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REVIEWS

Mechanisms Underlying Geroprotective Effects of Peptides

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We review the role of peptides in aging and the mechanisms underlying the geroprotective effect of peptide preparations. Geroprotective properties of peptides are associated with their influence on systems maintaining homeostasis in the body and regulation of mechanisms underlying aging. Peptides normalize synthesis of tissue-specific proteins and regulate expression of genes responsible for proliferation and differentiation of cells. Thus, peptides maintain normal physiological functions and decelerate aging.

Key Words: *peptides; geroprotective effect; aging; age-related diseases*

Much advances in theoretical and applied gerontology in the last decade open perspectives in the directional regulation of age-related processes. Recent gerontological studies are directed at prevention of accelerated aging and age-related diseases. This would increase the life span of humans and maintain normal physiological functions.

Natural and synthetic antioxidants, including the pineal hormone melatonin and vitamins E and C, possess geroprotective properties, protect cells in various organs and tissues from destructive changes, and are widely used in clinical practice [41, 42]. The search for new potent geroprotectors is stimilated by the appearance of various adverse factors and accelerated development of age-related diseases. These factors accelerate aging manifested in adaptive reconstruction, age-related changes in neurohormonal regulation, and suppressed synthesis of tissue-specific proteins. These processes are accompanied by a decrease in reserve capacities, accumulation of catabolic products, progressive destruction, functional disturbances in cells, and development of diseases. Theories concerning the mechanisms of aging postulate the existence of a relationship between factors that consecutively trigger the genetic program of cell degeneration. Changes in the expression of various genes regulating proliferation, differentiation, and death of cells play the major role in aging. Published data indicate that these processes are stochastic. The individual resistance of cells and organisms to stress plays an important role in aging.

Aging is characterized by complex molecular, genetic, and biochemical changes and disorganization of the peptidergic system regulating functional activity of organisms. Studies of organs and tissues undergoing age-related involution showed that they are characterized by suppressed secretion of physiologically active peptides and low intensity of protein synthesis. These data suggest that peptides play an important role in the mechanisms of aging. The peptide system is involved in the neuroimmunoendocrine interactions [9]. Moreover, the regulation of physiological functions is realized via tissue-specific peptides maintaining cellular homeostasis [11,12,37]. Peptide bioregulators are present in cells

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and tissues, formed during partial proteolysis, possess various biological properties, and coordinate functional activity and development of multicellular systems. Despite multilevel hierarchy, all mechanisms underlying the regulation of homeostasis by peptides coordinate biosynthetic processes in cells via modulation of gene expression.

The regulation of homeostasis by peptides plays an important role in aging of cells, tissues, organs, and organisms [2]. Morphofunctional manifestations of aging are involution of organs and tissues that belong to the major regulatory system, including nervous, endocrine, and immune systems [9, 18]. Previous studies revealed age-related hypoplasia or atrophy of the pineal gland (epiphysis), thymus, neurons in the brain cortex and subcortical structures, retina, vascular wall, and reproductive organs [31-33,36]. Age-related involution of organs and tissues leads to their functional disturbances. At the cellular level, these changes are manifested in impairment of specific protein synthesis. Suppressed synthesis of regulatory peptides in cells and changes in the sensitivity of target cells are the key events in aging. Taking into account that peptides have various biological properties [4], it can be assumed that the decrease in their production is accompanied by the impairment of regulatory mechanisms and functional disturbances in aging organisms. However, some authors reported that secretion of regulatory peptides is intensified during aging [27]. These age-related changes result from an imbalance between regulatory factors or reflect compensatory processes. The peptide regulation of homeostasis suggests processing of polypeptides. Peptidase activation leads to the formation of short peptide fragments at a particular site and time. These fragments possess a higher biological activity than parent compounds. Endo- and ectopeptidase activities in cells decrease with age, which is accompanied by the formation and accumulation of peptidase-resistant peptides. However, the cause of peptide accumulation in old cells remains unclear. Studies of age-related changes in the peptide regulation of homeostasis indicate that aging is accompanied by suppression of synthesis and secretion of regulatory peptides and decrease in target cell sensitivity.

Since endogenous peptides play an important physiological role in the regulation of aging, elaboration of new peptide geroprotectors is of particular interest. It should be emphasized that the endogenous dipeptide carnosine (β-Ala-His) was first shown to have geroprotective properties. The geroprotective effect of carnosine is primarily related to its antioxidant activity [5].

Polypeptide Geroprotectors

Peptide complexes were isolated from various cells, tissues, and biological fluids [11,12,14]. Physicochemical assays showed that these peptides differ in the composition, molecular weight, and electrochemical properties. Peptide complexes are widely used in medical practice for the therapy of various diseases. However, the mechanisms of their geroprotective effects require further investigations.

Aging is accompanied by involution of cells in the pineal gland and thymus (central endocrine and immune organs) and suppression of production of physiologically active proteins. It was hypothesized that peptides present in the pineal gland and thymus play an important role in aging.

Peptide bioregulators from the pineal gland (Epithalamin) and thymus (Thymalin) of young animals are used in medical practice for the correction of disturbances accompanying age-related involution of these organs [11,14]. Experiments with various strains of rats, mice, and *Drosophila melanogaster* showed that these preparations markedly increase the life span of animals and decelerate the development of age-related diseases [3,36]. Epithalamin decelerates the development of age-related changes in reproductive and immune systems and increase the life span of mice and rats by 30-40%. These changes are probably realized via stimulation of melatonin synthesis and secretion and activation of antioxidant protective systems. Epithalamin increases the average life span of *D. melanogaster* females and activates superoxide dismutase (SOD) in male flies.

Epithalamin and Thymalin are widely used in geriatrics [8,23-25]. Epithalamin normalizes the contents of estradiol, follicle-stimulating hormone, and cortisol in women during climax. This preparation decreases blood glucose level in patients with non-insulin-dependent diabetes mellitus. Treatment with Epithalamin in a combination with radiotherapy and chemotherapy increases lymphocyte count, improves the state, and attenuates the symptoms of intoxication in patients with hormone-dependent tumors [26]. Epithalamin increases plasma antioxidant and antiradical activities, decreases the content of lipid peroxidation products, and activates SOD and glutathione peroxidase in elderly patients. These data indicate that Epithalamin holds much promise for the correction of antioxidant status during accelerated aging and age-related diseases.

Thymalin increases the average life span of mice by 28% and decreases the incidence of neoplasms [14]. The thymus undergoes involution during aging and stress (accidental involution), which is accompanied by the decrease in immune reactivity and resistance to pathogenic factors. Thymalin

is used for the therapy of old and elderly patients with involution of the thymus [24].

Polymorphism of clinical manifestations of aging is associated with neurophysiological mechanism of functional changes in the regulation of integrative brain activity. Previous studies showed that age-related changes in cerebral circulation, bioelectric activity of the brain, and parameters of higher nerous activity depend on genetically determined resistance to brain disorders, diseases, traumas, and metabolic disturbances. Integrative activity of the brain during ontogeny determines not only the rate and type of aging, but also adaptive capacities of the organisms. These characteristics improve viability and contribute to the development of adaptive and regulatory mechanisms, which promotes the survival of species and increases the life span. Present notions of the mechanisms underlying neuroimmunoendocrine regulation of functions allow using the polypeptide preparation from animal brain cortex Cortexin for the therapy of patients with brain disorders and traumas. During studies of the efficiency of Cortexin in neurological practice, particular attention was given to old and elderly patients with brain disorders associated with organic damages to the cortex and circulatory disturbances [19]. High efficiency of Cortexin during the therapy of patients with acquired encephalopathies is probably associated with its direct effects on metabolic processes in nerve cells, which promotes the recovery of neuronal activity, contributes to the development of new associative between various brain regions, and improves integrative functions of the brain. It is now impossible to identify a particular function of the brain, which is selectively modulated by Cortexin. Simultaneous stimulation of locomotor activity, memory, learning abilities, emotions, and motivations by Cortexin suggests that the preparation affects subcortical nerve centers regulating and initiating these processes. The sedative, antistress, and antiepileptic effects of Cortexin are related to an increase in γ-aminobutyric acid and serotonin levels in brain neurons. Clinical activity of Cortexin is associated with functional modulation of dopaminergic synapses.

Aging is accompanied by specific functional changes in the vision system and its peripheral organ (eye). The therapy of retinal dystrophy in elderly and old patients is of considerable medical and social importance. The polypeptide preparation from the retina Retinalamin stimulates photoreceptors and cells in the retina, improves functional relationships between the pigment epithelium and outer segments of photoreceptors during dystrophic changes, and promotes the recovery of light sensitivity in the retina. The preparation normalizes vascular permeability, attenuates the symptoms of inflammation, and stimulates reparative processes in patients with retinal diseases and traumas [11,29].

The therapy of patients with chronic prostatitis and benign hyperplasia of the prostate (adenoma) is an urgent problem of geriatric urology and andrology. The polypeptide preparation from animal prostate gland Prostatilen possesses pronounced tissue-specific and geroprotective properties [1]. The therapeutic effect of Prostatilen is associated with normalization of cell proliferation and differentiation in the prostate, regulation of smooth muscle cells in the urinary bladder, and inhibition of platelet-vascular hemostasis.

Experimental and clinical observations indicate that the phenomenon of tissue specificity is important for understanding the geroprotective effect of polypeptide preparations [12, 14]. Probably, exogenous polypeptide preparations temporally compensate disturbances in physiological regulation, which results in the recovery of diminished or lost functions. Functional activity is then maintained at the normal level for a long time. These data indicate that compensatory functional systems can be restored in old organism [20]. We hypothesize that these changes are related to the ability of peptide preparations to normalize and maintain protein synthesis at the level observed in young organisms. The recovery of receptor proteins in cells normalizes their sensitivity to other humoral regulators.

Synthetic Peptide Geroprotectors

Studies of peptide functions and structure contribute to understanding of the mechanisms underlying their geroprotective effects. The synthetic analogue of immunoactive dipeptides obtained from Thymalin (Thymogen, Glu-Trp) stimulates differentiation of T lymphocytes, induces expression of differentiating antigens in these cells, and normalizes the count and ratio between T and B lymphocytes in the blood under pathological conditions [15,40]. Thymogen suppresses the growth of spontaneous and radionuclide-induced tumors and increases the life span of animals [25,35].

It should be emphasized that the isolation of individual peptides and estimation of their biological activity is an involved and long-term process, which requires studies of hundreds peptides and can not be performed over a short time. A new approach to the search and synthesis of physiologically active peptides consists in amino acid assay of complex polypeptide preparations, estimation of major tissue-specific amino acids in each prepara-

tion, and construction of the primary peptide structure on the basis of energetically advantageous conformations for ionized molecules. This approach allowed us to obtain peptide preparations regulating functional activity of the thymus, brain cortex, pineal gland, retina, vessels, heart, bronchi, prostate gland, and liver.

A comparative study of biological activity in complex polypeptide preparations and synthetic peptides revealed their similar effects on various organs and tissues under normal and pathological conditions.

Epithalon (Ala-Glu-Asp-Gly) was synthesized after amino acid assay of Epithalamin. Similarly to Epithalamin, Epithalon stimulates the growth of cultured nerve cells from subcortical structures. Being added into the nutrient medium at the larval stage of *D. melanogaster*, Epithalon prolongs the life span of adult flies [39]. It should be emphasized that under these conditions melatonin (control preparation) displays no biological activity. Epithalon stimulates the antioxidant system in flies, which probably underlies its influence on aging organism [28]. Epithalon produces pronounced antioxidant effects in old rats, which is related to the activation of antioxidant enzymes. Epithalon markedly stimulates melatonin synthesis in the evening time and normalizes circadian rhythms of cortisol secretion in old female rhesus macaques. It is important that Epithalon 3-fold intensifies melatonin production in old, but not in young monkeys [38]. This preparation normalizes the relationships between various endocrine cells and intensifies synthesis of extrapineal melatonin and serotonin after pinealectomy [22]. Administration of Epithalon to γ-irradiated rats enhanced functional activity of the pineal gland, which suggests that the preparation produces pronounced reparative effects on this organ [30]. Other experiments confirmed the effects of Epithalon on functional activity of subcortical brain structures. This preparation stimulates interleukin-2 (IL-2) gene expression in various hypothalamic structures in animals. It should be emphasized that Epithalamin and Retinalamin contain the same amount of tissue-specific amino acids. This is probably associated with similar morphofunctional and histogenetic characteristics of the pineal gland and retina and indicates that during ontogeny functional activity of these organs is regulated by the same peptidergic mechanisms [7]. Administration of Epithalon, which is structurally similar to the tetrapeptide constructed after amino acid assay of Retinalamin, to rats with hereditary pigment degeneration of the retina normalizes its functional activity and morphological structure.

The effects of other synthetic peptide Vilon (Lys-Glu) were studied in cultured cells of the thymus and spleen from newborn rats. This peptide in various concentrations inhibits the development of thymus cells, but stimulates the growth of spleen cells. Its effects are comparable with the activity of Thymogen (Glu-Trp). Vilon improves reparative regeneration and healing of wounds and normalizes metabolic processes in lymphocytes, macrophages, and fibroblasts. The molecular and genetic mechanisms of Vilon-induced changes include stimulation of IL-2 gene expression in lymphocytes, which intensifies differentiation of immunocompetent cells [15]. Aging and carcinogenesis are accompanied by a decrease in functional activity of T cells and disturbances in cell-mediated immunity. Studies of geroprotective and antitumor properties of peptides indicate that their effects are realized via thymomimetic regulatory mechanisms [15,34]. Antiangiogenic therapy of tumors attracts much recent attention, since the formation of new vessels plays an important role in the development and distribution of tumors [16]. Vilon decelerates the growth of transplanted M-1 sarcoma in rats, which is not associated with its direct cytotoxic effect on tumor cells. These data suggest that antitumor activity of Vilon is realized via stromal cells, including endotheliocytes. It should be emphasized that Vilon suppresses initiation and promotion of carcinogenesis.

Previous studies revealed the neurotrophic effect of Cortagen (Ala-Glu-Asp-Pro), which was constructed on the basis of amino acid assay of Cortexin. Intramuscular injection of Cortagen restores anatomical characteristics and functional state of fibers in damaged peripheral nerves [21].

The synthetic peptide Prostamax (Lys-Glu-Asp-Pro) was constructed on the basis of amino acid assay of Prostatilen. These peptide preparations normalize inflammation the prostate gland in rats with chronic bacterial prostatitis.

Experimental studies indicate that peptide bioregulators control gene expression and protein synthesis in cells [13]. The synthetic peptide bioregulator of liver functions Livagen (Lys-Glu-Asp-Ala) normalizes the intensity and rhythm of protein synthesis in monolayer hepatocyte cultures from old rats. These results suggest that the geroprotective effect of short peptides is associated with regulation of gene expression and biosynthesis impaired during aging. This property is of considerable importance, since the genetic apparatus plays the major role in the mechanisms of individual development and pathogenesis of diseases [6,10]. R. V. Petrov *et al.* [17] hypothesized that regulatory peptides are

medicinal preparations of a new generation that possess natural correcting properties and do not produce side effects.

Experimental and clinical observations show that peptide preparations possess geroprotective activity, which is related to normalization of homeostasis-maintaining systems and regulation of aging. Peptides intensify the synthesis of tissue-specific proteins and regulate expression of genes responsible for cell differentiation and proliferation. Moreover, peptides act as informational regulators of genetic stability. Thus, peptides maintain normal physiological functions and decelerate aging.

These data indicate that aging is an evolutionarily determined biological process of changes in gene expression, which is accompanied by the impairment of synthesis of tissue-specific regulatory peptides in various organs and tissues, structural and functional reconstructions, and development of diseases. Further studies of tissue mechanisms underlying geroprotective activity of peptides would open new perspectives in the regulation of aging, prevention of accelerated aging and age-related diseases, and increase in an active life span of humans.

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