Melatonin: circadian rhythm regulator, chronobiotic, antioxidant and beyond
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Abstract For many years, melatonin has been known to interact with circadian rhythms. New evidence indicates that melatonin acts as a free radical scavenger and antioxidant. Moreover, melatonin prevents apoptosis in different types of cells, because it induces mRNA levels of several antioxidant enzymes. It is evident that melatonin is involved in the cellular bioenergetic system as a mechanism that counteracts the progression of Alzheimer’s disease.

Introduction
Melatonin, the major secretory product of the pineal gland, has been known to interact with the neuroendocrine axis and circadian rhythms. Recently, it has been reported that melatonin acts as a free radical scavenger and antioxidant,1,2 and thus as an antiapoptotic agent.3 Mayo and colleagues4 reported that melatonin prevents apoptosis in undifferentiated and differentiated PC12 cells and suggested that the protective effect of melatonin may be associated with the increase in the mRNA levels of several antioxidative enzymes.

Pineal gland and melatonin

In humans, the pineal gland is 5 mm long, 1-4 mm thick, and weighs about 100 mg, in men and women.5 The pineal gland contains two major cell types: neuroglial cells and the predominant pinealocytes that produce melatonin. The pineal gland is a central structure in the cardiac system that is innervated by a neural multi-synaptic pathway originating in the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus. The SCN is the major circadian pacemaker of the mammalian brain and plays a central role in the generation and regulation of biological rhythms.6 The pineal gland produces melatonin in a marked circadian fashion,7 reflecting signals originating in the SCN. The human SCN innervates only a small number of hypothalamic nuclei directly.8 However, it may impose circadian fluctuations indirectly on the organism by means of melatonin released from the pineal gland.9

Melatonin biosynthesis and its regulation

The biosynthetic pathway of melatonin has been studied thoroughly. L-tryptophan is taken from circulation and converted to serotonin (5-HT) by tryptophan hydroxylase. 5-HT is metabolized by the rate-limiting enzyme arylalkylamine N-acetyltransferase (AA-NAT) to N-acetyl-5-hydroxytryptamine, and in turn by hydroxyindole-O-methyltransferase to melatonin. In all vertebrates, the activity of the rhythm-generating enzyme AA-NAT increases at night by a factor of 7-150, depending on the species. The molecular mechanisms regulating AA-NAT also are remarkably different among...
species. For instance, in rats, pineal AA-NAT is regulated at both the mRNA level and protein level; however, in sheep and rhesus macaque, the mRNA pineal AA-RAT levels show relatively little change over a 24-hr period, and changes in AA-NAT activity are regulated primarily at the protein level. In the human pineal gland, significant daily fluctuations in AA-NAT activity may be regulated mainly at the post transcriptional level.

Light intensity is the main environmental control of the pineal melatonin synthesis. Light perceived by the retina reaches the SCN through the retinohypothalamic tract, which has been revealed in the human hypothalamus. The SCN innervates the pineal gland, resulting in the rhythmic secretion of melatonin. The importance of ocular light as a temporal clue has been demonstrated in circadian studies of blind people, bilaterally enucleated, showing desynchronized melatonin and cortisol rhythm. Abundant evidence indicates that, in humans, the sympathetic stimulus is crucial for melatonin secretion. The primary neurotransmitter released from the postganglionic sympathetic terminals in the pineal gland is norepinephrine (NE); during darkness at night, NE is discharged onto the pinealocyte, where it couples (especially with beta 1-adrenoceptors). This process is potentiated further by stimulations of alpha 1-adrenoceptors. This leads to a marked rise in intracellular cAMP levels to denovo protein synthesis and eventually to the stimulation of AA-NAT.

Unlike other endocrine organs, the pineal does not store melatonin for later release after its synthesis; melatonin quickly diffuses out of the pinealocytes into the rich capillary bed within the gland and directly into the cerebrospinal fluid. Melatonin displays high lipid and water solubility, which facilitates passage across cell membranes. After release into the circulation, it gains access to various fluids, tissues, and cellular compartments (saliva, urine, cerebrospinal fluid, preovulatory follicles, semen, amniotic fluid, and milk).

Three mammalian melatonin receptors have been described: MT1, MT2, MT3. The first two are G-protein-coupled receptors, and their activation modulates a wide range of intracellular messengers (eg, cAMP, cGMP, or calcium concentrations). The MT1 receptor, with high affinity, is mainly expressed in the SCN and hypophyseal pars-tuberalis. The MT1 receptor with low affinity is expressed mainly in the retina. The MT3-binding site has been identified as a quinone reductase protein, but its physiological significance needs to be clarified.

A very large goiter may compress the superior cervical ganglia (SCG), thus altering melatonin synthesis in patients. After bilateral T1-T2 ganglionectomy in a patient with hyperhidrosis, melatonin levels in the cerebrospinal fluid (CSF) and plasma were reduced, and the diurnal rhythm was abolished. A circadian rhythm of β1-adrenergic receptors has been found in human pinealocytes. Propanolol, a β1-adrenergic receptor antagonist, causes a dose-dependent decrease in melatonin levels or completely abolishes nighttime surge. In turn, melatonin elicits two distinct, separable effects on the SCN: acute neuronal inhibition and phase shifting. The ability of melatonin to phase-shift in the circadian system has been investigated extensively in humans.

Jet lag

Jet lag is a considerable problem in the modern world with widespread air travel. When the internal body clock (or circadian rhythm) is not synchronized with the external “local” time (light-dark cycle), jet lag is experienced. The symptoms, which vary between individuals, include tiredness, inability to sleep at the new bedtime, inability to concentrate, disturbed sleep for several days after a long flight, headache, and gastrointestinal problems. All of these symptoms can interfere with normal activities. Jet lag also may cause considerable problems for training and performance in sports competitions and should be taken into account when planning journey times for sportsmen and women. Symptoms are more marked in older travellers, when more time zones are crossed, and when travelling in an easterly direction.

A recent Cochrane review assessed 10 trials comparing melatonin with a placebo and compared it with the hypnotic zolpidem. In 9 out of 10 trials, melatonin decreased jet lag resulting from crossing five or more time zones when taken close to the desired bedtime at the destination (10 P.M. to midnight). The time of dosing was very important, because if it is taken at the wrong time, melatonin could cause a delay in adaptation to the local time. The safety profile was very high in these trials, and the authors concluded that melatonin can be recommended safely to adults travelling across five or more time zones, particularly those who have experienced jet lag previously.

Delayed sleep phase disorder

According to the International Classifications of Sleep Disorders, individuals with delayed sleep phase disorder (DSPD) have difficulty falling asleep at their desired bedtime and an inability to wake spontaneously at the planned time in the morning. Weitzman and colleagues first defined this disorder and described its characteristics: long sleep onset latencies and late sleep onset times. This is caused by a delay of the major sleep period.

There is considerable evidence that DSPD arises from a delayed endogenous circadian rhythm. Sleep parameters, melatonin, and core body temperature rhythms have been delayed in individuals with sleep onset insomnia and DSPD when compared to control groups. If the core body temperature and melatonin circadian rhythms were phase delayed, then the “wake maintenance zone” would be delayed also. Although circadian rhythm phase delay is seen as the major contributor to DSPD, there are some
important behavioral and cognitive factors that should be addressed to improve treatment effectiveness.

Treatments that change the circadian rhythm phase or timing (such as morning bright light, exogenous melatonin, and chronotherapy) have been effective in treating of delayed circadian rhythm sleep disorders. Bright light is an effective intervention for phase advancing circadian rhythm.

The timing and duration of the light stimulus and the brightness and wavelength of light affects the magnitude of phase shift. The human phase response curve to light suggests that a phase advance of the circadian rhythm is achieved when the light stimulus is presented immediately after the normal circadian time (CT).28

Exogenous melatonin administration also is capable of shifting the circadian rhythm to a more desired time.29 In the evening phase, melatonin advances circadian rhythm when combined with optimal time of administration and greater doses: (3-5 mg) 4 to 8 hours prior to the onset of endogenous melatonin and smaller doses (0.3-0.5 mg) 3 hours before the beginning of melatonin production.30 A recent study31 showed that the addition of evening melatonin administration to morning bright light therapy produced a significantly greater phase advance than the morning bright light alone, suggesting that the two therapies are additive.

Although exogenous melatonin appears to be safe with short-term use (less than 3 months), there is little information available on its long-term administration.32 Because typical doses (3-5 mg) in many studies can elevate melatonin concentrations well above normal physiological plasma levels, it would be prudent to use much lower doses. Fortunately, the chronobiotic effects of low doses (0.3-0.5 mg) appear to be sufficient without requiring excessive supraphysiological levels.31 Adverse side effects reported following melatonin administration include headache, dizziness, nausea, and drowsiness.32 The effects of exogenous melatonin on sleep was the object of a recent meta-analysis report,33 which states: “this meta analysis supports the hypothesis that melatonin decreases sleep onset latency, increases sleep efficiency and total sleep duration. In spite of the heterogeneity of the data the present meta analysis does land statistical support to the notion that melatonin preparations can improve sleep quality with regard to sleep onset latency, sleep efficiency and sleep duration.”

Melatonin as an antioxidant, radical scavenger, and anti aging product

Melatonin is present in bacteria, plants, eukaryotes, fungi, and all phyla of multicellular animals; its original evolutionary role probably was to act as an antioxidant.34 Its antioxidant properties have been seen in tissue cultures and intact animals. One problem still discussed is whether melatonin acts in this manner directly or activates critical pathways involved in the disposition of free radicals.35 The evidence for a direct effect is seen when melatonin acts as a power-free radical scavenger in isolated cell-free-systems36; there are, however, reports that melatonin can act as a pro-oxidant in such systems.37

Melatonin is present within brain at a concentration of only 5% of that found in serum.38 Therefore, unless it is highly concentrated in a localized area, it can contribute little to scavenging when compared to predominant antioxidant species like glutathione and alfa-tocopherol.39

Melatonin receptors and enzyme induction

There are three major plasma membrane receptors for melatonin in the brain. However, the presence of additional melatonin binding sides in the nuclei of many cell types suggests the existence of a mechanism different from the mechanism mediated by the interaction with plasma membrane receptors.40 The specificity of melatonin may reside in its properties as a neurohormone, which affects transciptional events in the CNS.41

A wide range of antioxidant enzymes is induced by melatonin (eg, glutathione peroxidase, catalase, and superoxide dismutases).42 These protein changes are paralleled by altered levels of gene expression of oxidative enzymes.43 In addition, levels of some pro-oxidant enzymes (such as lipoxygenase and nitric oxide synthetase) are depressed after melatonin treatment.44

Presently, melatonin functions seem to fall into three categories: receptor-mediated, protein-mediated, and non-receptor-mediated effects. Receptor-mediated melatonin events involve membrane and nuclear receptors. Although membrane melatonin receptors are well-characterized in humans,45 some of the receptor-related antioxidant effects of melatonin seem to be related to its nuclear receptors.46 With this information, the interaction between membrane and nuclear melatonin signalling has been proposed.47

Melatonin and aging

One of the most recent theoretical advances in basic gerontology is the free radical theory of aging. This theory proposes that reactive oxygen species (ROS) (including superoxide [O₂⁻], hydroxyl (OH⁻) free radicals, hydrogen peroxide [H₂O₂] and possibly singlet oxygen) are generated as by-products of cellular respiration and other metabolic processes and damage cellular macromolecules. This results in mutations and genome instability, and all of these abnormalities can lead to the development of age-related pathological phenomena, including cancer, circulatory diseases, immuno depression, brain disfunction, and cataracts.48 Much evidence indicates that aging is characterized by a progressive deterioration of circadian time keeping.49
 Besides the age-related decline of melatonin production, age-related changes in the timing of the melatonin rhythm have been reported. The possible mechanism of age-related melatonin changes were associated with changes in the pineal gland morphology and its calcification; these data are in agreement with the assertion of decreased melatonin production with age. These findings suggest that the changes observed in the melatonin rhythm may be part of a general effect of aging in particular on the central clock of SCN and its regulation.

**Melatonin, mitochondria, and cellular bioenergetics**

Aerobic cells use oxygen for the production of 90%-95% of the total amount of ATP they use. The synthesis of ATP is the result of electron transport along the mitochondrial electron chain, resulting in the ultimate oxygen reduction and coupled to oxidative phosphorylation. Under normal conditions, a small percentage of oxygen may be reduced by one, two, or three electrons only, yielding superoxide anion, hydrogen peroxide, and the hydroxyl radical, respectively. The main radical produced by mitochondria is superoxide anion, and the intramitochondria antioxidant systems must scavenge this radical to avoid oxidative damage to the mitochondrial membrane, which leads to impaired ATP production.

During aging and some neurodegenerative diseases, oxidatively damaged mitochondria are unable to maintain the energy demands of the cells, leading to a further increased production of free radicals. Both defective ATP production and increased oxygen radical may induce mitochondrial-dependent apoptic cell death.

Melatonin has been reported to exert neuroprotective effects in several experimental and clinical situations involving neurotoxicity and excitotoxicity. Additionally, in a series of pathologies in which high production of free radicals is the primary cause of the diseases, melatonin is protective. The common features of these diseases is the existence of mitochondrial damage caused by oxidative stress.

**Melatonin and mitochondria**

Two main considerations support melatonin’s role in mitochondrial homeostasis: 1) mitochondria produce high amounts of ROS and RNS, and 2) mitochondria depend on the GSH uptake from the cytoplasm, although they have GPx and GRd to maintain GSH redox cycling. Thus, the antioxidant effect of melatonin and its ability to increase GSH levels may be of great importance for mitochondrial physiology. The protective effects of melatonin were analyzed in isolated mitochondria prepared from rat brain and liver tissue and were compared with those of other known antioxidants. After oxidative stress, virtually all GSH in mitochondria is oxidized to GSSG, and the activity of both GPx and GRd are reduced almost to zero. In this situation, melatonin counteracted these effects, restored basal levels of GHS and the normal activities of both GPx and GRd. To characterize the effects of melatonin on mitochondrial ETC activity, submitochondrial particles were used. Melatonin increased the activity of the C-I and C-IV complexes in a dose-dependent manner, as previously shown on intact mitochondria.

These results suggest a direct effect of melatonin on mitochondrial energy metabolism and provide a new homeostatic mechanism for regulation of mitochondrial function. The findings also identify a new mechanism of action of melatonin at the mitochondrial level. Thus, melatonin improves the bioenergetics of the cell, by providing more efficient nuclear and mitochondrial genomic repair mechanisms, increasing GSH levels, elevating ATP production, and improving ATP-dependent functions, including neurotransmission.

**Melatonin and Alzheimer’s disease**

Alzheimer’s disease (AD) is characterized by the presence of β-amyloid deposits and neurofibrillar tangles (NFT) in the brains of affected individuals. The development of early diagnostic tools and quantitative markers are crucial for exploring promising therapeutic strategies. Anti-inflammatory agents, antioxidants, vaccinations, cholesterol-lowering agents, and hormone therapy are examples of new approaches being developed for treating or delaying the progression of AD.

Recent evidence indicates that melatonin reduces the neuronal damage mediated by oxygen-based reactive species in experimental models of AD by acting as a free radical scavenger and antioxidant. Several clinical studies also have indicated that melatonin levels are decreased in AD patients. Thus, melatonin’s receptor-independent scavenging effects and receptor-mediated influences on enzyme activities (which counteract the effect of oxidative stress) may account for its possible beneficial effects in AD.

Increased awareness of the role oxidative stress plays in the pathogenesis of AD has highlighted the issue of whether oxidative damage is a fundamental step in pathogenesis or a result of the disease-associated pathology. Several studies have demonstrated the presence of lipid, protein, and DNA oxidation products in postmortem examinations of the brains of AD patients. By observing the specific markers of in vivo oxidative stress and the expression of apoptosis-related factors, researchers demonstrated that melatonin suppresses brain lipid peroxidation in transgenic mice, and reduces the expression of apoptosis-related factors in vivo.
Inhibitory role of melatonin in tau protein hyperphosphorylation

The cytoskeleton plays a crucial role in maintaining the highly asymmetrical shape and structural polarity of neurons essential for normal physiology. In AD, the cytoskeleton is assembled abnormally into NFT, and impairment of neurotransmission occurs. Microtubule-associated protein tau is capable of binding to tubulin to form the micro-tubules, essential structures for neuronal viability NFT.

The micro-tubule-stabilizing function of tau is diminished greatly by its hyperphosphorylation, which results in poor binging to tubulin. Glycogen synthase kinase-3, a downstream element of phosphoinositol-3 kinase, is one of the most active enzymes in phosphorylating tau in vivo. Protein kinase A (PKA) is another crucial kinase in AD-like tau hyperphosphorylation. Isoproterenol, the specific PKA activator, can induce tau hyperphosphorylation. Furthermore, melatonin enhances SOD activity and decreases the level of MDA. It has been suggested that isoproterenol may induce abnormal hyperphosphorylation of tau through not only the activation of PKA, but also by increasing oxidative stress.

Circadian rhythm disruptions in Alzheimer’s disease

The fragmented sleep-wake pattern that occurs in elderly people is even more pronounced in AD patients. Many patients also suffer often from circadian system related behavioral disturbances, such as daytime agitation and nightly restlessness.

Melatonin changes in preclinical and clinical Alzheimer’s disease

Many studies demonstrate that nocturnal melatonin levels are selectively decreased in AD, and daytime melatonin levels are increased in AD patients. A strong reduction was observed in postmortem CSF melatonin levels of AD patients; CSF melatonin levels in AD patients were only one-fifth of those in control subjects.

The melatonin levels in CSF decrease with the progression of AD neuropathology. More strikingly, CSF melatonin levels are already reduced in preclinical AD patients, who are cognitively intact and are showing only the earliest signs of AD neuropathology.

A significant high correlation exists between pineal melatonin content and CSF melatonin levels and between CSF and plasma melatonin amount, suggesting that reduced melatonin levels may be an early marker for the first stages of AD that could not be monitored another way. Melatonin deficiency is not only a consequence of the AD process; it may contribute to the pathogenesis of AD, because it acted as an antioxidant and neuroprotector in in vitro and in vivo experiments.

Mechanism underlying the melatonin changes in the progression of Alzheimer’s disease

The pineal gland shows molecular changes in preclinical and clinical AD. However, cells or afferent fibers are clear of the neuropathological hallmarks of AD (ie, neurofibrillary tangles, accumulation of neurofilaments, and hyperphosphorylated tau or β/4 amyloid deposition). The circadian melatonin rhythm disappears because of decreased nocturnal melatonin levels in AD preclinical and advanced patients. Moreover, the circadian rhythm of β-adrenergic receptor mRNA disappears in both patient groups, which suggests a dysfunction of the SCN innervation to the pineal. The biological SCN clock is affected severely. It shows prominent degenerative changes and the typical cytoskeletal alterations caused by pretangles and tangles. These degenerative changes in the SCN most likely result in a disrupted melatonin synthesis and may underlie the clinically common circadian rhythm disorders in AD.

The input of environmental light to the circadian timing system is also disrupted in AD. Besides the degenerative changes that are present in the SCN, several factors attenuate the input of environmental light to the circadian timing system; AD patients are exposed to less environmental light than their age-matched controls. Furthermore, the retina and optic nerve show degenerative changes, but without neurofibrillary tangles, neuritic plaques, or amyloid angiopathy.

Melatonin supplementation

In AD patients, melatonin has been suggested to improve circadian rhythmicity, decreasing agitated behavior, confusion, and sundowning in uncontrolled studies. Melatonin also may have beneficial effects on memory, possibly through protection against oxidative stress and neuroprotective capabilities. However, these suggestions need to be confirmed in well-controlled studies, and a few randomized placebo-controlled trials of melatonin administration to AD patients did not find improved in the sleep-wake pattern.

Conclusions

This contribution summarizes the actions of melatonin in reducing the effects of jet lag, delayed sleep phase disorder, and molecular damage caused by free radicals. In particular, melatonin has effects the reduction of oxidative damage in the CNS as it cross the blood-brain barrier. However, it is unlikely that all the actions by which melatonin reduces free radical damage have been uncovered. The simplest way to account for the multiple effects of melatonin is to hypothesize that it modifies early alterations of gene expression consisting in the depression of mRNAs for
immuno-related cytokines and in the elevation of mRNA for antioxidant enzymes. Also, transcription factors are activated after binding to cytoplasmic melatonin receptors or by changes caused by melatonin acting directly on molecular receptors. The reported beneficial effects of melatonin on oxidative stress-related damage were supported by the improvement of mitochondrial function, which was achieved by countering the oxidation of mitochondrial membrane and resulting in the amelioration of cell bioenergetic. This leads to a more efficient nuclear genomic repair mechanism, to increased GSH levels, the elevation of ATP production, and the improvement of ATP-dependent functions, including neurotransmission. All these mechanisms may represent the basis for the potential anti-aging properties of melatonin and its effective use in neurodegenerative diseases.

Melatonin can be considered an evolutionarily ancient neurohormone that has a very low toxicity and no carcinogenic properties which makes it a very safe compound that is available at a low cost. However more research is needed on the effects of therapeutic modulation of the melatonergic system on circadian haemodynamics and the possible impact on morbidity and mortality in humans.

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References


