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Peptide Regulation of Aging: 35-Year Research Experience

V. Kh. Khavinson and V. N. Anisimov

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The authors sum up the results of many-year studies of mechanisms of aging and efficiency of peptide bioregulators in the prevention of age-specific diseases. Data on the effects of peptides, evaluated by the up-to-date methods, are presented. A molecular model of complementary interactions between short peptides and gene promotor sites, underlying the initiation of protein synthesis, is proposed. Prospects of peptide bioregulators in prevention of early aging are discussed.

Key Words: *peptide bioregulators; aging; age-specific disease; cancer; prevention*

Aging is the most intricate problem of medicine and biology. The aging process is gradual involution of tissues and disorders in body functions. The symptoms of aging manifest at the end of the reproductive period and grow more intensive during subsequent aging. It is known that the species limit of animal and human life span is 30-40% longer than the mean lifespan. This fact is explained by the effects of untoward factors modifying the expression and structure of genes, which is paralleled by disorders in protein synthesis and reduction of body functions [10]. It is known that aging is associated with involution of the thymus (the central organ of the immune system) and of the epiphysis (the central organ of the neuroendocrine system) [10,11]. It is also associated with a significant reduction of protein synthesis in cells of different body tissues [11]. A special method for isolation, purification, and fractionation of low-molecular peptides from extracts of these organs was developed for restoration of the functions of the thymus, pineal gland, and other organs [10,11]. Low-molecular-weight peptides isolated from animal thymus (Thymalin) and epiphysis (Epithalamine) were studied on various biological models. These peptide preparations promoted a significant prolongation of the mean and in many cases of the maximum life span of mice and rats in numerous experiments (Table 1) and inhibited aging of the reproductive system, motor activity, and physical endurance in these animals [1-3,5,6,16-19,21-23]. A clear-cut correlation between the increase in the mean lifespan and the main indicator of cellular immunity (lymphocyte blast transformation reaction with phytohemagglutinin, characterizing the function of T-lymphocytes [17]), is worthy of note.

Significant prolongation of the mean lifespan of animals is to a certain measure caused by reduction of the incidence of spontaneous tumors under the effect of peptides isolated from the pineal gland and thymus. In addition, the peptides inhibited the growth of transplanted tumors, spontaneous and induced carcinogenesis in rats and mice of different strains, including transgenic animals [1-3,10,11,20,23].

Injection of epithalone (tetrapeptide) to female and male rats normalized free-radical processes, the

St. Petersburg Institute of Bioregulation and Gerontology, North-Western Division of the Russian Academy of Medical Sciences; N. N. Petrov Institute of Oncology, Ministry of Health and Social Development of the Russian Federation, St. Petersburg, Russia. ***Address for correspondence:*** aging@mail.ru. V. N. Anisimov

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majority of hormonal metabolic and behavioral parameters in animals exposed to permanent or natural illumination, and led to inhibition of the aging processes and development of age-specific diseases (including tumors), and prolongation of the life span [5,6,10,11,15,23].

Special series of experiments showed that short peptides isolated from different organs and tissues and their synthetic analogs (di-, tri-, tetrapeptides) are characterized by significant tissue-specific (gene-specific) activity in tissue culture and in experimental models in young and old animals [11].

Peptide treatment led to tissue-specific stimulation of protein synthesis in the cells of organs from which these peptides had been isolated. Stimulation of protein synthesis after peptide injection was detected in young and old animals [11]. The fact of the reproductive system recovery in old female rats after injection of the epiphyseal peptide preparation is particularly significant [3,23].

Hence, the advantages of short peptides were detected: they exhibit high biological activity, tissue specificity, but no species specificity and immunogenic activity. These characteristics make the regulatory peptides comparable to some peptide hormones [11,13].

The molecular weights, chemical characteristics, amino acid composition and sequence of low-molecular peptides from the thymus, epiphysis, and other organs have been studied for many years [10,11]. The resultant information was used for realization of chemical synthesis of some short peptides. Comparative analysis showed that biological activities of the natural and synthetic preparations are largely identical. For example, Glu—Trp dipeptide stimulated immunity [11,17]. Biological activities of natural and synthetic peptides was similar in standard tests in tissue cultures and animals [11]. Epithalamine (epiphyseal preparation) and epithalone (synthetic peptide), added to nutrient medium at the larval stage, significantly (up to 52%) prolonged the mean lifespan of the Drosophilidae males and females of different strains [10,11]. These results indicate good prospects of peptides as geroprotectors. The importance of search for new geroprotectors prompted preclinical studies at different structural levels.

At the level of the organism a significant variety of biological activities of short peptides (particularly thymic and pineal ones) was demonstrated in different animals. Their effects include antioxidant activity, effects on the endocrine system, proliferative activity, and apoptosis [10,11,15].

At the level of cell structures it was found that short peptides activated heterochromatin in blood lymphocyte cell nuclei in senile subjects [11,25].

The capacity of peptides to induce differentiation of polypotent cells is a fact of great importance. Addition of retinal peptides to polypotent cells of *Xernopus laevis* frog early gastrula ectoderm led to emergence of retinal and pigmented epithelial cells. This fact largely explains the clinical efficiency of retinal preparation in patients with degenerative diseases of the retina and in animals with genetically determined pigmented retinitis [11].

At the chromosome level the number of chromosome aberrations is used as a marker of DNA damage in an aging organism. Somatic mutations can emerge because of accumulation of stable aberrations and underlie the age-specific diseases, including malignant tumors. Significant antimutagenic and reparative activity of thymic and epiphyseal peptides is confirmed by reduced incidence of chromosome aberrations in bone marrow and corneal epithelial cells of early aging animals [11].

At the level of gene activity regulation it was found that Lys-Glu and Ala-Glu-Asp-Gly peptides, injected to transgenic mice, suppressed the expression of HER-2/neu gene (human breast cancer) 2-3.6 times in comparison with the control. This suppression of gene expression was paralleled by a significant reduction of tumor diameter [20].

Addition of Ala-Glu-Asp-Gly tetrapeptide to cultured human lung fibroblasts induced expression of telomerase gene, increased telomerase activity, and promoted a 2.4 times elongation of telomeres. Stimulation of gene expression was paralleled by a 42.5% increase in the number of mitoses (overcoming Hayflick's limit of cell divisions) [11,12].

The effects of Lys-Glu, Glu-Trp, Ala-Glu-Asp-Gly, Ala-Glu-Asp-Pro di- and tetrapeptides on the expression of 15,247 genes of mouse heart and brain before and after peptide injections were studied by the DNA microchip technology [4,11,14]. Clones from the Complementary DNA Library of Institute of Aging, USA, were used in the study. Unique data on changes in the expression of various genes under the effects of peptides were obtained in this experiment. An important conclusion of the study is that each peptide specifically regulates concrete genes. The results indicate the existence of a mechanism of peptide regulation of genetic activity. This experiment also showed that Lys-Glu dipeptide characterized by immunomodulating activity regulates the expression of IL-2 gene in blood lymphocytes [11].

At the molecular level there was an obvious gap between numerous proofs of the specific effects caused by the regulatory peptides in activation of gene transcription and limited schemes of the process underlying the selective binding of transcription factor to specific DNA sites. Nonspecific binding of proteins to DNA double strand has been proven by physicochemical methods [25]. As a rule, tens of macromo-

lecular activators and transcription factors are needed for stimulation of gene transcription in the cells of higher organisms.

We proposed a molecular model of interactions between regulatory peptides and DNA double strand at the gene promotor site [11,25].

The molecular model is based on the geometrical and chemical complementarity of the peptide amino acid sequence and sequence of DNA nucleotide pairs. A regulatory peptide recognizes a specific site of the DNA double strand if its own amino acid sequence is at a sufficient length complementary to the DNA nucleotide sequence; in other words, their interaction is specific due to coincidence of sequences.

Each sequence of nucleotide pairs in the DNA double strand forms a unique pattern of functional groups on the surface of the large groove of the DNA double helix. The peptide in an unfolded β-conformation can be complementary located in the DNA large groove along the helix axis. Published data on the molecular geometry of the DNA double strand and peptide β-sheet were used to find the sequence of nucleotide pairs for specific binding of DNA and Ala-Glu-Asp-Gly peptide. Screening has shown that this tetrapeptide can be located in the DNA large groove with the ATTTG (or ATTTC) nucleotide sequence at the main strand in accordance with the complementary location of their functional groups [9,11].

The molecular model was experimentally verified using synthetic preparations: DNA [poly(dA-dT):poly(dA-dT)] (double strand) and Ala-Glu-Asp-Gly tetrapeptide. It was proven by gel chromatography that Ala-Glu-Asp-Gly peptide formed a stable intermolecular complex with the DNA double strand [13,25].

Complementary binding of a peptide to a nucleotide sequence on the TATATA leading chain of the double strand can be realized by means of 6 hydrogen and 1 hydrophobic bonds between functional groups of both participants.

Normally DNA is a double strand with its two polymeric chains kept together by hydrogen bonds between base pairs at each chain. For the majority of biological processes involving DNA (transcription, replication), the double strand has to be separated into individual chains. It is known, for example, that local separation of the double strand chains precedes gene transcription by RNA polymerase. In order to start the transcription (matrix RNA synthesis), the DNA double strand has to be released from histones and the double strand chains have to be separated at the site of the beginning of matrix RNA synthesis.

A concentration-dependent hyperchromatic effect (increase of the optical density of solution at λ=260 nm) was detected by spectrophotometry in the ultraviolet spectra of solutions of synthetic DNA double strand and Ala-Glu-Asp-Gly tetrapeptide in a mixture of the peptide and double-stranded DNA. The hyperchromatic effect indicates partial destruction of hydrogen bonds between nucleotide pairs of the double strand and local separation of the double strand chains (allosteric conformational modification).

A special experiment showed that the chains of free DNA separate (melt) at 69.5°C. In the DNA-tetrapeptide system, the strand melted at 28°C and this process was characterized by a 2-fold reduction of entropy and enthalpy parameters [13]. This fact indicates thermodynamically easier separation of DNA chains at a temperature characteristic of the biochemical processes in the majority of living organisms. *In vitro* experiments indicate that a short peptide of a certain structure and amino acid sequence can participate in stimulation of gene transcription at the stage of DNA double strand separation. The biochemical aspect of this fact consists in similarity of the structure and amino acid sequence of the regulatory peptide and specific site of peptide chain of the macromolecular transcription factor.

Hence, studies of the biological activities of peptides at different structural levels and of physicochemical processes of their interactions reveled an obvious high physiological activity of peptide regulators. The main conclusion here is that the peptides regulate the gene expression. Preclinical studies have shown high biological activity and safety of synthetic peptides [10,11]. Injection of Lys-Glu, Ala-Glu-Asp-Gly peptides to animals promoted a reduction of tumor incidence and prolongation of the mean life span (Table 1). Ala-Glu-Asp-Pro peptide stimulated regeneration of the nerve, Lys-Glu-Asp-Trp peptide reduced blood glucose level in animals with experimental diabetes mellitus [11].

An important achievement is normalization of melatonin secretion in old monkeys after injection of epiphyseal peptide [11,24]. Circadian rhythm of production of hydrocortisone (the main adrenal hormone) normalized in the same monkeys after injection of the peptide.

Numerous reliable data on high geroprotective activities of tissue-specific natural and synthetic peptides have been accumulated, and hence, studies of the recent years are focused on the geroprotective activity of peptides in elderly and senile humans [7,8]. For example, annual courses of thymic and epiphyseal preparations led to a significant reduction of mortality due to improvement of the functions of the immune, endocrine, cardiovascular systems, brain, and improvement of bone tissue compactness [7]. The use of thymus preparations 2-fold reduced the incidence of acute respiratory diseases. The fact of restoration

TABLE 1. Effects of Peptide Bioregulators on the Lifespan, Estrous Function, and Development of Spontaneous Tumors in Laboratory Animals

Species, strain	Gender	Beginning of treatment	Life span, % of control		Population velocity of aging, % of control	Effect on estrous function	Effect on development of spontaneous tumors
			mean	maximum			
Epithalamine (peptide complex)							
Rats	Females	3.5 months	+25*	+6	-52*	Inhibition of age-associated extinction of estrous function	↓
Rats	Females	15 months	+6	+9	-72*		↓
C3H/Sn mice	Females	3.5 months	+31*	+14	-27*		↓
SHR mice	Females	3.5 months	+13	+5	-23*		↓
	Females	3.5 months	+11*	-2	+2		↓
	Females	12 months	+6	-3	N.s.		↓
Thymalin (peptide complex)							
C3H/Sn mice	Females	3.5 months	+28*	+11	N.s.	No effect	↓
SHR mice	Females	3.5 months	+12*	0	-9		↓
	Females	3.5 months	-2	-6	N.s.		↓
	Females	12 months	+13*	0	N.s.		↓
Glu-Trp peptide							
Rats	Females	4 months	+2	+14	-42*	No effect	↓
			Lys-Glu				
CBA mice	Females	6 months	+3*	+7	-23*	No effect	↓
Ala-Glu-Asp-Gly peptide							
			Rats				
	Male	4 months	+9	+1	+25*		↓
	Females	4 months	+10*	+3	-4	Inhibition of age-associated extinction of estrous function	↓
SHR mice	Females	3 months	0	+12	-29*		↓
CBA mice	Females	6 months	+5*	+42	-46*		↓
SAMR-1 mice	Females	2 months	+5	-8	+66		—
SAMP-1 mice	Females	2 months	+7*	+8	+59		—
HER-2/neu mice	Females	2 months	+13*	+4	-6		↓

Note. N.s.: not studied. ↓: decrease in the incidence and/or multiplicity and/or lengthening of the latent period of tumors. «—»: no effect. *$p<0.05$ compared to the control.

of melatonin secretion level in patients treated with epiphyseal preparation [8] is particularly important. These results open new prospects for solution of some demographic problems.

Studies of the mechanisms of aging showed that involution of the main organs and tissues, paralleled by reduction of protein synthesis in the cells, underlies this process. Treatment with peptides isolated from young animal organs are capable of inducing protein synthesis paralleled by recovery of the main functions.

Long-term use of peptides isolated from organs and synthesized from analogous amino acids in animals (as a rule, starting from the second half of life)

significantly prolongs the mean lifespan by 25-31% and it reaches the species-specific limit.

Short peptides (di-, tri, and tetrapeptides) are capable of complementary interaction with specific DNA binding sites at the gene promotor site, causing the double strand separation and activation of RNA polymerase. Detection of peptide stimulation of gene transcription indicates a natural mechanism maintaining the physiological functions of the body, based on complementary interactions of DNA with the regulatory peptides. This process underlies the development and functioning of living matter, while aging is an evolution-determined biological process of age-specific changes in genes expression and structure.

Preventive treatment with peptide preparations in humans led to a significant restoration of the main physiological functions and a significant reduction of mortality in different age groups during 6-12 years.

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