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Clinical aspects of the melatonin action: impact of development, aging, and puberty, involvement of melatonin in psychiatric disease and importance of neuroimmunoendocrine interactions

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Abstract. During the last decade we have learned much on physiological changes in the secretion of the pineal hormone melatonin (MLT) in man. Reportedly, there is little or no MLT secreted before age 3 months. Then MLT production commences, becmes circadian, and reaches highest nocturnal levels at the age of 1–3 years. During all of childhood nocturnal peak levels drop progressively by 80 % until adult levels are reached. This alteration appears to be the consequence of increasing body size in face of constant MLT production during childhood. The biological significance of this MLT alteration is presently unknown. Because of conceptual considerations, major depressive syndrome (MDS) and seasonal affective disorder (SAD) have been in the focus of pineal research for several years. Although in these disorders alterations in MLT levels could not be substantiated, light therapy, a consequence of this research, was discovered as an effective treatment for SAD and perhaps for MDS. In addition, there is some recent evidence for low MLT levels in schizophrenia. Finally, the potential effect of MLT in neuroimmunoendocrine interactions is presently explored. Reportedly, in vitro studies and animal experiments give evidence for a modulatory role of MLT in the immune response. However, the exact way of this possible action of MLT remains to be clarified. Clinical studies are too scant for a meaningful estimation of MLT's involvement in human neuroimmunoendocrine interactions.

Key words. Melatonin, human-pineal gland; aging; sexual maturation; SAD, major depressive syndrome; immunology.

Introduction

During the last decade much research has been carried out to unravel the action of melatonin (MLT) in man. The following review is intended to provide an overview of the background, questions raised, and results obtained in three clinically relevant topics which have been in the focus of discussion from time to time. Although the physiological significance of melatonin in man remains unknown, we have learned much about age-related changes in its secretion pattern. The first part of the paper addresses this issue. It is followed by a presentation of the huge efforts made to elucidate the possible role of melatonin in psychiatric disorders. The last part of the review is assigned to the newly-evolved field of the possible relation of melatonin to immunology.

Though the work on melatonin's potential influence on the circadian system and on cancer has attracted much attention, it is not included here because of space limitations. The former[7,45,68,109] and the latter[12,21,22] have been reviewed in recent years.

Impact of development, aging and puberty on melatonin levels

Onset of the circadian MLT secretion pattern

Although there are no data available on intrauterine MLT production by the human foetal pineal gland, evidence from animal studies[62,88,148] and histological findings[23,90] do not indicate such activity. However, because of the apparent free transport of MLT between the maternal and foetal compartment[61,151], foetuses are probably exposed to similar circadian MLT variation as their mothers.

Whereas literature on MLT during pregnancy and labor is not conclusive[18,87,98,131], a recent report indicates that in early pregnancy, women's MLT levels are not different from those of nonpregnant controls. However, during the course of pregnancy, an increase in MLT was noticed in day- and nighttime levels, with concentrations being twice as high in the third trimester than in the first[58]. During labor, the circadian MLT rhythm is preserved and reportedly not influenced by the strong physical stress of delivery[59]. MLT levels in the umbilical artery and the umbilical vein of newborns were not different from serum MLT of their mothers[60,64]. In addition, maternal and newborn serum MLT concentrations correlate excellently[94].

Several groups studied single diurnal and/or single nocturnal serum MLT levels in infants[9,44,139]. From these data it appears that diurnal MLT is low and does not change much during the first year of life; similarly, nighttime MLT is low or undetectable up to 2 to 3 months of age. It then increases steadily during the following months. This indicates a lack of circadian MLT rhythm after birth, the onset of the secretion pattern at an approximate age of 3 months, and subsequently a stepwise increase of the MLT amplitude.

This view is corroborated by studies on MLT and 6-hydroxymelatonin (6-OH-MLT) excretion[55,60]. Approximately 70 % of the total amount of MLT secreted by the pineal is metabolized by the liver to 6-OH-MLT; this compound is conjugated to sulfate or glucuronic acid and then excreted in the urine[63]. 1 % or less of the MLT produced goes unchanged into urine[28,106]. The excretion rate of both compounds has been proven to be a good indicator for MLT production and in adults it reportedly reflects blood MLT levels excellently[65,77,86]. However, because of the small percentage of unmetabolized MLT in urine, small alterations in the metabolic clearance for MLT could produce major changes in the MLT excretion; this may occur in pathological conditions or during human development and aging. In addition, specific measurements of MLT in urine may provide more problems than with 6-OH-MLT because the concentrations of urinary MLT are 3 powers of ten lower than those of 6-OH-MLT. Thus urinary 6-OH-MLT excretion is considered as a more reliable indicator of MLT production and serum MLT concentration than MLT excretion is.

Kennaway et al.[55] recently reported very low and arrhythmic 6-OH-MLT excretion in infants up to 9 to 12 weeks of age. Moreoever, the onset of the pineal rhythmicity appears to be related more closely to the date of conception and not to that of birth, indicating that it is a genetically determined event. Thus the time course of the onset of the circadian MLT rhythm corresponds to the development of other circadian variables such as sleep-wake rhythm[46], body temperature[52], cortisol[104] and TSH secretion[84].

In summary, the data presented suggest the following model of the onset of MLT secretion in man: during intrauterine development, foetuses do not produce noteworthy amounts of MLT. However, because of MLT's excellent placenta permeability, maternal MLT crosses the placenta freely and foetuses are exposed to the same MLT environment as their mothers. In late pregnancy, MLT levels may be slightly higher than in early pregnancy and in nonpregnant controls. During delivery, despite stress and medication, the circadian MLT signal is reserved. Shortly after birth all maternal MLT is cleared and fullterm infants endure a virtual lack of MLT for a period of 2–3 months. MLT production then increases and becomes circadian with a steadily rising MLT amplitude. The postpartal period of MLT deficiency is shorter in fullterm than in premature infants, indicating that the onset of MLT production, which occurs approximately 12 months after conception, is the result of a genetically determined maturation process.

Age-associated changes of the amplitude of the circadian MLT rhythm

Whether daytime MLT levels are subject to age-related changes is still a matter of discussion. A progressive decline in daytime serum MLT levles with aging is described by several authors[26,47]. In some additional reports, daytime levels tended to be higher in prepubertal children than in adults but no statistics are available on these data[8,42,96,128]. 6-OH-MLT excretion data also indicate such an alteration[25]. Other laboratories, however, including our own, were not able to observe any alterations in daytime MLT with advancing age[66,138,139]. The major problem with MLT levels during daytime is that they are low, mostly below the limit of detection of current assay systems. Thus age-dependent MLT alterations, if they exist, may be difficult to detect.

In a large cross-sectional study on single diurnal and nocturnal serum samples in endocrinally normal subjects (n = 367)[138,139], we observed the highest night-time MLT levels in very young children, aged 1–3 years. Mean MLT levels dropped progressively by 80% throughout childhood until adolescence. The decline progressed rather steadily with no sign of sudden irruptions, thus providing no sound evidence for a relationship between MLT and certain events of childhood (fig. 1). Although these data were originally challenged[34,39,100,123] several recent reports support our observations[8,29,42]. Among them is an extensive study on the circadian MLT secretion pattern in 62 children by Anita Cavallo[29]. This experiment showed a clear age effect on nocturnal MLT peaks but no further alterations of the circadian MLT secretion pattern during childhood and adolescence.

At seeming odds with these data are reports which describe a lack of any age-related alteration in the total amount of excreted 6-OH-MLT per time unit

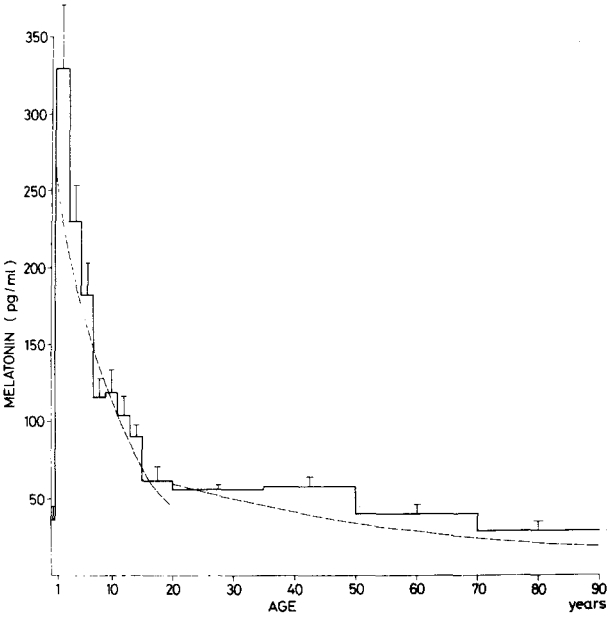

Figure 1. Average (±SEM) MLT concentrations in nocturnal serum samples of 367 endocrine normal subjects aged 3 days to 90 years; subjects are grouped according to age; the dotted line represents regression line[136].

in children[99,125]. However, if the excretion data are related to the childrens' body-size, age-dependency is evident[25,125,149].

In the above-mentioned cross-sectional study[138,139], night-time serum MLT concentrations also dropped significantly during adulthood (age-group 20–35 years vs. age-group 70–90 years); however, the difference in mean values accounted only for about 10% of the maximal levels measured in very young children. The major part of this additional decrease occured during senium (fig. 1). This may explain why some authors, who examined adults of a narrow age range[5,32], were unable to detect age-dependency in MLT concentrations, while others, who compared young subjects with elderly persons[47,96,128], did find lower MLT levels in the latter group.

Thus, the age-dependent decrease of nocturnal serum MLT after infancy consists of a steep fall from early childhood to adolescence and a moderate decline in old age.

The MLT decline during childhood can be explained by alterations in body size during development. The human body size increases by 500–800% from early childhood to adolescence but data on pineal size[108], pineal HIOMT content[147], and melatonin production[99,125,149] indicate only small alterations after infancy. Thus the MLT decline during childhood appears to be the result of a rather constant rate of hormone production against the backdrop of an increased volume of distribution of the hormone during development (fig. 2). This concept is also supported by a number of animal models (for review, cf. ref. 137).

The additional small decrease in MLT in elderly subjects may result from degeneration of the pineal body in old age, a feature frequently encountered with other endocrine glands. However, several other possible causes for the age-dependent alterations of serum MLT have been proposed, including reduction in the population or metabolism of pinealocytes, a reduction of the activity of norepinephrine-containing neurons in the pineal, reduced response of β-receptors in pinealocytes, and alterations in the clearance rate of MLT etc.[97,137].

Changing MLT levels and human sexual maturation

The age-related changes in human serum MLT concentrations certainly rise the question as to their physiological significance. For nearly a century the pineal has been implicated in human sexual maturation[57,85,117,129]. Primate and human sexual maturation is characterized by a long period of developmental arrest lasting from late infancy until the onset of puberty. During the first months of life, gonadotropin and sex steroid levels are high and gonads are active. By the end of the first year, these hormones drop to prepubertal levels and remain there until the onset of puberty. There is compelling evidence for an active, steroid-independent mechanism that lowers gonadotropins and sex steroids during the period of gonadal quiescence[101].

In agreement with the above-outlined longstanding hypothesis involving the pineal in human sexual maturation, we speculated as to whether the high MLT levels in children might be involved in the steroid-independent suppression of gonadotropins in prepubertal primates[138]. This view is supported by circumstantial evidence, i.e. the negative correlations of nocturnal serum MLT and gonadotropins in children and in young adults[138], by decreased nocturnal blood MLT levels in precocious puberty (fig. 3)[16,133], and by reports on

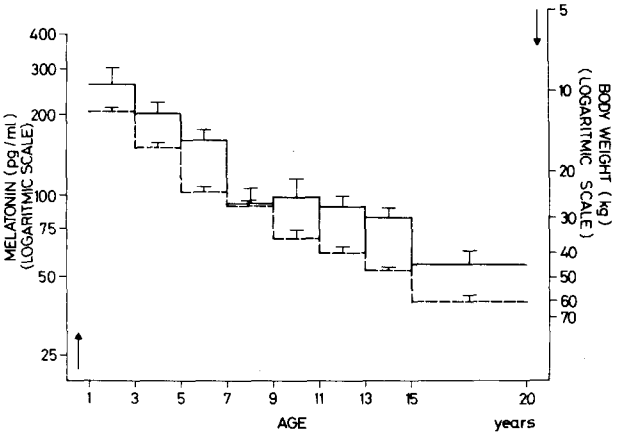

Figure 2. Nocturnal serum MLT levels (——, $\bar{x} \pm$ SEM) and body weight (– – –, $\bar{x} \pm$ SEM) of 208 children and adolescents (1–20 yers) grouped according to age[139].

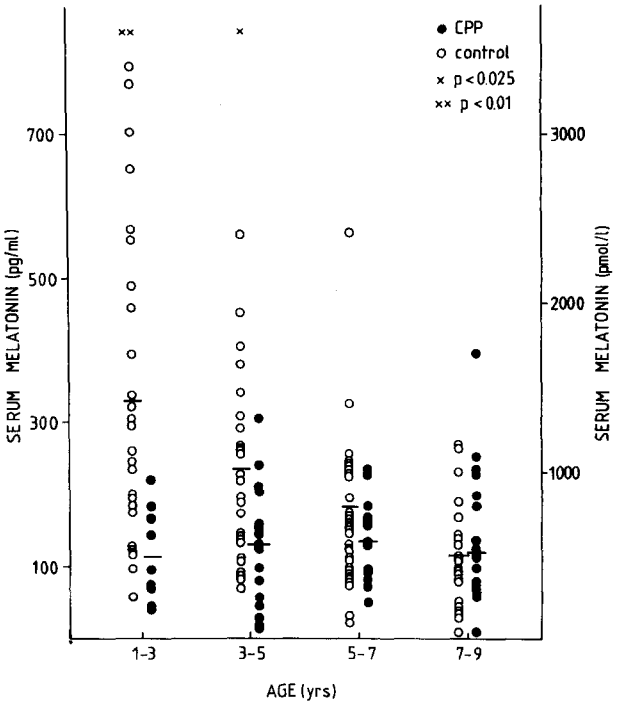

Figure 3. Individual nocturnal serum MLT levels in children with central precocious puberty (CPP) and age-matched controls; the bar represents the median of each group[133].

increased blood MLT concentrations in delayed puberty[6,33,105]. However, Plant et al.[102] were unable to find an increase in gonadotropins in prepubertal primates after pinealectomy (for review on MLT and human sexual maturation cf. ref. 132, 135). Thus, the biological significance of the steep decline of serum MLT during childhood is still obscure. The high serum MLT concentrations during childhood and the observation that some adults produce very little MLT at all[134] may be evidence for a distinct function of the pineal in children and may render research on children particularly fruitful in attempts to elucidate MLT's function in man.

Involvement of melatonin in psychiatric disease

Major depression

Major depressive syndrome is defined in the Diagnostic and Statistical Manual of Mental Disorders-III (DSM-III) as depressed mood or loss of interest, accompanied by several associated symptoms, such as weight loss or weight gain, fatigue and the almost daily occurrance of insomnia of hypersomnia[2]. In major depression a noradrenergic deficiency is postulated[115]. This hypothesis, in fact, is the link to much of the research on MLT levels and MLT secretion patterns in this mental state. MLT is secreted at night, primarily controlled by noradrenergic fibers. In accordance with the hypothesis of noradrenergic deficiency, the expectation of low MLT levels in major depression is reasonable. Early studies supported this expectation[13,32,89,143,144]. Wetterberg and coworkers reported reduced nocturnal MLT peak levels in acutely ill, depressed patients with an abnormal dexamethasone suppression test (DST)[14,144,145]. Furthermore, they observed a significantly higher hereditary factor for major depression among probands with low nocturnal MLT. Consequently, they developed the idea that low serum MLT might be a genetic trait marker for the susceptibility to depression[145]. Since the ratio of blood cortisol and blood MLT concentrations (C/M) of the depressed patients with abnormal DST at 02.00 h differed significantly from both patients with normal DST and controls, this C/M ratio was proposed to increase the diagnostic power for depression[145]. However, in all these studies patients and controls were neither individually matched nor free of drugs for a prolonged period. Both, personal factors[3,134] and antidepressant drugs[31,95] turned out to influence MLT levels.

Thompson et al.[127] performed the first study with 11 drug-free, depressed patients, individually matched to normal controls for parameters known to possibly influence MLT level, i.e. age, sex, menstrual status, season, weight and height[3,134]. Samples were taken on the hour for a whole day. The major result of this experiment is the absence of a difference in MLT levels between the groups. In a recent, well-designed comparison of 38 depressed patients and normal subjects, Rubin et al.[112] arrived at similar results, although patients tended to show higher MLT levels during daytime than controls. In addition, female patients displayed higher nocturnal serum MLT concentrations than their matched controls. In 1977, the excretion of MLT was measured by Jimmerson et al.[51] in 6 depressed patients, and no abnormalities were detected. By measuring 6-OH-MLT Watermann et al.[140] confirmed these findings in 31 prepubertal children with major depressive disorder and in controls. In addition, there was no difference in the excretion of 6-OH-MLT before and after recovery. Some authors have tried to find a relationship between MLT secretion patterns and patient characteristics, diagnosis and symptom patterns. Brown et al.[27] found significantly lower 23.00 h MLT in depressed female patients with melancholia than in depressed female patients without melancholia. In the largest report to date, by Rubin et al.[112], there were no consistent relationships between any of the MLT measurements and the patients' outpatient/inpatient status, presence or absence of DSM-III melancholia, Newcastle score, total socre in the Hamilton Depression Rating Scale or total score in the Beck Depression Inventory Scale. Nocturnal MLT peak and average measures correlated only modestly ($r = 0.28$ to 0.37) with the presence of psychotic features. Furthermore, in the study mentioned above on 6-OH-MLT excretion in 31 prepubertal children with major depressive symptoms[140], no difference was found among patients when they were grouped according to presence/absence of melancholia, endogenous subtypes, family history, or mono/bipolar depression.

There have been a few case studies on bipolar patients which suggest higher MLT levels during mania[56,69,146]. Recently, Kennedy et al. reported of a 29-year-old female with bipolar depression. MLT concentrations were determined hourly between 20.00 and 06.00 h during euthymia, depression and mania. Before each of the three examinations, the patient was free of drugs for at least 2 weeks. Although there was no MLT elevation during depression, a two-fold increase of MLT occurred during mania, compared with euthymia or depression[56]. However, more extensive studies are required before MLT alterations in mania can be established.

At the moment there are no consistent findings to confirm the low MLT hypothesis in depression. This is in agreement with recent reviews on this subject[3,4]. In addition, it is known that normal subjects secreting no MLT at all can be quite well psychiatrically[126].

Major depressive syndrome has also been associated with alterations in biological rhythms (for review of the circadian system in man cf. ref. 92, 93). Wehr et al.[142] described a phase advance in MDS; reportedly, the patients' circadian rhythms of body temperature and of rapid-eye-movement sleep were advanced with respect to the sleep schedule. When the sleep–wake cycle was

advanced by 6 h, one of the patients experienced a transient remission of MDS and 4 out of 7 patients advanced their time of awakening[142]. Because of MLT's stabile circadian secretion pattern, it can be used as a marker for the setting of the endogeneous clock[113]. In analyzing the 24-h secretion pattern of depressive patients with an abnormal dexamethason-suppression test, Wetterberg et al. reported a tendency towards a phase advance of the MLT peak in these subjects[145]. However, Claustrat et al.[32] and Cavallo et al.[30] could not confirm these findings. Presently, phase advance is not regarded as an important factor in the etiology and therapy of major depression[3].

Seasonal affective disorder

Seasonal affective disorder (SAD) is a form of major depression characterized by seasonally recurring depressive episodes in fall and winter and remissions in spring and summer[2, 126]. According to Research Diagnostic Criteria (RDC), more than half of these patients are bipolar, as they suffer from mania or hypomania in spring or summer[110].

Recent research in seasonal breeders identified the length of the photoperiod, i.e. the number of light hours in a day, as the crucial factor for regulating seasonal events. Light acts via suppression of the endogenous MLT secretion, i.e. a long photoperiod results in short duration of the nocturnal MLT elevation and a short photoperiod in the reverse. Thus, in photosensitive animals, the seasonal alteration in the photoperiod is mirrored in seasonal changes in the MLT secretion patterns[48, 124]. Although somewhat less sensitive, humans also react with MLT suppression to bright full spectrum light[70]. Based on these observations, Lewy et al. published a single case study. A patient with SAD showed remission of depressive symptoms after prolonged treatment with bright light for 6 h daily[67]. Subsequently, it was hypothesized the MLT would be involved in some way with SAD. An abnormal hormone secretion pattern was suggested and the therapeutic effect of phototherapy was attributed to suppression or modification of MLT secretion[111]. However, the studies by Wehr et al.[141] evidenced that suppression of MLT is not necessary for the antidepressant action of light. In seven SAD patients, treatment with 2500 lux of full spectrum bright light resulted in the same antidepressant effect, regardless of whether light was given in form of a long skeleton photoperiod (07.30 to 10.30 h and 20.00 to 23.00 h), imitating the summer type of light exposure or in form of a short skeleton photoperiod (09.00 to 12.00 h and 14.00 to 17.00 h), imitating the winter type of light exposure. However, only long skeleton photoperiod decreased MLT levels, as indicated by a reduced 24-h urine 6-OH-MLT excretion.

Similar to major depression, SAD was examined for disturbances of the circadian hormone secretion patterns. Sack et al.[114] suggested a phase delay of circadian rhythms in such patients, since the onset of the nocturnal MLT rise, defined as the time when plasma MLT concentrations exceeded 10 pg/ml, was delayed in 8 SAD patients. In the same study, morning light exposure was found to reduce depressive symptoms more effectively than evening light did. The superiority of morning therapy was explained primarily by the phase-advancing effect of morning light. In contrast to these results, Isaacs et al.[49] found that augmentation of light exposure during the middle of the day by supplementation with bright light (2500 lux) for 4 h alleviated depressive symptoms significantly more in 11 SAD patients than photoperiod extension (light exposure for 2 h before dawn and 2 h after dusk) did. Interestingly, light treatment at midday neither shifted circadian rhythms nor influenced the total amount of MLT secreted at night.

At present, light therapy is widely accepted as an effective treatment in SAD, but its mode of action appears not to involve alterations in the amount of MLT produced or its secretion pattern (for review cf. ref. 4, 24, 126).

Schizophrenia

Shortly after the development of suitable MLT assays, one of its first applications was in schizophrenia. Ferrier et al.[38] measured serum MLT concentrations in 21 male chronic schizophrenic patients, who had been drug-free for at least one year prior to the study, at 08.00 h and at 24.00 h. Nocturnal MLT levels were significantly lower in patients than in controls. However, since individuals were only matched for age and sex, the difference could be attributed to deviations in b.wt, which reportedly influenced adult MLT concentrations in some studies but not in others[5, 15, 25, 129]. Later, lower midnight MLT levels were also described in schizophrenics by Fanget et al.[35]. Again patients were not individually matched to controls and, in addition, they were being treated with different antipsychotic drugs. These shortcomings were surmounted in a recent well-designed study by Robinson et al.[107], who found a consistently reduced nocturnal MLT rise in chronic schizophrenic patients. This hormone pattern persisted up to two months after onset of neuroleptic drug therapy. From these data, a disturbance in the modulating principle for rhythmic behavior in chronic schizophrenic psychosis was deduced. Montelone et al.[91] confirmed the latter finding in 7 male patients diagnosed as chronic schizophrenics of the paranoid subtype. Patients were individually matched and had been drug-free for at least 3 weeks. The pathophysiological significance of the apparently abnormal MLT secretion pattern in schizophrenics is not clear and awaits elucidation.

Possible importance of MLT in neuroimmunoendocrine interactions

During the last decade, increasing evidence has been found for interactions between the neuro-endocrine and

the immune systems[17,19,119]. Hormones and neurotransmitters are present in the environment of immunocompetent cells, some of which have already been shown to carry receptors for these agents and to react to these mediators with modulation of their function[116,122]. On the other hand, cells of the immune system were found to be able to secrete hormones and hormone-like substances[118,120,121] which might play a role in the feedback regulation of the endocrine loop. It has been proposed that MLT may play a crucial role in this complex network of immune-neuroendocrine signalling.

It is well-known that serotonin, like other biogenic amines, is of importance for inflammatory reactions and some immune responses in experimental systems[10,40,43]. As serotonin is the precursor of MLT, it was considered as possible that MLT could influence immune reactions as well[50,83]. Furthermore, some studies reported circadian rythmicity of various immune functions[1,36,37,54] and one of the propositions was that the pineal gland influences the immune response through its cyclic, circadian release of MLT[83].

Experimental studies in animals

Data obtained from animal studies showed influences of MLT on several immune reactions. The effect of neonatal pinealectomy, compared to pinealectomy at the age of 6 weeks and sham-operated controls was tested in rats for Arthus reactivity, delayed hypersensitivity of the Jones-Mote type, antibody production per se, skin graft rejection, lymphocyte histology and the course of experimental encephalomyelitis[50]. Pinealectomy performed in 6-week-old animals caused a transient decrease in Arthus reactivity and in delayed-type hypersensitivity, both tested with bovine serum albumine as the antigenic stimulus. Furthermore, rats pinealectomized at the age of 6 weeks showed less expression of experimentally induced allergic encephalomyelitis than did the group of animals with neonatal pinealectomy or the sham-operated controls. At that time, discussion about the modes of action focused on a possible role of serotonin, the MLT precursor.

Later work described experimental settings where administration of propanolol in the evening, which is thought to suppress the beta-adrenergic stimulation of MLT production, or injections of p-chlorophenylalanin (PCPA), which inhibits serotonin production, resulted in a depression of the primary antibody response of mice to sheep red blood cells[79]. Spleen cells from these animals showed reduced reactivity in the autologous mixed lymphocyte reaction[79], which is used as a marker of the endogenous immune regulation. These effects were shown to be reversible by the subcutaneous administration of MLT in the evenings. The doses used in these studies were 10 mg/kg b.wt and must therefore have produced extremely high pharmacologic plasma concentrations. The levels of circadian MLT changes under propanolol or PCPA administration and MLT reconstitution were not described.

However, at the same concentrations, MLT was shown to antagonize the effect of corticosterone on the reduction of spleen cellularity and therefore on the number of antibody-producing cells per spleen. As no direct effect of MLT on mouse spleen cells was found in vitro after stimulation with either antigens or mitogens, it was proposed that the effect must be an indirect one, involving different systems. Further publications revealed that the effect of MLT on antibody production was dose-dependent and already significant at 10 µg/kg[80]. This effect of MLT was inhibited by the application of naltrexone, a specific opioid antagonist, used at doses from 0.5 mg/kg to 10 mg/kg b.wt, and therefore opioid peptides were considered to be possible mediators for these immuno-augmenting effects of MLT[80]. However, opioid peptides were shown to have different and even contradictory effects on the immune system[116]. Some more details about possible melatonin-opioid-immune interactions were described later[78].

Further reports showed that MLT diminishes some effects of stress on the immune system[81]. Mice were stressed by restraint and the application of MLT at doses of 20 µg/kg antagonized the stress-induced reduction of thymus weight and the stress-induced reduction of antibody production in spleen cells. Again, these effects of MLT were shown to be abolished by naltrexone at 1 mg/kg. Moreover, MLT reduced the mortality of stressed mice injected with sublethal doses of encephalomyocarditis virus, an extremely pathogenic virus in rodents[81]. As in vivo studies implicate many unknown factors, in vitro experiments were designed to elucidate some of the questions of these complex systems.

In vitro studies

Spleen cells from antigen-primed mice were incubated with MLT at doses from 0.1 to 50 nM. Supernatants derived from these cell cultures were tested for their capacity to antagonize the effects of stress, when reinjected into animals of the same strain[82]. It is reported that these supernatants were able to reconstitute the diminished thymus weight and the reduced number of antibody-producing cells in stressed animals. And again these effects could be abolished by naltrexone, which implies that the supernatants derived from the spleen cell cultures contained opioid agonists after incubation with MLT. MLT was effective at doses from 0.2 to 5 nM, thus physiologic doses were used for the first time. In the same study it was shown that activated human mononuclear blood cells also produced factors which could bind to mouse brain membranes in a way competitive to naloxone[82] after incubation with MLT in a cell culture, although the production of these putative opioids could not be demonstrated by the cells from all tested donors.

A recent publication described a direct effect of MLT on human lymphocytes from peripheral blood. It was shown that MLT affects cyclic AMP production in human lymphocytes in vitro[75]. MLT by itself did not have an effect on cyclic nucleotide production, yet it potentiated the effect of vasoactive intestinal peptide (VIP) in a significant way. VIP had been shown earlier to influence several functions of immunocompetent cells[41,122]. MLT and VIP were used at physiological doses and therefore one can speculate that MLT might play a role in modulating immune functions by affecting signal transduction pathways in immune cells. However, the manner of this action remains to be clarified. MLT binding sites have not yet been detected on human lymphocytes, nevertheless some reports inform of specific MLT binding sites in spleen membrane preparations of several animals[103,150].

Clinical trials in man

Clinical investigations concerning a possible importance of MLT in immune functions were all conducted in cancer patients. Many authors reported influences of the pineal gland or MLT on neoplastic diseases[11,12,20,22], whereas only a few reports focused on the theory of such influences via immunological mechanisms.

MLT serum levels were measured in cancer patients and controls in the morning and were correlated with measurements of lymphocyte subpopulations[72]. MLT serum levels were high in 10 patients and within the normal range in 17 others. The percentages of B lymphocytes, total T lymphocytes (CD3+), T helper/inducer (CD4+)- and T suppressor/cytotoxic (CD8+)-subtypes were evaluated and the CD4/CD8 ratio determined. No significant differences would be found between patients with high and low MLT levels. The application of MLT 20 mg/day intramuscularly for 2 months did not change the percentages of lymphocyte subpopulations measured significantly. However, to reveal functional changes, more detailed studies would be necessary to access information about the longitudinal course of activation states of immune cells.

During another clinical trial, cancer patients who had not responded to conventional therapies were treated by application of MLT 20 mg daily intramuscularly for 2 months, followed by a period where MLT was given orally in a dose of 10 mg/day[71]. Follow-up of neoplastic lesions were performed by radiological examination every 2 months and hormonal and immune status were evaluated by measuring serum levels of MLT, growth hormone, somatomedin-C and beta-endorphin and analyzing lymphocyte subpopulations. As no control groups were included in the study, the results cannot be clearly interpreted as specific effects of MLT treatment.

In a recent study, interleukin-2 and MLT were given to patients with untreatable non-small cell lung cancer[73]. The percentages of total lymphocytes, T-lymphocytes, CD25-positive cells (positive for receptor for interleukin-2), and eosinophils were evaluated. They increased significantly during the course of treatment. Mean serum levels of neopterin as a marker of macrophage activation, and of tumor necrosis factor and of soluble interleukin-2 receptor as a marker of T-lymphocyte activation, were significantly higher after therapy. These results are compatible with the effects of interleukin-2 administration[53,76]. In this study, no results of control groups are reported, therefore is is questionable to postulate a possible effect of MLT. Nevertheless, another publication reports of the effect of MLT on mean serum levels of neopterin as a marker of macrophage activation, during interleukin 2-immunotherapy of cancer[74]. Two groups of cancer patients received IL-2 subcutaneously twice a day. One of the groups had additional MLT application given orally once a day at a dose of 10 mg. It was shown that the increase of neopterin by IL-2-administration was significantly reduced in the MLT treated group. This interesting finding suggests a modulation of IL-2-induced macrophage activation by MLT.

To conclude the findings about the influence of MLT on immune reactions it can be said at least in some conditions, that this hormone seems to modulate the immune response. The exact mode of action remains to be clarified. However, evidence was found for an involvement of the opiatergic system. Specific receptors for MLT have not yet been found on cells of the immune system. A role of MLT in physiologic or pathologic immune responses in man cannot be definitely estimated. More trials in vivo and in vitro will be needed to elucidate some of the remaining questions.

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