REVIEWS

Extrapineal Melatonin and Its Role in the Neuroendocrine Regulation of Homeostasis

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The hormone of melatonin is the main regulator of biological rhythms. It was first found in the pineal gland in 1958. Melatonin is involved in the regulation of many vital physiological processes: maturation and development of genitalias, metabolism of pigments and free radicals, immune response, mood and sleep, and cell proliferation and differentiation. The pineal gland is not the only organ synthesizing melatonin. Extrapineal melatonin is widely dis-tributed in humans and animals. Melatonin-producing cells are found in the gastrointestinal and respiratory tracts, pancreas, adrenal and thyroid glands, thymus, cerebellum, urogenital system, placenta, and other organs. Melatonin is intensely synthesized in non-endocrine cells: mast cells, natural killers, eosinophilic leukocytes, platelets, and endotheliocytes. Such a wide distribution of melatonin reflects its key role as an intercellular neuroendocrine regulator and coordinator of many complex and interrelated biological processes.

Key Words: *melatonin; extrapineal synthesis; diffuse neuroendocrine system; tumor growth*

The existence of particular endocrine cells in the body was initially supposed by R. Heidenghain [24], N. K. Kul'chitskii [31], and F. Feyrter [22]. Only in 1968-1969, English pathologist and histochemist A. G. E. Pearse substantiated the existence of a specialized and highly organized cell system synthesizing peptide hormones and biogenic amines. His concept was based on numerous studies of endocrine cells in various organs, identification of substances produced by endocrine cells, and detailed cytochemical and ultrastructural analyses of these cells [44]. Many cells of various types present in the body can absorb monoamine precursors (5-hydroxytryptophan and L-dihydroxyphenylalanine), decarboxylate these substances, and synthesize biogenic amines. Therefore, A. G. E. Pearse used the term APUD (amine precursor uptake and decarboxylation) to designate these cells [44].

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The uptake of monoamine precursors by endocrine cells and their further transformation into biogenic amines play an important role. Therefore, we proposed to designate differentiated cells of this system as apudocytes [1,48], their precursors as apudoblasts, pathology of the APUD system as apudopathy, and tumors consisting of APUD cells as apudoma (A. G. E. Pearse). The term apudoma should be used for benign tumors and apudoblastoma for malignant tumors (endocrine cell cancers). Thus, we have a general terminology that is necessary for any nomenclature [8,48].

The APUD system includes more than 60 types of cells localized in the gastrointestinal tract, pancreas, urogenital system, epithelium of the respiratory tract, pineal gland, thyroid and adrenal glands, adenohypophysis, hypothalamus, carotid body, skin, sympathetic ganglia, thymus, placenta, and other organs [8,10,37, 48]. The appearance of radioimmunological methods and the development of immunohistochemistry revealed an unexpected phenomenon: the same biogenic

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amines and peptide hormones were identified in neurons and endocrine cells [45].

The accumulated data were beyond the scope of traditional concepts of the hierarchical dependence that was believed to be typical of two major regulatory systems, the nervous and endocrine systems. There is ample evidence that the mechanism of biological regulation is based on close coordinated functional interaction between the endocrine and nervous systems through common pathways of information reception and transfer at the subcellular, cellular, and tissue levels. Recent identification of physiologically active substances acting as neurotransmitters and neurohormones in the nervous system and locally or distantly as hormones in the endocrine APUD system allows us to consider these systems as the universal diffuse neuroendocrine system (DNES) [33,37,45,48,57].

DNES cells are localized in practically all organs and producing biologically active substances regulate the homeostasis by secreting various hormones by endocrine, paracrine, apocrine, neurocrine, neuroendocrine, and amphicrine pathways [33,39].

Recently considerable attention has been focused on one of DNES hormones, melatonin (MT). MT displays a wide range of biological activities and plays a key role in the regulation of biological rhythms [52], thus affecting significantly the nervous, endocrine, and immune systems and the body as the whole.

Evolution of Studies

At the end of the 1950s, A. Lerner *et al.* (Yale University) identified MT as the pineal substance lightening melanophores of frog skin [40]. They showed that MT is a 5-methoxy-N-acetylated metabolite of serotonin (5-hydroxytryptamine).

J. Axelrod and H. Weissbach demonstrated that N-acetyltransferase and hydroxyindole-O-methyltransferase are the key enzymes of MT synthesis [12]. In 1970, J. Axelrod was awarded the Nobel Prize on the discovery of pathways of the synthesis and metabolism of MT and other hormones and transmitters.

Identification of MT contributed to an increasing interest in physiology of the pineal gland. Recently a wide range of biological activities of pineal MT has been demonstrated. The most important properties of MT are the regulation of biological rhythms, antigonadotropic effects, stimulation of immune processes, scavenging of free radicals, and cytostatic and antiproliferative effects *in vitro* and *in vivo* [11,13,41,51,53]. Now MT is widely used as hypnotic, antidepressant, immunostimulator, and regulator of diurnal rhythms during long-term flights and night duty [52]. The pineal gland was believed to be the only source of MT in the body.

The progress in our knowledge about extrapineal sources of MT was associated with the development of the method for obtaining highly specific antibodies against indolylalkylamines [23,45].When these antibodies became available, MT, its precursors, and related catalytic enzymes were found in extrapineal tissues anatomically related to the optic system (the retina and Harderian gland) [17,18].

At the same time, radioimmunological methods and thin-layer chromatography identified MT in the plasma and urine of laboratory animals subjected to pinealectomy [28,43], which indicates extrapineal MT synthesis.

Taking into account that enterochromaffin cells of the gastrointestinal tract (EC cells) are the main serotonin story in the body [14,21], we first proposed that EC cells produce MT and then identified MT in these cells [47,50].

Enterochromaffin Cells as the Main Source of Melatonin in the Body

The presence of MT in EC cells of the gastrointestinal tract was studied by the following ways: determination of MT in the intestinal mucosa containing EC cells; identification of MT in EC cells by immunohistochemical methods; and estimation of the synthesis and accumulation of MT.

The presence of MT in the intestinal mucosa was confirmed by a classic biological test. Purified extracts from the mucosa of human appendices enriched with EC cells and injection of the same sterile extract into the frog lymph sac induced considerable paleness of frog skin [5,50]. This effect is typical of MT. Chromatographical analysis confirmed the presence of MT in mucosal extracts used in these biological tests [50]. Experimental studies of extracts prepared individually from appendices with catarrhal, phlegmonous, and gangrenous inflammations (the mean number of argentaffin EC cells in the mucosa of these appendices depends on the type of inflammation) showed that the rate of paleness development depends on the content of EC cells in the mucosa [47], and the correlation between the number of EC cells in the mucosa and the effect of MT is an indirect evidence that MT is localized exactly in these cells.

Chromatographical analysis revealed the presence of 5-hydroxytryptophan, 5-hydroxytryptamine (serotonin), 5-methoxytryptamine (mexamine), and MT in these extracts [47,49]. Synthetic MT and its precursors were used as indicators. The presence of MT precursors (tryptophan → serotonin → MT) also confirms MT synthesis in EC cells.

Immunohistochemical method with the use of antibodies against MT and its precursors (serotonin, N-

acetylserotonin, and mexamine) answered the question as whether MT is present in EC cells. An immunohistochemical assay using specific anti-MT antibodies and commercial antibodies against MT (CIDtech Research Inc. and Dianova) revealed the presence of MT-immunopositive cells and cells containing MT precursors in all portions of the gastrointestinal tract in humans and experimental animals (dogs, rabbits, rats, and mice) [34, 47,49]. A comparison of serial slices treated with specific antibodies or stained by argentaffin methods proposed by Masson showed that localization of argentaffin cells and MT-positive cells was similar [34].

Thus, the use of biological tests, thin-layer chromatography, histochemical staining, and immunohistochemical analysis demonstrated the synthesis of MT in intestinal EC cell. These results were then confirmed by other studies. G. A. Bubenik found MT in practically all portions of the gastrointestinal tract in rats [16] and demonstrated that MT and serotonin-producing argentaffin EC cells were localized in the same regions. The presence of hydroxyindole-O-methyltransferase, the key enzyme of MT synthesis, in the intestine [46] confirms the synthesis, rather than passive accumulation of MT.

A mathematical analysis allows us to consider that the number of EC cells in all portions of the intestine is much greater than that of MT-producing cells in the pineal gland [1]. G. Huether demonstrated that the content of MT in the gastrointestinal tract of birds and mammals 400-fold surpassed that in the pineal gland [26]. These data and the fact that EC cells contain 95% of endogenous serotonin (the main MT precursor) [59,60] allow us to consider EC cells as the major source of MT in humans and mammals.

Functional morphology of EC cells is well studied. EC cells is a classical example of apudocytes. The coexistence of biogenic amines (serotonin and MT) and peptide hormones (substance P, motilin, and enkephalins) is typical of these cells [56]. Recent studies showed colocalization of MT, motilin, and calretinin (in stomach parietal cells); MT and histamine (in mast cells); MT, somatostatin, and β-endorphin (in natural killers); and MT and prostaglandin F_2 (in thymic reticuloepithelial cells) [34,35].

Biological Role of Extrapineal Melatonin

MT in the body is produced by not only in pinealocytes, but also in extrapineal organs. Functionally, MT-producing apudocytes are the part of the APUD system and the DNES department (as the universal system of adaptation and maintenance of the homeostasis in the body).

There are central and peripheral MT-producing cells in DNES. Central MT-producing cells are localized in the pineal gland and optic system (the retina and Harderian gland). The rhythm of MT secretion in these cells coincides with the light-dark regimen [11, 52]. The peripheral population includes apudocytes of other organs. MT secretion in these organs does not depend on the illumination conditions. Cells synthesizing MT and its main precursor serotonin are localized not only in the gastrointestinal tract. These hormones were found in apudocytes of the respiratory tract, under the hepatic capsule, in the kidney cortex, between the adrenal cortex and medulla, and in paraganglia, gallbladder, ovaries, endometrium, placenta, and internal ear [1, 4,8,48].

Recently, MT was found in neuroendocrine cells (mast cells, natural killers, eosinophilic leukocytes, platelets, pancreatic acinar cells, thymic reticuloepithelial cells, and several endothelial cells) [35,36]. These data suggest a possible hormonal function of non-endocrine cells. Moreover, this phenomenon confirms evolutional antiquity of hormones, which probably appeared before divergence of the endocrine and exocrine functions. During ontogeny, biogenic amines appear at the early embryonic stages and play a role of intracellular hormones controlling cell division [1, 8]. A detailed analysis of hormonal functions of non-endocrine cells would provide a more comprehensive insight into intercellular adaptation and compensation mechanisms in the organism.

Thus, the following organs are the sources of extrapineal MT: the gastrointestinal and respiratory tracts, liver, kidneys, adrenal glands, paraganglia, gallbladder, ovaries, endometrium, placenta, and internal ear. MT is also synthesized in non-endocrine cells: mast cells, natural killers, eosinophilic leukocytes, platelets, pancreatic acinar cells, thymic reticuloepithelial cells, and several endothelial cells.

MT is known to be involved in the adaptive response and pathophysiological processes. However, the functional role of the peripheral part that includes extrapineal MT-producing apudocytes remains unclear. It can be only suggested that changes in the blood level of MT, which is regulated by the central population of these cells according to illumination conditions, affect peripheral MT-producing cells in various organs. However, pinealectomy induced no considerable changes in the content of endogenous MT in the intestine. Variations of the diurnal rhythm of MT levels in various portions of the gastrointestinal tract were not found [16]. Over the last 15 years, the effect of various pathologies and adverse factors (ionizing and nonionizing radiations, tumor growth, cytostatic therapy, autoimmune and gastrointestinal diseases, pharmacological and toxic effects, parenteral and dietary nutritional regimens, and others) on the behavior and functional morphology of extrapineal MT-pro-

ducing apudocytes and other major DNES cells have been extensively studied in the Laboratory of Radiational Pathomorphology (Medical Radiological Research Center, Russian Academy of Medical Sciences) and Laboratory of Histochemistry and Electron Microscopy (N. N. Blokhin Russian Oncological Research Center, Russian Academy of Medical Sciences). Our data indicate the involvement of extrapineal MT and other DNES hormones in endogenous pathogenic mechanisms of various diseases [2-4,6,8,35,38,48]. MT can be suggested to act as an endogenous scavenger of free radicals, whose role in the pathogenesis of many diseases is well established [29,55]. It was recently shown that MT is a potent inhibitor of hydroxyl radicals [53] and can protect tissues from free radicals-induced damage. This effect of MT is far superior to that of glutathione, a well-known scavenger of free radicals [54]. Free radicals are normal products of aerobic cell metabolism and can be detected in many tissues and organs [19]. High levels of free radicals were found in the intestine, brain, retina, and lungs [19,20,27], which are enriched with MT-producing cells. In addition, Harderian glands in some mammals contain considerable amounts of MT [18]. Harderian glands produce significant amounts of porphyrins that induce the generation of free radicals and are responsible for oxidative damages [25]. The hypothesis that MT protects Harderian glands from porphyrin-induced free radical damages [54] is confirmed by the fact that the content of MT in these glands strongly correlates with the content of porphyrins (for example, in male and female Syrian hamsters displaying considerable differences in the contents of porphyrins) [42].

Thus, taking into account the great population of MT-producing cells in many organs and wide range of the biological activity of MT (particularly, its role as universal regulator of biological rhythms), extrapineal MT can be suggested to play the key role as a paracrine signal molecule for local coordination of cell functions and cell-cell interactions. Extrapineal MT can act as a typical hormone and reach distant target cells with the blood flow. Many cells in various organs have MT receptors [30,58]. In both cases, some non-endocrine cells (mast cells and eosinophilic leukocytes) can absorb MT from the blood and intercellular space and transfer it to specific regions, where the hormone realizes its effects. In this context, of particular interest are the data of immunohistochemical analysis of the distribution of exogenous MT in the body [9,34]. Autoradiography and immunohistochemical analyses of the distribution of pulse injected ^{3}H-MT in endocrine and non-endocrine organs revealed a discrepancy between the distribution of this label and a histotopographic localization of MT-accumulating cells in these organs. Exogenous MT rapidly degraded and its metabolites were distributed over the body and incorporated primarily in cells with intense biosynthetic processes.

After administration of labeled MT, its binding in the adrenal glands was found only in the medulla. In the pancreas, the reaction to MT was revealed in single acinar cells and in the peripheral region of Langerhans islets 65 min and 3 h postinjection, respectively. In the spleen, exogenous MT was accumulated in cells liming sinusoid capillaries of the red pulp. In the duodenum, a specific histotopography of the distribution of MT-accumulating cells was demonstrated. MT was identified in the submucosal nerve plexus and mast cells of gastric and duodenal submucosa as soon as 5 min postinjection. These data show that endocrine and neuroendocrine cells of various organs can serve as MT target cells.

Extrapineal melatonin and tumor growth

Since MT possesses cytostatic properties [13], it is interesting to study MT as a possible modifier of carcinogenesis [2]. Considerable hyperplasia of EC cells and other MT-producing cells was observed at the early stages of tumor growth in humans (stomach, lung, colorectal, and breast cancers) and experimental animals (Lewis lung carcinoma and Ehrlich ascitic tumor), while at the late stages, the content of these cells decreased [2,35]. The behavior and functional morphology of other DNES cells also change during tumor growth. Tumors without metastases are accompanied by hypoplasia and decreased functional activity of gastric ECL cells (histamine), G cells (gastrin), and pancreatic A cells (glucagon). Hyperplasia and hyperfunction of these cells are observed in advanced cancer [4,8].

These data indicate that apudocytes, DNES cells, and their hormones are involved in the endogenous mechanisms of tumor growth. Many DNES hormones can regulate cell proliferation and differentiation. Functional exhaustion of neuroendocrine cells producing hormones with antiproliferative activities and enhanced secretion of hormones stimulating cells proliferation promote tumor growth and the development of metastases.

Apudocytes are the origin of specific tumors — apudomas and apudoblastomas (endocrine cell cancers) [8,48]. Hormone-producing transformed apudocytes were found in non-endocrine cancers, which is of great theoretical and practical importance [7]. Immunohistochemical approach showed that 30% of non-endocrine cancers of various localizations include endocrine cells. Moreover, 60% of such tumors include MT-producing cells [2,7]. Using immunohistochemi-

cal methods and radioimmunological assay, we studied the nocturnal urinary excretion of 6-sulfatoxymelatonin (MT degradation product), expression of the cell proliferation nuclear antigen in tumors, and the number of MT-immunopositive cells in 26 patients with colorectal, stomach, and lung cancers without metastases [15,32].

The expression of cell nuclear antigen reflecting cell proliferative capacity in tumors positively correlated with the urinary excretion of 6-sulfatoxymelatonin. The immunoreactivity of MT in tumors correlated negatively with the proliferative activity of tumor cells [15,32]. These parameters did not depend on the age of patients and the histological type and localization of the tumor.

Proliferative activity is an important characteristic of malignant tumor growth [55]. The cell proliferation antigen is the most valid marker. However, its immunohistochemical evaluation is possible only in tumor tissue obtained during the operation, which limits the use of this method. In light of this, the analysis of 6-sulfatoxymelatonin (as a marker of tumor proliferative activity) in the urine of patients is available and can be used for the diagnosis and monitoring of tumors during radiation and drug therapy, prediction of recurrences, radicalness of excision, and prognosis.

Despite numerous studies of the inhibitory effects of MT on tumor growth [15,33,41], its role in the regulation of proliferative activity of tumor cells remains unclear. Blood content of MT correlates with the level of 6-sulfatoxymelatonin in the urine. Therefore, the analysis of 6-sulfatoxymelatonin reflects the intensity of MT synthesis in the body and the degree of its binding by tumor cells.

Thus, over many years, MT a hormone of the APUD system, was considered as pineal hormone. The use of highly sensitive antibodies against indolylalkylamines allowed to identify MT in the pineal gland, retina, Harderian gland, cerebellum, mucosa of the gastrointestinal and respiratory tracts, liver, kidneys, adrenal glands, thymus, thyroid gland, pancreas, ovaries, carotid body, placenta, endometrium, and non-endocrine cells (mast cells, natural killers, eosinophilic leukocytes, platelets, and endotheliocytes).

Functionally, MT-producing cells are the part of the APUD system and the DNES department, the universal adaptation, regulation, and organism's defense systems). Taking into account a great number of MT-producing apudocytes in many organs and a wide range of biological activities of MT (especially, its main property as the universal regulator of biological rhythms), we can consider extrapineal MT as the key paracrine signal molecule that locally coordinates various cell functions.

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