Neuroendocrinology Letters ISSN 0172–780X

REVIEW ARTICLE

Extrapineal melatonin in pathology: New perspectives for diagnosis, prognosis and treatment of illness

Igor Kvetnoy
St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia

Correspondence to: Prof. Igor Kvetnoy
Institute of Bioregulation and Gerontology,
3 Dynamo Prospect, 197110 St. Petersburg,
Russia
TEL/FAX: +7 812 230 00 49
E-MAIL: kvetnoy@medport.ru

Submitted: *October 3, 2001*
Accepted: *October 31, 2001*

Key words: **melatonin; extrapineal synthesis; diagnosis; tumor growth**

Neuroendocrinology Letters 2002; ***23*** *(suppl 1):92–96 pii: NEL230702R12*

Abstract

During the last decade, attention was concentrated on melatonin - one of the hormones of the diffuse neuroendocrine system, which has been considered only as a hormone of the pineal gland, for many years. Currently, melatonin has been identified not only in the pineal gland, but also in extrapineal tissues - retina, harderian gland, gut mucosa, cerebellum, airway epithelium, liver, kidney, adrenals, thymus, thyroid, pancreas, ovary, carotid body, placenta and endometrium as well as in non-neuroendocrine cells like mast cells, natural killer cells, eosinophilic leukocytes, platelets and endothelial cells. The above list of the cells storing melatonin indicates that melatonin has a unique position among the hormones of the diffuse neuroendocrine system, which is present in practically all organ systems. Functionally, melatonin-producing cells are certain to be part and parcel of the diffuse neuroendocrine system as a universal system of response, control and organism protection. Taking into account the large number of melatonin-producing cells in many organs, the wide spectrum of biological activities of melatonin and especially its main property as a universal regulator of biological rhythms, it should be possible to consider extrapineal melatonin as a key paracrine signal molecule for the local coordination of intercellular relationships. Analysis of our long-term clinical investigations shows the direct participation and active role of extrapineal melatonin in the pathogenesis of tumor growth and many other non-tumor pathologies such as gastric ulcer, immune diseases, neurodegenerative processes, radiation disorders, etc. The modification of antitumor and other specific therapy by the activation or inhibition of extrapineal melatonin activity could be useful for the improvement of the treatment of illness. Introduction

About 30 years ago Pearse first suggested that a specialized, highly organized cell system should exist in organisms, whose main feature was the ability of component cells to produce peptide hormones and biogenic amines. The concept was based on an extensive series of experiments on distinguishing endocrine cells in different organs, identifying endocrine cell-generated products and making a thorough cytochemical and ultrastructural analysis of these cells [1]. Different types of cells widely dispersed throughout the organism have a common ability of absorbing monoamine precursors (5-hydroxytryptophan and L-dihydroxyphenylalanine) and decarboxylating them, thus producing biogenic amines. That ability accounts for the term APUD, an abbreviation of "Amine Precursor Uptake and Decarboxylation" used by Pearse to designate the cell series [2]. Presently, the APUD series includes over 60 types of cells located in gut, pancreas, urogenital tract, airway epithelium, pineal gland, thyroid gland, adrenals, adenohypophysis and hypothalamus, carotid body,

skin, sympathetic ganglia, thymus, placenta and other organs [3-5]. Meanwhile the appearance of radioimmunological methods and rapid development of immunohistochemistry resulted in establishing a completely unexpected phenomenon, i.e. the same biogenic amines and peptide hormones were identified in neurons and endocrine cells [6]. The accumulated data did not fit the traditional concepts of hierarchical dependence within two main regulatory systems, viz. nervous and endocrine ones. It became more and more evident that the mechanism of biological regulation should be founded on the coordinated functional interaction between the endocrine system and the central and peripheral nervous system based on the common type of information perception and transmission at subcellular, cellular and tissue levels. Recent data on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones; and, locally or distantly as hormones within the endocrine system, enables both system to be incorporated into the universal diffuse neuroendocrine system - DNES [5-9]. It should be possible to unite in the organisms the structurally isolated nervous and endocrine systems by means of functional relationships between biogenic amines and regulatory peptides and, to a certain extent, to provide a basis for the concept of integrated functions. The DNES cells, located practically in all organs and producing biologically active substances, are regulators of homeostasis acting via neurocrine, endocrine and paracrine mechanisms [8, 10]. In recent years the attention has especially been focused on one of the hormones of DNES - melatonin (MT). Featuring a wide activity spectrum, this hormone plays a key role for the control of biological rhythms [11], thus essentially affecting the nervous, endocrine and immune systems as well as the organism as a whole.

Pineal melatonin

In the late 50s Dr. Aaron Lerner and his team from Yale University first identified MT as the pineal substance bleaching frog skin, and MT was found to be the 5-methoxy-N-acetylated derivative of serotonin (ST) - 5-hydroxytryptamine [12]. Two years later Axelrod and Weissbach [13] have determined the key enzymes of MT synthesis being N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT). The identification of MT stimulated researchers' interest in the physiology of the pineal gland and a wide spectrum of biological activities of pineal MT was shown. The main of them are the following: control of biological rhythms, antigonadotrophic effects, stimulation of immune processes, scavenging of free radicals, cytostatic and antiproliferative effects *in vitro* and *in vivo* [14-18]. However, the pineal gland was considered to be the only source of MT. At the same time the important data were obtained which indicated that after the removal of pineal gland, MT would still be identified in blood plasma and urine of laboratory animals [19, 20], indicating a significant extrapineal synthesis of MT.

Extrapineal melatonin

As soon as highly sensitive techniques of analysis and identification became available, MT and its precursors as well as catalytic enzymes began to be found in extrapineal tissues, primarily those anatomically connected with the visual system - retina and Harderian gland [21, 22]. It is to be noted that progress of knowledge about extrapineal sources of MT was based on developing a technique of obtaining highly specific antibodies to indolealalkylamines [23].

Taking into account the fact that gut enterochromaffin cells (EC cells) are the main ST depot in the organism [24, 25], we were the first to identify MT production for these cells [26, 27].

In MT identification for EC cells three steps were followed. 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 in EC cells had to be identified by immunohistochemical method and, finally we wanted to see if the hormone could be stored or synthesized in EC cells.

Using re-make of Lerner's classical biological test, the presence of MT in gut mucosa was discovered [26]. When purified extraxts of human appendix's mucosa (as known, especially rich in EC cells) were applied onto frog's skin, and the sterile extract was injected into the lymphatic sac, the skin colour became definitely lighter (the effect characteristic for MT). Chromatographic analysis confirmed that MT was present in the mucous extracts used in the bio-tests [26].

Chromatographic analysis of the test extracts using as indicators synthetic MT and its main precursors, showed the presence in gut mucosa of 5-hydroxytryptophan, 5-hydroxytryptamine (ST), 5-methoxytryptamine (mexamin) and MT [27]. The fact that gut extracts contained the immediate precursors of MT which are generated in the chain tryptophan > ST > MT also supported the suggestion of MT being synthesised in gut EC cells.

However the ultimate answer to the question whether MT was present in EC cells was obtained thanks to the application of the immunohistochemical techniques using antibodies against MT and its immediate precursors: ST, N-acetylserotonin and mexamin. The immunohistochemical study, using of our own antibodies as well as two commercial antibodies to MT (CIDtech Research Inc., Mississauga, Ontario, Canada; and Dianova, Gamburg, Germany) showed the presence of immunopositive cells to MT and its precursors throughout the gastrointestinal tract in both humans and experimental animals: dogs, rabbits, rats and mice [27-29].

Thus, the integrated application of methods of biological testing, thin-layer chromatography, histochemical stain and immunohistochemical analysis, enabled the first-ever demonstration of the possibility, in principle, of MT synthesis in gut EC cells. Soon these results were confirmed by Bubenik [30], who using immunohistochemical method detected MT in practically all parts of the rat gastro-intestinal tract. It was emphasised that MT distribution corresponded to localization of ST producing argentaffin EC cells. The fact that key MT-

synthesizing enzyme HIOMT was localized in gut [31] confirmed the occurence of its synthesis rather than just passive accumulation.

Mathematical analysis showed that the total number of EC cells throughout the gut would be significantly larger than the possible number of MT producing cells of the pineal gland. Huether [32] showed that the avian and the mammalian gastrointestinal tract contains at least 400x more MT than the pineal gland. These data, and the fact that EC cell account for 95% of all endogenous ST [33], being the principal precursor of MT, allow to consider gut EC cells as the main source of MT in the organism of humans and animals.

Today it becomes increasingly more evident that the pineal gland is not the only site of MT production. The MT content in the organism depends not only on the pineal gland secretion, but also on extrapineal sources of its synthesis, regulated by different outer and inner factors.

It seems, also as within the whole DNES, two compartments may be distinguished in MT producing cell population, viz. central and peripheral. The central compartment includes MT producing cells which are associated with the pineal gland and the visual system (retina, Harderian gland) whose secretion rhythm complies with the rhythm light-darkness [11,18]. The peripheral compartment seems to account for all cells located outside the above areas, and its function probably does not depend on the degree of illumination.

Actually the cells synthesizing MT exist not only in gut. Availability of this hormone was observed in APUD cells of airway epithelium, along the border between the cortex and medulla of adrenals, in thyroid gland, beneath the hepatic capsule, in kidney cortex, paraganlia, gall bladder, ovary, endometrium, placenta, inner ear [5]. We have found MT also in non-endocrine cells [34], i.e. in mast cells, natural killer cells, eosinophilic leukocytes, pancreatic acinar cells, reticulo-epithelial cells of thymus, some endothelial cells.

Thus, currently the extrapineal localization of MT shows a wide spectrum of cells in the different organs. The list of the cells producing and storing MT indicates that MT has a unique position among the hormones of the DNES, being found in practically all organ systems. Functionally, MT producing cells are certain to be part and parcel of the DNES as a universal system of response, control and organism protection.

Thus, taking into account the large number of MT-producing cells in many organs, the wide spectrum of biological activities of MT and especially its main property as a universal regulator of biological rhythms, it should be possible to suppose that extrapineal MT may play a key role as a paracrine signal molecule for the local coordination of intercellular relationships. Extrapineal MT may also act as a typical hormone, reaching widely spread target cells through the bloodstream. It has now been shown that many cells in different organs have MT receptors [35, 36]. In both cases, some non-endocrine cells as mast cells and eosinophilic leukocytes may take up MT from the blood or intercellular space for transport to sites where it exerts its effects.

Extrapineal melatonin in pathology and oncology

During last ten years our team studied the functional morphology and behaviour of extrapineal MT-producing cells as well as other main APUD cells in different pathologies and environmental conditions (e.g. ionizing and non-ionizing radiation, tumour growth and cytostatic therapy, autoimmune and gastrointestinal diseases, pharmacological and toxicological influence, etc.). The obtained data testify to active participation of extrapineal MT equally with other hormones in the pathogenesis of various diseases [4, 29, 34].

The oncopathology is our main research interest. Therefore, taking into account the fact, that MT has expressed cytostatic and antitumor properties, we have studied the behaviour of MT producing cells in tumor growth [37, 38]. Actually, we showed an increase of the number of EC and other MT- producing cells for initial stages and a decrease of the number of these cells for late stages of carcinogenesis. Generally, the functional activity of MT-producing cells, which produce the hormone with antiproliferative effect may arrange the conditions which are unfavourable for fast tumour growth and metastases formation. It seems, that these data open the perspectives for the elaboration of new approaches for improvement of antitumor therapy.

For example, we induced a functional modification of mast cells which form an endogenous "radioprotective shield" around the tumour by accumulating MT and ST [37, 39]. In particular, we observed an increase in tumour cell sensitivity to ionizing radiation after administration of ketotyphen, a drug which prevents the release of mediators from mast cells. Ketotyphen injections before radiation therapy of tumours increased radiosensitivity by 26% in terms of growth rate and 20% in terms of proliferative activity [39].

It is well-known now that the special type of tumors - apudomas- are developing from APUD cells. Most of them are carcinoids - the typical neoplasms from EC cells. At the same time the presence of hormone-producing cells in the non-endocrine carcinomas has a great theoretical and applied significance. Using immunohistochemical method, it was shown that about 30% of all non-endocrine carcinomas of different histological types and localization contain endocrine cells and about 60% of such tumors have MT producing cells in its composition [38].

In our special study, using either immunohistochemical method or radioimmunoassay we have studied the excretion of 6-sulphatoxymelatonin (aMT6s) in urine, the expression of proliferating cell nuclear antigen (PCNA) and the number of MT immunopositive cells in different primary human gut and lung tumors (cancer of colon, rectum, stomach, and lung) without metastases. Our results showed strong positive correlations between the expression of PCNA in tumors and aMT6s excretion in urine. On the contrary, strong negative correlations were observed between MT-immunoreactivity and proliferative activity of tumor cells [40]. These parameters were

independent of the age of the patients as well as the histological type and localization of tumor.

It is now well established that the proliferative activity of tumor cells plays a key role in neoplastic growth, invasiveness and metastatic formation [41]. Therefore the measurement of this property has been suggested to be effective in judging the malignant potential of various carcinomas. From this point of view PCNA is one of the most suitable markers. However, it is necessary to empasize that PCNA is an immunohistochemical marker of proliferative activity and its determination is possible only in tissue specimens of tumors obtained during surgery.

Our finding of strong correlations between proliferative activity of tumors and aMT6s excretion in urine which were obtained by combination of immunohistochemical and radioimmunological methods establishes a new non-invasive method allowing a determination of the degree of tumor proliferation at different stage of malignant disease in daily clinical practice.

In spite of many studies of the inhibiting effect of MT on tumour growth [for review see 42-44], the mechanism of its role in the regulation of proliferative activity of tumor cells remains unclear. Taking into account the direct connection between the contents of MT in blood and aMT6s in urine, the determination of the latter appears to represent a reliable marker of the degree of MT synthesis in the organism. Therefore it may be possible to assume the following two variants of the participation of MT in tumour development which have a great significance for prognosis in cancer patients.

The first variant. A high urinary excretion of aMT6s could be an evidence of an increase of MT secretion by pinealocytes and extrapineal sources in blood, that in turn leads to a decrease of binding of MT in the tumor. Due to a deficiency of MT in the tumor the proliferative activity of tumour cells is increasing and metastatic potential becomes even stronger.

The second variant. A decrease of urinary aMT6s excretion parallels a reduced secretion of MT from cellular sources into blood; MT binding in the tumor increases under such conditions and via paracrine mechanisms may result in a suppression of tumor cell proliferation. As compared to the first variant the second one is more favourable for the prognosis of the patient. Hence it follows that the maintenance of urinary aMT6s within normal limits or above in cancer patients could be regarded as an unfavourable sign for prognosis which may give evidence for defects within endogenous adaptive mechanisms.

Conclusions

It is necessary to underline, that extrapineal localization of MT in different organs and tissues was studied using the complex of up-to-date methods of the morphological investigation, viz. histochemistry, immunohistochemistry, radioautography, electron microscopy, and computer image analysis. A detailed description of the research methodology and applied reagents were described in special papers [9, 29].

In conclusion, we want to underline that our great wish is to bring the above views forward to stress once more the great significance of Pearse's concept. His theory revealed new perspectives in many different fields of biology and medicine, also for elucidation of the functions of extrapineal MT.

Acknowledgements

I would like to thank my mentor Prof. Nathan Raikhlin (Oncological Research Centre, Moscow) for encouraging me in 1972 to study the extrapineal sources of melatonin, and his permanent interest and patronage of this field of study. I am especially indebted to Drs. Christian and Hella Bartsch (Tübingen University) and my wife and co-worker Dr. T. Kvetnaia (St. Petersburg Institute of Bioregulation and Gerontology) for long-term very fruitful collaboration to study the diagnostic and prognostic significanse of MT in neoplastic disease. I address my great thanks to Prof. Gregory Brown (Clarke Institute of Psychiatry, Toronto) for giving of ultraspecific MT antibody.

I am very grateful to the Royal Microscopical Society (UK) for its high evaluation of my research on extrapineal melatonin and DNES by awarding me the Pearse Prize in 1996.

REFERENCES

1 Pearse AGE. Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C-cells and calcitonin. Proc Roy Soc B 1968; **170**:71-80.

2 Pearse AGE. The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 1969; **17**:303-13.

3 Andrew A. The APUD concept: where has it led us? Brit Med Bull 1982; **38**:221-325.

4 Raikhlin NT, Kvetnoy IM, Osadchuk MA. APUD-system (general pathological and oncoradiological aspects). Obninsk: MRRC Press; 1993.

5 Raikhlin NT, Kvetnoy IM. The APUD system (diffuse endocrine system) in normal and pathological states. Physiol Gen Biol Rev 1994; **8**:1-44.

6 Polak JM, Bloom SR. Immunocytochemistry of the diffuse neuroendocrine system. In: Polak JM, Van Noorden S, editors. Immunocytochemistry: modern methods and applications. Bristol: John Wright & Sons; 1986. p. 328-48.

7 Sundler F, Bottcher G, Ekblad E, Hakanson R. The neuroendocrine system of the gut. Acta Oncol 1989; **283**:303-14.

8 Kvetnoy IM, Sandvik AK, Waldum HL. The diffuse neuroendocrine system and extrapineal melatonin. J Mol Endocrinol 1997; **18**:1-3.

9 Kvetnoy IM, Yuzhakov VV, Raikhlin NT. APUD cells: modern strategy of morpho-functional analysis. Microsc Analysis 1997; **48**:25-7.

10 Larsson LI. On the possible existence of multiple endocrine, paracrine and neurocrine messengers in secretory system. Invest Cell Pathol 1980; **3**:73-85.

11 Reiter RJ. Melatonin: that ubiquitously acting pineal hormone. News Physiol Sci 1991; **6**:223-8.

12 Lerner A, Case J, Takahashi J. Isolation of melatonin, the pineal gland factor that

lightens melanocytes. J Amer Chem Soc 1958; **81**:6084-6.
13 Axelrod J, Weissbach H. Enzymatic O-methylation of N-acetyl-serotonin to melatonin. Science 1960; **131**:1312-8.
14 Banerjee S, Margulis L. Mitotic arrest by melatonin. Exp Cell Res 1973; **78**:314-8.
15 Reiter RJ. The pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1980; **1**:109-31.
16 Maestroni G, Conti A. The melatonin-immune system-opiod network. Adv Pineal Res 1990, **4**:233-41.
17 Reiter RJ, Poeggeler B, Tan DX, Chen LD, Manchester LC, Guerrero JM. Antioxidant capacity of melatonin: a novel action not requiring a receptor. Neuroendocrinol Lett 1993; **15**:103-16.
18 Arendt J. Human responses to light and melatonin. Adv Pineal Res 1994; **8**:439-41.
19 Ozaki Y, Lynch H. Presence of melatonin in plasma and urine of pinealectomised rats. Endocrinology 1976; **99**:641-4.
20 Kennaway DJ, Frith RG, Phillipou G, Matthews CD, Seamark RF. A specific radioimmunoassay for melatonin in biological tissue and fluids and its validation by gas chromatography-mass spectrometry. Endocrinology 1977; **110**:2186-94.
21 Bubenik GA, Brown GM, Grota LJ. Immunohistochemical localization of melatonin in the rat Harderian gland. J Histochem Cytochem 1976; **24**:1173-7.
22 Bubenik GA, Purtill R, Brown GM. Melatonin in the retina and Harderian gland. Ontogeneity, diurnal variations and melatonin treatment. Exp Eye Res 1978; **27**:323-33.
23 Grota LJ, Brown GM. Antibodies to indolealkylamines: serotonin and melatonin. Canad J Biochem 1974; **52**:196-203.
24 Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffine cell system, as 5-hydroxytryptamine. Nature 1952; **169**:800-1.
25 Barter R, Pearse AGE. Mammalian enterochromaffin cells as the source of serotonin (5-hydroxytryptamine). J Pathol Bacteriol 1955; **69**:25-31.
26 Raikhlin NT, Kvetnoy IM, Tolkachev VN. Melatonin may be synthesised in enterochromaffine cells. Nature 1975; **255**:344-5.
27 Raikhlin NT, Kvetnoy IM. Melatonin and enterochromaffine cells. Acta Histochem 1976; **55**:19-25.
28 Raikhlin NT, Kvetnoy IM, Kadagidze ZG, Sokolov AV Immunomorphological studies on synthesis of melatonin in enterochromaffine cells. Acta Histochem Cytochem 1976; **11**:75-7.
29 Kvetnoy IM, Yuzhakov VV, Raikhlin NT. APUD cells: modern strategy of morpho-functional analysis. Microsc Analysis 1997; **48**:25-7.
30 Bubenik GA Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm Res 1980; **6**:313-23.
31 Quay WB, Ma YH. Demonstration of gastrointestinal hydroxyindol-O-methyltransferase. IRCS Med Sci 1976; **4**:563.
32 Huether G. The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. Experientia 1993; **49**:1-6.
33 Verhofstad AAJ, Steinbusch HWM, Joosten HWJ, Penke B, Varga J, Goldstein M. Immunocytochemical localization of noradrenaline, adrenaline and serotonin. In: Polak JM, Van Noorden S, editors. Immunocytochemitry. Practical Applications in Biology and Pathology. Bristol: Wright PSG; 1983. p. 143-68.
34 Kvetnoy IM. Extrapineal melatonin: location and role within diffuse neuroendocrine system. Histochem J 1999; **31**:1-12.
35 Vanecek J, Vollrath L. Localization and characterization of melatonin receptors. Adv Pineal Res 1990; **4**:147-54.
36 Krause DN, Dubocovich ML. Melatonin receptors. Annu Rev Pharmacol Toxicol 1991; **31**:549-68.
37 Kvetnoy IM, Kvetnaia TV, Yuzhakov VV. Role of extrapineal melatonin and related APUD-series peptides in malignancy. In: Bartsch C, Bartsch H, Blask DE, Cardinali D, Hrushesky W, Mecke D, editors. The pineal gland and cancer: neuroimmunoendocrine mechanisms in malignancy. Heidelberg : Springer; 2001. p. 259-74.
38 Kvetnoy IM, Kvetnaia TV, Konopljannikov AG, Tsyb AF, Yuzhakov VV. Melatonin and tumor growth: from experiments to clinical application. In: Kvetnoy IM, Reiter RJ, editors. Melatonin: general biological and oncoradiological aspects. Obninsk: MRRC Press; 1994. p. 17-23.
39 Kvetnoy IM, Yuzhakov VV, Sandvik AK, Waldum HL. Melatonin in mast cells and tumor radiosensitivity. J Pineal Res 1997; **22**:169-70.
40 Bartsch C, Kvetnoy IM, Kvetnaia TV, Bartsch H, Molotkov AO, Franz H, et al. Nocturnal urinary 6-sulphatoxymelatonin and proliferative cell nuclear antigen-immunopositive tumor cells show strong positive correlations in patients with gastrointestinal and lung cancer. J Pineal Res 1997; **23**:90-6.
41 Sagar SM, Klassen GA, Barclay KD, Aldrich JE. Tumour blood flow: measurement and manipulation for therapeutic gain. Cancer Treat Rev 1993; **19**:299-349.
42 Blask DE, Wilson ST, Lemus-Wilson AM. The oncostatic and oncomodulatory role of the pineal gland and melatonin. Adv Pineal Res 1994; **7**:235-42.
43 Karasek M, Pawlikowski M. Pineal gland, melatonin and cancer. Neuroendocrinol Lett 1999; **20**:139-44.
44 Pawlikowski M, Winczyk K, Karasek M. Oncostatic action of melatonin: facts and question marks. Neuroendocrinol Lett 2002; **23**:(suppl 1):24–29.