. However, they differ in the primary structure, total geometric size of molecules, number and topographic characteristics of potentially binding groups, formal charges, and energetically beneficial conformations (Fig. 2). Probably, these peptides cannot complementary bind to similar receptors. There is little likelihood that the same biological effect of the two compounds is mediated by different mechanisms and receptors. More likely the soliton signals initiated by these peptides and having similar characteristics can affect the same type of receptors. We believe that

structurally different ligands involved in the production of soliton signals for target cells in the aqueous medium can cause similar biological effects. Indirect experimental evidence of our hypothesis is temperature dependencies of the near-infrared spectrum for solutions of preparations (5180 $cm^{-1}$, Fig. 3). We revealed regions with the same type of non-monotony for both peptides. Previous studies showed that these nonmonotonous dependencies indirectly reflect the existence of water solitons with different characteristics before and after the interaction with the ligand molecules (individual amino acids and peptides). It should be emphasized that similar nonmonotonicity probably reflects the same nature of signals transduced in water [5,6]. These data suggest that the receptor (membrane sensor) perceives only critical changes in specified frequency-and-amplitude ranges of the general wave soliton homeostasis for the target cells. From the viewpoint of nonlinear physics, even minor changes can produce considerable effect [6]. The regulation can be performed by weak exogenous factors. It is important that the result can greatly exceed or qualitatively differ from the inducing factor. These data can explain the effects of BAS in the presence of a great excess of endogenous ligands. Our findings explain the influence of BAS in ULD (*e.g.*, homeopathy) and a variety of effects produced by external radiation. The individual mechanisms of these processes can have a common principle that suggests changes in dynamic characteristics of water in the intercellular space of target tissues.

## REFERENCES

1. L. A. Blyumenfel'd, *Biofizika*, **38**, No. 1, 129-132 (1993).
2. E. B. Burlakova, *Vestn. Ros. Akad. Nauk*, **64**, No. 5, 425-431 (1994).
3. E. B. Burlakova, *Ros. Khim. Zh*., **43**, No. 5, 3-11 (1999).
4. S. D. Varfolomeev and S. V. Zaitsev, *Kinetic Methods in Biochemical Assays* [in Russian], Moscow (1982).
5. E. I. Grigor'ev, V. Kh. Khavinson, I. N. Kochnev, *et al*., *Byull. Eksp. Biol. Med*., **133**, No. 5, 525-529 (2002).
6. I. N. Kochnev, A. I. Khaloimov, E. I. Grigor'ev, *et al*., *Biofizika*, **47**, No. 1, 12-19 (2002).
7. T. V. Lelekova, P. Ya. Romanovskii, P. N. Aleksandrov, and I. P. Ashmarin, *Byull. Eksp. Biol. Med*., **108**, No. 7, 8-10 (1989).
8. V. G. Morozov, V. Kh. Khavinson, and V. V. Malinin, *Peptide Thymomimetics* [in Russian], St. Petersburg (2000).
9. O. A. Ponomarev, I. P. Susak, E. E. Fesenko, and A. S. Shigaev, *Biofizika*, **47**, No. 3, 395-410 (2002).
10. L. A. Sazanov and S. V. Zaitsev, *Biokhimiya*, **57**, No. 10, 1443-1459 (1992).
11. V. Kh. Khavinson, E. G. Rybakina, V. V. Malinin, *et al*., *Byull. Eksp. Biol. Med.*, **133**, No. 5, 574-577 (2002).
12. E. E. Fesenko, V. I. Popov, V. V. Novikov, and S. S. Khutsyan, *Biofizika*, **47**, No. 3, 389-394 (2002).
13. P. P. Yamskova and I. A. Yamskov, *Ros. Khim. Zh*., **43**, No. 3, 74-79 (1999).
14. V. Kh. Khavinson, N. Goncharova, and B. Lapin, *Neuroendocrinol. Lett*., **22**, 251-254 (2001).
15. B. P. Roques, *J. Peptide Sci*., **7**, No. 7, 63-73 (2001).