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Review Article

Melatonin, Receptors, Mechanism, and Uses

Omar SH, Nabi Saba[1]

Phytomedicine and Pharmaceutical Biotechnology Research Lab., Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkhla University, Hat Yai, Songkhla- 90112, Thailand. [1]*College of Pharmacy, Qassim University, Buraydah-51452, Saudi Arabia*

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ABSTRACT

Melatonin, a hormone produced primarily by the pineal gland, is also secreted from the gut and eye during darkness. There are three classes of melatonin receptors, MT_1, MT_2, and MT_3, in various regions of the human brain, gut, ovaries, and blood vessels, but most consistently found in the suprachiasmatic nucleus (SCN) of the hypothalamus and the pars tuberalis of the anterior pituitary. Melatonin has endocrine, autocrine, and paracrine actions, which are mostly receptor mediated. Primary clinical uses include the regulation of circadian rhythms and sleep disorders, although it has other endocrine, immunomodulatory, and oncostatic effects. This review summarizes the current state of melatonin research with an emphasis on its receptors, pharmacological effects, and clinical therapeutic uses.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone that is primarily produced by the pineal gland, located behind the third ventricle in the brain.[1] The pineal gland is a small organ shaped like a pine cone (hence its name). The gland varies in size among species; in humans it is roughly 1 cm in length, whereas in dogs it is only about 1 mm long. It consists of two types of cells: pinealocytes, which predominate and produce both indolamines (mostly melatonin) and peptides, and neuroglial cells. In older animals, the pineal gland often contains calcium deposits, "brain sand."

The earliest indication that the pineal gland contained a biologically active substance came from the work showing that bovine pineal extracts applied to frog skin could produce a skin lightening response.[2] Melatonin was first identified in bovine pineal extracts on the basis of its ability to aggregate melanin granules and thereby lightens frog skin.[3]

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Correspondence:
Syed Haris Omar; E-mail: syedharisomar@gmail.com

Occurrence and biosynthesis

Melatonin is present in a number of lower organisms and plants, such as bacteria, algae, fungi, vegetables, fruits, rice, wheat, bananas, beets, cucumbers, tomatoes,[4] and herbal medicines; it is also found in insects, vertebrates,[5] and human milk.[6] In addition to the pineal gland, melatonin is synthesized in the retina,[7] bone marrow,[8] gastrointestinal tract,[9-11] gut[12], and bile,[13] with the gut appearing to produce proportionally more melatonin than the pineal gland.[14] The pineal body is the only endocrine gland directly influenced by the external environment via the retina; in fact, the gland converts environmental signals into neuroendocrine messages.[15-17] The information is transmitted from the retinal photoreceptors to the suprachiasmatic nuclei, then to the paraventricular nuclei and, through the intermediolateral cell column, to the superior cervical ganglia [Figure 1].

Noradrenergic fibers originating from superior cervical ganglia have their terminals in the pineal body.[15,17,18] These fibers stimulate either β- or α-adrenergic receptors of the pinealocyte.[18-20] The activation of these receptors synergistically increases intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP). In fact, α1-adrenoceptor activation has proved to significantly potentiate β-adrenergic stimulation of both cAMP and cGMP. The increase in intracellular cAMP enhances N-acetyltransferase (NAT) activity.[18,20,21] Thus, serotonin, which is produced in two steps (hydroxylation and decarboxylation) from tryptophan within the pinealocyte,[22] is converted to N-acetylserotonin by NAT.[23] N-acetylserotonin is finally converted into the hormone melatonin (5-methoxy-*N*-acetyltryptamine) by the pineal-specific enzyme hydroxyindole-*o*-methyltransferase (HIOMT).

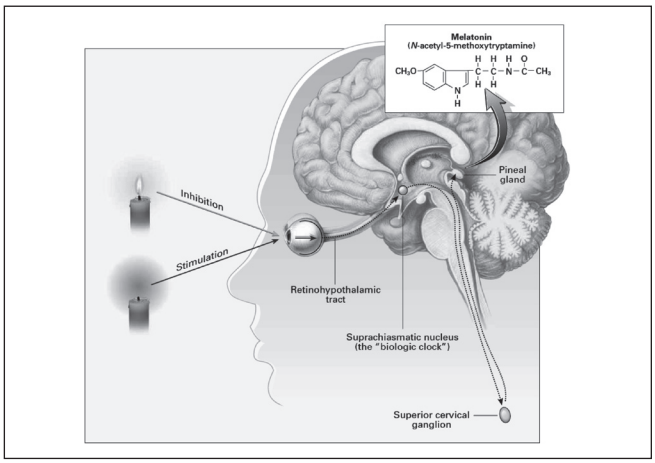

Figure 1: Retino-pineal pathway: noradrenergic fibres originating from superior cervical ganglia have their terminals in the pineal[61]

Thus, NAT activity is under the control of the retinopineal pathway [Figure 2] and represents the rate-limiting factor of hormonal synthesis.[23,24]

Early investigations in this area suggested that the *o*-methylation of N-acetylserotonin (the immediate precursor of melatonin) by the enzyme HIOMT determined the quantity of melatonin produced on a nightly basis.[25] Further results suggested that this may be invalid and a case was made for the enzyme that N-acetylates serotonin–NAT being rate limiting in melatonin production.[26] This idea persisted for several decades but recent findings have again shifted the emphasis to HIOMT as possibly being responsible for controlling the amount of melatonin produced in the pineal gland

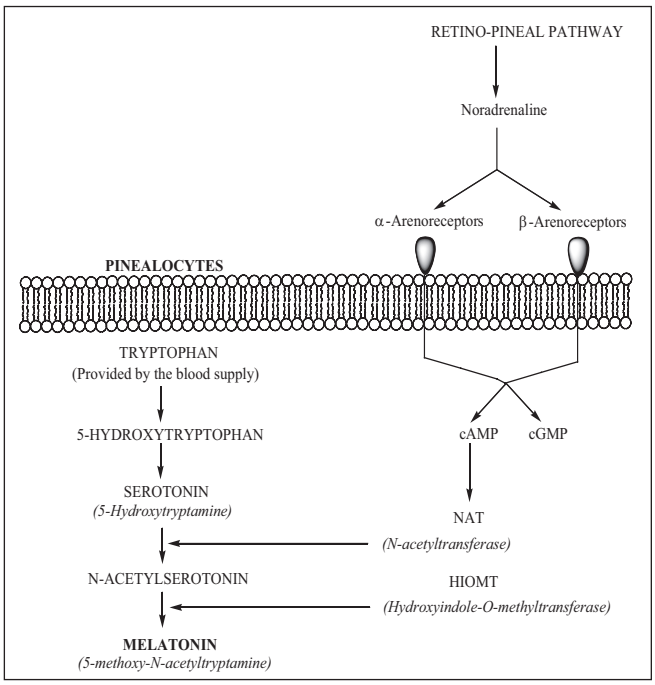

Figure 2: Pathway for secretion of melatonin within the pinealocyte: the *N*-acetyltransferase (NAT) activity, which is under the control of the retino-pineal pathway (through the release of noradrenaline), represents the rate-limiting factor in the synthesis of melatonin.

at night, at least under some conditions.

In support of HIOMT being the rate-limiting enzyme in pineal melatonin production, Ribelayga *et al*.[27] noted that melatonin levels in the Siberian hamster pineal gland do not correlate with the activity of the serotonin acetylating enzyme. Instead, levels of melatonin fluctuate in synchrony with the activity of HIOMT. Furthermore, it was observed that, in the same species, stimulation of the pineal gland with α- and β-receptor agonists causes a clear dichotomy between the responses of NAT and melatonin whereas the HIOMT activity correlates directly with melatonin.[28] This disconnect between the activity of pineal NAT and the quantity of melatonin in the gland has also been noted in sheeps.[29] The arylalkylamine N-acetyltransferase (AA-NAT) activity during the dusk period does not correspond to a simultaneous elevation in the melatonin content in humans.[30]

Further evidence for NAT not limiting melatonin production has been provided by Liu and Borjigin[31] who used a genetic mutant rat model, the Long Evens cinnamon (LEC) rat. Despite the apparent large deficiency in the NAT activity, the melatonin rhythm in the pineal gland remained essentially unaltered. Moreover, Liu and Borjign[31] also observed that the concentrations of N-acetylserotonin, the product of the N-acetylation of serotonin, had essentially no relationship with melatonin levels. Thus, in the presence of unusually high levels of N-acetylserotonin, melatonin remained depressed. This argues in favor of a mechanism downstream from N-acetylserotonin (i.e., HIOMT activity) being a major determinant of melatonin production.

Although melatonin is the major, or at least the most studied, secretion product of the pineal gland, recent studies have also ascribed importance to other pineal indoles.[32,33] Melatonin shows strong binding to guanosine triphosphate-binding proteins and quinone reductase, but also demonstrates somewhat weaker binding affinity to calmodulin,[34] as well as to nuclear receptors of the retinoic acid receptor family, RORα1, RORα2, and RZRβ.[35,36]

Melatonin is secreted into the blood with an endogenous and individual rhythm synchronized by the dark–light cycle; the plasma concentrations of the hormone reach a peak during the night-time, at least in the absence of light, and the persistence of high concentrations of melatonin is proportional to the duration of darkness.[37-40] Exposure to light rapidly inhibits melatonin synthesis by the epiphysis and its secretion into the blood. Thus, because of changes in the duration of night and day, the rhythm of melatonin release is also influenced by the cycle of the seasons. Indeed, the pineal gland, through its hormonal secretion, informs the whole organism about the current phase both of the day and of the year.[38]

Melatonin receptors

Melatonin has endocrine, autocrine, and paracrine actions,[41] and some of these actions are receptor mediated, while others are direct. Three mammalian melatonin receptors have been identified: MT_1[42] MT_2[43], MT_3.[44] Melatonin acts on the target cells either directly or via G-protein coupled receptors and are denoted as MT_1 and MT_2 whereas the newly purified MT_3 protein belongs to the family of quinone reductases. The MT_1 and MT_2 melatonin receptors are classified as unique subtypes based on their molecular structure and chromosomal localization.[43,45-47]

In mammalian tissues, the distribution of melatonin receptors appears to be widespread.[41] Autoradiography and radio-receptor assays have demonstrated the presence of melatonin receptors

in various regions of the human brain,[48] gut,[14] ovaries,[49] and blood vessels.[50] The receptors are most consistently found in the suprachiasmatic nucleus (SCN) of the hypothalamus and the pars tuberalis of the anterior pituitary, although current research suggests that few tissues are devoid of melatonin receptors.[41]

MT_1 receptors are high-affinity receptors that fall into the G-protein coupled receptor superfamily, and the binding of melatonin to these receptors results in the inhibition of the adenylate cyclase activity in target cells.[51] There are two subgroups of the MT_1 receptors, MT_{1a} receptors and MT_{1b} receptors.[52] The MT_1 melatonin receptor, which is expressed in the SCN and cardiac vessels is involved in modulating circadian rhythms[53,54] and constricting cardiac vessels.[55] Besides these specific regions, the MT_1 is expressed in other regions of the brain and peripheral tissues.[56-60]

The MT_2 receptors are low-affinity receptors that are coupled to phosphoinositol hydrolysis.[61] MT_2 receptors are involved in retinal physiology,[18] modulating circadian rhythms,[53] dilating cardiac vessels,[55] and affecting inflammatory responses in the microcirculation,[62] as reviewed.[63-65] Unlike the MT_1, these receptors are more restricted in their localization, which includes the cerebellum, SCN of the hypothalamus, retina, kidney, ovary, cardiac vessels, and various cancerous cell lines, as reviewed.[63-65]

A protein that displays a binding profile similar to that of the MT_2 receptor[66,67] now denoted as MT_3 was affinity purified from the Syrian hamster kidney.[44] It was shown that this protein shares 95% homology to human quinone reductase 2, an enzyme involved in detoxification.[44] The activation of MT_3 receptors inhibits leukotriene B_4-induced leukocyte adhesion and decreases intraocular pressure.[45]

Melatonin receptors (nomenclature as agreed by NC-IUPHAR Subcommittee on melatonin receptors)[68-69] are activated by the endogenous ligands melatonin and N-acetylserotonin [Table 1]. Melatonin, 2-iodo-melatonin, S20098, GR196429, LY156735, and TAK375[70] are nonselective agonists for MT_1 and MT_2 receptors. 2-Iodo-[^{125}I]-melatonin can be used to label all three melatonin receptor subtypes. (−)-AMMTC displays 400-fold greater agonist potency than (+)-AMMTC in rat MT_1 receptors.[71] Luzindole is a nonselective melatonin receptor antagonist with some selectivity for the MT_2 receptor.[53] The MT_3 binding site of the hamster kidney was identified as the hamster homologue of human quinone reductase 2 (ENSG00000124588).[44,72] MT_3 has recently been identified as quinone reductase 2 (QR2), which is of significance since it links the antioxidant effects of melatonin to a mechanism of action.[73] MT_1/MT_2 heterodimers present different pharmacological profiles from MT_1 and MT_2 receptors.[74]

Table 1: Melatonin receptors' pharmacology and functions

Currently accepted name *Previous names*	MT_1 Mel_{1a} ML_{1A} MEL_{1A}	MT_2 Mel_{1b} ML_{1B} MEL_{1B}	MT_3 ML_2
Structural information *Full agonists*	350 aa (human) Melatonin (M5250), 2-Iodomelatonin (I1899), N-propionyl melatonin, N-butanoyl melatonin, 6-Chloromelatonin (C0331 2-Methyl-6,7-dichloromelatonin, S20098, GR 196429, 8M-PDOT, (–)-AMMTC, S26131	363 aa (human) Melatonin (M5250), 2-Iodomelatonin (I1899), N-propionyl melatonin, N-butanoyl melatonin, 6-Chloromelatonin (C0331), 2-Methyl-6,7-dichloromelatonin, S20098, GR 196429, 8M-PDOT, (–)-AMMTC, IIK7 (I5531)	Not known 2-Iodomelatonin (I1899), 6-Chloromelatonin (C0331), Melatonin (M5250), N-Acetylserotonin (A1824), 5-MCA-NAT (G0294)
Partial agonists	5-Methoxyluzindole, N-acetyltryptamine (A7342)	5-Methoxyluzindole, N-acetyltryptamine (A7342)	Not known
Antagonists	Luzindole (L2407), S20928	Luzindole (L2407), S20928, 4P-PDOT, 4P-ADOT, K185 (K1888)	Luzindole (L2407), Prazosin (P7791), Prazosin (P7791)
Signal transduction mechanisms	Gi (cAMP modulation) Gq/11 (increase IP3/DAG)	Gi (cAMP modulation) cGMP modulation	Gq/11 (Increase IP3/DAG)
Radioligands of choice	2-[^{125}I]-Iodomelatonin [3H]-Melatonin	2-[^{125}I]-Iodomelatonin [3H]-Melatonin	2-[^{125}I]-Iodomelatonin 2-[^{125}I]-MCA-NAT
Tissue expression	Suprachiasmatic nucleus, retina, cerebellum, arteries, pars tuberalis	Suprachiasmatic nucleus, retina, cerebellum, arteries	Brain, kidney, testis
Physiological function Inhibition of neuronal firing, phase shift circadian rhythms, not known inhibition of prolactin secretion, vasoconstriction dopamine release, vasodilation			
Disease relevance Insomnia, phase shift circadian rhythms, decrease in intraocular pressure, increase in immune function			

Abbreviations

4P-ADOT: 4-Phenyl-2-acetamidotetralin

(–)-AMMTC: N-Acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole

4P-CADOT: 4-Phenyl-2-chloroacetamidotetralin

GR 196429: N-[2-[2,3,7,8-Tetrahydro-1H-furo(2,3-g)indol-1-yl]ethyl] acetamide

IIK7: N-Butanoyl-2-(2-methoxy-6H-isoindolo[2,1-a]indole-11-yl) ethanamine

K185: N-Butanoyl-2-(5,6,7-trihydro-11-methoxybenzo[3,4] cyclohept[2,1-a]indol-13-yl)ethanamine

Luzindole: 2-Benzyl-N-acetyltryptamine

5-MCA-NAT: 5-Methoxycarbonylamino-N-acetyltryptamine

Melatonin: 5-Methoxy-N-acetyltryptamine

4P-PDOT: 4-Phenyl-2-propionamidotetralin

8M-PDOT: 8-Methoxy-2-propionamidotetralin

S20098: N-[2-(7-Methoxy-1-naphthalenyl) ethyl]acetamide

S20928: N-[2-Naphth-1-yl-ethyl]-cyclobutyl carboxamide

S26131: N-(2-{7-[3-({8-[2-Acetylamino)ethyl]-2-naphtyl}oxy)propoxy]-1-naphthyl} ethyl)acetamide

Mechanism of action and therapeutic uses

Sleep disorders

Many studies[75-78] suggest a relationship among sleep, pineal function, and melatonin levels. Nocturnal melatonin levels and the quality of sleep both decline at puberty.[75] Serum melatonin concentrations were found to be significantly lower, with later peak nighttime concentrations, in some elderly subjects with insomnia than in age-matched controls without insomnia.[79] When administered in pharmacological doses, melatonin acts as a powerful "chronobiotic," maintaining synchronicity.[80] Dijk and Cajochen[81] have suggested that exogenous melatonin may be valuable in maintaining sleep consolidation in the elderly, some of whom experience fragmented sleep in the early morning hours. Melatonin also promotes sleep in young people with normal melatonin rhythms, if administered at a time of day (e.g., 1200–1800 h) when plasma melatonin levels are low.[82] The ingestion of melatonin (0.1–0.3 mg) during daytime, which increased the circulating melatonin levels close to that observed during night, induced sleep in healthy human subjects.[82]

It had previously been proposed that the decline in melatonin secretion observed toward the end of the first decade of life depends not on the individual's age *per se* but on his or her pubertal stage.[83] Now, Salti *et al*.[84] describe findings on eight male and eight female subjects (age 8.7–16.8 years) that confirm it is pubertal stage and not chronologic age that counts. It cannot now be stated whether the puberty-associated decline in plasma melatonin has endocrine consequences, or whether it is simply a response to the other endocrine changes occurring concurrently, or perhaps a temporally coincident, but otherwise unrelated, phenomenon.

The first human experiments of melatonin, conducted in the early 1970s, provided evidence of a sleep-inducing effect of melatonin in humans.[85,86] A number of randomized controlled trials have been conducted to examine the effect of melatonin in the treatment of various types of insomnia,[87-92] such as sleep maintenance insomnia,[93] terminal insomnia,[92] sleep-onset insomnia,[94] and psychophysiological insomnia,[95] as well as circadian rhythm disorders such as time zone change (jet lag) syndrome,[96-100] shift work sleep disorder,[101-105] delayed sleep-phase syndrome,[106-110] and non-24-h sleep wake disorder (associated with blindness).[108, 111,112]

Sleep disorders due to jet lag

When melatonin is taken at the destination, between 10:00 pm and midnight, it can correct the sleep disturbances, mental inefficiency, and daytime fatigue (cumulatively known as "jet lag") that occurs after flights across several time zones.[76-78] Daily doses between 0.5 and 5 mg were similar in effectiveness, except that at the higher doses people fell asleep more quickly. Doses >5 mg were no more effective than doses ≤5 mg. Slow-release melatonin (2 mg) was less effective than regular-release tablets.[77] The biological rhythm disorganization caused by the rapid change in environment (and associated light/dark cues) apparently can be corrected by melatonin. The benefit is likely to be greater as more time zones are crossed and is less for westward flights.[77] Unlike taking melatonin on immediate arrival, taking melatonin before travel can actually worsen symptoms.[78] Parry[113] has reviewed the use and effectiveness of melatonin as a "dark pulse" at night, with appropriately timed bright light to reduce symptoms of jet lag.

One study demonstrated that phase advancement after melatonin administration (3-mg doses just before bedtime) as well as faster resynchronization occurred in all 11 subjects traveling from Tokyo to Los Angeles compared with controls. Melatonin increased the phase shift from 1.1 to 1.4 h per day, causing complete entrainment of 7–8 h after 5 days of melatonin intake.[114] Melatonin resulted in a 50% reduction in the subjective assessment of jet lag symptoms in 474 subjects taking 5 mg regular-release melatonin.[115] No "hangover" effects were noted as assessed by mood and performance tests administered the morning after treatment.[116]

Insomnia

Insomnia has various etiologies and is the most common sleep disorder, affecting 6–12% of the adult population. Primary insomnia has been conceptualized as sleep disturbance not arising from a medical, psychiatric, circadian, behavioral, or pharmacologic cause.[117] Chronic primary insomnia has been characterized as a state of hyperarousal. Summaries of the epidemiological evidence conclude that 10–13% of the adult population suffers from chronic insomnia, and an additional 25–35% has transient or occasional insomnia.[118,119] It is estimated that 75% of population-based chronic insomnia is associated with psychiatric and medical diseases or with primary sleep disorders, and primary insomnia accounts for approximately 25% of all chronic insomnia.[120] Thus, primary insomnia is estimated to occur in 1–2% of the general population,[120] while it accounts for as much as 25% of all chronic insomnia cases.[117] For those with insomnia characterized by poor sleep at least three nights/week and subjective daytime impairment, the problem persists from 2–6 years[121]; durations over 2 years were typical for more than half of those reporting moderate-to-severe symptoms.[121] In retrospective reports, individuals with severe symptoms report having sleep difficulties for at least 1 year, with 40% suffering for more than 5 years. In various longitudinal reports, up to 80% of individuals with severe insomnia experienced no remission over time.[122]

Insomnia is more prevalent in older populations, and several authors have noted that the elderly are prone to a number of concomitant risk factors, such as increased prescription drug use, somatic disorders, neurological decline, reduced exposure to outdoor light, and polyphasic sleep–wake patterns.[123] Lack of physical activity also may play a role in the age-related increase in insomnia prevalence.[124] Longitudinal data suggest that reduced physical activity, independent of social engagement or interaction, represents a strong risk factor for the development of insomnia in elderly individuals.[124]

The administration of melatonin (2 mg slow-release) daily for 3 weeks to 12 elderly insomniac subjects, who were receiving various medications for chronic illnesses, improved the quality and duration of sleep.[87] The administration of melatonin (3 mg nightly) for up to 6 months to insomnia patients in addition to a benzodiazepine augmented sleep quality and duration and decreased sleep onset latency and the number of awakening episodes in elderly insomniacs.[125]

Nocturnal melatonin levels are reduced in primary insomnia.[126] Supplemental melatonin has been used successfully as a hypnotic for delayed sleep-phase syndrome, a type of insomnia characterized by wakefulness and the inability to fall asleep before 2:00–3:00 am. In two small studies of 8 and 14 subjects, 5-mg doses of melatonin given at 10:00 pm resulted in an advance of the sleep phase (shortening of time to sleep) by about 1.5 h[127,128] and reduced sleep duration by about 30 min,[127] suggesting a lowered sleep requirement as a consequence of improved sleep quality.

Narcolepsy

Narcolepsy, associated with hypocretin (orexin; a neuropeptide associated with wakefulness) deficiency, characterized by irresistible episodes of sleep, excessive daytime sleepiness, and disturbed sleep at night, is generally treated by an α1 noradrenergic stimulant modafinil, which has no amphetaminic properties.[129]

Melatonin has been used to alter sleep architecture in narcolepsy, which is characterized by disturbed circadian sleep/wake rhythm and rapid-eye-movement (REM) sleep deficit. Changes in REM sleep patterns similar to those of narcolepsy also occur in animals and humans after the removal of the pineal gland.[130]

Pharmacologic doses of melatonin (50 mg) dramatically increased REM sleep time in three narcoleptics and normals, and greatly intensified subjective dream phenomena.[130] In another study of 14 patients with neuropsychiatric sleep disorders and reduced REM sleep duration, 7 received 3 mg melatonin and 7 received placebo daily, administered between 10:00 and 11:00 pm for 4 weeks. Patients on melatonin experienced significant increases in REM sleep percentage (baseline/melatonin) and improvements in subjective measures of daytime dysfunction compared to placebo.[131] Studies of narcolepsy using varying doses of melatonin (2–20 mg/daily) have reported improved sleep quality, accelerated sleep initiation, and improved sleep maintenance without significantly altering memory, in contrast to benzodiazepines.[132,133]

Sleep disorders in children

Melatonin has been used successfully to treat serious sleep disorders in hyperactive and neurologically compromised children, such as those with attention-deficit hyperactivity disorder. In one study, doses of 2.5–5 mg melatonin at night provided prompt sedation and improved sleep quality in all 15 subjects, with no side effects.[111] Irritability was reduced, children were more alert and sociable, and developmental gains were reported in children treated with melatonin.[111]

Endocrine effects of melatonin

Several studies demonstrate that melatonin significantly affects the synthesis and function of hormones, including testosterone, estrogens, progesterone, prolactin, gonadotropins, and growth hormone (GH).

Melatonin's effect on the pituitary gland

A close, reciprocal relationship exists between the pineal gland and the pituitary/adrenal axis. There is a phase relationship between melatonin and synthesis of prolactin and growth hormone.[134] The diurnal concentrations of melatonin positively correlate with those of prolactin;[135,136] nocturnal increase and morning decrease in prolactin levels are preceded by similar changes in melatonin levels,[137] and melatonin administration stimulates prolactin secretion.[136,138] Moreover, the administration of 5 mg melatonin in healthy women resulted in a rapid and prominent prolactin release, similar to that observed at night in patients with hyperprolactinemia.[139] In a study of 28 idiopathic hyperprolactinemia and 14 healthy women, an increase in the melatonin serum concentration in hyperprolactinemic patients in comparison with healthy women during the night was increased.[140] In a study of seven normal subjects, a prolactin-stimulating effect was noted in women given 2 mg melatonin at 4:00 pm and 8:00 pm, with no significant effect on luteinizing hormone (LH) or thyroid-stimulating hormone (TSH).[141]

The relationship between melatonin and growth hormone is poorly understood. The administration of melatonin caused either enhancement of spontaneous and exercise-induced GH secretion in 80 healthy male subjects[142] and 7 healthy male subjects, respectively,[143] or had no effect in 14 healthy volunteers.[138] In one study of six healthy young adult males, given 3 mg melatonin, 10 mg flumazenil, or placebo at 5:00 pm, melatonin decreased LH levels without altering testosterone levels.[144]

Melatonin is present in human semen[145] and Van Vuuren *et al*.[146] demonstrated melatonin-binding sites in human spermatozoa. Melatonin in concentrations of 150–450 pg/mL was reported to have an inhibitory effect on sperm motility *in vitro*.[147] In a small, 6-month, double-blind, crossover study of 3 mg melatonin or placebo given orally at 5:00 pm to eight healthy men, melatonin appeared to impair sperm production in two young men.[148] In this study, the samples were analyzed for volume, sperm concentration, total sperm count, motility, and morphology as described by Jequier and Crich.[149]

The administration of melatonin at a dose of 1 mg in five subjects with hyperpigmented skin decreased serum LH levels.[150] Other research confirms a decrease in serum LH and increase in serum prolactin concentrations after the daily administration of 2 mg melatonin but not placebo in 12 subjects (10 men, 2 women) at 5:00 pm for 1 month.[151]

Menopause is associated with a decline in melatonin secretion and increased pineal calcification.[152] In menopausal women, hot flashes are often synchronous with pulsatile release of LH.[153,154] Suhner *et al*. reasoned that by suppressing hot flashes, melatonin might be an effective treatment for menopausal sleep disturbances. In 4 subjects, they found 3 mg melatonin at bedtime significantly decreased nocturnal LH secretion after 2 weeks.[155] Another study confirmed a decrease in serum LH after the administration of 0.5 mg melatonin in 20 postmenopausal women for 4 weeks.[156]

Effect of melatonin on the adrenal gland

Melatonin modulates the activity of the adrenal gland and peripheral activity of corticosteroids. Pinealectomy causes adrenal hypertrophy, which is reversed by melatonin administration.[157] Some have proposed that melatonin acts as a corticotropin-releasing factor inhibitor, and that disinhibition of the pituitary/adrenal axis in major depression in which melatonin levels are low[158] results from a lack of this modulating influence by the pineal gland.[159]

The interrelationship between melatonin and cortisol rhythms was first proposed by Wetterberg[158] in patients with Cushing's disease, based on the coincidence of low melatonin and high cortisol levels in depressive patients.[159,160] Melatonin antagonizes several effects of exogenous corticoids: immune depression,[161] hypercatabolism, thymic involution, and adrenal suppression.[162] These findings have led to the suggestion that melatonin might work as an antiadrenocortical or antistress factor.[162] The melatonin/corticoid relationship is significant because chronic hypercortisolemia has been linked to several aspects of aging and age-associated phenomena, including glucose intolerance, atherogenesis, impaired immune function, and cancer.[163]

In addition to high absolute levels of corticoids, disorganization of the normal rhythm of corticoid release is also pathogenic. Corticoids are normally high in the early morning and daytime and low at night. Properly timed exogenous melatonin may entrain or reorganize this

critical endocrine rhythm, resulting in long-term systemic benefit. Indeed, the immune-enhancing and anticorticoid effects of melatonin, or putative mediators of melatonin action, appear to depend on nocturnal administration.[161,164] This may represent an integral immune-recovery mechanism by which melatonin acts as a kind of buffer against the harmful effects of stress on immune homeostasis.[161]

Effect of melatonin on the thyroid gland

The inhibitory effect of melatonin on thyroid gland function found in animals does not seem to occur in humans.[165,166] Mazzoccoli *et al*.[167] showed interactions between the hypothalamic–pituitary–thyroid axis and melatonin in the control of body temperature in humans. They suggested that changes in serum thyroid stimulating hormone (TSH) levels are smaller and those in free thyroxine (FT_4) are greater at night, when melatonin levels are higher, indicating that the response of anterior pituitary to hypothalamic thyroid releasing hormone (TRH) and of thyroid to TSH may be influenced by pineal hormone modulation of the hypothalamic–pituitary–thyroid axis function and might influence the circadian rhythm of body temperature.[167]

In a placebo-controlled study of perimenopausal and menopausal women, 3 mg melatonin for 6 months resulted in a highly significant improvement in thyroid function, positive changes in gonadotropins levels (reflective of younger persons), and abrogation of menopause-related depression.[168]

Other hormone effects of melatonin

Pang *et al*.[169] examined the plasma level of immunoreactive melatonin, estradiol, progesterone, FSH and β-human chorionic gonadotropin (βhCG) during pregnancy and shortly after parturition in 105 Chinese women. In pregnant women, there were significant negative correlations between melatonin and estradiol, melatonin and progesterone, βhCG and progesterone, and βhCG and estradiol, with positive correlations between melatonin and follicle-stimulating hormone (FSH) and progesterone and estradiol. The findings suggest that gonadal steroids inhibit and FSH potentiates circulatory melatonin in pregnant women.[169]

A total of 32 women received medication (melatonin) for 2–4 months, and 8 nonmedicated controls were evaluated for one cycle. Out of 32, melatonin was administered in a dosage of 300 mg to 12 women for 4 months. In 16 women melatonin was also combined with the synthetic progestin norethisterone (NET). In addition, two women were medicated with 300 mg MEL alone, and two were medicated with 300 mg MEL/0.15 mg NET on days l–21 for 2 months. The result suggested that exogenous melatonin decreases plasma progesterone and estradiol levels in healthy women.[170]

In a study of 22 postmenopausal women of whom 14 were on hormone replacement therapy, placebo or melatonin (1 mg) was administered randomly at 8:00 am on two nonconsecutive days in a double-blind fashion. The finding suggested that in aged women, the administration of 1 mg of melatonin reduces glucose tolerance and insulin sensitivity.[171] In a study, 18 healthy men were treated (postoperatively) with 6 or 12 mg melatonin or placebo in basal conditions ($N = 6$ subjects) or concomitantly to the administration of insulin (0.15 IU/kg body weight in an intravenous bolus) ($N = 6$ subjects) or angiotensin II (increasing doses of 4, 8, and 16 mg/kg/min, at intervals of 20 min). The finding indicate an involvement of melatonin in the regulation of the oxytocin response to hypoglycemia in normal men.[172] A recent study supports that melatonin caused reduction in serum insulin, serum cortisol, serum ACTH and serum TSH levels while increasing the serum gastrin level through administration of 75 g/day of melatonin spaced in five different doses of 15 g each and given to 10 patients of Type 2 diabetes and 10 normal patients for 1 month. These 20 patients, residing in two different areas, aged between 45 and 52 years, were divided in four groups containing 5 patients each.[173]

Immunomodulatory effect of melatonin

Maestroni and Pierpaoli in 1981 who gave first evidence related to a possible immunological role of melatonin that exposure of mice to continuous illumination or evening administration of β-adrenergic blockers (both of which inhibit melatonin formation) was associated with depressed immune function.[174] Melatonin has been proved to have immune enhancing effects. An increase in the human leukocyte natural killer (NK) activity reported to occur upon chronic melatonin treatment[175] inhibits apoptosis in some immune cells and regulate the gene expression of several immunomodulatory cytokines including interleukin 1β.[176] Melatonin acts on specific membrane receptors expressed on immunocompetent cells with MT_2 receptors which favor a T helper (Th) type 1 response. Physiologically, nocturnal melatonin concentration correlates with the rhythmicity of Th1/Th2 ratio.[177] Th1/Th2 balance is of crucial importance in the immune system homeostasis. MEL stimulates the immune response during viral and bacterial infections, to also strengthen the immune reactivity as a prophylactic procedure.[178] The evidences show that there is a possible interaction between melatonin and the immune system.[177-179] Maestroni *et al*. first showed that the inhibition of melatonin synthesis causes the inhibition of cellular and humoral responses in animals.[180] The nocturnal rise in serum melatonin in young adult humans correlates with the increased thymic production of thymosin alpha-1 and thymulin.[181]

The potential intracrine and paracrine role of melatonin in immune regulation is indicated by synthesis of a biologically relevant quantity of melatonin from human peripheral blood mononuclear cells[182] and it has been shown to have multiple immunomodulatory effects: T lymphocytes have cell surface G-protein-linked and nuclear melatonin receptors (ability to directly bind to DNA and regulate the expression of adjacent genes), and melatonin has been shown to stimulate the production of helper T-cell type 1 cytokines by T lymphocytes.[183] The regulation of IgG isotype switching *in vivo* will depend upon the types of T helper cell (Th) and B cell interaction, and the involvement of T-cell-derived lymphokines. Th1 clones were shown to elicit IgG2a antibody secretion, whereas Th2 clones induce the production of IgG1 antibodies secretion of interleukin-2 (IL-2), IFN-g, IL-4, and IgG isotypes. Results described here suggest that melatonin possibly acts on Th2-type cells, as evidenced by predominant secretion of IL-4, IgG1 antibody, but not IL-2, IFN-g, and IgG2a subtype production.[184] Shaji *et al*. showed that melatonin specifically enhanced the secretion of antigen-specific IgG1 antibodies and decreased the yield of IgG2a isotype and suggested that the anti-inflammatory action of melatonin due to the induction of Th2 lymphocytes that produce IL-4, thereby inhibiting the function of Th1 cells acts by selectively activating a Th2-like immune response.[185]

β-adrenoceptor blockers that depress melatonin secretion exert immunosuppressive effects, but only when given in the

evening.[186,187] This is when melatonin and its immune-enhancing effects are highest. A preliminary report of 11 patients with AIDS who took 40 mg/day melatonin for 4 weeks showed beneficial effects on immune parameters (number of lymphocytes, eosinophils, T lymphocytes, NK cells, CD25- and DR-positive lymphocytes) and suggested that the combined neuroimmunotherapy with low-dose subcutaneous IL-2 and melatonin improve the immune status also in AIDS patients with CD4 cell counts below 200/mm^3, who generally do not respond to IL-2 alone.[188] It has been recommended that the dose be timed not only periodically within each day (at night only) but also periodically within the month, with treatment periods of 3–4 weeks, followed by a week-long "washout" period.[186]

In a study of 8 days in 23 subjects (5 normal control subjects, 6 patients with nocturnal asthma and 12 patients with non-nocturnal asthma) received melatonin in concentrations between 10^{-12} and 10^{-5} M suggested differential immunomodulatory effects of melatonin based on asthma clinical phenotype and indicate an adverse effect of exogenous melatonin in asthma.[189] On the other hand, in a rat model of adjuvant-induced arthritis, both prophylactic and therapeutic melatonin administrations inhibited the inflammatory response.[190] This inhibition was accompanied by enhanced thymocyte proliferation and interleukin (IL)-2 production by melatonin. In another animal study, melatonin was shown to possess both cellular and humoral immune-enhancing effects, and immune responses were augmented even in the absence of previous immunosuppression.[191]

Oncostatic effect

Numerous studies suggest an association between melatonin levels and cancer progression and shown reduced levels of melatonin in patients with certain types of cancers compared with age-matched controls.[192-195] *In vitro* studies have reported a reduction in the growth of malignant cells and/or tumors of the breast,[196-200] prostate,[201-206] and other tumor sites[207-211] by both pharmacological and physiologic doses of melatonin. A number of studies have investigated a potential link between night shift work and cancer. Four studies have directly investigated the association between night shift work and the development of breast[212-214] and colon[215] cancer. Hansen[212] reported an increased risk of breast cancer associated with occupational shift work exposure (primarily women working in catering jobs or as air cabin attendants) in a large study (over 6000 cases) in Denmark. Davis *et al*. have reported an increased risk of breast cancer in women who engaged in graveyard shift work (beginning work after 7:00 pm and ending work before 9:00 am)[213] and Schernhammer *et al*.[214] reported similar results in nurses who worked rotating shifts.

Experimental studies have suggested that light exposure during darkness increases the risk of cancer progression via the elimination of the nocturnal melatonin signal and its suppression of tumor linoleic acid uptake and metabolism to 13-hydroxyoctadecadienoic acid.[216] Thus, experimental studies strongly suggest a carcinogenic effect of light at night.[217]

A number of mechanisms have been proposed to explain such direct anticancer activity: melatonin may have an antimitotic activity by its direct effect on hormone-dependent proliferation through interaction with nuclear receptors; it affects cell-cycle control; and it increases the expression of the tumor-suppressor gene p53.[61,200,218,219]

Breast cancer

Many studies on the antiproliferative effects of melatonin have focused on breast cancer, possibly because melatonin has been shown to modulate the activity of several aspects of endocrine physiology.[220,221] Patients with breast cancer often have low melatonin levels. There is a correlation between a decrease in nocturnal melatonin and an increase in tumor size in individuals with primary breast cancer.[222] In one study, 14 metastatic breast cancer patients who were unresponsive to tamoxifen alone were given 20 mg melatonin daily in the evening along with tamoxifen. A response was achieved in 28% of these patients.[222]

Light of sufficient intensity, duration, and spectral quality suppresses melatonin production at night; the short wavelength (around 465 nm in humans) is most effective.[223,224] Epidemiological studies indicate light at night is a potential risk factor for breast cancer.[214,225] The circadian amplitude of melatonin was reduced by more than 50% in patients with breast cancer versus patients with nonmalignant breast disease.[226] The increased incidence of breast cancer or colorectal cancer seen in nurses engaged in the night shift work suggests a possible link with the diminished secretion of melatonin associated with increased exposure to light at night.[227] Recently, studies show that the tumor growth response to exposure to light during darkness is intensity dependent and that the human nocturnal, circadian melatonin signal not only inhibits human breast cancer growth but that this effect is extinguished by short-term ocular exposure to bright, white light at night.[228,229]

Melatonin appears to impact breast cancer, by down-regulating estrogen receptors, inhibiting estrogen-stimulated breast cancer growth, and complementing the oncostatic action of anti-estrogen drugs (tamoxifen), leading to the suggestion that melatonin is a "natural anti-estrogen."[230] An experimental study demonstrated that the combined use of melatonin (Mlt) and 9-cis-retinoic acid (9cRA) produces additive or synergistic effects in the prevention of breast cancer via preventing (N-nitroso-N-methylurea) NMU-induced mammary tumor formation, which are more efficacious than 9cRA alone. This combination of Mlt and 9cRA could be a potentially useful clinical treatment regimen for breast cancer since it allows the use of lower doses (0.4 mg/kg) of retinoic acid, thus, avoiding the toxic side effects (hypertriglyceridemia and hypercholesterolemia, headache, dry skin, arthralgias, and rash)[231,232] associated with the use of high dose (4.0 and 2.0 mg/kg) retinoids.[233]

The direct anti-estrogenic effects of melatonin on breast cancer cells were evidenced from *in vitro* studies[234,235] mostly on MCF-7 human breast cancer cells. The molecular mechanisms involved in the effects of melatonin on MCF-7 cells are as follows: (1) melatonin inhibits proliferation only in estrogen receptor (ER) α-positive cells,[234] (2) melatonin blocks the mitogenic effects of estradiol as well as counteracts the estradiol-induced invasiveness of MCF-7 cells,[198] (3) melatonin potentiates the sensitivity of MCF-7 cells to anti-estrogens such as tamoxifen,[236] (4) the transfection of MT_1 melatonin receptors to MCF-7 cells (ERα positive) or MDA-MB-231 cells (ERα negative) significantly enhances the growth-suppression effects of melatonin only in MCF-7 cells, that is to say, in those also expressing ER,[237] and (5) melatonin inhibits the expression of estrogen-regulated genes such as pS2 or cathepsine.[238] Melatonin has been shown to shift forskolin- and estrogen-induced elevation of cAMP levels by 57% and 45%, respectively,[239] thereby affecting signal-transduction mechanisms in human breast cancer cells.

Prostate and colorectal cancers

Melatonin may also play a special role in prostate and colorectal cancers. The circadian amplitude of melatonin is reduced by two-thirds in patients with prostate cancer compared to those who have benign prostate disease,[226] and similar phenomena have been observed in patients with colorectal cancer.[240] In prostatic carcinoma, melatonin exerts complex interactions with androgen receptors and affects intracellular trafficking; melatonin does not affect cell growth in the absence of dihydrotestosterone.[202]

Research conducted by Barni *et al*. suggests that low-dose subcutaneous IL-2 (3 million IU/day for 6 days/week for 4 weeks) plus melatonin (40 mg/day orally) may be effective as a second-line therapy to induce tumor regression and to prolong percent survival at 1 year in metastatic colorectal cancer patients progressing under 5-FU and folates.[241] Lissoni *et al*.'s study shows that both immunotherapy with high-dose IL-2 (18 million IU/day subcutaneously for 3 days) and neuroimmunotherapy with low-dose IL-2 (6 million IU/day subcutaneously for 5 days) plus MLT (40 mg/day orally) preoperatively are tolerated biotherapies, capable of neutralizing surgery-induced lymphocytopenia in 30 cancer patients, but suggest that the neuroimmunotherapy may induce a more rapid effect on postoperative immune changes with respect to IL-2 alone.[242] The antiproliferative and proapoptotic actions of melatonin on experimental colon carcinoma are probably mediated by melatonin MT_1 and MT_2 receptors.[243]

Affect of melatonin on other cancer types

Melatonin has been shown to play a critical role in a variety of normal skin functions, such as hair growth cycling, fur pigmentation, and melanoma control. In one study, four different doses of melatonin ranging from 5 to 700 mg/m^2/day were applied topically as a monotherapy to 40 patients with metastatic malignant melanoma for 5 weeks.[244] A partial response was achieved in six patients, the disease was stabilized in six more patients, and an overall response rate of 30% was achieved.[244]

Neri *et al*. conducted a trial in 22 patients with documented progressing renal cell carcinoma (RCC), assessing the effect of a long-term regimen (12 months) with human lymphoblastoid interferon (IFN) and melatonin. There were a total of seven (33%) remissions – four complete (involving lung and soft tissue) and four partial, with a median duration of 16 months. Nine patients achieved stable disease, and five progressed.[245]

A total of 50 patients with brain metastases due to solid tumors were treated with supportive care alone (steroids plus anticonvulsant agents) and with supportive care plus melatonin (20 mg/day at 8:00 pm orally). Nine of the 24 patients who received melatonin survived 1 year compared with 3 of 26 who did not receive melatonin.[246] In another study of 30 patients with brain glioblastomas, 14 patients received radiotherapy (RT) alone (60 Gy) and 16 received RT plus melatonin (MLT, 20 mg/daily orally) until disease progression. Both the survival curve and the percent of survival at 1 year were significantly higher in patients treated with RT plus MLT than in those receiving RT alone (6/14 vs.1/16).[247]

In a clinical trial, 63 non-small-cell lung cancer (NSCLC) patients with metastatic disease who did not respond to initial therapy with cisplatin were randomly placed on either 10 mg melatonin daily at 7:00 pm or supportive care alone.[248] The data from this study showed that the mean survival time was significantly higher for patients treated with melatonin than those receiving supportive care alone.[248] A second trial with 60 patients evaluated the efficacy of immunotherapy with low-dose IL-2 plus melatonin versus chemotherapy in advanced NSCLC. This study included 60 patients with locally advanced or metastatic NSCLC who were randomized to receive immunotherapy (IL-2 and melatonin) or chemotherapy (cisplatin and etoposide). The data from this study showed that immunotherapy with low-dose IL-2 plus melatonin was better tolerated and more effective in terms of the mean progression-free period and the percentage survival at 1 year was significantly higher in patients treated with immunotherapy than in those with NSCLC treated with chemotherapy containing cisplatin.[249]

Some have suggested that melatonin be administered to patients at earlier stages of cancer, in parallel with standard oncologic treatment regimens.[250] However, some questions remain concerning the anticancer effects of melatonin,[251] and data from stringent, large, randomized clinical trials are required before melatonin can be universally accepted as cancer treatment.

Safety of melatonin

In general, although melatonin is one of the least toxic substances known, it is not recommended for people with autoimmune diseases or immune system cancers because of its ability to stimulate immune function. Morera *et al*.[252] published in a review and showed that the 35-year (1966–2000) bibliographic search using the Medline database showed the range of dose involved in adverse reactions to be between 1 mg and 36 mg. The adverse reactions were as follows: one patient with autoimmune hepatitis, one case of confusion due to overdose, one case of optic neuropathy, four subjects with fragmented sleep, one psychotic episode, one case of nistagmus, four cases of seizures, one case of headache, and two cases of skin eruptions. Attention should be paid on the necessity of enquiring about the drugs that patients are taking, because this product is not harmless for health.[252,253]

Conclusion

This review summarizes conditions under which melatonin has been found beneficial, including sleep disorders and cancer. Melatonin also appears to exert significant effects on the endocrine and immune systems. Melatonin is distributed widely in nature, from unicellular organisms to plants, fungi, animals, and humans. Melatonin synthesis is not restricted to the pineal gland, and also takes place in other areas such as the eye, lymphocytes, GI tract, bone marrow, skin, and gonads, where it acts in a paracrine or an autocrine manner.

Melatonin can be used as a chronobiotic that is capable of normalizing the disturbed circadian rhythms, including sleep–wake rhythms and imbalances imposed by jet lag or shift work.

Over the last 30 years, a number of reports have documented an immunomodulatory effect of melatonin on the immune system. Several studies indicate that melatonin levels may be linked with breast cancer risk. For example, women with breast cancer tend to have lower levels of melatonin than those without the disease and melatonin as adjuvant to conventional treatments has shown beneficial additive effects. Studies indicated that the prostate cancer and brain glioblastomas are associated with lower melatonin levels compared to disease-free individuals. More cancer types, different doses, administration timings, routes of administration, and combinations of melatonin with other anticancer agents should be investigated.

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