

Peptides, Genome, Aging

V. Kh. Khavinson

Institute of Bioregulation and Gerontology, pr. Dinamo 3, St. Petersburg, 197110 Russia

e-mail: ibg@gerontology.ru

Pavlov Institute of Physiology, Russian Academy of Sciences, nab. Makarova 6, St. Petersburg, 199034 Russia

Mechnikov Northwestern State Medical University, ul. Kirochnaya 41, St. Petersburg, 193015 Russia

Abstract—During investigation of the mechanism of the action of short peptides, it was established that they regulate gene expression and protein synthesis. These peptides stimulate proliferation and differentiation and suppress cell apoptosis, which leads to the restoration of the functions of various organs. Administration of peptides helps to reduce the incidence of cancer and to increase the average and maximum lifespan of animals. Most experiments identified that the peptides are able to enhance the physiological resource of cells, tissues, and the body up to 20–42%. The use of peptide preparations in patients contributed to increased vital resource and led to reduced mortality of elderly and senile aged persons.

Keywords: peptides, gene expression, protein synthesis, longevity

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INTRODUCTION

Fundamental investigation of the role of short peptides in the physiological regulation of body functions during aging has been a topical task in gerontology for the past decade. This is extremely important for knowledge of the mechanism of action of peptides and evidence of their safety and efficacy in creating new drugs.

Theoretical and experimental studies of peptides have made it possible to clarify the molecular mechanism of their biological activity and function. For this, the effects of peptides on gene expression, proliferation, apoptosis, differentiation, organ functions, carcinogenesis, and animal longevity were studied. Clinical research assessed the effect of the peptides on human vital resources and survival of elderly and senile aged people [16, 25, 26, 28, 30, 35, 36, 38, 43, 44, 61, 64, 65, 69, 70].

REGULATION OF GENE EXPRESSION

It was established that the FITC-labeled di-, tri-, and tetrapeptides enter the cytoplasm, the nucleus, and the nucleolus of HeLa cells [23]. It is known that the nucleus of eukaryotic cells has a system of nuclear pores formed by protein complexes—the nucleoporins. The inner diameter of nuclear pores is about 50 nm. Consequently, they are permeable to low molecular weight freely diffusing substances with a

molecular weight up to 3500 Da. Thus, the short peptides (Table 1) by their physical and chemical characteristics (charge, size, and hydrophobicity) can pass through the cytoplasmic and nuclear membrane of a cell and interact with DNA [47].

According to the physical methods (UV spectroscopy, circular dichroism, viscosimetry, atomic force microscopy) and molecular simulation, signal peptides are capable of binding to DNA in solution in vitro [31, 39, 41, 42, 48, 58]. This process takes several hours and occurs practically without electrostatic forces. As a result of complex formation, which is carried out in the groove of DNA with the involvement of nitrogenous bases, peptide destabilization of the secondary structure of the macromolecule is observed. Using spectrophotometry, a concentration-dependent hyperchromic effect was detected in the ultraviolet region of the spectrum (increased absorbance of the solution at a wavelength of 260 nm) in a mixture of the peptide AEDG and double-stranded DNA. The hyperchromic effect indicates partial destruction of the hydrogen bonds between the nucleotide pairs of the double helix and a local separation of chains of the double helix (allosteric conformational change).

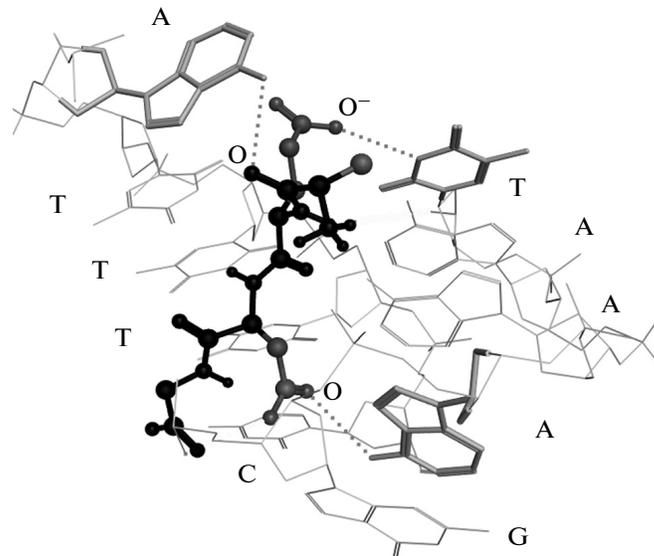
The experiment established that the separation of chains (melting) of free synthetic DNA occurs at +69.5°C. The spiral melted at +28°C in the system of DNA with the peptide AEDG and was characterized

Table 1. Basic peptides synthesized in the St. Petersburg Institute of Bioregulation and Gerontology [47]

Name (structure)	Biological activity	Molecular mass, Da
Thymogen (EW)	Immunomodulator	333
Vilon (KE)	Stimulant of tissue regeneration	275
Normoftal (KE)	Regulation of the functions of the retina	275
Kartalaks (AED)	Regulation of the functions of joints	333
Pinealon (EDR)	Regulation of brain functions	418
Honluten (EDG)	Regulation of the functions of the respiratory system	319
Vezugen (KED)	Regulation of vascular function	391
Kristagen (EDP)	Immunomodulator	358
Ovagen (EDL)	Regulation of the liver function	375
Epithalon (AEDG)	Regulation of the neuroendocrine system	390
Prosta-Max (KEDP)	Regulation of the functions of the prostate gland	488
Livagen (KEDA)	Regulation of the liver function	462
Kortagen (AEDP)	Regulation of brain functions	430
Pankragen (KEDW)	Regulation of pancreatic functions	576
Kardiogen (AEDR)	Regulation of myocardial function	490
Testagen (KEDG)	Regulation of the functions of the testes	448
Bronhogen (AEDL)	Regulation of the functions of the bronchi	446

by decreased indicators of entropy and enthalpy of the process by about two times. This important fact indicates the feasibility of a thermodynamically assisted way of DNA strand separation at a temperature range characteristic for biochemical reactions. This also indicates that separation of DNA chains at the physiological temperature is not denaturation and is characteristic for the initiation of protein synthesis process.

The theoretical and experimental results allowed proposing a model of interaction of peptides with DNA. They point to the formation of a stable DNA-peptide complex [59]. Analysis of the main physicochemical parameters of the complex (the number of hydrogen bonds, hydrophobic and electrostatic interactions, energy minimization of the DNA-peptide) was performed with molecular simulation and made it



The interaction of the peptide AEDG with nitrogenous DNA bases (sequence ATTTC). The dotted line designates the hydrogen bonds between the atoms of the peptides and DNA; the nitrogenous bases of DNA forming peptide hydrogen network are in thick lines. Letters refer to the nitrogenous bases (A—adenine, T—thymine, G—guanine, C—cytosine) and the atoms of the peptides (N—nitrogen, O—oxygen).

possible to determine the quantitative characteristics of the DNA-protein complex [Molecular Operating Environment; Chemical Computing Group Inc. (2012), 1010 Sherbooke St. West, Suite no. 910, Montreal, QC, Canada, H3A 2R7, 2012]. A three-dimensional model of AEDG peptide interaction with the ATTTC DNA site was created on the basis of these calculations (figure) [40].

The specific binding of peptides to oligonucleotides was revealed, which may be of particular importance for the epigenetic mechanism of regulation of gene expression [34, 37]. The interaction of short peptides namely with single-stranded DNA can directionally control gene expression. Furthermore, the binding of short peptides (AEDG) with DNA is accompanied by local untwisted strands of DNA that may give rise to a single-stranded target appearance for binding of a peptide with DNA.

It was established that short peptides modulate the action of endonucleases. Most likely, modulation of the action of endonucleases by peptides occurs due to site-specific binding of the peptide–DNA that protects DNA from enzymatic hydrolysis. Modulation of endonuclease action by peptides, in turn, is modulated by histones. Histones of chromatin in the nucleus can affect the binding of short peptides with DNA. Along with this, some peptides can apparently control the hydrolysis of DNA by endonucleases and at the level of peptide interaction with the enzyme [24, 34, 37].

It was found that short peptides activate heterochromatin in the cell nuclei of senile aged people and contribute to “liberation” of genes repressed by heterochromatinization of euchromatin regions of chromosomes that occurs with aging (Table 2) [27, 34, 66].

Structural chromatin condensation closely correlates with the functional heterogeneity. It was established that during aging heterochromatinization is enhanced, which correlates with the inactivation of previously active genes. Tightly condensed heterochromatic chromosomal regions are genetically inactivated and are replicated late. Decondensed (euchromatic) regions of chromosomes function actively. It is known that active chromatin is a necessary condition for the transcriptional activity of genes. In the cell nucleus, there are two types of chromatin: light euchromatin and dense heterochromatin adjacent to the nuclear membrane. Gene transcription occurs in the light phase—in euchromatin. The amount of heterochromatin in the nucleus increases during aging. Regulatory peptides increase the amount of euchromatin in the nucleus. This means that a larger number of genes becomes available for the transcription factors and transcription occurs more intensively and protein synthesis increases. The higher the content of euchromatin in the nucleus, the more intensive protein synthesis in a cell. The results of this experiment led to the conclusion that heterochromatinization is a reversible process.

Table 2. Effect of peptides on chromatin in lymphocytes in the elderly

Group	Associating acrocentric chromosomes (in a cell)	Deheterochromatinization of facultative heterochromatin (in a cell)	Total heterochromatin	Structural heterochromatin of chromosome 1
Norm (20–40 years)	1.33 ± 0.06	7.7 ± 0.4	Stable condition	Stable condition
Control group (75–88 years)	1.17 ± 0.05*	5.9 ± 0.2*	Heterochromatinization	Heterochromatinization
Peptide KE	2.39 ± 0.11**	9.9 ± 0.6**	Deheterochromatinization	Heterochromatinization
Peptide AEDG	2.32 ± 0.12**	8.4 ± 0.5**	Deheterochromatinization	Deheterochromatinization

* $p < 0.05$ compared to the norm (20–40 years); ** $p < 0.001$ compared with the control group (75–88 years).

Peptides KE and AEDG, when administered to the organism of transgenic mice, suppressed 2–3.6 times expression of the gene *HER-2/neu* (human breast cancer) compared with the control group. This suppression of gene expression was accompanied by a significant decrease in the diameter of the tumor [19].

The addition of the peptide AEDG to a culture of human lung fibroblasts was established to induce the expression of the telomerase gene; telomerase activity promotes telomere elongation 2.4 times [4, 32, 33]. Activation of gene expression was accompanied by an increase in the number of cell divisions at 42.5% demonstrating overcoming the Hayflick limit of cell divisions [31].

Using DNA-microarray technology, the effect of the peptides KE, EW, AEDG, and AEDP was studied on the expression of 15 247 genes of the heart and brain of mice. It was established that each peptide specifically regulates the expression of a specific group of genes. The results of the experiment point to an existing mechanism of peptide regulation of genetic activity. The experiment revealed that the dipeptide KE having immunomodulating activity regulates the expression of the *IL-2* gene in blood lymphocytes [2, 3, 6].

In cell cultures of human bronchial epithelial, the tripeptide ADEL activates the expression of bronchial epithelium differentiation genes *Nkx2.1*, *SCGB1A1*, *SCGB3A2*, *FoxA1*, and *FoxA2*. The peptide also increases the expression of the genes *MUC4*, *MUC5AC*, and *SftpAI*, the decreased activity of which correlates with the development of chronic bronchitis. The tetrapeptide KEDW in human pancreas cell cultures increased the expression of differentiation genes *PDX1*, *NGN3*, *PAX6*, *FOXA2*, *NKX2.2*, *NKX6.1*, and

PAX4 and reduced the expression of the genes *MNX1* and *HOXA3*.

It was established that the tripeptide EDG regulates mRNA expression of different genes in the model of induced gastric ulcer in rats. Peptide EDG lowered synthesis of mRNA genes encoding proteins of cellular metabolism SOD, TNF- α , and Cox-2 [49].

Thus, the specific (complementary) peptide-DNA interactions can epigenetically control the genetic functions of the cell, and, most likely, this mechanism played an important role in the very early stages of the origin of life and further evolution.

REGULATION OF PROTEIN SYNTHESIS— MARKERS OF CELL PROLIFERATION, DIFFERENTIATION, AND APOPTOSIS

The addition of peptides led to tissue-specific stimulation of protein synthesis in the cells of organs from which these peptides were isolated. The effect of protein synthesis enhancement after administration of peptides was detected in young and old animals [36]. It was found that short peptides stimulate tissue-specific expression of differentiation factors CXCL12, Hoxa3, and WEGC1: peptide KEDW in pancreatic cells, peptide ADEL in the bronchial epithelium, and peptide KED in fibroblasts. The inducing effect of the peptides on the expression of differentiation factors is most pronounced in the “old” cell cultures, which may be one of the mechanisms of their geroprotective actions [51]. In addition, the peptide KEDW increased expression of metalloproteinases (MMP2, MMP9), serotonin, glycoprotein CD79 α , antiapoptotic protein Mcl-1, and proliferation factors PCNA

and Ki67, as well as decreased the expression of the proapoptotic protein p53 in the “old” pancreatic cell cultures [29, 57]. The peptide ADEL regulates the synthesis of the protein Ki67, Mcl-1, p53, CD79, and NOS-3 in human bronchial epithelial cell cultures in different passages. This contributes to the activation of cell renewal and increased functional activity of the cells of the bronchial epithelium.

The tripeptide KED in a cell culture of human cortical thymocytes strengthened differentiation of thymocytes in the direction of regulatory T-cells, increased their proliferative activity, and decreased apoptosis. Furthermore, the peptide KED stimulated proliferation (Ki67) and the anti-apoptotic (Mcl-1) activity of mature regulatory T-cells. The effect of the peptide KED on stem CD34⁺ cells of bone marrow was studied. It was shown that the peptide KED stimulated the expression of CD14 myeloid cells marker and B-lymphocytes antigen CD19 in the bone marrow [54, 67].

Addition of the AEDG peptide to a culture of pinealocytes resulted in stimulation of the synthesis of the arylalkylamine-*N*-acetyltransferase (AANAT) enzyme and the transcription factor pCREB involved in the synthesis of melatonin from serotonin. Furthermore, the content of melatonin increased in the culture medium under the action of AEDG [5, 7, 9, 13, 17, 50].

The addition of peptides AEDG and KE in embryonic cultures of retinal cells contributed to the induction of differentiation of various types of retinal neurons (activation of Brn3, Pax6, Prox1, and Vsx1 proteins expression) and pigment epithelium (activation of transterritin protein synthesis) [46]. The addition of retinal peptides to the pluripotent cells of the ectoderm of early gastrula of the frog *Xenopus laevis* led to the emergence of retinal cells and pigment epithelium. Adding other short peptides to the pluripotent cells of the ectoderm in the same experimental model led to the emergence of a variety of tissues [45, 53].

It was found that the peptide AEDR strengthened the expression of cyto- and caryoskeleton proteins in the cell culture of embryonic fibroblasts. This peptide increased the expression of cytoskeletal proteins (actin, tubulin, and vimentin) 2–5 times and that of nuclear proteins (lamin A, lamin C) by 2–3 times. Thus, the molecular mechanism of this tetrapeptide is based on its ability to activate the synthesis of cyto- and caryoskeleton proteins, which enhances cell proliferation and reduces apoptosis [52].

These experiments demonstrated that peptides are able to induce differentiation and proliferation and suppress cell apoptosis depending on the structure of the substance added. The results of analysis of these studies provide a basis to conclude the feasibility of targeted induction of cell differentiation and use of the

cell reserve of various organs and tissues of the body. This is the material substrate to enhance vital resources of the organism and to increase the average longevity to the species limit.

IMPACT ON LONGEVITY

Peptide preparations of the thymus (the drug Timalin) and the epiphysis (the drug Epithalamin) contributed to a significant increase in the average longevity of animals by 25–40% compared with the control group [21]. In some experiments, a slight increase in the maximum longevity was also recorded. The most significant effect of increasing the maximum longevity was observed in the CBA mice lineage when the peptide AEDG was administered and reached 42.3%. A clear correlation of the increase in the mean longevity and the main indicator of cell immunity, the T-lymphocyte blast transformation reaction on phytohemagglutinin, should be emphasized.

IMPACT ON CARCINOGENESIS

Peptides isolated from the epiphysis and thymus had reliable antitumor activity. This was accompanied by reduced frequency of spontaneous and radiation- or carcinogen-induced malignancies in animals by 1.4–7 times [1, 8, 10–12, 14–16, 18, 22]. It should be emphasized that this unprecedented level of reducing the number of tumors was observed in the majority of experiments. The results of these studies, taking into account the general mechanism of carcinogenesis in all mammals, are of great practical importance for prevention of tumors in humans.

USE IN HUMANS

The use of thymus peptide drugs (the preparation Timalin, peptides EW and KE) was found to be efficient in many diseases and conditions associated with a decrease in cellular immunity and phagocytosis: in radiation therapy and chemotherapy in cancer patients, in acute and infectious-inflammatory diseases, with the use of massive doses of antibiotics, under the suppression of the regeneration processes in post-traumatic and postoperative periods in cases of various complications, in obliterating diseases of limb arteries, in chronic liver disease, in prostate cancer, in complex treatment of certain forms of tuberculosis, and in treating leprosy [36, 60, 62, 68].

A decreased rate of aging and mortality was established for elderly people with accelerated aging of the cardiovascular system in randomized comparative study during a 15-year observation period. It was established that long-term use of the preparation Epi-

thalamine (6 courses for three years) reduced the rate of aging of the cardiovascular system, restored an age-dependent decline in physical performance, and had a normalizing effect on the circadian rhythm of melatonin synthesis and carbohydrate and lipid metabolism. Reduction in mortality rate according to Kaplan–Meier survival curves also points to the geroprotective effect of the peptide [61].

A significant neuroprotective effect is provided by the peptide preparation Cortexin isolated from the cerebral cortex. This preparation improves memory processes, stimulates reparative processes in the brain, and accelerates the recovery of its functions after stressful factors. The drug is effective in traumatic brain injury, impairments in cerebrovascular circulation, viral and bacterial neuroinfections, encephalopathy of various genesis, and acute and chronic encephalitis and encephalomyelitis. A particularly high efficacy of the brain peptide preparation was observed in elderly and senile patients [36].

The peptide preparation Retinalamin isolated from the retina of animals has bright clinical efficacy [46, 55]. This unique drug was first created in medical practice and applied in patients with various degenerative diseases of the retina: diabetes retinopathy, involuntal dystrophy, retinitis pigmentosa, and other pathologies. Of particular importance was the ability of the preparation to restore the electrical activity of the retina that generally correlated with improvement of visual function [36].

A distinct effect was observed in patients after administration of the peptide preparation Prostatilen (Samprost) isolated from the prostate gland of animals. The drug proved to be effective in chronic prostatitis, adenoma, and complications after surgery on the prostate gland, as well as in various age-related impairments of the prostate [36].

Furthermore, it was found that short synthetic peptides exhibit resistance to hydrolysis in the gastrointestinal tract and blood. Oral administration of the peptide KEDW in elderly patients with diabetes mellitus type 2 helped to reduce the level of plasma glucose and the insulin resistance index [63]. Oral administration of the peptides EDR and EDP in athletes contributed to the normalization of functions of the antioxidant system [20, 56], increased level of adaptation to physical load, fitness of the organism, and energy metabolism. In addition, the tripeptide EDR under oral administration proved to be an effective means in the treatment of CNS pathology (the consequences of traumatic brain injury). The peptide KED in combination with general treatment in patients of elderly and senile ages with atherosclerosis proved to be significantly effective. Oral application of the tripeptide AED contributed to restored functional activity of the

musculoskeletal system in patients. Oral administration of short peptides was found to be effective in various pathologies (diabetes, atherosclerosis of vessels, disruption of the central nervous system, accelerated aging due to high physical load).

Thus, long-term study and use of peptide preparations showed their high efficacy in patients of different age groups. The undoubted advantage of this group of peptide geroprotective bioregulators is the absence of any side effects [36].

In conclusion, it should be emphasized that the described peptide bioregulators bind selectively with specific sites of DNA in vitro. These peptides epigenetically regulate the expression of genes (oncogenes, telomerase gene, interleukin genes, and transcription factor genes) and protein synthesis—markers of cell differentiation, proliferation, and apoptosis, and increase the length of telomeres in somatic cells. Peptides enhance vital resources and average longevity.

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