Expression of AIF and CGRP Markers in Epiphysis and Thymus during Aging

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Abstract—The expression of apoptotic inducing factor (AIF), a marker of mitochondrial apoptosis, and calcitonin gene related peptide (CGRP), a neuropeptide, in the autopsy material of the epiphysis and thymus from individuals older than 60 years. The expression of AIF and CGRP was detected in both organs; however, it did not change with age, which indicates the possible preservation of signal functions in the organs of neuroimmunoendocrine system during its aging. A correlation between the AIF and CGRP expression was detected in epiphysis, while this dependence is absent in the thymus. It is possible that some common regulatory molecule that connects two signaling pathways is expressed in the epiphysis (as opposed to the thymus).

Keywords: epiphysis, thymus, signal molecules, aging.

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INTRODUCTION

Age-related changes in the adaptive ability of the organism are characterized by the morphofunctional reorganization of the organs of the neuroimmunoendocrine system (thymus and epiphysis). Information about the fundamental mechanisms of age involution on both organ and tissue and on the molecular level is required to correct the aging processes [5]. In recent years, a great deal of attention has been paid to the search for signal molecules, which play a key role in the morphofunctional involution of the organs [8, 13, 14]. The violation of the expression of the signal molecule may be an indicator of the cellular aging [4]. Apoptosis-inducing factor (AIF) and calcitonin gene-related peptide (CGRP) are of interest for the studying the mechanisms of age pathology.

AIF protein is a key factor, which introduces a cell in apoptosis in a caspase-independent way. The intensity of apoptotic processes in the organism increases with age, which results in a decrease in the pool of cells and their ability to maintain functional activity [6, 11]. Delayed or excess apoptosis is involved in the pathogenesis of age-associated diseases, such as Alzheimer's and Parkinson's disease [19], diabetes mellitus, and myocardial infarction [17], while the inability of dividing cells to enter into apoptosis promotes the development of cancer [9, 10, 12, 18].

CGRP peptide plays the role of a neurotransmitter during the transmission of the signal from afferent somatic nerve fibers to peripheral nerve fibers and skeletal muscles. In addition, CGRP is a cardioprotector and can prevent the aging of the endotheliocyte precursor cells in patients with hypertension, while the accelerated aging of the endothelium in these patients may be associated with a decrease in CGRP expression. There are data on the participation of CGRP in the immune response. White dendritic epidermocytes (Langerhans cells) and epithelial dendritic cells, which are capable to present antigens for stimulation of the cellular immunity, are associated with the nerve cells that express CGRP, which suppresses the presentation of antigens (by Langerhans cells) to type-1 T-helpers (*Th1*). CGRP stimulates the presentation (by Langerhans cells) of antigens for the cell response of type-2 T-helpers (*Th2*). Thus, contact between Langerhans cells with CGRP produced in situ by neurons can promote the development of *Th2* cellular immunity [9]. CGRP has a mitogenic effect and can influence the functioning of different types of skin cells. In the cell culture, CGRP increases the expression of *IL*-1α, *IL*-8, and *TNF*-α, and is able to increase the secretion of neuron growth factor in keratinocytes [9]. Change in the CGRP expression, especially in aging and elderly people, can result in the development of allergic reactions [15, 16], oncological diseases [17], and the violation of neural transmission and other pathologies [12]. Its expression was verified in the bronchoalveolar epithelium [15], skin, pancreas [7], and nerve and other tissues. Vasodilating, proinflammatory, and proangiogenic effects are the best-studied effects of the CGRP. Change in CGRP expression during aging can result in the violation of homeostasis of the indicated systems and be a reason for age pathology.
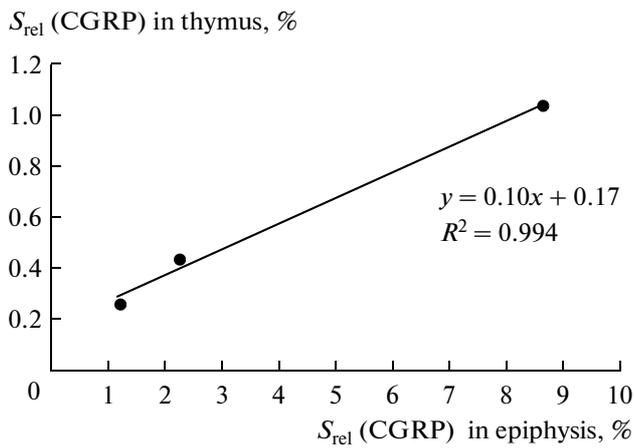
S_{rel} (CGRP) in thymus, %

$$y = 0.10x + 0.17$$
$$R^2 = 0.994$$

S_{rel} (CGRP) in epiphysis, %

Fig. 1. Dependence of area of CGRP expression in thymus on area of CGRP expression in epiphysis.

S_{rel} (AIF), %

$$y = 1.13x + 1.56$$
$$R^2 = 0.987$$

S_{rel} (CGRP), %

Fig. 2. Dependence of area of AIF expression in epiphysis on area of CGRP expression in epiphysis (men).

The data on multiple studies allow us to conclude that AIF and CGRP are key markers of age-related diseases because these signal molecules are localized in nearly all tissues of the organism and have a wide spectrum of functional activity. However, age-related peculiarities of the AIF and CGRP expression in the tissues of the thymus and epiphysis have not been yet determined. The aim of this study was to estimate the dynamics of the change in the AIF and CGRP expression in the thymus and epiphysis in different age groups, as well as the study of the interdependence of their expression [2, 3].

MATERIALS AND METHODS

Autopsy materials from the epiphysis and thymus were obtained from 18 individuals (eight men and ten women) aged 60−100. According to the World Health Organization (WHO) classification, material was obtained from patients from three age groups, including aging ($n = 6$), elderly ($n = 6$), and long-living ($n = 6$) patients. The fragments of the thymus and epiphysis were fixed in a neutral formalin solution (pH = 7), dehydrated, and poured in paraffin according to a standard method. The sections (3 μm thick) were prepared from paraffin blocks on a Leica 540 M microtome, then applied to glasses treated with poly-L-lysine (Sigma). The sections were stained with hematoxylin for the overview study.

The verification of AIF and CGRP expression was conducted by the immunohistochemical method with the avidin−biotin system of visualization. Hybridization using primary monoclonal rabbit antibodies to AIF (1 : 500, Abcam) and CGRP (1 : 500, Abcam) was conducted at room temperature for 30 min. Biotinylated antirabbit immunoglobulins from a universal kit (Dako) incubated at room temperature for 30 min were used as secondary antibodies. The visuali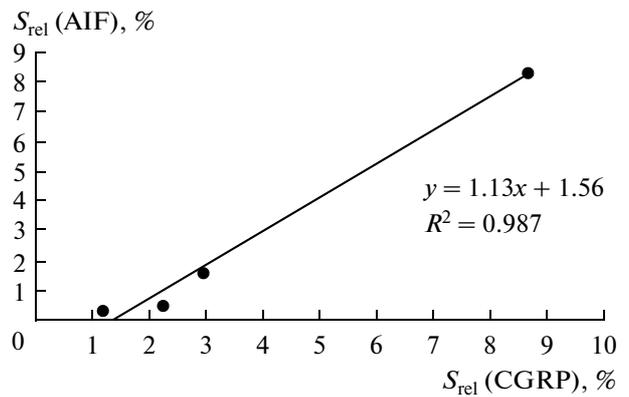zation of coloring was performed with a complex of avidin and biotinylated peroxidase (ABC-kit) with the subsequent development of horseradish peroxidase by diaminobenzidine (Dako). Immunohistochemical study was conducted on a Nicon E400 microscope. Three to ten fields of view (zoom 400) were photographed for each preparation.

In order to estimate the intensity of the coloring, morphometric studies were conducted using a system of computer analysis of microscopic pictures (Nikon) and a licensed program (Morphology 5.0). At the same time, the relative area of expression (S_{rel}) and optical density (P) were estimated. The relative area of expression was calculated in percentages as the ratio of the area of expression to a total area of the sample. The optical density was calculated as the ratio of the optical density of colored regions in a background optical density of the sample and was expressed in percentages. In order to estimate the level of expression in general, the index of expression level (IEL) was introduced, which is expressed as a product of the relative and relative optical areas of expression and is a nondimensional quantity.

A statistical treatment of the results was performed using SPSS 17.0 and Excel 2007 programs. To estimate the significance of differences between three age groups, a single-factor analysis of variance with Bonferroni criterion was used [1]. Regression analysis was used to analyze the type of expression parameter distribution depending on age. The verification of hypotheses of a correlation between the age and level of expression was estimated by means of Pearson's correlation criterion.

RESULTS AND DISCUSSION

The tendency of AIF IEL to increase with aging was detected in the thymus, which is caused by an increase in the area of expression at a constant optical density. An analysis of the dependence between the AIF and CGRP parameters of expression in the thymus (with elimination of the age parameter) demon-
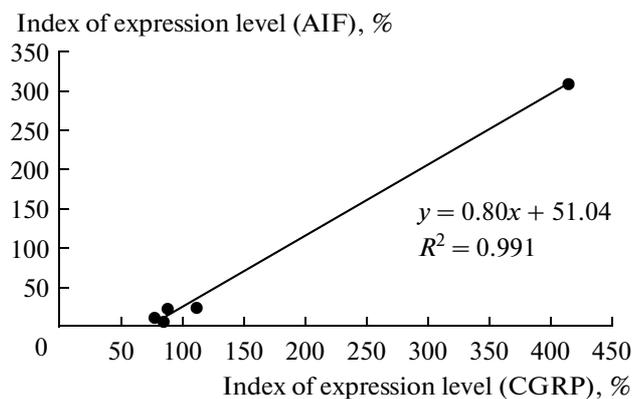
Index of expression level (AIF), %

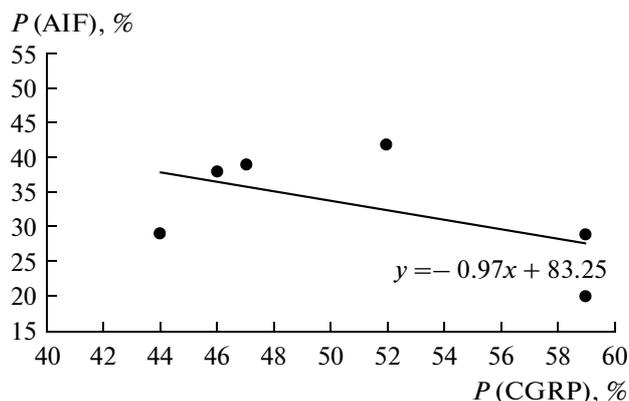**Fig. 3.** Dependence of AIF index of expression level in epiphysis on CGRP index of expression level in epiphysis (men).

Fig. 4. Dependence of density of AIF expression on density of CGRP expression in epiphysis (women).
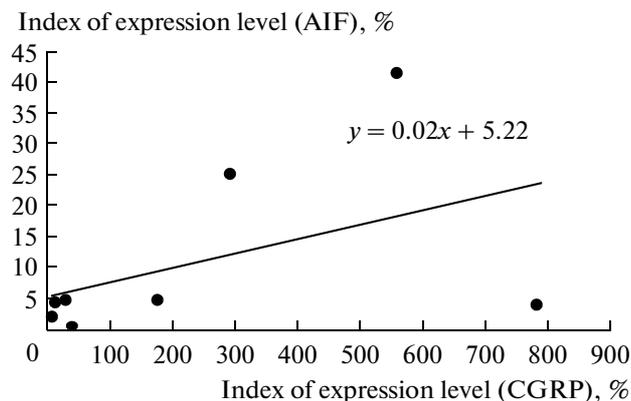
Index of expression level (AIF), %

Fig. 5. Dependence of AIF index of expression level on CGRP index of expression level in thymus (women).
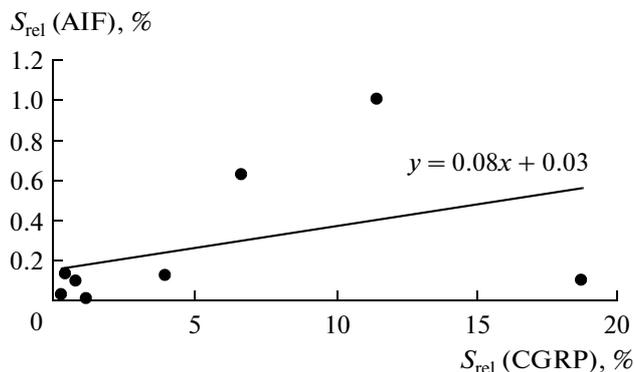

Fig. 6. Dependence of area of AIF expression on area of CGRP expression in thymus (women).

strated that the AIF IEL correlates with the CGRP IEL in pairs ($p < 0.05$), while this association attenuates with age and does not depend on sex.

In a subgroup of men, the area of the CGRP expression in the thymus and epiphysis significantly correlates in pairs ($p < 0.05$, Fig. 1). In addition, a statistically significant dependence between the area of the AIF and CGRP expression in the epiphysis wass observed ($p < 0.01$, Fig. 2), as well as between expression indices of these signal molecules ($p < 0.01$, Fig. 3), but this correlation was stronger than in the thymus.

In a subgroup of women, the optical density of the AIF and CGRP expression in the epiphysis negatively correlated ($p < 0.05$, Fig. 4). In the thymus, the IEL and the area of the CGRP expression practically did not change with an increase in the IEL and the area of the AIF expression, but a statistically significant correlation between the IEL ($p < 0.001$) and areas of expression ($p < 0.001$, Figs. 5, 6) was observed.

CONCLUSIONS

A significant correlation between the AIF and CGRP expression was detected in the epiphysis, while this dependence was not found in the thymus. Most likely, this fact indicates that the level of CGRP expression in the thymus does not influence the intensity of mitochondrial apoptosis. It seems possible that some common mediator (a common regulatory molecule) that connects both signal pathways is expressed in the epiphysis (in contrast to the thymus).

There was a significant correlation between the level of CGRP expression in the thymus and epiphysis, which confirms the hypothesis about the existence of a common regulatory molecule for the CGRP and AIF in the epiphysis and its absence in the thymus. It is important to note that the expression of CGRP is a feature known to be inherent to nerve tissue. CGRP secreted by nerve cells has mitogenic features and is able to induce the emission of neuron growth factor by adjacent tissues. The presence of a negative correlation in the epiphysis in the optical density of the AIF and CGRP expression (in the absence of such in the area and index of expression level) indicates a balance of the processes of apoptosis and proliferation in the nerve tissue. The CGRP expression in the thymus and epiphysis indicates the unity of the organs of neuroimmunoendocrine system.

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