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REVIEWS

Morphofunctional and Molecular Bases of Pineal Gland Aging

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Abstract—The review analyzes the morphology, molecular and functional aspects of pineal gland (PG) aging and methods of its correction. The PG is the central organ that regulates the activity of the neuroimmunoendocrine, antioxidant and other systems of the body. Functional activity of the PG is decreased with aging, causing a decrease in the melatonin level. Molecular and morphological studies have demonstrated that the PG displays no pronounced atrophy with aging. Long-term experience has shown that epithalamine, a peptide extraction of PG, as well as epithalon, a synthetic tetrapeptide made on its basis, restore melatonin secretion in the PG and have a regulatory effect on the neuroimmunoendocrine and antioxidant systems of the body.

Keywords: pineal gland, aging, signal molecules, melatonin, peptides, epithalamine, epithalon.

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Together with the hypophysis and hypothalamus, the epiphysis (pineal gland, PG) is one of the highest centers of the endocrine system of the body [1]. The aging rate of the neuroimmunoendocrine system and age-related pathology development are connected with involution of the PG, which, in turn, is determined to a greater degree by a decrease in its functional activity and to a lesser degree, by morphological and molecular changes [2, 3].

Morphological Changes in the PG with Aging

The study of autopsy samples of the PGs obtained from 2700 subjects aged from several months to 90 years can be used as the initial point for the analysis of published data on age-related changes in the PG. It was shown that PG weight increases from 80–100 to 150-160 mg in the age interval 10–50 years [4]. PG weight is inversely proportional to sexual activity; therefore, this index is higher in pubertal and post-menopausal periods than in the period of sexual maturity. The increase in the gland weight during aging was related to an increase in the volume of fibrous tissue and the number of cysts without a significant decrease in the number of pinealocytes in an organ's parenchyma. Changes in the connective tissue composition have been observed: the number of collagen fibers is increased, and the number of argyrophilic fibers is decreased [5].

According to the computed tomography data, the volume of the PG characterizing its functional activity decreased at the age interval from 21 to 60 and then stabilized [6]. These data are in good correlation with the study [7], which shows that there are no pronounced differences in PG structures at a young and elderly age.

Sequential changes in three prevaling types of PG structure (cellular, trabecular, and alveolar) occur [8].

The cellular type of structure of the PG is typical of a young age. PG is represented by a continuous field of pinealocytes.

The trabecular type of the PG, glandular tissue with differently directed stromal septum, is typical of middle-aged subjects. Replacement of the trabecular type by the alveolar type of PG structure is typical of elderly subjects. Trabeculae subdivide PG in alveolar structure into circular or polygonal lobules separated by stromal rings.

In addition, one can see age-related changes in the vessels of the PG. After birth, PG vessels are located among pinealocytes. Expansion of argyrophilic stroma around the vessels that takes place with age is the reason of the transition from a cellular to trabecular type of structure of the PG. A dense network of vessels enlaces the parenchymal lobules of the PG determining its alveolar structure. In subjects over 60 years of age, one can see the appearance of outgrowths of the reticular frame of connective tissue in the development of sclerosing processes in the PG. The blood circulation in the PG is decreased as compared to subjects aged 40–45 years. Age-related decrease in trophism in the PG results in involution changes in its cells, pinealocytes.

Accumulation of lipofuscin and lipid inclusions, as well as fragmentation and budding of nuclei, was observed in pinealocytes with aging [5, 9]. Electron microscopic study of the PGs of subjects aged from 2 days to 86 years showed that lipofuscin pigments are located not only in the cytoplasm of pinealocytes, but also in the areas of capillaries [10]. The authors believe that the origin of these inclusions is mainly connected

with secretory activity of the gland rather than its atrophy. It was shown that involution of pinealocytes started at 50 years and reached a maximum at 80 years. This involution is expressed in a decrease in the volumes of nuclei and nucleoli, the number of secretory granules, and also in sclerotic changes in the arterioles and capillaries of the PG [11]. A decrease in the nucleoplasmic ratio in the case of equal sizes of cells reflects a decrease in the age-related functional activity of pinealocytes. According to other data, a decrease in the size of the nuclei of pinealocytes starts at the age of 40 years [12]. The total number of pinealocytes decreases by 18% with aging; age-related changes in second-type cells are expressed as a change in nucleus shape, an increase in the propportion of heterochromatin, clarification of the mitochondrial matrix, and formation of autophagosomes [13]. In addition, it was shown that the main age-related change in pinealocytes is an increase in the surface of their nuclei, which results in an increase in the contact surface of the nucleus membrane and cytoplasm and, then, in the enhancement of the synthesis of protein [14]. Data on the presence of cells with two nuclei with a double DNA content in subject older than 30 years prove this fact.

A positive correlation between the level of calcium in the PGs in subjects aged from 3 months to 65 years and a subject's age and a negative one between the amount of calcium and night concentration of melatonin (MT) hormone in the PG have been found [5]. However, the dependence between the age and accumulation of brain sand consisting of calcium deposits in pinealocytes is still under discussion.

Researchers showed an increase in the number of calcifications in the PG [15], this was supposed to be connected with an age-related decrease in the activity of Ca^{++}-ATPase [2]. This dependence was not observed in other studies [6], or calcifications were not found in PG even in long-living persons [10], or they were verified in the PG of children [16].

In recent years, it was widely assumed that formation of calcium deposits and lipofuscin in pinealocytes is connected primarily with the functional activity of the PG than with its genuine atrophy. Computed tomography results obtained in 70 subjects with epilepsy aged 9–58 years prove this hypothesis [17]. The rate of calcification in the PG was not related to the age and was higher in the case of the localization of the epileptic focus in the right temporal lobe than in the left one. As a result of interhemispheric asymmetry and innervation of the PG, epileptic seizures in the right lobe results in more pronounced stimulation of PG, and the number of calcium deposits in the right lobe was higher than in the left one. Accelerated accumulation of brain sand in the PG of gerbils in immobilization stress proves the dependence of the number of calcifications in the PG from the level of its stimulation [18].

These data make it possible to draw the conclusion that morphological changes in the PG are characterized by an increase in the content of connective tissue, which is typical of all organs with aging and cannot prove the "pronounced atrophy" of the PG.

The term pronounced atrophy includes significant morphological age-related changes in an organ and determines an irreversible decrease in its functional activity. A good example of pronounced atrophy of an organ with aging is the thymus, for which a sharp decrease in weight and sizes in the substitution of the main part by a fat component was shown in subjects older than 70 years. This substitution is the cause of an irreversible decrease in the thymus's functional activity.

It should be noted that the PG weight does not change with aging. Other described morphological signs of aging (the presence of calcifications, lipofuscin, a decrease in the nucleus–cytoplasm ratio) prove a decrease in its functional activity rather than involution–dystrophic changes in the PG. This is a supposition for the possible restoration of PG functions.

Molecular Changes in the PG in Aging

To study the molecular basis of PG aging, an extended immunohistological analysis of PG samples obtained in the autopsy of middle-aged, elderly, and long-lived subjects was carried out.

Immunohistological analysis of the PG showed that the expression of proapoptotic protein P53 by pinealocytes hardly decreases in the age interval from 60 to 70 years and does not significantly change after 70 years. Expression by pinealocytes of the proliferation marker Ki-67 does not change in middle-aged, elderly, and long-lived subjects. The obtained data make it possible to assume that PG involution takes place due to a weakening of the proliferative activity of the pinealocytes and the contribution of apoptosis into this process is nonsignificant [19, 20].

Important markers to characterize intercellular interactions, whose intensity decreases in aging, are enzymes belonging to matrix metalloproteinases (MMPs). Our studies gave the possibility to verify metalloproteinases MMP2 and MMP9 in PG tissue in subjects over 60 years of age. This proves the active remodeling of intercellular space in PG aging. At the same time, an age-related decrease in the synthesis of MMP2 and MMP9 by pinealocytes indicates inhibition of processes of cleavage of collagen of IV type, fibronectin, tenascin-C, and elastin. Correlation analysis showed that the age-related decrease in the number of MMP2 and MMP9 is described by one linear equation, which indicates a single mechanism of an age-related decrease in the synthesis of enzymes participating in intercellular interactions.

It was established that the calcitonin gene-related peptide (CGRP), which acts as a neurotransmitter, immunomodulator, and cardioprotector, is secreted in the PG. It was shown that CGRP expression displays a tendency towards an increase in the transition from middle to elderly age; this parameter is lower by a factor of 2.5 in long-lived persons as compared to middle-aged

people. Correlation analysis showed that synthesis of CGRP peptide in the PG does not depend on the age, proving the ability of PG for synthesis of biologically active molecules in subjects over 60 years old.

Thus, expression of signal molecules of PG reflected the intensity of apoptosis, proliferation, secretory activity, and the capacity of pinealocytes for intercellular interactions is slightly decreased with aging, which is a supposition of the ability to maintain the activity of the PG in involution.

Functional Changes in the PG with Aging

Functional changes in the the PG with aging are expressed by a disturbance in synthesis by the pinealocytes of MT [21], which displays a wide range of biological activity related to both antioxidant and neuroimmunoendocrine systems. MT is secreted rhythmically during the 24-h period, mainly in the evening and nighttime in humans and all vertebrates [22]. Regulation of MT synthesis by the PG is closely related to its ability to affect circadian rhythms of the body. Control of MT secretion is mediated by the suprachiasmatic nuclei (SCN) located in the frontal hypothalamus above the chiasma and numerous nervous sympathetic pathways connected with the PG. The circadian clock of the body is located in the SCN; this clock synchronizes MT secretion by the PG for 24 h with the level of illumination in the environment, information on the changes of which are transmitted to the SCN from the retina via the retina–hypothalamus tract [23].

The circadian rhythm generated by the SCN and mediated by changes in MT secretion by the PG regulates one of the most important processes in the body, the sleep–awakening cycle. Under the effect of MT, the activities of different parts of the human brain were shown to correspond with its state during sleep, although the subjects were awake in the experiment [24].

Recovery of memory and ability to learn was observed in experiments with administration of MT in mice with amnesia induced by D-galactose. According to the authors, the obtained effect is connected with the MT's ability to stimulate the activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase, and decrease the levels of free radicals and active forms of oxygen (AFO) in brain tissues [5].

One of the most significant MT effects is the antioxidant effect based on a decrease in the amount of AFO interacting with biologically significant molecules in cells of different organs and tissues. It was shown that the content of lipid peroxidation products and markers of oxidative DNA damage increases upon PG ectomy in mice [5]. Correlation between the increase in the maximal concentration of melatonin and total antioxidant status during nighttime is one more proof of the antioxidant effect of MT [25].

It was shown that MT participates in the regulation of the cardiovascular system [26]. Administration of MT by elderly persons with a decreased function of the PG resulted in normalization of blood pressure and a decrease in the frequency of heart contractions and an increase in the variability of the heart rhythm [5, 27].

The PG is known to regulate many components of the endocrine system. MT affects the median eminence of the hypothalamus, where releasing hormones and statins regulating the adenohypophysis activity are synthesized. Through a mediated effect on the adenohypophysis, MT inhibits synthesis of the gonadotrophic and thyroid hormones, gluco- and mineralocorticoids [9]. Direct and mediated antigonadotrophic MT effects expressed in a decrease in sex hormone synthesis and deceleration of the production of the follicle-stimulating and lactotropic hormones in the hypothalmic–pituitary system are studied in detail [9]. Recently, the MT effect on the pancreas was also shown. For example, an increase in the incidence of pancreatic diabetes incidence was observed in the hereditary hypertrophy of the PG. According to several authors, this was mediated by an increase in the tolerance to glucose and a decrease in insulin synthesis [28].

MT is assumed to be the hormone playing the leading role in body adaptation, and the PG, an organ mediating the effects of the stress-limiting body system [29]. Presumably, the mechanism of protective MT function in stress consists in the deceleration of corticoliberin formation in the hypothalamus, and corticotrophin, in the PG; these substances at high concentrations are able to cause damage. In addition, MT promotes a decrease in the activity of the sympathoadrenal system. The stress-protective MT effect may be connected with its effects on the nervous, endocrine, antioxidant, and chronobiological systems.

The described range of biological effects of PG MT proves its leading role in maintaining homeostasis; age-related changes in the rhythm and level of MT secretion by the PG can become the cause of premature aging.

A decrease in the MT concentration in the blood, blood plasma, and saliva during the evening and nighttime in persons over 60 years old has been shown [5, 9]. At the same time, the MT concentration in the blood, blood plasma, and saliva during daytime does not differ significantly from those in young persons.

The concentration of nocturnal urinary 6-sulfatoxymelatonin (6-SOMT), an MT metabolite, in middle-aged and elderly subjects was lower as compared with young ones. This parameter did not depend on the age in the day and evening time [31]. A decrease in MT content in PG tissue in elderly subjects was shown in an immunohistochemical study of autopsy samples of the PGs [5].

Association between the circadian cycle of MT secretion and the circadian sleep-wake cycle was observed to be disturbed with aging. Going to sleep is synchronized with an increase in the blood MT content, and awakening, with its decrease in young subjects. A negative relationship was found in middle-aged

and elderly subjects: going to sleep takes place against a background of relatively low MT concentrations; and awakening, at an increased blood MT content [24].

A decrease in MT secretion by the PG does not take place in all elderly subjects [5]. The PG function was decreased in 71% of the tested elderly subjects, but it remained stable in 29% [5]. The blood MT level in elderly subjects with an intact function of the PG corresponded to values typical of young subjects. The authors draw the conclusion that age-related damage in PG functions is of an individual character, like the aging of the body as a whole.

The data obtained in the study [32] prove the heterogeneity of PG aging. A decrease in urinary excretion of 6-SOMT and a decrease in the amplitude of the circadian rhythm of blood MT content were found in middle-aged and elderly subjects; however, these parameters were significantly higher and close to the values obtained for young subjects. The obtained results were explained by the effects of natural selection: subjects with a high activity of the PG can live longer.

A preserved melatonin production function of the PG in a quarter of the elderly and long-lived subjects shows that functional involution of the PG in some cases does not occur, and correction of the decreased function of the PG in aging is possible.

In addition to the PG, other systems regulating the circadian rhythms of the body (SCN in the hypothalamus, retina-hypothalamic tract, superior cervical sympathetic ganglia innervating the PG, eye lens and retina) participate in an age-related decrease in melatonin production by the PG. Destruction of SCN neurons often takes place in aging of the body, which results in the damage of the circadian rhythm of the MT synthesis in the PG and a decrease in the number of MT receptors [33]. Damage of the delivery of information on changes in the illumination in SCN can also result in a decrease in the MT synthesis in the PG. Age-related changes in the eye lens and retina promote this process.

The PG is tightly related with other brain structures, and involution changes in this gland are mainly determined by age-related changes in the catecholaminergic system. The concentrations of β-adrenergic receptors in the PG and other brain structures decreases in aging. At the same time, the capacity for increasing the number of adrenergic receptors in response to adrenergic deprivation decreases with aging [2, 8]. A decrease in the functional activity of the PG with aging is supposed to be connected with a decrease in the reactivity of β-adrenoreceptors of pinealocytes interacting with noradrenalin [2]. Synthesis and the ability to release noradrenalin from postganglionic sympathetic fibers are also decreased with aging. A decrease in the functional activity of the PG during its aging is determined not only by changes in the PG itself, but also by involution changes in a number of brain structures and the light-optical system connected with the PG. The main manifestation of the age-related involution of the PG is a decrease of evening and nocturnal secretion of MT, which results in the damage of neuroimmunoendocrine interactions at the body level and is one of the main reasons for the development of age-related pathology. One can suppose that involution changes in the PG functions can be corrected because morphological signs of PG aging are expressed to a lesser degree than functional ones.

The Possibility of Restoration of PG Functions Using Peptide Bioregulators

Despite the decrease in the functional activity of the PG with aging expressed in changes in MT secretion, morphological and molecular changes in the PG during involution are expressed slightly, giving the possibility to restore the PG activity.

The 35-year experience of studies carried out in St. Petersburg Institute of Bioregulation and Gerontology, Russian Academy of Medical Sciences prove that peptide complex epithalamine extracted from animal PG [34–38] and the tetrapeptide epithalon synthesized on its basis are of pronounced geroprotective effect on the PG.

Epithalamine is a complex of peptides with molecular masses lower than 10 kDa without an admixture of MT and other indoles. Study of the pharmacological characteristics of peptide extraction from the PG showed that it participates in the regulation of the hormone status through the effect on the hypothalmic–pituitary system [35]. On the basis of the study of the amino acid content of epithalamine and in accordance with the processing regulation theory, epithalon (AE-0, Ala–Glu–Asp–Gly) was synthesized. Epithalon displays biological effects similar to those of epithalamine but expressed at lower concentrations [38].

Epithalamine, a peptide PG extraction, and epithalon, its synthetic analog, display a wide range of physiological effects and participate in the regulation of pineal MT synthesis, circadian rhythms of the body, functional state of the brain, as well as the cardiovascular, endocrine, and immune systems; they have antioxidant, oncostatic, and stress-protective effects [5].

The main part of pineal peptide effects are connected with its ability to activate the synthesis of pineal MT, which is especially expressed in an age-related decrease in the melatonin-forming function of the PG. Thus, a significant part of the effects of epithalamine and epithalon is mediated by the MT effect.

The effect of a course of epithalamine or epithalon administration on the melatonin-forming function of the PG depends on the initial degree of preservation of this function in elderly subjects [5]. PG peptide preparations had no significant effect in elderly subjects with unchanged function of the PG or in young subjects. The MT concentration increased by a factor of two under the effect of epithalamine and epithalon in elderly subjects with involution changes in the PG. These data prove the modulatory effect of PG peptide preparations

on the melatonin-forming function of the PG during its aging.

An increase in the sensibility of the retina and SCN to stimuli (which decreases with age) and restoration of the catecholaminergic function of the PG may underlie the modulating effects of epithalamine and epithalon on the MT synthesis in the PG [39]. It was shown that administration of epithalamine to old mice normalized the level of neurotransmitters in the hypothalamus [33].

Epithalamine and epithalon, like MT, display pronounced immunomodulatory effects underlying their heroprotective activity.

The melatonin-forming function of the PG decreases with aging, promoting changes in circadian rhythms of the body, e.g., circadian rhythms of changes in the blood cortisol content, in primates [9, 40]. Normalization of blood cortisol in animals was observed after a course of epithalon; circadian changes in the amplitude of this parameter were close to the values obtained in young monkeys [9]. At the same time, an evening increase in the blood MT concentration was observed, proving a mediated effect of epithalon through restoration of the melatonin-forming function of the PG. A similar restoration effect of epithalon on the periodicity of the functioning of the hypothalmic–pituitary–adrenal system was seen in old primates [9]. The stimulating effects of epithalamine and epithalon on the functional state of the brain included an increase in the activity of intracellular enzymes regulating metabolic processes: succinate dehydrogenase, ketoglutarate dehydrogenase, and pyruvate dehydrogenase. Effects of PG peptide preparations at the level of the body included an improvement of learning abilities and widening of the dietary habits in rats after PG ectomy [41].

Numerous antioxidant effects of epithalamine and epithalon were observed in elderly subjects and in animal studies. For example, the activity of superoxide dismutase increased; the amounts of lipid peroxidation products, diene conjugates, and AFO decreased in the blood sera of elderly subjects under the effect of peptide preparations. Epithalamine caused a more pronounced effect on the activity of antioxidant enzymes as compared to the MT effect [42]. This effect may be related to the stimulating influence of epithalamine on the MT production [9] and also to its direct influence on the enzyme activities of the antioxidant system.

Epithalamine displays an oncostatic effect on ascitic tumor cells of sarcoma-37 and adenocarcinoma of the mammary gland. The latent period of neoplasma development increased upon long-term administration of epithalamine [43]. A decrease in the frequency of tumor development and prolongation of their latent period in mice treated with epithalamine were shown in one more study [44]. The inhibitory effect of epithalamine on tumor cell growth may be related to either the oncostatic effect of MT, whose synthesis increases under the influence of PG peptide preparations, or the direct effect of epithalamine on the sensitivity of the hypothalamus to estrogens [45].

Epithalamine produces a complex effect on the parameters of the cardiovascular system in humans. For example, in subjects over 60 years of age, the functional age of whose cardiovascular system exceeded the calendar age by more than 5 years, this parameter decreased by more than 3 years after epithalamine administration [5].

PG peptide preparations participate in the regulation of the functions of the pancreas, as well as the reproductive and hypothalmic–pituitary systems. Epithalamine and epithalon restore the reproductive function in old male and female rats [44]. The PG peptide preparations promote an increase in the blood lutenizing hormone and blood testosterone concentrations in old males [35] and restoration of the periodicity of the estrous cycles in female rats. This last effect of epithalamine is based on its ability to increase the sensibility threshold of the hypothalamus to estrogens [45]. Administration of epithalon at doses that do not change the blood corticosterone concentration resulted in the restoration of the decreased sensitivity of the hypothalmic–pituitary system to corticosteroids in aging animals [38]. Epithalon also promoted restoration of the early phase of insulin secretion by the pancreas in old monkeys [9]. Probably, the sensitivity of β-cells of the islets of Langerhans to a high concentration of glucose increased.

Epithalamine increased the tolerance to glucose and improved the parameters of lipid metabolism in animals with experimental alloxan-induced diabetes mellitus, i.e., produced effects that were opposite to the effects observed after PG ectomy [38]. An increase in the MT concentration and decrease in the glucose concentration in the blood plasma were shown in monkeys treated with epithalon [9]. A decrease in the blood content of cholesterol and low-density and very-low-density lipoproteins was observed after clinical administration of epithalamine in patients with non-insulin-dependent diabetes mellitus [46].

The antioxidant effect of the PG peptide preparations and their ability to regulate the functioning of the sympathoadrenal and pituitary–adrenal systems underlie the stress-protective effects of epithalamine and epithalon. A decrease in the increment of the adrenaline and nonadrenaline concentrations was observed after physical exercises in men [9]. Epithalamine promoted a quicker normalization of hemodynamic parameters after traumatic shock in humans [5]. The ability of epithalamine to activate the synthesis of peptides of the thymus and spleen and, at the cell level, stimulate the cascade of cGMP-dependent signal system play an important role in its stress-protective effect [5].

The presented data prove that PG peptides display unique broad effects involving practically all organs and systems of the body. The effects of MT and PG peptides are mainly unidirectional; these biological substances take part in regulation of functions of the antioxidant, nervous, and endocrine systems, as well as many other

systems. The example of the antioxidant system shows that epithalamine and epithalon produce a more pronounced effect on the activity of its enzymes that highlights the possible role of peptides in the regulation of MT synthesis and their mediated influence through the regulation of hormonal activity of the PG.

CONCLUSIONS

Review of published data and our own data on PG aging give the possibility to distinguish two aspects of the problem: estimation of morphological signs of PG aging and a decrease in functional activity with aging. Introduction of this conventional division is worthwhile because, as a rule, the presence of significant morphological changes in an organ and a decrease in its functional activity are irreversible and hardly to correct, e.g., in the thymus.

In aging, PG structure undergoes no significant morphological changes. The observed increase in the percentage of connective tissue, calcification, and lipofuscin has no correlation with the age and can be considered among the characteristics of the functional state of the PG.

Estimation of the age-related time course of signal molecules of the human PG showed a decrease in the proliferative capacity of pinealocytes; however, their ability to synthesize biologically active molecules is not age-related. The capacity for intercellular interactions in the PG is preserved in subjects over 60 years of age, which also proves a high degree of preservation of PG tissue during involution. The functional activity of the PG decreases with aging, which is expressed in the damage of MT secretion.

According to the data of long-term studies, the PG peptide preparations epithalamine and epithalon promote the restoration of the melatonin-forming function of the PG and regulate the antioxidant, neuroimmunoendocrine, and other systems of the body.

Thus, partial restoration of functions of the PG during age-related involution is possible due to preservation of morphological and molecular structures of the PG and the use of PG peptide preparations

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