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INVITED NEL REVIEW

Gastrointestinal Melatonin: Cellular Identification and Biological Role

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Abstract Melatonin, a pineal hormone, because of its wide activity spectrum, is a subject of much current interest for biologists and physicians. It has been demonstrated that pineal gland is not an exclusive source of melatonin synthesis. Melatonin synthesis has been found in different sites of the organism, and a major source of extrapineal melatonin is the gastrointestinal tract. The role of melatonin in gastrointestinal functions is considered in the present review.

Abbreviations:

MT	melatonin,
NAT	N-acetyltransferase,
HIOMT	hydroxyindol-O-methyltransferase,
ST	serotonin,
GIT	gastrointestinal tract,
CNS	central nervous system,
DNES	diffuse neuroendocrine system,
NSAID	non-steroidal anti-inflammatory drug,
ATP	adenosine-triphosphate,
cAMP	cyclic adenosine-monophosphate,
RNA	ribonucleic acid,
DNA	desoxyribonucleic acid,
GABA	γ-amino-butyric acid

Introduction

Today melatonin (MT) is a well-known ubiquitously acting hormone, a key regulator of biological rhythms. Originally MT was found in pineal gland in 1958. [1]. During about 20 years after this discovery it was clearly demonstrated that MT plays a great role in much vitally important physiological processes, such as control of biological rhythms, maturing and development of genitals, pigment metabolism, immune response, metabolism of free radicals, monitoring of mood and sleep, cell proliferation and differentiation. Now it has been securely established, that pineal gland is not an exclusive organ where MT is synthesized. Extrapineal MT is widespread in the organism of human and animal: MT-producing cells are found in gastrointestinal tract, airway epithelium, pancreas, suprarenal glands, thyroid gland, thymus, urogenital tract, placenta and other organs [2]. Moreover, an active synthesis of MT has been demonstrated in the non-endocrine cells, such as mast cells, natural killer cells, eosinophilic leukocytes, platelets, endothelial cells and others [3]. Such a wide distribution of MT in the organism determines its key role as intercellular neuroendocrine regulator and coordinator of many complex and interrelated biological processes. The highest content of extrapilneal MT is found in gastrointestinal tract (GIT): MT level in GIT organs exceeds its nighttime peak in the pineal gland at 400-fold [4]. Therefore the investigation of MT is of great importance to gain a better understanding of its functions and role in organism as a whole.

What we know about MT?

In the late 50s a group of American dermatologist A. Lerner from Yale University identified MT in bovine pineal gland extracts [1]. A little later MT was found to be 5-methoxy-N-acetylated derivative of serotonin (N-acetyl-5-methoxytriptamine), with the key enzymes of its synthesis being N-acetyltransferase (NAT) and hydroxyindol-O-methyltransferase (HIOMT) [5].

The discovery of MT stimulated researchers' interest and for a number of years the pineal gland was considered to be the only source of MT.

The most important physiological actions of MT include control of circadian and seasonal rhythms, stimulation of many metabolic processes, inhibiting effect on pigment metabolism, anti-gonadotropic effects, sedative and hallucinogenic action on the central nervous system (CNS), and an inhibitory effect on cell proliferation and division. MT stimulates oxygen consumption and production of carbon dioxide, as well as glucose uptake by tissues, increases concentration of ATP and creatine phosphate and contributes to storage of glycogen in tissues [6,7].

The intensity of MT metabolism depends on the level of illumination. The activity of HIOMT in rat pineal glands after keeping animals in darkness for 50 days is 10 times more than in animals kept during the same period under constant light. The level of this enzyme in the pineal gland at night is 3.5 times higher than at the day. Similarly, concentration of MT in pinealocytes increases at night, whereas the level of serotonin (ST) in them is 7–9 times lower at night than during the day. Probably at night and under conditions of artificial darkness ST is used for increased synthesis of MT. Therefore, there is observed evident dependence of MT synthesis on circadian rhythms [8].

The latest investigations on light/dark cycle and MT revealed that mechanisms underlying the daily and photoperiodic variations in MT level are related to regulation of MT receptor mRNA and protein at genetic level [9,10].

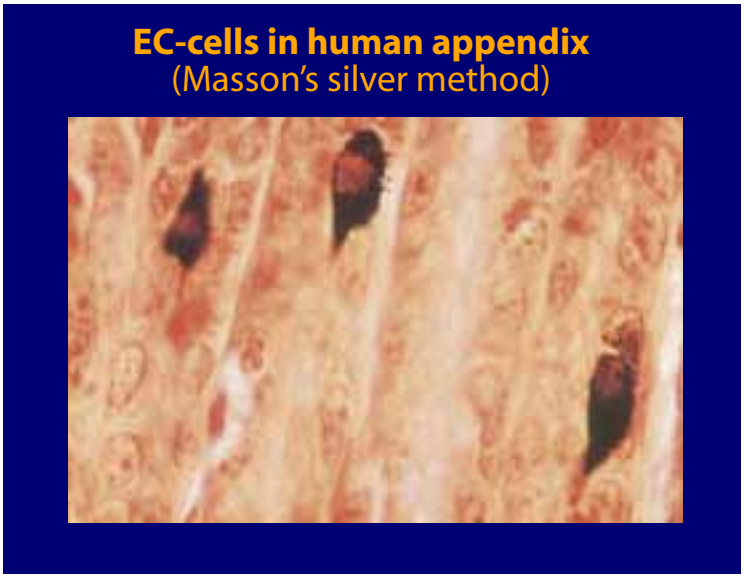
The effects of MT on reproductive function in mammals have been identified in many species. In fact, MT is known to regulate annual fluctuations in breeding capability in animals maintained under natural photoperiodic conditions [11,12]. From the other side, in literature occurs an opinion on the anti-gonadotropic effect of MT. For example, the anti-gonadotropic effect of MT is confirmed by the data on the delay of spontaneous vaginal opening, decrease in the weight of ovaries and lower frequency of estral cycle phases in immature female rats receiving MT per os with water for 28 days [13], as well as by inhibitory action of MT on testosterone secretion [14]. Actually, MT should not be thought of as either strictly an anti- or a progonadotropic factor but rather as a hormonal messenger of the pineal gland that appraises the reproductive system, as well as other organ systems, of the photoperiod environment. The following using of this information by a specific system is organ dependent [15].

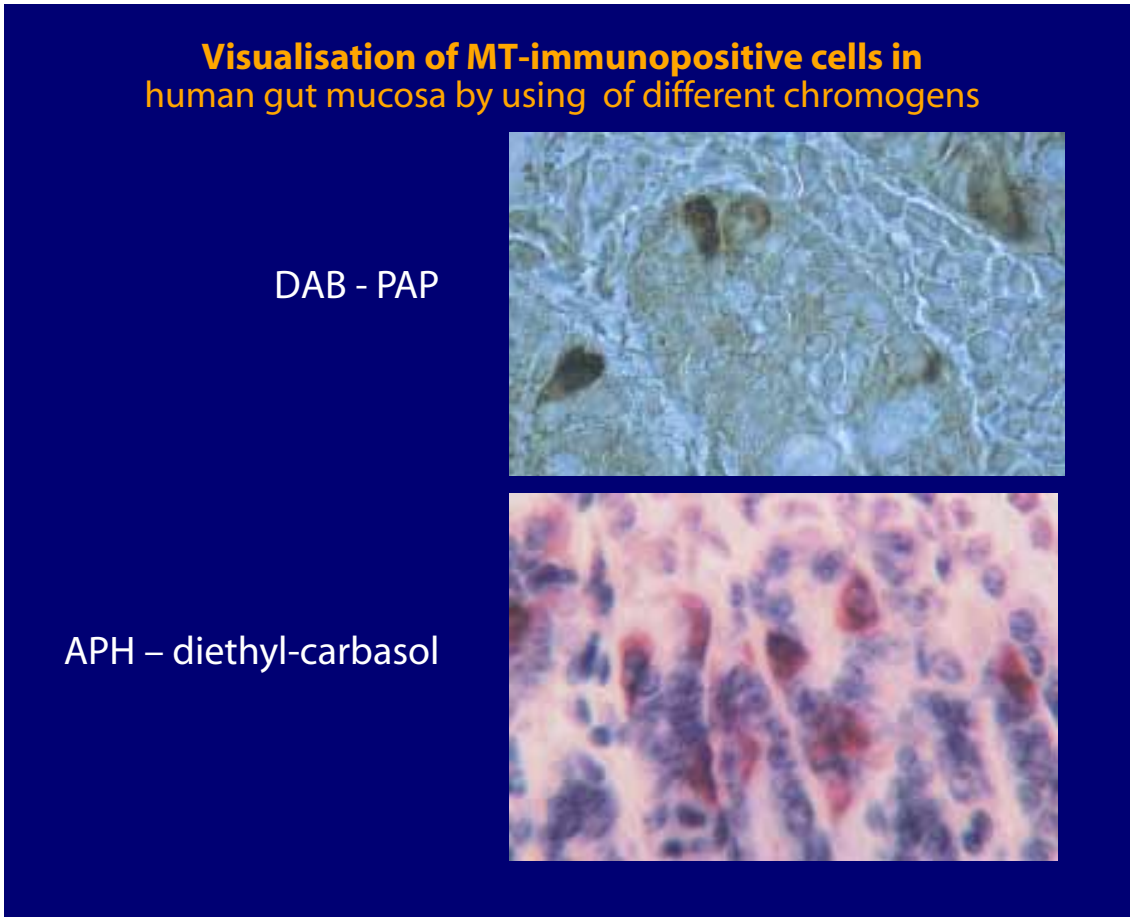
MT, as well as other biogenic amines, possesses the neurotransmitter function in addition to its hormonal effects. Thus, it affects the permeability of postsynaptic membranes of the synaptic system and participates in this way in nerve impulse transmission. The transmitter function is essential for the important role of biogenic amines in the functioning of the nervous system: from visceral functions and to higher integrative functions of CNS such as behavior, learning and memory [16,17,18]. According to [1], MT is directly involved in transmission of the nerve impulse through the synapse and, at higher doses, it decreases the functional

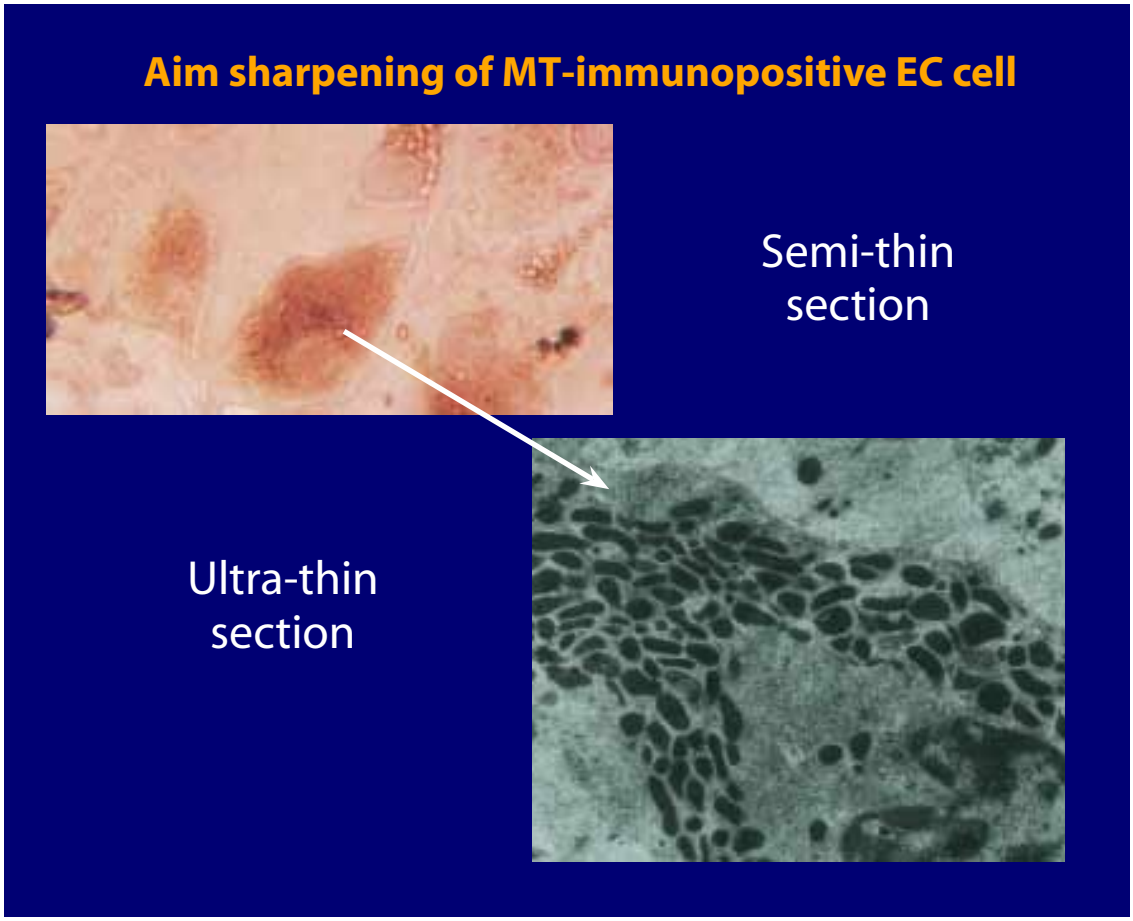
activity of the cerebral cortex and subcortical brain structures by decreasing cortical neuron performance.

In the literature of recent years appeared the data on the localization of MT not only in cytoplasm, but in the cell nucleus too [19]. Moreover, MT was found to have a capacity to scavenge free radicals and, therefore, to protect cell (in particular, its genome) from oxidative stress [20,21]. Now it is well established that MT protects macromolecules from oxidative damage in all subcellular compartments. This confirms to the protection by MT of lipids and proteins, as well as both nuclear and mitochondrial DNA. MT achieves this widespread protection by means of its ubiquitous actions as a direct free radical scavenger and an indirect antioxidant. Thus, MT directly scavenges a variety of free radicals and reactive species including the hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite anion, and peroxynitrous acid. Furthermore, MT stimulates a number of antioxidative enzymes including superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase. These multiple actions make MT a potentially useful agent in the treatment of neurological disorders that have oxidative damage as part of their pathogenesis, such as Alzheimer's disease [22]. Furthermore, now MT has been used successfully in sleep disorders [23, 24] and to treat epilepsy [25].

It is known that at the very early stages of embryogenesis the biogenic amines play the role of specialized intracellular signaling molecules regulating processes of cell division [26]. Control of cell proliferation rate is the most important function of biogenic amines in general and MT in particular. It is also extremely important in the postnatal phase of life [7]. The prominent inhibitory effect of MT on cell division [27] has been demonstrated same as for colhicine, which acts as a protecting substance. These data correlate with evidence of a certain anti-tumor effect of pineal gland extracts and MT [6,28,29].







However, it should be noted, that MT not always acts as anti-tumor drug; sometimes its employment may prove deleterious, and the literature data on this problem are contradictory [30-35].

Among MT's versatile functions, immunomodulation has emerged as one of major effects of this hormone in vertebrates [36]. Many studies have shown that MT plays a fundamental role in neuroimmunomodulation [37–39].

Featuring a wide activity spectrum, MT plays the role of a neuroendocrine signal coordinator and information transducer, thus essentially affecting the nervous, endocrine and immune systems and the organism as a whole.

Extrapineal MT: where and why?

As soon as highly sensitive techniques of analysis and identification became available, MT and its catalytic enzymes began to be found in extrapineal tissues, primarily those anatomically connected with the visual system. It must be noted that progress of knowledge about extrapineal sources of MT was based on developing a technique of obtaining highly specific antibodies to indolealalkylamines [40]. The use of such antibodies in a number of studies enabled the scientists to detect histologically, using the immunofluorescence method, the presence of MT in the retina, Harderian gland, and some other sites of the central nervous system in addition to the pineal gland [41,42]. In the same years, using biological and radioimmunological methods, as well as thin-layer chromatography, important data were obtained which indicated that after the removal of the pineal glands, MT would still be identified in blood plasma and urine of laboratory animals and humans [43–45]. This indirectly supported the suggestion of extrapineal sources of MT synthesis. Thus, a new era of MT research has begun.

During the last three decades MT synthesis was found in many various organs, tissues and cells: in the gut, liver, kidneys, adrenals [46,47], in lymphocytes [48], in mast cells, natural killer cells, eosinophilic leukocytes, thymic epithelial cells, some endothelial cells, placenta and endometrium [49]. The above list of the cell localization sites can signify that there are considerable prospects for the future search for potential MT producers. Further research into the nature of MT synthesis and deposition in non-endocrine cells seems to be very necessary.

Today the pineal gland is undoubtedly not the exclusive site of MT production. The MT content in organism and its concentration in blood are accounted for not only by the pineal gland secretion, but also by extrapineal sources of its synthesis, changes in the volume of extracellular fluid, hormone binding with blood proteins, metabolism and excretion rates depending on different outer and inner regulatory factors. Functionally, MT-producing cells are certain to be part and parcel of the diffuse neuroendocrine system (DNES) as

a universal system of response, control and organism protection. Also, as within the whole DNES, two compartments may be distinguished in the MT-producing cells, viz central and peripheral. The central compartment includes the MT-producing cells, which are associated with the visual system (pineal gland, retina, Harderian gland, and possibly others) whose secretion rhythm complies with the rhythm light-darkness. The peripheral compartment of the MT-producing cells includes other cells located in different organs, mainly gastrointestinal enterochromaffin cells (EC cells).

MT localization in retina was found immunocytochemically [50,51]. The fact that pinealectomy did not result in any alterations of retinal MT level, allowed to consider proved this hormone synthesis in retina as independent on pineal gland[52–54]. Furthermore, the presence of key enzymes of MT biosynthesis - NAT [52,55,56] and HIOMT [57–59] were shown in retinal tissue, as well as MT synthesis from labeled precursors (tryptophan and ST) has been demonstrated [60-62]. There are presented the evidences of MT synthesis in the layer of photoreceptor cells [63, 51], that seems to be more likely in cytoplasm of these cells [64, 65].

Light is a crucial factor for MT biosynthesis in the retina, as well as in the pineal gland [66]. Thus, it is interesting to note that light influences only one of two enzymes participating in MT synthesis, namely, the NAT [67, 52, 68, 56, 69]. Activity of another enzyme - HIOMT does not depend on light action [59, 56]. Hence one can conclude that N-acetylation of ST represents the key step of MT biosynthesis in the retina, as well as in the pineal gland, [69].

Thus, the level of MT biosynthesis in the retina is determined by two essential factors: availability of its main precursor – ST and NAT activity [70,71]. Therefore, the rhythm of retinal MT biosynthesis is defined and regulated by circadian "clock", localized in eye; it is in agreement with circadian rhythm of pineal gland, but does not depend on that [66]. It must be noted that MT biosynthesis in retina appears to be more complicated than in pineal gland. In the process of retinal MT synthesis there are involved additionally certain factors, such as cAMP [55,72], Ca^{2+} ions [73,74], dopamine [75,76], GABA [77,78], and some else, that have not cleared up finally [66].

Available data permit to consider that regulation of physiological processes in complex "retina - retinal pigment epithelium", submitting to light regime, is an essential function of retinal MT [69]. It enables to assume that in retina MT carries out a transductive function of coordinator in receiving, primary processing and transmission of visual and nervous information.

Harderian gland (especial type of intra-orbital lacrimal glands in birds and some mammalians) is one of the sources of extrapineal MT synthesis [4,79,19]. Evidently, MT synthesis in Harderian gland occurs as well as in pineal gland; at least, one of two key enzymes of MT biosynthesis – HIOMT – was found in Harderian gland [80, 81].

MT synthesis in Harderian gland been shown to comply with circadian rhythm, typical for pineal gland, but does not depend on it [82–84]. Moreover, by the data [54], there was observed a compensatory increase of MT content in Harderian gland of rats some weeks after pinealectomy. Physiological role of MT in Harderian gland is not determined exactly as yet.

Concerning of the peripheral compartment of MT-producing cells, as it was mentioned above, these cells are widely distributed in the organism, but the total number of them are located predominantly in the gastrointestinal tract.

Gut enterochromaffin cells are the main source of MT in organism

The integrated application of methods of biological testing, thin-layer chromatography and immunohistochemical analysis, enabled the Russian scientists to be first to demonstrate the active MT synthesis in human gut enterochromaffin cells (EC cells) [46]. Three parts were followed in MT identification for EC cells. Initially it had to be found out whether MT was present in gut mucosa – in the same wall layer which houses EC cells; then MT location had to be identified in EC cells and, finally, we wanted to see if the hormone could be stored or synthesized in EC cells.

Arranging the re-make of classical Lerner's experiment for pineal glands, we repeat it only for appendix mucosa and the presence of MT in the extract receiving from 1500 human appendixes was obtained - when purified extracts of mucous membrane of human appendixes (especially rich in EC cells) were applied onto frog skin, and the sterile extract was injected into the lymphatic sac, the skin color was observed to become definitely lighter, which is characteristic of MT impact. The clearing effect of MT on the frog skin is detected in very low concentrations (10^{-12} g/ml) [85,46]. Control tests showed that 0.006% MT solution in ethanol had a similar effect, and chromatographic analysis confirmed that MT was presented in the mucous extracts used in the bio-tests [86]. Experimental studies of extracts prepared separately from appendixes with simple, phlegmonous and gangrenous inflammation (the mean number of argentaffin EC cells in crypts depends on the type of inflammation) showed that the frog skin-bleaching rate was related to the EC-cell content of the mucous membrane. Correlation between the frog skin-bleaching rate which is MT-specific and the number of EC cells seemed to be an indirect confirmation of MT being present precisely in EC cells.

Chromatographic analysis of the tested extracts using as indicators of synthetic MT and its main precursors, showed the presence of 5-hydroxytriptophan, 5-hydroxytriptamin (serotonin), 5-methoxytriptamin (mexamin) and MT. The fact that the extracts contained immediate precursors of MT which are gener-

ated in the chain tryptophan > serotonin > melatonin also supported the suggestion of MT being synthesized in EC cells.

However the ultimate answer to the question whether MT was presented in EC cells, was obtained owing to the application of the immunohistochemical techniques using antisera against MT and its immediate precursors: serotonin, N-acetylserotonin [87,88]. Analysis of the coloring results using Coon's indirect immunofluorescent method and the immunoperoxidase method showed the presence of immunoreactive cells to MT and its precursors throughout the gastrointestinal tract in both human and common animals (dogs, rats and mice). When serial sections were compared, one of which was treated with a specific antiserum, and the other was stained using the argentaffin method according to Masson, coincidence was observed of localization of argentaffin cells and the cells positively responding to the antiserum to MT.

Soon other scientists confirmed these results. Using the immunohistochemical method MT was detected in practically all parts of the rat gastro-intestinal tract [89]. It was emphasized that the MT distribution corresponded to localization of ST-producing argentaffin cells. The fact that the MT-synthesizing enzyme HIOMT was localized in the gastrointestinal tract confirmed the occurrence of synthesis rather than just passive accumulation [90].

Mathematical analysis showed that the total number of EC cells throughout the gastro-intestinal tract would be significantly larger than the possible number of MT-producing cells of the pineal gland. This, as well as the fact that EC cells account for 95% of ST production (ST being the principal precursor of MT), made it possible to consider EC cells as the main source of MT production in organism [91].

To date, the functional morphology of EC cells has been studied sufficiently well. However, not much is known about their functional role and factors inducing EC cells activity. EC cells can serve as a "classic" example of receptor-effector endocrine cells, in which the biogenic amines and peptide hormones co-exist. In semi-thin sections impregnated with silver according to Masson, the cell's apical part looks like a thin antenna-shaped tentacle spreading into the tract opening; the basal part is extended and filled with numerous secretory granules. In ultra-thin sections, the secretory granules are of specific shape, size and high electron density of osmiophilic contents. The above ultrastructural features enable a clear distinction to be made between EC cells and other types of endocrine cells. The results of immunohistochemical investigations indicate the existence of several subpopulations of enterochromaffin cells. Such peptide hormones as motilin, substance P and enkephalines have been identified in EC cells. No answer has been obtained yet to the question, which of the subpopulations (or all of them) is able to synthesize MT from ST.

Hence, EC cells represent one of the most numerous groups of peculiar cells in digestive organs, which synthesize a whole series of biogenic amines and peptide hormones. Now many experts agree that EC cells could be considered as a main source of MT in the organism.

Melatonin regulates many gut functions

Besides, the presence of MT in EC cells [46], receptors for MT and enzymes involved in its synthesis from tryptophan were detected in GIT tissues also [90,92]. There are few experimental and clinical data regarding the role of MT in the regulation of GIT functions. It is hypothesized that MT plays an important role in physiological activity of GIT. Disturbances in MT secretion of various genesis may result in GIT diseases.

MT is present in all portions of human and animal GIT (from the esophagus to the rectum). Maximum amounts of this hormone were found in the mucosa, while the submucosal and muscle layers contain the lowest concentrations of MT [89,93,94]. Studies with pinealectomized animals demonstrated that the content of MT in their GIT organs does not differ from the control [93]. The data suggest that MT is synthesized in GIT organs. EC cells were shown to contain MT [46], as well as the enzyme HIOMT, that catalyzes transformation of N-acetylserotonin into MT [90]. The distribution of receptors for MT in GIT tissues is similar to its localization: the density of MT receptors in the mucosa is much higher than that in the submucosal and muscle layers. The number of intracellular receptors for MT decreases in the following order: nucleus > microsomes > mitochondria > cytoplasm [94, 95]. Experiments with laboratory animals showed that the content of MT in GIT organs 400-fold surpasses its nighttime concentration in the pineal gland [4]. However, the contribution of MT produced in GIT into the total amount of circulating hormone is relatively small. It was reported that 90% MT diffusing from GIT tissues into the portal vein are metabolized during the first passage through the liver [96]. This fact is confirmed by an 80% decrease in the concentration of circulating MT in pinealectomized animals [43]. It remains unclear whether MT secretion in GIT tissues is characterized by circadian rhythmicity, which is typical of hormone production in the pineal gland. Some authors reported diurnal/nocturnal differences in the content of MT in GIT [84,94,97], while others failed to reveal these peculiarities [43,93]. Since GIT tissues contain MT of both local and circulation origins, these diurnal/nocturnal differences in hormone content in GIT are probably associated with MT diffusing from the circulation. No diurnal/nocturnal differences in the content of MT in the plasma of pinealectomized animals confirm the absence of circadian rhythmicity of hormone production in GIT tissues [44]. MT pro-

duction in the pineal gland is synchronized by the light/dark cycle. By contrast, the synthesis and secretion of MT in GIT tissues is regulated by eating and food composition. Experiments with animals and volunteers revealed a sharp increase in the content of MT in GIT organs and circulation in response to food intake [98,99]. Peroral treatment of humans and animals with pharmacological doses of L-tryptophan at daytime produced a significant increase in the concentration of circulating MT, which was comparable with nocturnal MT peak [100–102].

L-tryptophan administration to pinealectomized animals increased plasma MT content compared to that in controls not treated with the amino acid [100,102]. The increase in plasma MT content in pinealectomized animals was less pronounced than that in sham-operated animals (insignificant) [100,102]. Portal vein ligation abolished an increase in the concentration of circulating MT in sham-operated animals receiving L-tryptophan and pinealectomized animals [100]. All these results confirm the data firstly presented by Kvetnoy' team that GIT tissues synthesize MT. In addition to this, local synthesis of MT in GIT depends on the content of tryptophan in food. *In vitro* addition of L-tryptophan or its metabolites (MT precursors) into the perfusate did not lead to MT synthesis in GIT tissues [103]. Therefore, *in vivo* synthesis of MT by GIT tissues is under central regulation. This process can be also controlled by intestinal contents.

Similarly to various hormones, whose synthesis and presence were revealed in the CNS and GIT, the effects of MT are mediated by the humoral, neurocrine, paracrine, and autocrine mechanisms [104,94,105]. Probably, the effects of MT synthesized in GIT are primarily mediated by the paracrine mechanism, while pineal gland MT produces changes via the humoral and neurocrine pathways. Besides biorhythmic, antioxidant, and immunomodulating activities, MT affects motor functions of GIT, microcirculation, and mucosal cell proliferation [106].

In vitro and *in vivo* experiments with animals showed that MT inhibits motor activity of GIT. The degree of inhibitory effects is directly proportional to the tone and intensity of contractions in the stomach, duodenum, and small and large intestines [107,108,109, 104]. MT inhibits motor activity of GIT organs stimulated with various agents, e.g., ST [107,104], KCl [110], and carbachol (cholinoreceptor agonist) [110]. Therefore, the inhibitory effect of MT on muscle contractions is mediated by various mechanisms, including binding to specific [109] and ST-inhibiting receptors [105, 109] and regulation of activity of Ca^{2+} channels and Ca^{2+}-activated K^+ channels in cell membranes [110]. Besides direct effects of MT on muscle cell membranes, this hormone blocks nicotinic acetylcholine receptors on cells in the submucosal nervous plexus of the small intestine in guinea pigs [105]. These data indicate the neurocrine mechanism of MT effects on motor activity of GIT. Experiments with pinealectomized rats showed that pinealectomy suppresses interdigestive rhythmic contractions of the large and small intestines, while MT administration normalizes the rhythmicity of myoelectric complexes [111,112]. Therefore, MT modulates motor functions of the intestine and acts as the major regulator of motor activity in GIT organs. The feedback mechanisms underlying synthesis and secretion of MT and ST in animals [113,114], as well as the relationship between their effects [109,115,104,116], are of particular interest in this respect. Immunohistochemical studies showed that rat GIT portions with maximum concentration of MT contain greater amounts of ST compared to those with low MT content [114]. Intraperitoneal injection of MT at physiological doses stimulated ST secretion in rat CNS [117,114]. Treatment of mice with physiological concentrations of ST increased the content of MT in CNS and various GIT portions [116]. The administration of exogenous MT to animals with an implanted container, which provided a gradual release of ST, attenuated markedly the increase in MT content in GIT tissues and CNS compared to that in animals treated with MT [116]. Experiments with isolated pineal glands perfused with MT at physiological concentrations showed that MT at high doses (10^{-3}) stimulates ST secretion; while at low doses (below 10^{-6}) the hormone inhibits this process. It was demonstrated that MT regulates ST production by pinealocytes by stimulating or inhibiting ST transformation into 5-hydroxyindole acetic acid [118]. *In vitro* preincubation of GIT portions from rats with various concentrations of MT dose-dependently decreased the stimulatory effect of ST at physiological and pharmacological doses on motor activity of the portions isolated (stomach, duodenum, jejunum, ileum, and large intestine) [119, 107, 109]. *In vitro* experiments showed that MT abolishes ST-induced contraction of vascular smooth muscles in various animal organs [115]. These data indicate the existence of the MT-ST system in GIT and CNS. It was shown that the mechanisms underlying feedback regulation of secretion and effects of various agents present in GIT and CNS are the same [120]. Besides the acetylcholine-norepinephrine system, the ST-MT system modulates motor activity of GIT and microcirculation at the paracrine level [119,109,99,110]. Impaired ST production contributes to the pathogenesis of gastrointestinal diseases, e.g., the irritable bowel syndrome and gastroesophageal reflux disease [121]. The reciprocal regulation of MT and ST production suggests that MT is involved in the pathogenesis of these disorders. Measurements of MT and ST concentrations in the plasma of infants from the first days after birth to 1 year demonstrated the absence of MT and circadian rhythmicity of its production over the first 3 month of life (except for the first week after birth when the plasma contained MT passed from the maternal body through the placenta). However, plasma ST concentration at this period was higher than that in

infants greater than 3 months old [122]. It is hypothesized that the absence of circadian rhythmicity of MT production and ST-MT misbalance contribute to evening intestinal colic in newborns that coincides with the nocturnal ST peak [122].

The existence of similar relationships between gastrin and MT is suggested. This assumption is based on experiments with animals receiving for a long time omeprazole to modulate hypergastrinemia [123]. MT abolished gastrin-induced acceleration of mucosal cell proliferation and stimulation of GIT motor activity [123]. Taking into account the similarity of chemical structures of MT and gastrin antagonist benzotript, it was hypothesized that MT counteracts the effect of gastrin by binding (i.e., blocking) to its receptors [123]. Thus, the effects of MT on GIT organs are probably mediated via binding to MT receptors or blockade of gastrin receptors. The existence of the gastrin-MT system regulating functional state of GIT mucosal cells is confirmed by the following facts. First, MT and gastrin produce the opposite effects on intracellular cAMP concentration in GIT mucosal cells. Gastrin increases intracellular cAMP concentration [124], while MT decreases this parameter [123]. Second, MT and gastrin cause opposite changes in motor activity of GIT. And third, MT inhibits proliferation of GIT mucosal cells induced by hypergastrinemia [123]. In available literature, we found no data on the effect of MT on hydrochloric acid secretion by the gastric mucosa. Since MT inhibits production of cAMP playing an important role in hydrochloric acid secretion by parietal cells and acts as gastrin antagonist, it can be suggested that this hormone suppresses HCl production. ST inhibits hydrochloric acid secretion (basal and stimulated with carbacholine, pentagastrin, and histamine) [125, 126] and stimulates gastrin production by G cells [127]. The data indicate that ST-induced inhibition of HCl production results from its vasoconstrictor properties, but not from the effect on receptor-mediated mechanisms of this process. Therefore, our assumption that MT inhibits hydrochloric acid production is based on (but not in contradiction with) the notion of antagonistic relationships between ST and MT regarding their effects on GIT functions.

Experiments with chicken and duck pineal glands showed that histamine stimulates cAMP production [128,129], which indicates its important role in the regulation of pineal gland activity, including MT secretion that depends on cAMP level [130]. Recent studies confirmed the stimulatory effect of histamine on MT secretion by isolated chicken pineal glands [131]. It was shown that chicken pineal gland contain histamine present in mastocyte-like cells and enzymes involved in its synthesis and inactivation [131]. Studies of rat pineal gland innervation revealed histaminergic nerve fibers [132]. It was noted that the interregulation of secretion of substances present in GIT and CNS is realized by the same mechanisms [120]. Therefore, histamine probably regulates secretion of MT by EC cells in GIT (similarly to CNS). Accounting of the anatomical similarity of MT-producing EC cells and histamine-producing ECL cells allows to suggest that MT regulates histamine production by the paracrine mechanism.

It is known that cholecystokinin is an important regulator of GIT motor activity [133]. Pinealectomy suppresses interdigestive rhythmic motor activity of the intestine, which is normalized by exogenous MT [111,112]. The data suggest the interrelation between MT and cholecystokinin [112]. This hypothesis was confirmed by the fact that pinealectomy abolished cholecystokinin-induced stimulation of motor activity in the intestine. Treatment of pinealectomized animals with MT and cholecystokinin restored the effect of cholecystokinin [112]. These results indicate that MT probably mediates the influence of cholecystokinin on motor activity of GIT.

The effect of MT on microcirculation is associated with relaxation of vascular smooth muscles [115,134]. The mechanism underlying MT-induced relaxation of vascular smooth muscles includes the regulation of Ca^{2+} and K^+ influxes in cells by modulating functioning of Ca^{2+} and Ca^{2+}-activated K^+ channels in cell membranes rather than the stimulation of Ca^{2+} release from intracellular stores [115,134]. Intragastric administration of MT to rats with ischemic gastric ulcers decreased significantly the incidence of ulceration and the size of ulcerative lesions [135,136]. MT decreased the content of free radicals in the plasma and enhanced blood supply to the stomach wall [136]. Doppler ultrasonography used in experiments on rats with 40% ethanol-induced gastric ulcers showed that MT decreased the incidence of ulceration, increased blood flow in the stomach wall, and normalized blood supply to the gastric mucosa inhibited by ST [137]. Therefore, the antiulcer effect of MT is related not only to its antioxidant properties, but also to the improvement of microcirculation [137].

Experiments with animals showed that pinealectomy stimulates proliferative activity of cells in various organs, including the GIT system [138–140]. The effect of MT on proliferation of GIT mucosal cells is realized by the humoral and neurocrine pathways: vagotomy and local sympathectomy attenuated pinealectomy-induced acceleration of proliferation of small intestine crypt cells. However, proliferative activity of these cells remained above the control [140]. MT is probably one of the most potent regulators of cell proliferation in the GIT mucosa. It was shown that proliferative activity of mucosal cells in the small and large intestines of rats remained high for at least 6 months after pinealectomy (considerable period of the rat life span). Cell proliferation in pinealectomized animals was not normalized for a long period, which indicates an important role of MT in the regulation of proliferative processes in the GIT mucosa. The phenomenon of MT-induced inhibition of cell proliferation was studied in *in vitro* and *in vivo* experiments

in the field of oncology [141]. However, these experimental and clinical observations were performed with pharmacological doses of MT used as monotherapy or in combination with interleukin [142–145]. The data that various physiological doses of MT produce the opposite effects on mucosal cell proliferation in the small intestine are of considerable interest in this respect. MT inhibited proliferative activity of cells, if its concentration in the perfusate was similar to diurnal levels of circulating hormone. However, MT at a concentration similar to its nocturnal level in the circulation stimulated cell proliferation [146,147]. These experimental facts are consistent with published data showing circadian rhythmicity of cell proliferation in various organs with maximum activity in the nighttime period [148]. On the one hand, circadian rhythmicity of MT secretion plays an important role in rhythmic processes in the body and on the other hand regulates cells proliferation. MT modulates cell proliferation probably by stimulating prostaglandin E_2 production, which was demonstrated in experiments with gastric ulcers in rats induced by piroxicam (nonsteroid antiinflammatory drug) administration [149]. The inhibition of prostaglandin E_2 synthesis was abolished by intragastric administration of MT at doses of 1, 3, and 7.5 mg/kg. Subcutaneous injection of MT at the same doses had no effect on prostaglandin E_2 synthesis in the gastric mucosa. The data indicate that this effect realized by the paracrine mechanism was associated with the MT fraction synthesized in the GIT mucosa [149]. It was hypothesized that the mechanism underlying MT-induced stimulation of prostaglandin E_2 synthesis involves the activation of cyclooxygenase, which potentiates production of prostaglandins, prostacyclin, and thromboxane from polyunsaturated fatty acids [150]. Since prostaglandins E and thromboxane inhibit secretion of hydrochloric acid and pepsin, but stimulate production of bicarbonates in the gastric mucosa [151], it can be suggested that endogenous MT synthesized in the GIT mucosa produces similar effects on gastric secretion. Enprostil (synthetic prostaglandin E_2 analogue) displays antigastrin activity and decreases blood gastrin concentration via the direct regulation of gastrin production by G cells [152]. Thus, MT-induced stimulation of prostaglandin E_2 synthesis indicates that this hormone is involved in mucosal protection damaging factors.

Thus, it seems to be as a fact, that MT produces various effects on GIT organs. Studies of its role in the regulation of GIT functional activity are of considerable theoretical and practical importance since MT holds much promise as a potent drug.

General conclusions

Summing up this review, it is necessary to reveal the main points of data analyzed. The first, MT is an ubiquitously acting hormone with a wide spectrum of effects. Among these are control of biological rhythms, influence on reproductive cycle, immune response, scavenge of free radicals, monitoring of mood and sleep, cell proliferation and differentiation and so on. MT secretion is related to the photoperiod in a circadian model of low activity during light phase and high activity in a dark time.

The second, MT synthesis takes place not only in the pineal gland, but also to a greater extent this process is observed in widely distributed cells in the whole organism, therewith the gastrointestinal tract is a major source of extrapineal MT [153], where it is synthesized essentially by intestinal EC cells [46].

And the third, in the recent years there has been found, that MT can protect gastrointestinal mucosa from ulceration by its antioxidant action, stimulation of the immune system and by fostering microcirculation and epithelial regeneration [153]. Moreover, MT may interact with receptors and subsequently stimulate the synthesis of gastroprotective hormones and also exerts a direct defense on the epithelium, enhances submucosal blood flow and prevents the damage induced by ischemia followed by reperfusion. Studies have shown that treatment with MT reduces the severity of the lesions induced by NSAIDs on gastric mucosa suggesting a beneficial role of MT in preventing this gastropathy related to antiinflammatories [154] Therefore, MT can be considered as a potential gastroprotective agent in various pathologies of the digestive tract.

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