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Melatonin in humans—where we are 40 years after its discovery

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Abstract Although the pineal gland was well known for more than 2000 years and has been documented since Galen (130–200 AD), for many centuries different theories concerning its function were presented. The organ was believed to be a sphincter (ancient Greeks), the point at which the soul preeminently controls the body (Descartes, 1596–1650), a rudimentary organ (up to the 19th century), and a gland having endocrine function. Endocrine function of the pineal was postulated already at the end of 19th century and the beginning of the 20th century with the notion of antigonadotropic pineal influence, and the functional cooperation between the pineal and hypothalamo-hypophysial axis, but no secretory substance responsible for the gland function was known. In 1958 Lerner and coworkers succeeded in isolating from bovine pineal glands the compound termed melatonin because of its blanching effect on melanophores. This discovery constituted the milestone for further pineal research. Since then the knowledge of the structure and function of the pineal gland has tremendously increased, especially during the last two decades. However, it should be stressed that many problems in pineal research still must be solved. In this paper, the recent knowledge on the role of melatonin in humans is briefly presented.

THE STORY BEHIND

Short history of the pineal gland

Although the pineal gland has been well known for more than 2000 years, the history of pineal research is full of alternating periods of stagnation and progress. The organ was first mentioned by Galen (130–200 AD), and its first pictorial representation is derived from Vasalius (*De Humani Corporis Fabrica, Libri Septem*, 1563) [see 1, 2].

There were several theories concerning the pineal function, including those from ancient times that are characterized by much speculation due to the strong influence of prevailing philosophical systems. The most famous is the concept of Rene Decartes (1596–1650) who is commonly cited as stating that the pineal is "the seat of the soul." It should be stated, however, that according to Decartes, in principle, the soul cannot be localized in any precise part of the body but exercises its function more particularly in the pineal, the only unpaired part of the brain [see 1, 2].

Despite some progress in pineal anatomy and physiology during the Renaissance, we entered the 20th century with the pineal as a functionless vestigial organ. Although the work at the beginning of the 20th century, especially that of Marburg, gave the first indication of the relationship between the pineal and reproductive system, we needed an additional 50 years before placing the pineal gland within the endocrine system [see 1, 2]. In the development of modern pineal research, the book 'The Pineal Gland' published by Kitay and Altschule in 1954 on the basis of critical review of some 1,800 references seems to play an important role [3]. The authors concluded that the pineal is primarily concerned with reproduction, with light-pigmentation responses, and possibly with behavior.

However, the real milestone in pineal research was the discovery of melatonin, the substance regarded now as a pineal hormone. Melatonin was discovered in 1958 by Aaron Lerner and colleagues [4] after four years of searching for an amphibian skin lightening factor known to be present in pineal glands. This discovery gave strong impetus to further studies on its importance and role, both in animals and in humans.

Biosynthesis and metabolism of melatonin

Melatonin is produced in mammals mostly in the pineal gland, although several other organs (e.g., retina, gastrointestinal tract, Harderian gland, blood platelets) may produce the hormone as well [5]. Moreover, secretion of melatonin is not restricted to mammalian species but it is also produced in nonmammalian vertebrates, in some invertebrates and in many plants [5, 6].

The synthesis of melatonin is presented in Figure 1. The first step in melatonin formation is uptake of the essential amino acid L-tryptophan from the circulation into the gland. Within the pinealocyte L-tryptophan is catalyzed by tryptrophan-5-hydroxylase (L-tryptophan, tetrahydropteridine: oxygen oxidoreductase, EC 1.14.16.4) to 5-hydroxytryptophan which is then decarboxylated

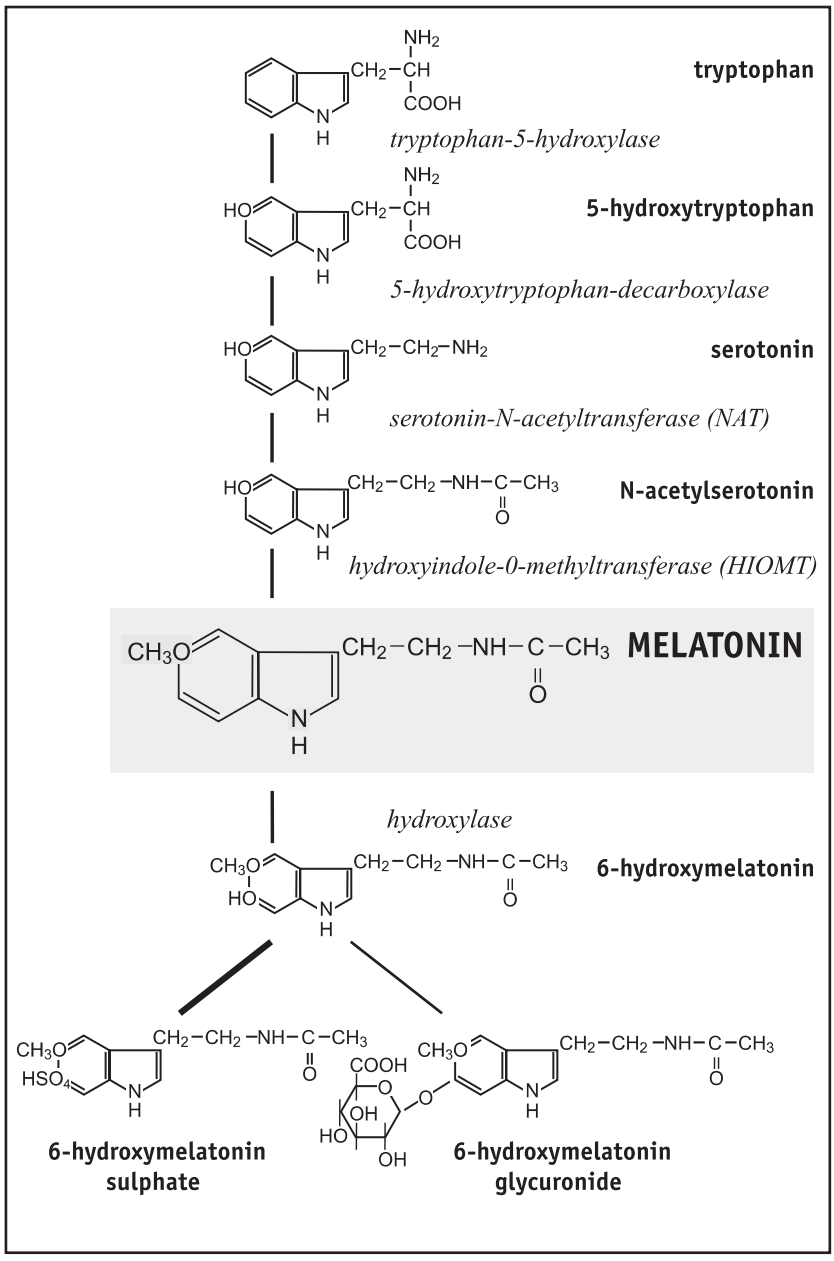

Fig. 1. Biosynthesis and metabolism of melatonin.

to serotonin by L-aromatic amino acid decarboxylase (aromatic L-aminoacid carboxylase, EC 4.1.1.28). The key enzyme in melatonin synthesis, N-acetyltransferase (acetyl CoA:aryl-amine N-acetyltransferase, EC 2.3.1.5), completes the next step, i.e., N-acetylation of serotonin to N-acetylserotonin. The final step in the pathway is the O-methylation of N-acetylserotonin to melatonin by hydroxyindole-O-methyltransferase (S-adenosyl-L-methionin: N-acetyl-serotonin-O-methyl-tranferase, EC 2.1.1.4) [7, 8].

Melatonin is metabolized primarily in the liver and secondarily in the kidney. It undergoes 6-hydroxylation to 6-hydroxymelatonin, followed by sulfate or glucuronide conjugation to 6-hydroxymelatonin sulfate (90%) or 6-hydroxymelatonin glucuronide (10%).

Melatonin forms also some minor metabolites, such as cyclic 2-hydroxymelatonin, N-gamma-acetyl-N-2-formyl-5-methoxykynurenamine and N-gamma-acetyl-5-methoxykynurenamine [8].

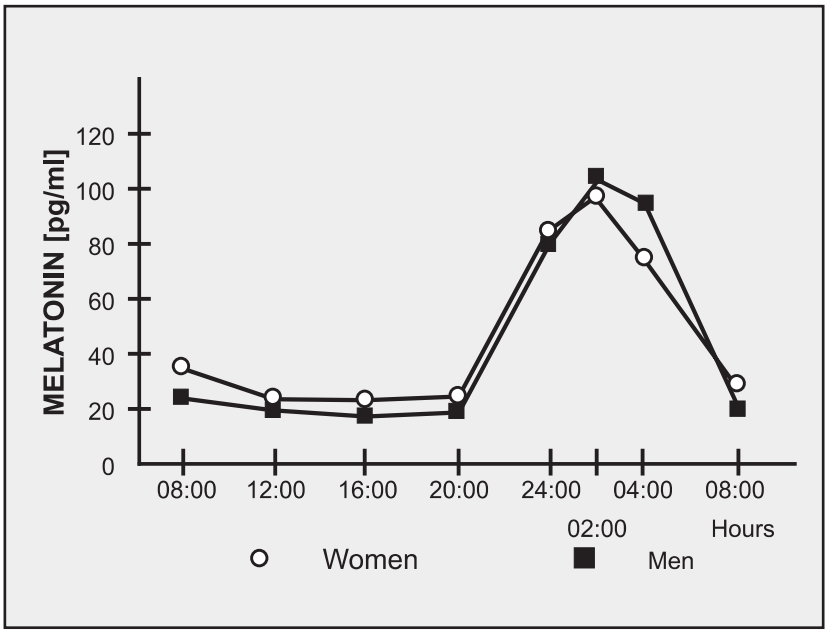

Fig. 2. Diurnal rhythm of melatonin concentrations in 10 healthy women and 10 healthy men.

Diurnal rhythm of melatonin and its regulation

Melatonin exhibits very characteristic diurnal rhythms of synthesis and secretion. During the day serum concentrations of the hormone are low (10-20 pg/ml), significantly increasing at night (80-120 pg/ml) with a peak between 24:00 and 03:00 h (Fig. 2). The onset of secretion is usually around 21:00–22:00 h and the offset at 07:00–09:00 h. Very close relationship to melatonin rhythm shows its major urinary metabolite—6-hydroxymelatonin sulfate [8]. Such diurnal profile of melatonin secretion is characteristic not only for mammals but has been found even in unicellular alga, the dinoflagellate *Gonyaulax polyedra* [9]. In humans the rhythm in melatonin concentrations appears soon after birth, at 6–8 weeks of life, and seems to be well established at 21–24 weeks of life [10].

The production of melatonin is controlled by lighting conditions. Photosensory information arrives at the pineal via the polyneuronal pathway that begins in the retina and involves the retinohypothalamic tract, suprachiasmatic nuclei, paraventricular nuclei, medial forebrain bundle, reticular formation, intermediolateral cell column of the spinal cord, superior cervical ganglia, internal carotid nerve, and nervii conarii (Fig. 3). The stimulus which signals the nighttime rise in melatonin seems to originate in the suprachiasmatic nuclei [8, 11].

Postganglionic sympathetic nerve fibers that end in the pineal release noradrenalin, which plays a crucial role in the control of melatonin synthesis. Noradrenalin binds to pinealocyte α-adrenergic receptors (and partially α-adrenergic receptors), activating adenylate cyclase through GTP-binding protein in the cell membrane, and increases cAMP levels leading to stimulation of the activity of N-acetyltransferase, and subsequently to synthesis of melatonin. Stimulation of α-adrenergic receptors potentiates the β-stimulation, and in this mechanism participate calcium ions, phosphatidylinositol, diacylglicerol, and protein kinase C [12].

The peak in melatonin production in the pineal gland occurs always during the daily dark period, irrespective of whether mammals are nocturnally or diurnally active. However, melatonin synthesis is rapidly suppressed in the dark phase by acute exposure to light of sufficient intensity, although the response of the pineal gland to different light irradiances varies greatly among species [8, 13]. For example, light irradiance of as little as 0.0005 $\mu W/cm^2$ suppressed melatonin nocturnal synthesis in the laboratory-raised rat [14], but as much as 1850 $\mu W/cm^2$ was needed to suppress melatonin nocturnal synthesis in the wild-captured Richardson's ground squirrel [15]. It has been suggested in early experiments that domestic intensity light did not suppress melatonin nocturnal secretion in humans. However, it has been demonstrated lately that if sufficient intensity and duration of light is used at night (725 $\mu W/cm^2$ for 2 hours) also human melatonin secretion could be suppressed [16]. Moreover, substantial indi-

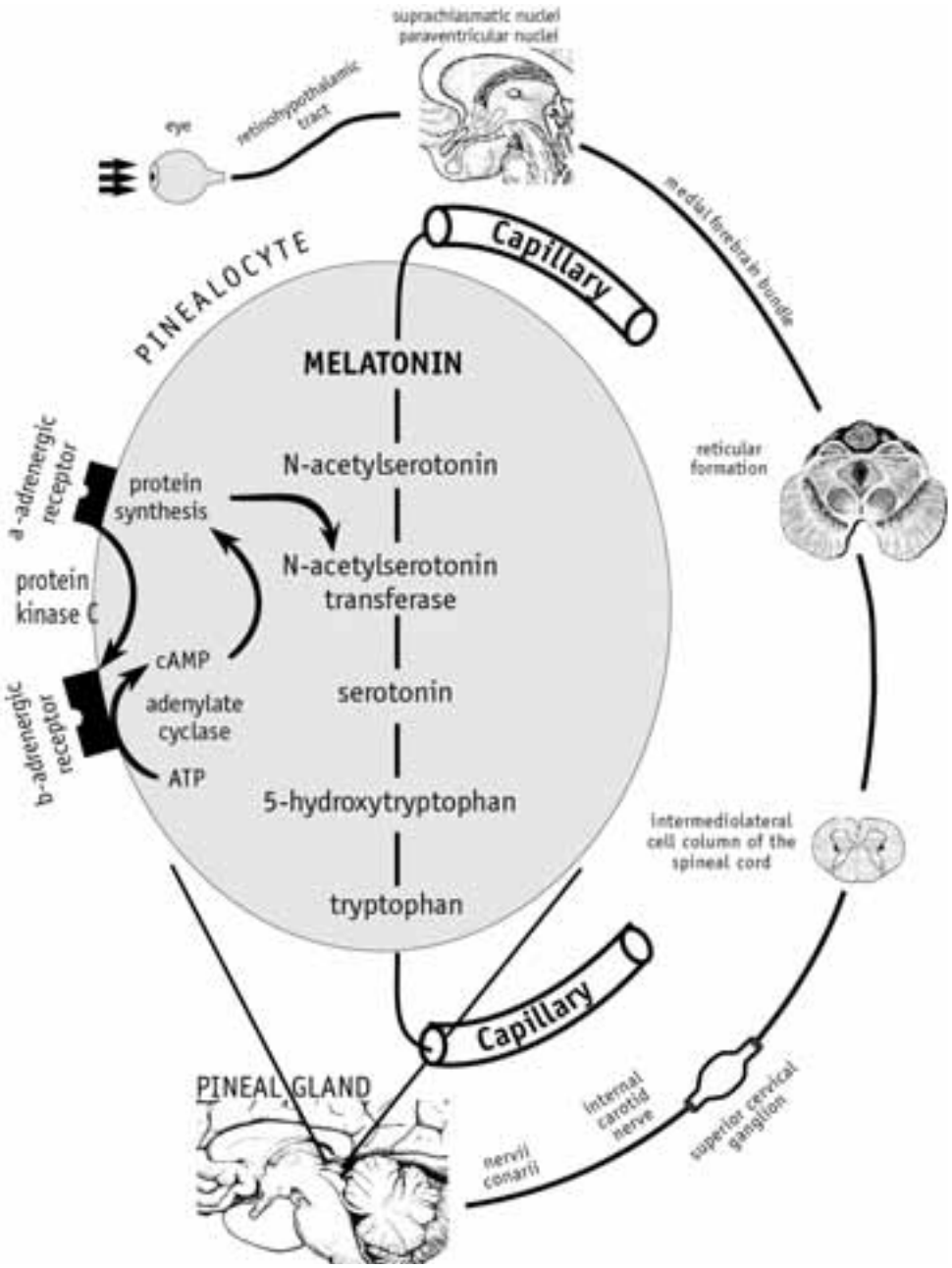

Fig. 3. Diagram of the neural connections between the retina and the pineal gland; and noradrenergic contron of melatonin secretion.

vidual variations in human sensitivity to light that may be both genetically and environmentally determined exist [8]. Brainard et al. [17] demonstrated that as little as 1.6-5 μW/cm^2 of monochromatic green light at 509 nm and 29 μW/cm^2 of broadband white light can significantly suppress melatonin secretion in normal volunteers. In order to explain why in the earlier studies much higher light intensity was required, the authors postulate that it is necessary to examine ocular mechanism, which mediates this photic effect. The following factors may determine whether a specific light stimulus can regulate melatonin secretion in humans: (i) size, brightness and spectral quality of the light source, (ii) gaze behavior relative to the light source, (iii) age of the ocular lens, (iv) pupillary dilatation, (v) the sensitivity of the operative photopigments and photoreceptors, (vi) photoreceptor in the retina and (vii) the ability of the circadian system to integrate photic stimuli spatially and temporally [17].

Significance of melatonin in humans

For many years studies on the pineal gland and melatonin were restricted mainly to the experiments with animals and employed mostly biochemical and histological parameters. Little information was available on the significance of melatonin in humans, mostly due to lack of sensitive and specific assay systems for this hormone. Establishment of specific radioimmunoassays for melatonin in the late 1970s attracted many researchers, including clinicians, and caused remarkable development in research on the role of this hormone in humans, although many functions of the pineal gland and melatonin still remain to be confirmed. Having ignored the potential significance of melatonin for a long time, there are now many indications that it may be of some importance in human physiology and may be of some therapeutical significance.

Melatonin in human physiology and pathology

Melatonin levels throughout the life span

As mentioned earlier melatonin exhibits typical patterns of diurnal secretion with low levels during the daytime and high levels at night. In newborns very little melatonin is detectable, and the diurnal rhythm of secretion is fully established at the 6th month of life. Nocturnal melatonin concentrations rapidly increase thereafter reaching a lifetime peak between the 4th and 8th years, and then decreasing, most significantly, around

puberty. Values remain relatively stable until 35–40 years, and subsequently decline reaching around 70's levels similar to daytime concentrations [8, 18]. There are, however, large individual variations in melatonin concentration [19].

Melatonin and the reproductive system

The relationship between the pineal and reproductive system is well established in animals, but in humans it is more difficult to demonstrate. However, some studies suggest that melatonin may play a role in the physiological development of normal puberty [20, 21]. Precocious puberty or delayed puberty is often associated with abnormal melatonin levels [22, 23]. High concentrations of melatonin have been reported in men with hypogonadism [24] and infertility [25] and in women with hypothalamic amenorrhea [26, 27] and anorexia nervosa [28].

Melatonin and pituitary hormones

The data on the relationship between melatonin and pituitary hormones are inconsistent. The diurnal concentrations of melatonin positively correlate with those of prolactin [29, 30]. Moreover, nocturnal increase and morning decrease in prolactin levels are proceeded by similar changes in melatonin levels [31], and melatonin administration stimulates prolactin secretion [30, 32]. Although these data suggest participation of melatonin in the control of prolactin secretion, it does not seem probable that melatonin plays an important role in this mechanism.

Results on the relationship between melatonin and growth hormones are controversial. Stimulation of growth hormones (due to insuline-induced hypoglycemia, arginine infusion, clonidine administration or growth hormone releasing hormone stimulation) was found to be associated with inhibition of circulating melatonin in children [21]. Administration of melatonin caused either enhancement of growth hormone secretion [33] or did not exert any effect in adults [32]. Although age and puberty related variations in growth hormone response to melatonin has been reported [34], the role of melatonin in mechanisms of regulation of growth hormone secretion seems to be secondary and not important.

No sufficient data are available on the relationship between melatonin and secretion of other pituitary hormones. Administration of melatonin did not change levels of LH, FSH and TSH [32, 34].

Melatonin and endocrine disorders

No relationship between melatonin and hypothalamic-pituitary-adrenal axis seems to exist since in patients with low ACTH levels diurnal melatonin rhythm was not abolished, and in patients with abnormal melatonin profiles ACTH and cortisol levels were not changed [35]. Moreover, circadian rhythm of melatonin in children with congenital adrenal hyperplasia did not differ from that in healthy children of the same age [32]. Normal nocturnal levels of melatonin have been found in patients with acromegaly, prolactinomas or empty sella syndrome [36]. However, in Cushing's syndrome decreased melatonin concentrations were observed [37].

There are experimental data suggesting a relationship between the pineal and hypothalamo-hypophysial-thyroid axis in animals [38]. However, no sufficient data are available on the existence of such a relationship in humans. In patients with hypothyroidism and hyperthyroidism, the circadian rhythm of melatonin was not altered [39].

Melatonin and the immune system and neoplastic disease

Most of the available information on the stimulatory effect of melatonin on the immune system concerns animal work [40]. However, there are also data suggesting that also in humans melatonin may have immunoenhancing activity. The peak time of some immune system constituents (e.g. lymphocytes, NK cells) closely corresponds to that of melatonin [8]. Melatonin increased *in vitro* the ability of monocytes to destroy skin cancer cells by about 70% [41]. One month of melatonin administration in patients suffering from advanced solid tumors with melatonin resulted in increases of inteleukin-2 (51%), interferon-ã (41%), and tumor necrosis factor-á (28%) [42]. The existence of specific binding sites for melatonin in human T-lymphocytes suggests, among others, the possibility of direct mechanism of the regulation of the immune function by melatonin [43].

The relationship between the pineal gland and neoplastic growth is well documented in experimentally induced animal tumors as well as in different types of human cancer cells cultured *in vitro* [44]. Alterations in melatonin concentrations in humans have been demonstrated in several reports. However, the studies produced inconsistent patterns of melatonin profiles in human malignancy. It should be also stressed that for a long time there was no strict uniform paradigm for such studies, and in many reports melatonin was assayed at one time point only, in the morning. In studies in which detailed diurnal profiles of melatonin were measured, depressed nocturnal concentrations of this hormone have been demonstrated in patients with primary breast cancer [45, 46], prostate cancer [47], colorectal carcinoma [48], and adenocarcinoma of uterine corpus [49]. Moreover, stage-dependency in melatonin depression was found in breast cancer [46].

Melatonin and sleep disorders

The number of reports on melatonin concentrations in sleep disorders is surprisingly low considering its use in therapy of insomnia. In major sleep disorders such as narcolepsy, delayed sleep phase syndrome, and Klein Levine syndrome only a small delay in the melatonin rhythm was observed [8]. However, nocturnal melatonin concentrations were significantly lower in patients suffering from chronic primary insomnia [50]. Moreover, a correlation has been found between disturbances of rhythm of 6-sulphatoxymelatonin excretion and poor sleep quality in elderly subjects. Urinary 6-sulphatoxymelatonin concentrations have been found to be significantly lower in elderly insomniacs than in age-matched controls, and their onset and peak times have been delayed [51]. Based on findings that the timing of the sleep gate was correlated with the onset of nocturnal melatonin secretion [52], Lavie et al. [53] suggest that from the accumulated data it is evident that melatonin characteristics are not those of a typical hypnotic or sedative. Melatonin affects sleep in a -much more subtle way. The authors propose that the role of melatonin in the induction of sleep does not involve the active induction of sleep, but rather is mediated by an inhibition of a wakefulness-producing mechanism.

Melatonin and psychiatric and neurological disorders

Abnormalities in melatonin secretion have been observed in some psychiatric disorders. Decline in the amplitude of the melatonin rhythm has been found in depression both in adults [54] and in children [55]. In a 29 year-old patient suffering from bipolar mood disorder, much higher melatonin nocturnal levels have been found when she was manic than when she was depressed [56]. Unusually low melatonin levels have also patients with schizophrenia [57, 58], and completely abolished in paranoid schizophrenics [59]. Lower melatonin concentrations have been also found in the obsessive-compulsive disorder [59]. Depressed melatonin concentrations have been observed in alcoholics, even after long abstinence, suggesting that chronic use of alcohol might permanently alter the pineal ability to produce melatonin [60]. Although it was suggested that melatonin may play a role in seasonal affective disease, there is only evidence for delayed melatonin rhythm in winter in patients suffering from this disease [61]. Although light appears to be efficient in treatment of seasonal affective disease, it does not seem to work through melatonin [8].

Melatonin levels were reduced in cluster headaches [62], whereas the circadian rhythm of the hormone was not altered in epileptic patients [63].

In old demented patients (senile dementia Alzheimer's type or multinfarct dementia) the circadian profile of melatonin was clearly flattened, when compared to mentally healthy people of the same age [64]. Disappearance of daily variations in melatonin concentrations and lower levels of the hormone were found in the pineal glands of patients who died of Alzheimer's disease, in comparison with a non-Alzheimer control group. However, when more accurately age-matched controls were compared with the Alzheimer's subjects, no differences between the two groups were observed [65]. It has been recently reported that although in many cases of Alzheimer's disease a lack of nocturnal elevation of melatonin levels has been observed, as many as 68% out of 18 Alzheimer's patients demonstrated existence of circadian rhythm, similar to healthy age-matched subjects [66].

Melatonin as a free radical scavenger

It has been discovered, recently, that melatonin is involved in the antioxidative defense system of the organism, designed to protect molecules from damage by toxic oxygen radicals [5, 18, 67]. Melatonin is both lipophilic and hydrophilic and therefore easily passes all morphophysiological barriers; it enters all cells and may carry out its antioxidant function with equal efficiency in multiple cellular compartments, i.e. in the nucleus, cytosol and membranes [67]. The question is still open whether melatonin is an efficient free radical scavenger also in physiological concentration or whether the observations made to date are of pharmacological importance only. However, it should be stressed that compared to two well-known scavengers, glutatione and mannitol, melatonin is 4x and 14x more effective, respectively [68]. Free radical scavenging ability of melatonin has implications for a variety of diseases, including age-associated neurodegenerative diseases and cancer initiation.

Melatonin and the magnetic field

In numerous animal studies the magnetic field has been shown to alter melatonin secretion [69]. However, the data on its influence in humans are scarce and contradictory [70]. Although it has been shown in some studies that the magnetic field may reduce melatonin levels [70], in others no changes have been found [71, Karasek et al. unpublished data). It is probable that differences may depend on different parameters (strength, frequency, duration, applied vector, etc.) of the applied magnetic field.

Melatonin and other disorders

Melatonin concentrations in blood and cerebrospinal fluid were significantly lower in infants who

died of sudden infant death syndrome than those in infants who died of other cases [72]. Lowered melatonin secretion was reported in patients with coronary heart disease [73] and in hypertension [74]. Melatonin reduced also blood pressure and the pulsatility index of the internal carotid artery in healthy subjects [75].

Melatonin and aging

A role for the pineal gland in aging has been implicated recently [76–79]. It is based on the fact that the pineal hormone melatonin has been shown to influence a wide variety of bodily functions. Melatonin is present in all species, from algae to human, and exhibits a very characteristic circadian rhythm. The levels of melatonin, especially its nocturnal amplitude, significantly decrease with age. A prominent theory of aging attributes the rate of aging to accumulated free-radical damage [80]. Melatonin is known as a very potent free radical scavenger [18, 78]. Loss of melatonin in advanced age may lead to disturbances in the circadian pacemaker, which causes internal temporal dysfunction and may induce a variety of chronopathologies leading to generalized deterioration of health and early death [77]. Although there is no definitive proof that age-related decrease in melatonin levels is directly related to aging, some results implicate that melatonin may have beneficial effects in delaying the aging processes.

Possibility of melatonin's therapeutic significance

It has been proposed that melatonin may be of some therapeutic significance because of the following properties:

- aids sleep,
- relieves jet lag,
- may help prevent cancer:
  - improves quality of life,
  - counteracts the toxic effects of chemotherapy,
  - prolongs survival,
- helps in cardiovascular diseases:
  - lowers blood pressure,
  - reduces cholesterol levels,
- delays aging.

Generally, melatonin has been proven to be useful in circadian rhythm disorders, such as sleep disturbances, jet lag, shift work, and blindness. Of these above-mentioned melatonin actions, the first two have received the most attention, and there are convincing clinical data that melatonin may help in sleep disturbances and in the alleviation of jet lag.

Several studies reported that melatonin treatment may help sleep both in adults [81, 82] and children [83] with insomnia, and it is especially successful in delayed sleep phase insomnia [84, 85] and in sleep disorders in elderly people [86].

Crossing several time zones during transcontinental travel causes many symptoms, including insomnia, lack of concentration, headache, fatigue and irritability. Timed treatment with melatonin significantly alleviates jet lag, and the improvement is greater with the number of time zones, and in an eastward direction compared to westward [8].

Although the data on use of melatonin in shift work and in blind people are rare, it has been suggested that it may improve sleep and increase daytime alertness when administered at the desired bedtime during a night shift and may stabilize sleep onset and sometimes improve quality and duration of sleep in blind people [8].

Other possibilities for therapeutic usefulness of melatonin are not definitively proved.

There are some indications that melatonin may be helpful in therapy of advanced cancer of various types. Although it has not been proved that melatonin may be considered as an oncostatic agent, it has been demonstrated that in patients who failed to respond to standard anticancer therapy or in patients with untreatable solid neoplasms melatonin administration resulted in stabilization of the disease in some cases and caused an improvement in performance status [87, 88]. It has been also reported that melatonin may enhance the efficacy of antitumor properties of cytokines (especially interleukin-2) and/or reduce their toxicity [89]. Moreover, in breast cancer melatonin may amplify the therapeutic efficacy of tamoxifen [90]. Although more clear-cut clinical trials are necessary, available data suggest a possible therapeutic role for melatonin in human malignancy, at least in terms of survival time and the quality of life.

There are some occasional data that melatonin may have some benefits in heart diseases because it has been demonstrated that it may reduce blood pressure [91] and may reduce cholesterol levels [92]. Based on very well known inhibitory action of melatonin on reproduction in animals, a contraceptive pill containing melatonin and norethisterone has been developed [92]. However, it should be stressed that the dose of melatonin in these pills is very high (75 mg).

The most controversial is the possible use of melatonin as an agent delaying aging. This action of melatonin is based on decline in its concentration in aging and its role in protecting from free-radical damage [18, 78]. Although it is estimated that millions of people all over the world are taking melatonin in

order to delay aging, there is a lack of definitive clinical trials. However, some data suggest that melatonin can safely improve some aspects of sleep, memory, and mood in the elderly with mild cognitive impairment, even in short-term use (10 day trials) [93]. There is no definitive proof that age-related decrease in melatonin levels is directly related to aging. Further studies are needed before one can advise taking melatonin in order to prolong life span.

Very recently is has been reported that melatonin may have a beneficial, therapeutic effect in Alz heimer's disease [94, 95].

Despite these beneficial effects of melatonin, the hormone should be administered therapeutically with caution and properly timed. Recent studies have shown that, when administered during the day, melatonin produces significant decrements in some aspects of neurobehavioral performance (two-choice visual reaction and response time, unpredictable talking, and extended two-choice visual reaction and response time), although not as conspicuous as following administration of other drugs that possess sleep-inducing properties, such as benzodiazepines [96]. According to Rogers et al. [96] these decrements in neurobehavioral performance could represent a potential health risk associated with the use of melatonin.

Final comment

Although from the discovery of melatonin remarkable developments in understanding the role of this hormone have been made, its many functions still remain to be confirmed. The same is true as to the therapeutic potential of melatonin and its significance to human health. Although we still know much more about the role of melatonin in animals than in humans, studies of the last decade without any doubt allowed accepting the hormone as an important constituent also in human physiology. The possibility of therapeutic use of melatonin is now widely tested. It seems that its beneficial use has been until now proved in alleviation of jet lag and in some sleep disturbances. Although data on potential use of melatonin in cancer and Alzheimer's disease have brought about interesting results, it seems that more studies are needed before introducing melatonin even as an additional therapy of these diseases.

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