Peptide Bioregulators Inhibit Apoptosis

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The effects of peptide bioregulators epithalon and vilon on the dynamics of irradiation-induced apoptotic death of spleen lymphocytes in rats indicate that these agents inhibit physiologically programmed cell death. The antiapoptotic effect of vilon was more pronounced, which corroborates the concept on tissue-specific effect of peptide bioregulators.

Key Words: *peptide bioregulators; apoptosis; aging*

Aging of living organisms is accompanied by cell loss [7]. Age-related elimination of the cells is genetically controlled and characterized by similar cytological and biochemical manifestations in diverse organs and tissues, described as the physiologically programmed cell death (apoptosis). The term "apoptosis" was coined in [11] to denote the peculiar type of cell death, which differs morphologically from necrosis. It is shown that apoptotic cell death induced by various endogenous and exogenous stimuli of very low intensity develops according to a specific program promoting elimination of the nonviable cells with critical DNA abnormalities [6].

In mammals, *bcl-2* gene is the major inhibitor of apoptosis [10]. This is directly manifested by massive death of nerve and hemopoietic cells during embryonic development of *bcl-2* knockout mice [14]. These data corroborate the hypothesis that stimulation of apoptosis shortens the lifetime of the organism [8].

In mammals, the age-related involution of the pineal gland, thymus, gonads, and other organs and tissues is accompanied by apoptosis of parenchymatous cells [2]. In humans, the pathological induction of apoptosis provokes severe diseases accompanied by massive cell degeneration (Alzheimer disease) and premature aging [12]. Therefore, the search for new agents stabilizing apoptosis in normal organs at a genetically determined level is an actual problem of modern gerontology. In this respect, peptide bioregulators such as physiologically active peptides isolated from various organs and tissues known for their pronounced homeostatic effects are of particular interest [1]. Moreover, of importance is the fact that peptides isolated from the pineal gland [4,5] and thymus increase lifetime [1].

Taking into consideration the important role of apoptosis in the mechanisms of aging, our aim was to study the effect of epithalon and vilon, the synthetic peptide bioregulators, on the physiological death of lymphocytes. Epithalon (Ala—Glu—Asp—Glu) and vilon (Lys—Glu) were constructed and synthesized on the basic of amino acid analysis of epithalamine (a polypeptide preparation from the pineal gland) and thymalin (a thymic polypeptide), respectively, with due account for the data on amino acid sequence of other thymic peptides and cytokines [1].

MATERIALS AND METHODS

Experiments were carried out on 50 male Wistar rats weighing 120-140 g. The animals were maintained under standard vivarium conditions. Apoptosis was induced by irradiation.

The animals were subdivided into intact and four experimental groups of 10 animals each. The experimental rats were γ-irradiated in an GUB-2000 cobalt apparatus (200 rad/min, 6 Gy total dose). Group 1 rats (control) were not injected. Groups 2 and 3 rats were intraperitoneally injected with epithalon and vilon, respectively, during 5 days starting from postirradiation

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day 2 in a daily dose of 0.5 μg in 0.5 ml physiological saline. Group 4 rats were injected with physiological saline according to the same scheme. Specimens were taken from intact and experimental rats on postirradiation day 8. Splenic lymphocytes were chosen as the test object because of their rapid renewal [3]. The specimens of the spleen were fixed for 24 h in acid Bouin fixative and embedded in paraffin. The specimens were stained with hematoxylin and eosin for identification of apoptotic cells, splenic sections (5 μ) were impregnated as described elsewhere [13], to reveal condensed chromatin in dying cells. Morphometric study was performed using an Imstar S. A. image analysis system and Morphostar-2 and Colquant (Imstar S. A.) software. The total number and the number of apoptotic cells in lymphoid follicles were determined. The mean section area of the reactive center was 0.06 mm^2. The cells were counted in 60 fields (×900) under an Axiophot-2 immersion microscope (Zeiss). The total tested area included no less than 1000 lymphoid cell nuclei. The apoptotic index (AI) was calculated as the percentage of apoptotic cells. The data were analyzed statistically using parametric and nonparametric tests.

RESULTS

In all groups apoptotic lymphocytes were clearly identified by specific morphologic signs [3]: chromatin margination (selective impregnation [13]), cytoplasm condensation and thickening with the formation of «apoptotic bodies», and others.

In intact rats AI was 0.84±0.09%. Irradiation markedly stimulated apoptosis: AI increased 3.15-fold (to 2.65±0.10%, $p<0.05$). The peptide bioregulators significantly inhibited lymphocyte apoptosis. Compared to the control, epithalon and vilon decreased AI 2.12-fold (1.25±0.15%) and 3.4-fold (0.78±0.12%), respectively ($p<0.05$). Hence, vilon produced a more potent apoptosis-inhibiting effect compared to epithalon. In group 3 rats, AI decreased by 37.6% compared to group 2 rats. This fact corroborates the observation that peptide bioregulators are most active in their tropic tissues [1].

From biological and evolutionary viewpoints, apoptosis is a conservative process strictly controlled at the genetic level. Nevertheless, various exogenous and endogenous signals can affect cell viability [9]. The proliferotropic action of many hormones was described elsewhere [2,6]. Our data indicate that peptide bioregulators can regulate cell renewal in various organs. Stabilization of the apoptotic rate at a genetically determined level characteristic of a particular tissue is probably a target of peptide bioregulators. When apoptosis is stimulated (for instance by irradiation), the peptide bioregulators play an inhibitory role in this process.

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