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# Melatonin

## Nature's most versatile biological signal?

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Melatonin is a ubiquitous molecule and widely distributed in nature, with functional activity occurring in unicellular organisms, plants, fungi and animals. In most vertebrates, including humans, melatonin is synthesized primarily in the pineal gland and is regulated by the environmental light/dark cycle via the suprachiasmatic nucleus. Pinealocytes function as 'neuroendocrine transducers' to secrete melatonin during the dark phase of the light/dark cycle and, consequently, melatonin is often called the 'hormone of darkness'. Melatonin is principally secreted at night and is centrally involved in sleep regulation, as well as in a number of other cyclical bodily activities. Melatonin is exclusively involved in signaling the 'time of day' and 'time of year' (hence considered to help both clock and calendar functions) to all tissues and is thus considered to be the body's chronological pacemaker or 'Zeitgeber'. Synthesis of melatonin also occurs in other areas of the body, including the retina, the gastrointestinal tract, skin, bone marrow and in lymphocytes, from which it may influence other physiological functions through paracrine signaling. Melatonin has also been extracted from the seeds and leaves of a number of plants and its concentration in some of this material is several orders of magnitude higher than its night-time plasma value in humans. Melatonin participates in diverse physiological functions. In addition to its timekeeping functions, melatonin is an effective antioxidant which scavenges free radicals and up-regulates several antioxidant enzymes. It also has a strong antiapoptotic signaling function, an effect which it exerts even during ischemia. Melatonin's cytoprotective properties have practical implications in the treatment of neurodegenerative diseases. Melatonin also has immune-enhancing and oncostatic properties. Its 'chronobiotic' properties have been shown to have value in treating various circadian rhythm sleep

**Abbreviations**
AA-NAT, arylakylamine *N*-acetyltransferase; AD, Alzheimer's disease; aMT6S, 6-sulfatoxymelatonin; AFMK, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine; AMK, $N^1$-acetyl-5-methoxykynuramine; CRSD, circadian rhythm sleep disorders; CYP, cytochrome $P_{450}$ isoforms (hydroxylases and demethylases); GC, glucocorticoids; GI, gastrointestinal; GnRH, gonadotropin-releasing hormone; IL, interleukin; $MT_1$, $MT_2$, melatonin membrane receptors 1 and 2; NE, norepinephrine; NO, nitric oxide; RORα, RZRβ, nuclear receptors of retinoic acid receptor superfamily; SCN, suprachiasmatic nucleus.

disorders, such as jet lag or shift-work sleep disorder. Melatonin acting as an 'internal sleep facilitator' promotes sleep, and melatonin's sleep-facilitating properties have been found to be useful for treating insomnia symptoms in elderly and depressive patients. A recently introduced melatonin analog, agomelatine, is also efficient for the treatment of major depressive disorder and bipolar affective disorder. Melatonin's role as a 'photoperiodic molecule' in seasonal reproduction has been established in photoperiodic species, although its regulatory influence in humans remains under investigation. Taken together, this evidence implicates melatonin in a broad range of effects with a significant regulatory influence over many of the body's physiological functions.

## Introduction

Melatonin occurs ubiquitously in nature and its actions are thought to represent one of the most phylogenetically ancient of all biological signaling mechanisms. It has been identified in all major taxa of organisms (including bacteria, unicellular eukaryotes and macroalgae), in different parts of plants (including the roots, stems, flowers and seeds) and in invertebrate and vertebrate species [1–5]. In some plants, melatonin is present in high concentrations. Melatonin is a potent free radical scavenger and regulator of redox-active enzymes. It has been suggested that dietary melatonin derived from plants may be a good supplementary source of antioxidants for animals [2]. In animals and humans, melatonin has been identified as a remarkable molecule with diverse physiological actions, signaling not only the time of the day or year, but also promoting various immunomodulatory and cytoprotective properties. It has been suggested to represent one of the first biological signals which appeared on Earth [6].

In vertebrates, melatonin is primarily secreted by the pineal gland. Synthesis also occurs, however, in other cells and organs, including the retina [7–9], human and murine bone marrow cells [10], platelets [11], the gastrointestinal (GI) tract [12], skin [13,14] and lymphocytes [15]. Melatonin secretion is synchronized to the light/dark cycle, with a nocturnal maximum (in young subjects, $\approx 200$ pg·mL$^{-1}$ plasma) and low diurnal baseline levels ($\approx 10$ pg·mL$^{-1}$ plasma). Various studies have supported the value of exogenous administration in circadian rhythm sleep disorders (CRSD), insomnia, cancer, neurodegenerative diseases, disorders of the immune function and oxidative damage [16–19].

## Melatonin in plants

To date, the presence of melatonin has been demonstrated in more than 20 dicotyledon and monocotyledon families of flowering plants. Nearly 60 commonly used Chinese medicinal herbs contain melatonin in concentrations ranging from 12 to 3771 ng·g$^{-1}$ [4]. It is interesting to note that the majority of herbs used in traditional Chinese medicine for retarding age-related changes and for treating diseases associated with the generation of free radicals also contain the highest levels of melatonin [4]. The presence of melatonin in plants may help to protect them from oxidative damage and from adverse environmental insults [1,20]. The high concentrations of melatonin detected in seeds presumably provide antioxidative defense in a dormant and more or less dry system, in which enzymes are poorly effective and cannot be up-regulated; therefore, low-molecular-weight antioxidants, such as melatonin, can be of benefit. Melatonin was observed to be elevated in alpine and mediterranean plants exposed to strong UV irradiation, a finding amenable to the interpretation that melatonin's antioxidant properties can antagonize damage caused by light-induced oxidants [5].

Many plants represent an excellent dietary source of melatonin, as indicated by the increase in its plasma levels in chickens fed with melatonin-rich foods [21]. Conversely, removal of melatonin from chicken feed is associated with a fall in plasma melatonin levels [22]. From this, it is evident that melatonin acts not only as a hormone but also as a tissue factor. Additionally, melatonin is an antioxidant nutrient. Although its redox properties are difficult to preserve in food, it has been suggested that certain of its metabolites, especially a substituted kynuramine formed by oxidative pyrrole-ring cleavage, may be stable enough to serve as a dietary supplement without a significant loss of its antioxidant effects [5].

## Melatonin biosynthesis, catabolism and regulation

The enzymatic machinery for the biosynthesis of melatonin in pinealocytes was first identified by Axelrod [23]. Its precursor, tryptophan, is taken up from the

blood and converted, via 5-hydroxytryptophan, to serotonin. Serotonin is then acetylated to form *N*-acetylserotonin by arylakylamine *N*-acetyltransferase (AA-NAT), which, in most cases, represents the rate-limiting enzyme. *N*-acetylserotonin is converted into melatonin by hydroxyindole *O*-methyltransferase (Fig. 1). Pineal melatonin production exhibits a circadian rhythm, with a low level during daytime and high levels during night. This circadian rhythm persists in most vertebrates, irrespective of whether the organisms are active during the day or during the night [6]. The synthesis of melatonin in the eye exhibits a similar circadian periodicity. The enzymes of melatonin biosynthesis have recently been identified in human lymphocytes [15], and locally synthesized melatonin is probably involved in the regulation of the immune system. Among various other extrapineal sites of melatonin biosynthesis, the GI tract is of particular importance as it contains amounts of melatonin exceeding by several hundred fold those found in the pineal gland. GI melatonin can be released into the circulation, especially under the influence of high dietary tryptophan levels [12] (Fig. 1).

In mammals, the regulation of pineal melatonin biosynthesis is mediated by the retinohypothalamic tract, which projects from the retina to the suprachiasmatic nucleus (SCN), the major circadian oscillator [24].

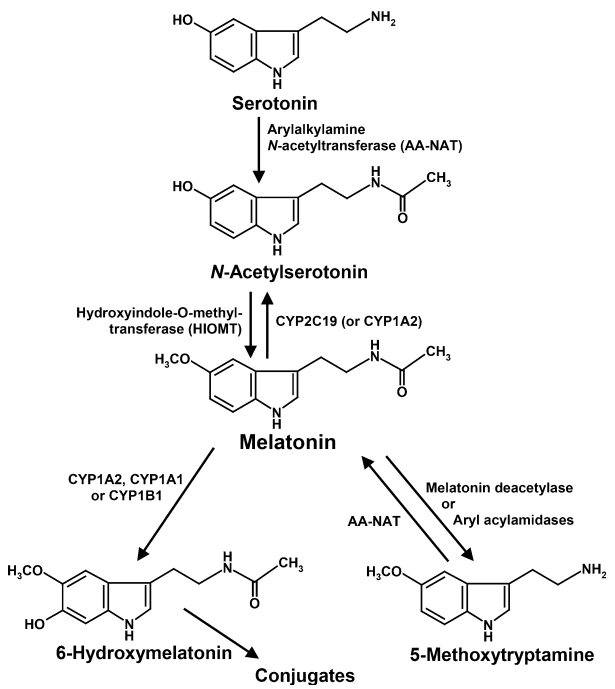


**Fig. 1.** Formation of melatonin, its major pathways of indolic catabolism, and interconversions between bioactive indoleamines. CYP, cytochrome $P_{450}$ isoforms (hydroxylases and demethylases).

Special photoreceptive retinal ganglion cells containing melanopsin as a photopigment [25] are involved in this projection [26]. Fibers from the SCN pass through the paraventricular nucleus, medial forebrain bundle and reticular formation, and influence intermediolateral horn cells of the spinal cord, where preganglionic sympathetic neurons innervating the superior cervical ganglion are located [24]. The postganglionic sympathetic fibers of the superior cervical ganglion terminate on the pinealocytes and regulate melatonin synthesis by releasing norepinephrine (NE). The release of NE from these nerve terminals occurs during the night. NE, by binding to β-adrenergic receptors on the pinealocytes, activates adenylate cyclase via the α-subunit of $G_s$ protein. The increase in cAMP promotes the synthesis of proteins, among them the melatonin-synthesizing enzymes, and in particular the rate-limiting AA-NAT [27]. During the light phase of the daily photoperiod, the SCN electrical activity is high and, under these conditions, pineal NE release is low. During scotophase, the SCN activity is inhibited and pineal melatonin synthesis is stimulated by increases in NE [28]. Melatonin synthesis in the pineal gland is also influenced by neuropeptides, such as vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide and neuropeptide Y, which are partially coreleased and seem to potentiate the NE response [29]. Up-regulation of melatonin formation is complex and also involves AA-NAT activation by cAMP-dependent phosphorylation and AA-NAT stabilization by a 14-3-3 protein [30]. It is also subject, however, to feedback mechanisms by expression of the cAMP-dependent inducible 3′,5′-cyclic adenosine monophosphate early repressor and by $Ca^{2+}$-dependent formation of the downstream regulatory element antagonist modulator [29,30]. Once formed, melatonin is not stored within the pineal gland but diffuses out into the capillary blood and cerebrospinal fluid [31].

Although melatonin is synthesized in a number of tissues, circulating melatonin in mammals, but not all vertebrates, is largely derived from the pineal gland. Melatonin reaches all tissues of the body within a very short period [32,33]. Melatonin half-life is bi-exponential, with a first distribution half-life of 2 min and a second of 20 min [6]. Melatonin released to the cerebrospinal fluid via the pineal recess attains, in the third ventricle, concentrations up to 20–30 times higher than in the blood. These concentrations, however, rapidly diminish with increasing distance from the pineal [31], thus suggesting that melatonin is taken up by brain tissue. Melatonin production exhibits considerable interindividual differences [33]. Some subjects produce more melatonin during their lifetime than others, but

the significance of this variation is not known. Studies of twins suggest that these differences may have a genetic basis [34].

Circulating melatonin is metabolized mainly in the liver where it is first hydroxylated in the C6 position by cytochrome $P_{450}$ mono-oxygenases (isoenzymes CYP1A2, CYP1A1 and, to a lesser extent, CYP1B1) (Fig. 1) and thereafter conjugated with sulfate to be excreted as 6-sulfatoxymelatonin (aMT6S); glucuronide conjugation is extremely limited [6]. CYP2C19 and, at lower rates, CYP1A2 also demethylate melatonin to *N*-acetylserotonin, being otherwise its precursor [35]. The metabolism in extrahepatic tissues exhibits substantial differences. Tissues of neural origin, including the pineal gland and retina, contain melatonin-deacetylating enzymes, which are either specific melatonin deacetylases [36] or less specific aryl acylamidases; as eserine-sensitive acetylcholinesterase has an aryl acylamidase side activity, melatonin can be deacetylated to 5-methoxytryptamine in any tissue carrying this enzyme [36,37] (Fig. 1). Melatonin can be metabolized nonenzymatically in all cells, and also extracellularly, by free radicals and a few other oxidants. It is converted into cyclic 3-hydroxymelatonin when it directly scavenges two hydroxyl radicals [38]. In the brain, a substantial fraction of melatonin is metabolized to kynuramine derivatives [39]. This is of interest as the antioxidant and anti-inflammatory properties of melatonin are shared by these metabolites, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) [22,40,41] and, with considerably higher efficacy, $N^1$-acetyl-5-methoxykynuramine (AMK) [42–44]. AFMK is produced by numerous nonenzymatic and enzymatic mechanisms [1,5,41]; its formation by myeloperoxidase appears to be important in quantitative terms [45] (Fig. 2).

Inasmuch as melatonin diffuses through biological membranes with ease, it can exert actions in almost every cell in the body. Some of its effects are receptor mediated, while others are receptor independent (Fig. 3). Melatonin is involved in various physiological functions, such as sleep propensity [54–56], control of sleep/wake rhythm [56], blood pressure regulation [57,58], immune function [59–61], circadian rhythm regulation [62], retinal functions [63], detoxification of free radicals [64], control of tumor growth [65], bone protection [66] and the regulation of bicarbonate secretion in the GI tract [12].

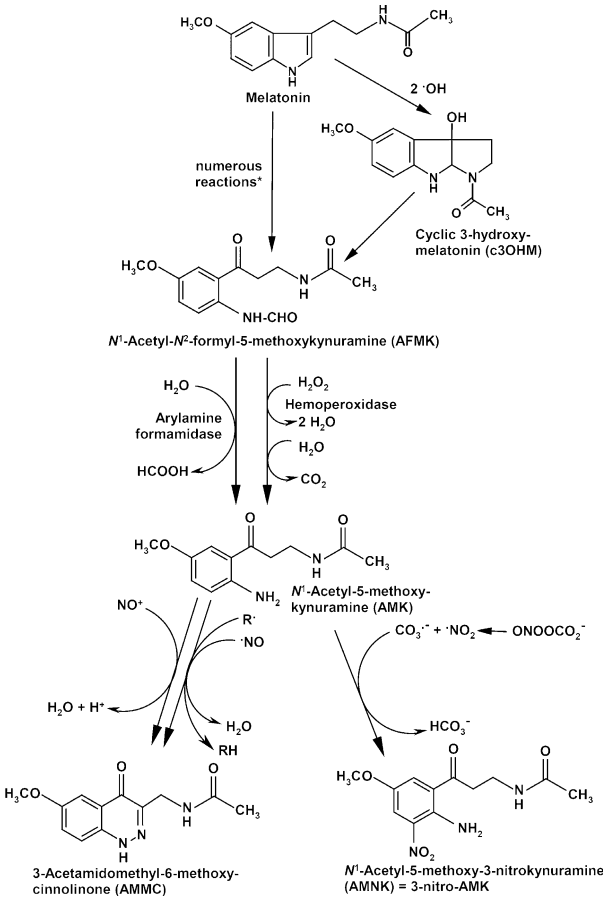


**Fig. 2.** The kynuric pathway of melatonin metabolism, including recently discovered metabolites formed by interaction of $N^1$-acetyl-5-methoxykynuramine (AMK) with reactive nitrogen species. *Mechanisms of $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK) formation [1,5,36,37,40,45–53]: (1) enzymatic: indoleamine 2,3 dioxygenase, myeloperoxidase; (2) pseudoenzymatic: oxoferryl-hemoglobin, hemin; (3) photocatalytic: protoporphyrinyl cation radicals + $O_3^{\bullet-}$, $O_2(1\Delta_g)$, $O_2$ + UV; (4) reactions with oxygen radicals: $\bullet OH + O_2^{\bullet-}$, $CO_3^{\bullet-} + O_2^{\bullet-}$; and (5) ozonolysis.

## Melatonin receptors, other binding sites and signaling mechanisms

Several major actions of melatonin are mediated by the membrane receptors $MT_1$ and $MT_2$ (Fig. 3) [94–96]. They belong to the superfamily of G-protein coupled receptors containing the typical seven transmembrane domains. These receptors are responsible for chronobiological effects at the SCN, the circadian pacemaker. $MT_2$ acts mainly by inducing phase shifts and $MT_1$ acts by suppressing neuronal firing activity. $MT_1$ and $MT_2$ are also expressed in peripheral organs and cells, and contribute, for example, to several immunological actions or to vasomotor control [97]. $MT_1$ seems to mediate mainly vasoconstriction, whereas $MT_2$ mainly causes vasodilation. A frequently observed primary effect is a $G_i$-dependent decrease in cAMP. In other effects, $G_o$ is involved. Decreases in cAMP can have relevant downstream effects, for

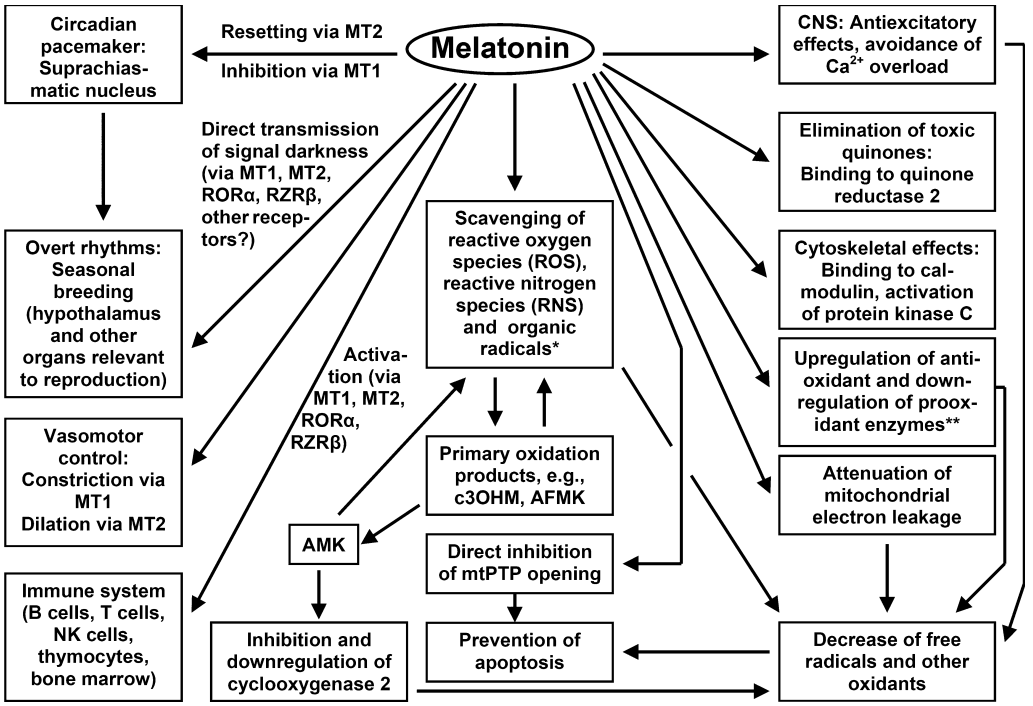


**Fig. 3.** The pleiotropy of melatonin: an overview of several major actions. AFMK, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine; AMK, $N^1$-acetyl-5-methoxykynuramine; c3OHM, cyclic 3-hydroxymelatonin; $MT_1$, $MT_2$, melatonin membrane receptors 1 and 2; mtPTP, mitochondrial permeability transition pore; RORα, RZRβ, nuclear receptors of retinoic acid receptor superfamily. *Several reactive oxygen species (ROS) scavenged by melatonin: •OH, $CO_3^{\bullet-}$, $O_2(^1\Delta_g)$, $O_3$, in catalyzed systems also $O_2^{\bullet-}$ species [1,5,36–38,40,46,49,51,52,67–72] reactive nitrogen species (RNS) scavenged by melatonin: •NO, $\bullet NO_2$ (in conjunction with •OH or $CO_3^{\bullet-}$), perhaps peroxynitrite ($ONOO^-$) [5,40,70,72–75]; organic radicals scavenged by melatonin: protoporphyrinyl cation radicals, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) cation radicals, substituted anthranylyl radicals, some peroxyl radicals [1,5,36,47,49,67]; radical scavenging by c3OHM, AFMK and AMK [38,40,41,47,49,76–78]. **Antioxidant enzymes up-regulated by melatonin: glutathione peroxidase (GPx) (consistently in different tissues), glutathione reductase (GRoad), γ-glutamylcysteine synthase, glucose 6-phosphate dehydrogenase [5,5,49,79–85]; hemoperoxidase/catalase, Cu-, Zn- and Mn-superoxide dismutases (SODs) (extent of stimulation cell type-specific, sometimes small) [5,49,83,84,86]; pro-oxidant enzymes down-regulated by melatonin: neuronal and inducible nitric oxide synthases [52,87–90], 5- and 12-lipoxygenases [91–93].

example on $Ca^{2+}$-activated $K^+$ channels [97]. A third binding site, initially described as $MT_3$, has been subsequently characterized as the enzyme quinone reductase 2 [98]. Quinone reductases participate in the protection against oxidative stress by preventing electron transfer reactions of quinones [99]. Melatonin also binds with relevant, but somewhat lower, affinities to calmodulin [100], as well as to nuclear receptors of the retinoic acid receptor family, RORα1, RORα2 and RZRβ [101,102]. RORα1 and RORα2 seem to be involved in some aspects of immune modulation, whereas RZRβ is expressed in the central nervous system, including the pineal gland. Direct inhibition of the mitochondrial permeability transition pore by melatonin [103] may indicate that another, mitochondrial-binding, site is involved, although at the present time this has not been confirmed. Although antioxidative protection by melatonin is partially based on receptor mechanisms, as far as gene expression is concerned some other antioxidant actions do not require receptors. These include direct scavenging of free radicals and electron exchange reactions with the mitochondrial respiratory chain (Fig. 3).

## Melatonin as an antioxidant

Since the discovery that melatonin is oxidized by photocatalytic mechanisms involving free radicals, its scavenging actions have become a matter of particular interest [1,37]. Melatonin's capability for rapidly scavenging hydroxyl radicals has stimulated numerous investigations into radical detoxification and antioxidative protection. Evidence has shown that melatonin is considerably more efficient than the majority of its naturally occurring analogs [46], indicating that the substituents of this indole moiety strongly influence reactivity and selectivity [5]. Rate constants determined for the reaction with hydroxyl radicals were

$1.2 \times 10^{10}$–$7.5 \times 10^{10}$ $M^{-1} \cdot s^{-1}$, depending on the method applied [67–69,104]. Regardless of the differences in the precision of determination, melatonin has been shown independently, by different groups, to be a remarkably good scavenger for hydroxyl radicals. Contrary to most of its analogs, melatonin is largely devoid of pro-oxidant side-effects (Fig. 3).

Contrary to initial claims in the literature that almost all melatonin is metabolized in the liver to aMT6S followed by conjugation and excretion, recent estimates attribute $\approx 30\%$ of overall melatonin degradation to pyrrole ring cleavage [45]. The rate of AFMK formation may be even higher in certain tissues because extrahepatic $P_{450}$ mono-oxygenase activities are frequently low and, consequently, smaller amounts of aMT6S are produced.

AFMK appears to be a central metabolite of melatonin oxidation, especially in nonhepatic tissues [5,47,49]. It should be noted that the kynuric pathway of melatonin metabolism includes a series of radical scavengers with the possible sequence of melatonin $\rightarrow$ cyclic 3-hydroxymelatonin $\rightarrow$ AFMK $\rightarrow$ AMK. In the metabolic steps from melatonin to AFMK, up to four free radicals can be consumed [47]. However, the complete cascade should be only expected under high rates of hydroxyl radical formation. Otherwise, melatonin forms AFMK directly and the conversion to AMK is, according to present knowledge, predominantly catalyzed enzymatically. Recent studies have shown a greater number of free radicals eliminated than predicted from the cascade, and many previously unknown products are now being characterized [77] (J. Rosen & R. Hardeland, unpublished results). The potent scavenger, AMK, consumes additional radicals in primary and secondary reactions [42,77]. Interestingly, AMK interacts not only with reactive oxygen but also with reactive nitrogen species [78].

Melatonin antioxidant capacity also includes the indirect effect of up-regulating several antioxidative enzymes and down-regulating pro-oxidant enzymes, in particular 5- and 12-lipo-oxygenases [91–93] and nitric oxide (NO) synthases [52,87–90] (Fig. 3). The attenuation of NO formation is significant as it limits the rise in the levels of the pro-oxidant metabolite, peroxynitrite, and of free radicals derived from this compound (i.e. $NO_2$, $CO_3^-$ and OH radicals). It also helps to reduce the inflammatory response [5].

Inasmuch as mitochondria are the major source of free radicals, the damage inflicted by these radicals contributes to major mitochondria-related diseases. Electron transfer to molecular oxygen at the matrix site, largely at the iron–sulphur cluster N2 of complex I, is a main source of free radicals [105]. This process also diminishes electron flux rates and therefore the ATP-generating potential. Melatonin increases mitochondrial respiration and ATP synthesis in conjunction with the rise in complex I and IV activities [106–109].

The effects of melatonin on the respiratory chain may represent new opportunities for the prevention of radical formation, in addition to eliminating radicals already formed. A model of radical avoidance, in which electron leakage is reduced by single electron exchange reactions between melatonin and the components of the electron transport chain, was proposed by Hardeland and his coworkers [53,110]. According to this model, a cycle of electron donation to the respiratory chain at cytochrome *c* should generate a melatonyl cation radical which can compete, as an alternate electron acceptor, with molecular oxygen for electrons leaking from N2 of complex I, thereby decreasing the rate of $O_2^-$ formation. In the proposed model, not only are electrons largely recycled to the respiratory chain, but most of the melatonin is also regenerated in the cycle. Inasmuch as the recycled electrons are not lost for the respiratory chain, the potential exists for improvements in complex IV activity, oxygen consumption and ATP production.

Similarly, the highly reactive melatonin metabolite, AMK, may undergo single-electron transfer reactions [42]. The mitochondrial protection by AMK was proposed [51] and experimentally confirmed [108]. In a manner similar to the action attributed to melatonin, AMK exerts its effects on electron flux through the respiratory chain and seems to improve ATP synthesis.

## Melatonin's antioxidant action: clinical significance

Neurodegenerative diseases are a group of chronic and progressive diseases that are characterized by selective and often symmetric loss of neurons in motor, sensory and cognitive systems. Clinically relevant examples of these disorders are Alzheimer's disease (AD), Parkinson's disease, Huntington's chorea and amyotrophic lateral sclerosis [111]. Although the origin of neurodegenerative diseases mostly remains undefined, three major and frequently inter-related processes (glutamate excitotoxicity, free radical-mediated nerve injury and mitochondrial dysfunction) have been identified as common pathophysiological mechanisms leading to neuronal death [85]. In the context of oxidative stress, the brain is particularly vulnerable to injury because it is enriched with phospholipids and proteins that are sensitive to oxidative damage and has a rather weak antioxidative defense system [112]. In the case of AD, the increase in β-amyloid protein- or peptide-induced

oxidative stress [113], in conjunction with decreased neurotrophic support [114], contributes significantly to the pathophysiology of the disease. AD has been also related to mitochondrial dysfunction [115]. Collectively, most evidence convincingly supports the notion that the neural tissue of AD patients is subjected to an increased oxidative stress [116,117]. Therefore, attenuation or prevention of oxidative stress by administration of suitable antioxidants should be a possible basis for the strategic treatment of AD.

Melatonin has assumed a potentially significant therapeutic role in AD inasmuch as it has been shown to be effective in transgenic mouse models of AD [118,119]. To date, this has to be regarded merely as a proof-of-concept rather than as an immediately applicable procedure. The brains of the AD transgenic mice exhibit increased indices of oxidative stress, such as accumulation of thiobarbituric acid-reactive substances, a decrease in glutathione content, as well as the up-regulation of apoptosis-related factors such as Bax, caspase-3 and prostate apoptosis response-4. The mouse model for AD mimics the accumulation of senile plaques, neuronal loss and memory impairment found in AD patients [120]. Melatonin administration decreased the amount of thiobarbituric acid-reactive substances, increased glutathione levels and superoxide dismutase activity, and counteracted the up-regulation of Bax, caspase-3 and prostate apoptosis response-4 expression, thereby significantly reducing oxidative stress and neuronal apoptosis [120]. Melatonin inhibited fibrillogenesis both *in vitro* [121] and at pharmacological concentrations in the transgenic mouse model *in vivo* [118]. Administration of melatonin to AD patients has been found to improve significantly sleep and circadian abnormality and generally to decelerate the downward progression of the disease [122–128]. It also slowed evolution of disease [122,123,127]. In the absence of any other therapies dealing with the core problem of AD, the potential value of melatonin urgently deserves further investigation.

Oxidative stress has been suggested as a major cause of dopaminergic neuronal cell death in Parkinson's disease [129]. Melatonin protects neuronal cells from neurotoxin-induced damage in a variety of neuronal culture media that serve as experimental models for the study of Parkinson's disease [85,117]. In a recent study, melatonin attenuated significantly mitochondrial DNA damage in the substantia nigra induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and its active metabolite, 1-methyl-4-phenylpyridine ion: free radical generation was reduced; and the collapse of the mitochondrial membrane potential and cell death were antagonized [130]. Administration of high doses of melatonin (50 mg per day) increased actigraphically scored total night-time sleep in parkinsonian patients [131].

## Melatonin as an oncostatic substance

There is evidence that tumor initiation, promotion and/or progression may be restrained by the night-time physiological surge of melatonin in the blood or extracellular fluid [65]. Numerous experimental studies have now provided overwhelming support for the general oncostatic effect of melatonin. When administered in physiological and pharmacological concentrations, melatonin exhibits a growth inhibitory effect in estrogen-positive, MCF human breast cancer cell lines. Cell culture studies have suggested that melatonin's effects in this regard are mediated through increased glutathione levels [65]. Melatonin also inhibits the growth of estrogen-responsive breast cancer by modulating the cell's estrogen signaling pathway [132]. Melatonin can exert its action on cell growth by modulation of estradiol receptor $\alpha$ transcriptional activity in breast cancer cells [133]. Another antitumor effect of melatonin, also demonstrated in hepatomas, seems to result from $MT_1/MT_2$-dependent inhibition of fatty acid uptake, in particular, of linoleic acid, thereby preventing the formation of its mitogenic metabolite, 13-hydroxyoctadecadienoic acid [65].

In several studies, melatonin has demonstrated oncostatic effects against a variety of tumor cells, including ovarian carcinoma cell lines [134], endometrial carcinoma [135], human uveal melanoma cells [136,137], prostate tumor cells [138] and intestinal tumors [139,140]. The concomitant administration of melatonin and cisplatinium etoposide increased both the survival and quality of life in patients with metastatic nonsmall cell lung cancer [141]. Melatonin not only exerts objective benefits concerning tumor progression, but also provides subjective benefits and increases the quality of life of patients by ameliorating myelotoxicity and lymphocytopenia associated with antitumoral therapeutic regimens [142]. Although melatonin is mostly anticarcinogenic and an inhibitor of tumor growth *in vivo* and *in vitro*, in some models it may promote tumor growth [143].

Oxidative stress has been implicated to participate in the initiation, promotion and progression of carcinogenesis [144]. In terms of reducing mutagenesis, the anticarcinogenic actions of melatonin are primarily attributed to its antioxidative and free radical scavenging activity [145]. Melatonin secretion is disturbed in patients suffering from various types of cancer [146,147]. To what extent the variations in melatonin

concentrations in cancer patients are causally related to the disease remains to be defined. The increased incidence of breast cancer or colorectal cancer seen in nurses engaged in night shift work suggests a possible link with the diminished secretion of melatonin associated with increased exposure to light at night [148]. This hypothesis received experimental support in a recent study [149]. Exposure of rats bearing rat hepatomas or human breast cancer xenografts to increasing intensities of white fluorescent light during each 12-h dark phase resulted in a dose-dependent suppression of nocturnal melatonin blood levels and a stimulation of tumor growth. Blask and coworkers [149] then took blood samples from 12 healthy, premenopausal volunteers. The samples were collected under three different conditions: during the daytime; during the night-time following 2 h of complete darkness; and during the night-time following 90 min of exposure to bright fluorescent light. These blood samples were then pumped directly through the developing tumors. The melatonin-rich blood collected from subjects while in total darkness severely slowed the growth of the tumors. The results are the first to 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 [149].

## Melatonin's immunomodulatory function

Studies undertaken in recent years have shown that melatonin has an immunomodulatory role. Maestroni and his coworkers first demonstrated that inhibition of melatonin synthesis results in the attenuation of cellular and humoral responses in mice [150]. Exogenous melatonin has been shown to counteract immunodeficiencies secondary to stress events or drug treatment and to protect mice from lethal encephalitogenic viruses [151]. Melatonin has also been shown to protect hematopoietic precursor cells from the toxic effect of cancer chemotherapeutic agents [152]. Melatonin enhances the production of interleukin (IL)-2 and IL-6 by cultured mononuclear cells [153] and of IL-2 and IL-12 in macrophages [154]. The presence of specific melatonin-binding sites in the lymphoid cells provides evidence for a direct effect of melatonin on the regulation of the immune system [155,156]. Melatonin's immuno-enhancing effect depends not only upon its ability to enhance the production of cytokines, but also upon its antiapoptotic and antioxidant actions [117]. Melatonin synthesized by human lymphocytes stimulates IL-2 production in an autocrine or a paracrine manner [15]. The nocturnal melatonin levels were found to correlate with the rhythmicity of T-helper cells [15]; indeed, melatonin treatment augmented the number of $CD4^+$ cells in rats [157]. Correlation of serum levels of melatonin and IL-12 in a cohort of 77 HIV-1-infected individuals has revealed that decreased levels of serum melatonin found in HIV-1-infected individuals can contribute to the impairment of the T helper 1 immunoresponse [158]. Inasmuch as melatonin stimulates the production of intracellular glutathione [81], its immuno-enhancing action may be partly a result of its action on glutathione levels.

The immuno-enhancing actions of melatonin have been confirmed in a variety of animal species and in humans [61,159]. Melatonin may play a role in the pathogenesis of autoimmune diseases, particularly in patients with rheumatoid arthritis who exhibit higher nocturnal serum melatonin levels than healthy controls [160]. The increased prevalence of auto-immune diseases at high latitudes during winter may be caused by an increased immunostimulatory effect of melatonin during the long nights [160]. It has been suggested that melatonin provides a time-related signal to the immune system [60]. In a recent study, melatonin implants were found to enhance a defined T helper 2-based immune response under *in vivo* conditions (i.e. the increase of antibody titres after aluminium hydroxide), thus demonstrating melatonin's potential as a novel adjuvant immunomodulatory agent [161].

## Melatonin as a hypnotic

Melatonin promotes sleep in diurnal animals, including healthy humans [162]. The close relationship between the nocturnal increase of endogenous melatonin and the timing of sleep in humans suggests that melatonin is involved in the physiological regulation of sleep [163–165]. The temporal relationship between the nocturnal increase of endogenous melatonin and the 'opening of the sleep gate' has prompted many investigators to propose that melatonin facilitates sleep by inhibiting the circadian wakefulness-generating mechanism [55,166]. $MT_1$ receptors present in SCN presumably mediate this effect.

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 [167]. Administration of melatonin (3 mg, orally) for up to 6 months to insomnia patients as an add-on to hypnotic (benzodiazepine) treatment augmented sleep quality and duration and decreased

sleep onset latency, as well as the number of awakening episodes in elderly insomniacs [168].

A reduced endogenous melatonin production seems to be a prerequisite for effective exogenous melatonin treatment of sleep disorders. A recent meta-analysis of the effects of melatonin in sleep disturbances, including all age groups (and presumably individuals with normal melatonin levels), failed to document significant and clinically meaningful effects of exogenous melatonin on sleep quality, efficiency or latency [169]. It must be noted that a statistically nonsignificant finding indicates that the alternative hypothesis (e.g. melatonin is effective at decreasing sleep onset latency) is not likely to be true, rather than that the null hypothesis is true (which in this case is that melatonin has no effect on sleep onset latency) because of the possibility of a type II error. By combining several studies, meta-analyses provide better size effect estimates and reduce the probability of a type II error, making false-negative results less likely. Nonetheless, this seems not to be the case in the study of Buscemi *et al.* [169], where sample size was constituted by less than 300 subjects. Moreover, reviewed papers showed significant variations in the route of administration of melatonin, the dose administered and the way in which outcomes were measured. All of these drawbacks resulted in a significant heterogeneity index and in a low quality size effect estimation (shown by the wide 95% confidence intervals reported) [169].

In contrast, another meta-analysis, undertaken by Brzezinski *et al.*, using 17 different studies involving 284 subjects, most of whom were older, concluded that melatonin is effective in increasing sleep efficiency and reducing sleep onset time [170]. Based on this meta-analysis, the use of melatonin in the treatment of insomnia, particularly in aged individuals with nocturnal melatonin deficiency, was proposed.

## Melatonin as a chronobiotic molecule

Melatonin has been shown to act as an endogenous synchronizer either in stabilizing bodily rhythms or in reinforcing them. Hence, it is called a 'chronobiotic' [171] (i.e. a substance that adjusts the timing or reinforces oscillations of the central biological clock). The first evidence that exogenous melatonin was effective in this regard was the finding that 2 mg of melatonin was capable of advancing the endogenous circadian rhythm in humans and producing early sleepiness or fatigue [172]. Lewy *et al.* [173] found an alteration of the dim light melatonin onset (i.e. the first significant rise of plasma melatonin during the evening, after oral administration of melatonin for four consecutive days). Since then, many studies have confirmed that exogenous melatonin administration changes the timing of bodily rhythms, including sleep, core body temperature, endogenous melatonin or cortisol [174]. Intake of 5 mg of fast-release melatonin, for instance, has been found to advance the timing of the internal clock up by $\approx 1.5$ h [175]. In a recent study, daily administration of a 'surge sustained' release preparation of 1.5 mg of melatonin phase-advanced the timing of sleep without altering the total sleep time [176], thereby showing that melatonin acts in this context on the timing mechanisms of sleep, rather than as a hypnotic.

The phase shifting effect of melatonin depends upon its time of administration. When given during the evening and the first half of the night, it phase-advances the circadian clock, whereas circadian rhythms during the second half of the night or at early daytime are phase delayed. The melatonin dose for producing these effects varies from 0.5 to 10 mg [173]. The magnitude of phase advance or phase delay depends on the dose [175]. Melatonin can entrain free-running rhythms, both in normal individuals and in blind people. As melatonin crosses the placenta, it may play an active role in synchronizing the fetal biological clock [6].

Phase-shifting by melatonin is attributed to its action on $MT_2$ receptors present in the SCN [177]. Melatonin's chronobiotic effect is caused by its direct influence on the electrical and metabolic activity of the SCN, a finding which has been confirmed both *in vivo* and *in vitro* [178]. The application of melatonin directly to the SCN significantly increases the amplitude of the melatonin peak, thereby suggesting that in addition to its phase-shifting effect, melatonin acts directly on the amplitude of the oscillations [178]. However, amplitude modulation seems to be unrelated to clock gene expression in the SCN [179].

## Implications of melatonin's chronobiotic actions in CRSD

A major CRSD is shift-work disorder. Human health is adversely affected by the disruption and desynchronization of circadian rhythms encountered in this condition [180,181]. The sleep loss and fatigue seen in night shift workers has also been found to be the primary risk factor for industrial accidents and injuries. Permanent night shift workers exhibit altered melatonin production and sleep patterns [182]. However, a number of studies indicate that many shift-workers retain the typical circadian pattern of melatonin production [183].

Shifting the phase of the endogenous circadian pacemaker to coincide with the altered work schedules of shift-workers has been proposed for improving

daytime sleep and night-time alertness. It has been found that night shift nurses who had the ability to shift the onset of nocturnal production to the new time schedule exhibited improved shift-work tolerance [184]. Research studies have suggested that melatonin monitoring and wrist actigraphy could be useful in resolving issues related to circadian adaptation to night shift work.

A number of studies have investigated melatonin's potential for alleviating the symptoms of jet lag, another CRSD. Melatonin has been found to be effective in 11 placebo-controlled studies for reducing the subjective symptoms of jet lag, such as sleepiness and impaired alertness [185]. The most severe health effects of jet lag occur following eastbound flights, because this requires a phase advancement of the biological clock. In a recent study, phase advancement after melatonin administration (3-mg doses just before bedtime) occurred in all 11 subjects traveling from Tokyo to Los Angeles as well as faster resynchronization compared with controls. Melatonin increased the phase shift from $\approx$1.1–1.4 h per day, causing complete entrainment of 7–8 h after 5 days of melatonin intake [186]. Melatonin has been found to be useful in causing 50% reduction in subjective assessment of jet lag symptoms in 474 subjects taking 5 mg of fast-release tablets [185]. Therefore, with few exceptions, a compelling amount of evidence indicates that melatonin is useful for ameliorating 'jet-lag' symptoms in air travelers (see the meta-analysis in the Cochrane database) [187].

One of us examined the timely use of three factors (melatonin treatment, exposure to light, physical exercise) to hasten the resynchronization in a group of elite sports competitors after a transmeridian flight across 12 time zones [188]. Outdoor light exposure and physical exercise were used to cover symmetrically the phase delay and the phase advance portions of the phase-response curve. Melatonin taken at local bedtime helped to resynchronize the circadian oscillator to the new time environment. Individual actograms performed from sleep log data showed that all subjects became synchronized in their sleep to the local time in 24–48 h, well in advance of what would be expected in the absence of any treatment [188]. More recently, a retrospective analysis of the data obtained from 134 normal volunteers flying the Buenos Aires to Sydney transpolar route in the last 9 years was published [189]. The mean resynchronization rate was 2.27 $\pm$ 1.1 days for eastbound flights and 2.54 $\pm$ 1.3 days for westbound flights. These findings confirm that melatonin is beneficial in situations in which re-alignment of the circadian clock to a new environment or to impose work–sleep schedules in inverted light/dark schedules is needed [181,190].

A number of clinical studies have now successfully made use of melatonin's phase-advancing capabilities for treating delayed sleep phase syndrome. Melatonin, in a 5-mg dose, has been found to be very beneficial in advancing the sleep-onset time and wake time in subjects with delayed sleep phase syndrome [191–193]. Melatonin was found to be effective when given 5 h before melatonin onset or 7 h before sleep onset.

Circadian rhythmicity is disrupted with ageing at various levels of biological organization [165,194]. Age-related changes in the circadian system result in a decreased amplitude of the circadian rhythm of sleep and waking in a 12 h light/12 h dark cycle, and phase advancement of several circadian rhythms. Melatonin administration in various doses (0.5–6.0 mg) has been found to be beneficial in improving subjective and objective sleep parameters [195]. The beneficial effects of melatonin could be a result of either its soporific or phase-shifting effects, or both. The efficacy of melatonin to entrain 'free running' circadian rhythms in blind people has also been demonstrated [196,197].

One seldom-considered possibility, concerning melatonin's mechanism of action, relates to its immunomodulatory properties. The linkage between sleep deprivation and susceptibility to illness has been commonly noted. Conversely, many infections cause increased somnolence. Whether the increased sleep associated with infections is just an epiphenomenon or is the result of the enhanced immune response is uncertain. Epidemiological studies have shown an association between increased mortality rates and sleep durations that are either longer or shorter than those seen in normals [198]. It seems now rather clear that cytokines released by activated immunocompetent cells during infections may affect sleep duration. Cytokines, including tumor necrosis factor, IL-1, IL-6 and interferons, may act as sleep inducers, while the anti-inflammatory cytokines tend to inhibit sleep [199]. Besides, the increased somnolence associated with acute infections seems to depend on cytokines, such as IL-1 and IL-6, that are also important for the physiological regulation of sleep. Thus, both the ability of melatonin to stimulate the production of inflammatory cytokines and to entrain circadian rhythms might be related somewhat to its sleep-facilitating properties.

## Melatonin in depression

A number of studies have shown altered melatonin levels in depressed patients. Melatonin studies in relation to patients with mood disorders have been

reported in numerous investigations [200]. In many of those studies, low melatonin levels occurred in patients with major depressive disorder, although increases in melatonin have also been documented [201,202]. Phase-shift of melatonin is a major feature of major depressive disorder, and low melatonin levels have been described as a 'trait marker' for depression [203]. Reduced amplitude of melatonin secretion was found in a group of bipolar depressive patients during the recovery phase [204]. Indeed, the amplitude of melatonin secretion has been suggested as 'state dependent' in bipolar patients [205]. It is interesting that male and female $MT_1$ knockout ($MT_1$–/–) mice tested in the acoustic startle/prepulse inhibition, open field and Porsolt forced swim tests displayed dramatically impaired prepulse inhibition in the acoustic startle response [206]. Both male and female $MT_1$–/– mice significantly increased the time spent immobile in the forced swim test, an indication of depressed-like behavior. Therefore, the lifetime lack of $MT_1$ signaling contributes to behavioral abnormalities, including impairments in sensorimotor gating and increases in depressive-like behaviors. $MT_1$ receptor signaling may be important for normal brain and behavioral function [206].

Treatment of patients with major depressive disorder with antidepressants indicates that plasma melatonin levels and urinary aMT6S excretion increase with improvement of the clinical state [207–209]. As melatonin has been used successfully in the treatment of CRSD [181], it has the potential value of being used as a therapeutic agent in the treatment of mood disorders. Melatonin treatment (3 mg) significantly improved sleep, but did not improve the clinical state of depressive disorders [210]. Agomelatine, an $MT_1/MT_2$ melatonin agonist and selective antagonist of 5-$HT_{2C}$ receptors, has been demonstrated to be active in several animal models of depression. In a double-blind, randomized multicenter multinational placebo-controlled study, including 711 patients suffering from major depressive disorder, agomelatine (25 mg) was significantly more effective (61.5%) than placebo (46.3%) in the treatment of major depression disease [211]. Recently, this finding has been confirmed by two more studies. The efficacy of agomelatine compared with placebo was noted after 6 weeks of treatment (at a dose of 25 mg per day) in patients with major depressive disorder who met Diagnostic and Statistical Manual of Mental Disorders, version IV (DSM-IV) criteria [212]. In another clinical study, agomelatine, at a dose of 25 mg per day, was found to be significantly better than placebo in treating not only depressive symptomatology but also in treating anxiety symptoms [213]. From these studies, it is evident that agomelatine has emerged as a novel melatonergic antidepressant and may have value for the treatment of depression.

## Melatonin in meditation

Apart from the regulatory effects of melatonin on the photoperiod, other less well-studied effects involve melatonin's influence on mental states. Romijn's suggestion that the pineal should be recognized as a 'tranquilizing organ' [214] is consistent with the well-documented sedating effects of melatonin. Two studies have demonstrated increases in overnight samples of urinary aMT6S [215] and in night-time plasma melatonin [216] following meditative practice. Psychosocial interventions may not only modulate melatonin levels, but may also be mediated by the hormone. In this context, the pineal can be understood as a psychosensitive organ. Meditation is considered to be an effective relaxation technique that has a greater benefit than other relaxation procedures [217]. The fact that the reported effects on various bodily symptoms of meditation and melatonin are similar prompted investigators to suggest that meditation exerts its beneficial effects by increasing melatonin secretion [215,216]. As psychosocial factors play a significant role in stress and stress-related health problems, influences of meditation on stress management, including benefits to the immune system and, perhaps, consequences for aging, and the development of cancer may be related to melatonin. The common effect of relaxation exerted by both meditation and melatonin is consistent with stress reduction observed after either intervention.

The link between meditation and increased melatonin secretion is not without controversy. No changes in melatonin levels were noted in a group of breast cancer and prostate cancer patients following meditation practice [218]. In other subjects, meditation decreased circulating melatonin (e.g. plasma melatonin was significantly reduced 3 h after morning meditation) [219]. The discrepancies found can be in part attributed to the time of melatonin measurement, in other words night [215,216] or morning [219] melatonin levels. This should be seen as a chronobiological effect, reflecting, perhaps, an increased circadian amplitude. Further studies are needed to substantiate the role of melatonin at the interface between psyche and soma.

## Clinical significance of GI melatonin

It is now known that melatonin is not only present [220], but also synthesized in the enterochromaffin cells

of the GI tract and can be released to the circulation, especially in response to food intake [12]. As noted above, the presence of melatonin in the GI tract is greater by orders of magnitude than in the pineal gland or in the circulation. In the intestine, melatonin has been demonstrated to increase duodenal mucosal secretion of bicarbonate through its action on the $MT_2$ receptor [221], this alkaline secretion being an important mechanism for duodenal protection against gastric acid. An inverse relationship between melatonin and the incidence of stomach ulcers has been observed in the stomach tissue and plasma of pigs [222]. Exacerbation of duodenal ulcers in human patients is correlated with low urinary melatonin levels [223]. The antioxidant action of melatonin has also been hypothesized to be one of the primary reasons for its gastroprotective efficacy [224]. Moreover, melatonin inhibits contraction of the smooth muscles of the stomach, ileum and colon [12]. Melatonin has also been detected at a high concentration in the bile (1000 times higher than its daytime concentrations in the blood); it has been hypothesized that melatonin in the bile prevents oxidative damage to the intestinal epithelium caused by bile acids [224].

## Melatonin in cardiovascular diseases

Studies undertaken in humans suggest that melatonin influences autonomic cardiovascular regulation [225–227]. Decreases in nocturnal serum melatonin concentration or in urinary aMT6S levels have been reported in patients with coronary heart disease [228–230] or cardiac failure [231]. Melatonin administration increases the cardiac vagal tone and decreases circulating NE levels [225,226].

Melatonin is effective at reducing blood pressure in hypertensive patients. In a double-blind, placebo-controlled study conducted on 14 normal healthy men, it was noted that the administration of 1 mg of melatonin reduced systolic, diastolic and mean blood pressure; NE levels also decreased following melatonin administration [226]. In another double-blind, placebo-controlled study, melatonin given orally (2.5 mg per day) for 3 weeks to patients with essential hypertension reduced significantly both systolic and diastolic blood pressure [58].

The hypotensive action of melatonin may involve either peripheral or central mechanisms. Melatonin's vasodilating action is supported by a decrease of the internal artery pulsatile index, which reflects the downstream vasomotor state and resistance [226]. In fact, vasoregulatory actions of melatonin are complex insofar as vasodilation is mediated via $MT_2$ receptors, whereas $MT_1$-dependent signaling leads to vasoconstriction [97]. The local balance between these receptors is obviously different, and constriction prevails in the cerebral vessels investigated to date. However, this effect is accompanied by a considerably enhanced dilatory response to hypercapnia [232]. The findings demonstrated that melatonin attenuates diurnal fluctuations in cerebral blood flow and diminishes the risk of hypoperfusion. The overall effect of melatonin on arterial blood pressure could be mediated centrally by mechanisms controlling the autonomic nervous system [227]. It has been suggested that the reduction of nocturnal blood pressure by repeated melatonin intake at night is attributable to its effect on amplification of the circadian output of the SCN [58]. The normalization of circadian pacemaker function in the regulation of blood pressure by melatonin treatment has been proposed as a potential strategy for the treatment of essential hypertension [233].

## Melatonin effects on bone

A direct osseous effect of melatonin has been demonstrated by the finding that it inhibits *in vitro* the increased calcium uptake in bone samples of rats treated with pharmacologic amounts of corticosterone [234]. A direct activity of melatonin was demonstrated in rat pre-osteoblast and osteoblast-like osteosarcoma cell lines [235]. In the presence of nanomolar concentrations of melatonin, pre-osteoblast cells underwent cell differentiation. After melatonin exposure, both cell lines showed an increased gene expression of bone matrix sialoprotein as well as other bone marker proteins, such as alkaline phosphatase, osteopontin and osteocalcin. In another study on human bone cells and osteoblastic cell lines exposed to melatonin, methoxyindole increased cell proliferation in a dose-dependent manner. In these cells, melatonin increased procollagen type Ic-peptide production without modifying alkaline phosphatase or osteocalcin [236]. Melatonin seems to cause inhibition of bone resorption and augmentation of bone mass by down-regulating receptor activator of nuclear factor κB-mediated osteoclast activation [237].

Osteoclasts generate high levels of superoxide anions during bone resorption and this may contribute to the degradative process. In view of the very strong antioxidative efficiency of melatonin and its metabolites for free radical scavenging, the effect of melatonin in preventing osteoclast activity in bone may depend, in part, on its antioxidant properties. The first indication that melatonin administration was effective for decreasing bone loss *in vivo* was obtained in ovariectomized rats [238]. In rats receiving melatonin in the

drinking water (25 $\mu g \cdot mL^{-1}$ water), a reduction in urinary deoxypyridinoline increase after ovariectomy (an index of bone resorption) was seen within 30 days after surgery, indicating a possible effect of melatonin in delaying bone resorption after ovariectomy. Subsequent studies corroborated the *in vivo* preventive effect of melatonin on bone loss [237,239–241].

The effect of melatonin on bone metabolism in ovariectomized rats receiving estradiol replacement therapy was also assessed [242]. Ovariectomy augmented, and melatonin or estradiol lowered, urinary deoxypyridinoline excretion. Moreover, the efficacy of estradiol to counteract ovariectomy-induced bone resorption was increased by melatonin. Therefore, postovariectomy disruption of bone remodeling could be prevented in rats by administering a pharmacological amount of melatonin (in terms of circulating melatonin levels), providing that appropriate levels of circulating estradiol were present [242].

Another line of evidence for a melatonin effect on the skeleton derived from studies on experimental scoliosis in animals. Scoliosis developed in pinealectomized chickens [243], with anatomical characteristics similar to those of human idiopathic scoliosis [244]. Pinealectomy induced malformation of the spine and reduced the mechanical strength of vertebrae in Atlantic salmon [245]. The possibility that melatonin and its receptors could be involved in hereditary lordoscoliosis in rabbits was also entertained [246]. Interestingly, serum melatonin levels in adolescents with idiopathic scoliosis were significantly lower than in controls [247].

Glucocorticoids (GC) are among the hormones that significantly affect bone remodeling. Prolonged exposure to GC at pharmacological concentrations induces osteoporosis associated with an increased risk of bone fracture [248–250]. The adverse effects of GC excess on the skeleton may be mediated by direct actions on bone cells, actions on extraskeletal tissues, or both [251]. While high doses or long-term GC therapy cause bone resorption and decrease bone mineral density [252,253], other studies demonstrated that GC treatment increased bone mass by a relatively greater suppression of bone resorption than of bone formation [254–256]. Thus, differences in steroid formulation, doses and duration of administration, as well as in the age and strain of the animals, may affect the final outcome of the treatments. In a recent study, the effect of melatonin (25 $\mu g \cdot mL^{-1}$ of drinking water, $\approx 500$ $\mu g$ per day) on a 10-week-long treatment of male rats with a low dose of methylprednisolone (5 $mg \cdot kg^{-1}$ subcutaneously, 5 days per week) was examined [257].

Bone densitometry and mechanical properties, calcemia, phosphatemia, serum bone alkaline phosphatase activity and C-telopeptide fragments of collagen type I were measured. Most densitometric parameters augmented after methylprednisolone or melatonin administration and, in many cases, the combination of corticoid and melatonin resulted in the highest values observed. Rats receiving the combined treatment showed the highest values of work to failure in femoral biomechanical testing. Circulating levels of C-telopeptide fragments of collagen type I, an index of bone resorption, decreased after melatonin or methylprednisolone, both treatments summating to achieve the lowest values observed [257]. The results were compatible with the view that low doses of methylprednisolone or melatonin decrease bone resorption and have a bone protecting effect.

## Melatonin's role in energy expenditure and body mass regulation

Melatonin is known to play a role in energy expenditure and body mass regulation in mammals [258]. Visceral fat levels increase with age, whereas melatonin secretion declines [125,229,259–263]. Daily melatonin supplementation to middle-aged rats has been shown to restore melatonin levels to those observed in young rats and to suppress the age-related gain in visceral fat [264,265]. In one of our laboratories, melatonin treatment prevented the increase in body fat caused by ovariectomy in rats [242]. In a study on melatonin or methylprednisolone, both treatments were effective at decreasing body weight in middle-aged rats through effects that summated when melatonin and methylprednisolone were conjointly administered. Melatonin's effects are partly mediated through $MT_2$ receptors present in adipose tissue [266].

In human adults, obesity is not accompanied by significant modifications of melatonin secretion [267]. In childhood and adolescence, significant changes in body composition take place. The possible correlation of obesity in prepubertal children and adolescents with melatonin secretion was recently examined by measuring diurnal, nocturnal and total melatonin secretion in 50 obese children and adolescents and 44 normal controls matched on age, gender and maturational stage [268]. Secretion of melatonin was assessed by measuring the 24 h urinary output of the predominant melatonin metabolite, aMT6S. A factorial ANOVA indicated that nocturnal aMT6S excretion and amplitude were significantly higher in the obese individuals. A significant interaction of weight and age was detected (i.e. the effect of weight was significant in the pubertal group only). Total nocturnal and diurnal aMT6S excretion was significantly higher in girls. Further

statistical analysis segregated by gender indicated that the increase in total and nocturnal aMT6S excretion and amplitude found in obesity occurred only in boys and at the pubertal age. Therefore, obese pubertal males have a greater urinary excretion of aMT6S and therefore a greater secretion of melatonin. The increase in melatonin in pubertal obese males might be one of the possible mechanisms accounting for delayed puberty in many of these subjects [268].

## Melatonin in reproduction and sexual maturation

Available evidence indicates that melatonin regulates the reproductive function in seasonal mammals by its inhibitory action at various levels of the hypothalamic–pituitary–gonadal axis. The pulsatile secretion of gonadotropin-releasing hormone (GnRH), from a small number of neurons in the hypothalamus, control luteinizing hormone and follicle-stimulating hormone secretion that, in turn, regulates the functional activity of gonads [269,270]. Melatonin has been shown to down-regulate GnRH gene expression in a cyclical pattern over a 24-h period [271]. Exposure of GT1-7 neurons of the hypothalamus to melatonin resulted in the down-regulation of GnRH mRNA levels, 12 h after exposure. Melatonin exerts its inhibitory effect by acting on G-protein coupled melatonin receptors $MT_1$ and $MT_2$ and nuclear orphan receptors ROR$\alpha$ and RZR$\beta$ [271].

Earlier studies have concluded that neurons found in the pre-optic area and/or the mediobasal hypothalamus and pituitary [272,273] are the main sites through which melatonin exerts its reproductive actions. Melatonin micro-implants in the area of pre-optic and mediobasal hypothalamus of mice caused complete gonadal involution [269]. $MT_1$ and $MT_2$ receptors are expressed in the pituitary gland where melatonin inhibits GnRH-induced calcium signaling and gonadotrophin secretion mainly in neonatal pituitary cells [274].

In women, an influence of melatonin on reproductive function can be inferred from the studies indicating high melatonin levels in hypothalamic amenorrhea, which would support a casual relationship between high melatonin concentration and hypothalamic–pituitary–gonadal hypofunction [275]. Normal melatonin rhythms are closely related to those of reproductive hormones during infancy and reciprocally correlated during puberty. The demonstration of melatonin receptors in reproductive organs [276,277], and the localization of sex hormone receptors in the pineal gland [278–281], further support the inference that melatonin plays an important role in these inter-relationships.

In seasonal breeders, reproductive performance is timed by variations in the photoperiod [282], effects that are mediated by corresponding changes in melatonin [283,284]. Whether melatonin suppresses gonadal functions, as in many rodents, or stimulates them, depends on the species-specific season of reproduction. In sheep and ewes, gonadal activity is initiated during the fall and is inhibited during summer. Melatonin exerts a stimulatory effect on the reproductive axis in this species [285]. It mediates the influence of photoperiod on luteinizing hormone pulsatile secretion. Removal of the pineal gland disrupts the photoperiod-induced reproductive responses to seasonal changes in the duration of night and day [286]. Insertion of melatonin implants in the form of slow-release capsules has been shown to be effective at increasing sheep production and in promoting fur growth. Administration of melatonin induces the same effects as photoperiodic changes on seasonal reproduction. In ewes, the summer melatonin pattern entrains the circannual reproductive rhythm, whereas the winter pattern does not [287].

Melatonin may mediate the moderate seasonal fluctuations observed in the human reproductive function [288,289]. The increased conception rate seen in northern countries during the summer season has been reported to be caused by changes in luteinizing hormone and melatonin secretion in these individuals. The nocturnal plasma melatonin concentration on day 10 of the menstrual cycle has been found to be higher in winter than in summer, whereas plasma luteinizing hormone levels are higher in summer than in winter [290]. Although humans are not seasonal breeders, seasonal changes in reproductive performance do occur and melatonin secretion may be involved.

Melatonin has been implicated in sexual maturation. Melatonin exerts an inhibitory role on the hypothalamus and on pubertal maturation. The decline of serum melatonin below a threshold value ($\approx 115$ pg·mL$^{-1}$) may constitute the activating signal for the hypothalamic pulsatile secretion of GnRH and subsequent onset of pubertal changes [291]. The hypothalamic–pituitary–gonadal axis, which is already active during fetal life, remains quiescent until the age of $\approx 10$ years and is reactivated again at this time with the increase in the amplitude and frequency of GnRH pulses. Stimulating the pulsatile secretion of luteinizing hormone and follicle-stimulating hormone is crucial for pubertal changes and therefore the decline in melatonin concentration below the threshold value is very important for the initiation of puberty. Support for this has been obtained from clinical studies. Children with precocious puberty have lower nocturnal serum melatonin levels [292]. On the other hand, children with delayed

puberty exhibit higher nocturnal melatonin concentrations [268,293]. In a case of hypothalamic hamartoma (a benign malformation of the brain), decreased secretion of melatonin, together with precocious puberty, has been found [294]. The decreased secretion of melatonin was attributed to the bulk of hamartoma tissue interrupting the neural connection between SCN and the pineal gland. The low concentration of melatonin would result in premature activation of the hypothalamic GnRH secretion and the occurrence of precocious puberty [294]. Recent studies on neonatal gonadotrophs show that the tonic inhibitory effects of melatonin on GnRH-induced calcium signaling and gonadotrophin secretion provide an effective mechanism for protecting premature initiation of pubertal changes. The inhibitory effects of melatonin on GnRH action gradually decline as a result of decreased expression of functional melatonin receptors [274].

## Conclusions

Melatonin is distributed widely in nature, ranging from unicellular organisms, plants, fungi and animals to humans. It acts as a photoperiod messenger molecule, transducing photoperiod changes to reproductive organs, and plays a vital role in the seasonal control of reproduction in certain animals. Melatonin participates in reproductive function by acting at hypothalamic, pituitary and gonadal levels. Melatonin may have a significant role in the onset of human puberty. Melatonin can be used as a chronobiotic that is capable of normalizing the disturbed bodily rhythms, including sleep–wake rhythms. It has been found to be effective in treating CRSD and is very helpful in treating subjects suffering from shift-work disorder. Melatonin is implicated in mood disorders. Changes in the amplitude and phasing of the melatonin rhythm have been described in patients with major depressive, bipolar affective and seasonal affective disorders. The melatonin agonist, agomelatine, has been found to be effective in causing clinical remission in patients with major depressive and bipolar disorders. Melatonin may mediate some of the tranquillizing effects of meditation, thereby acting at the interface between psyche and soma. Melatonin synthesis is not restricted to the pineal gland, but also takes place in other areas such as the eye, lymphocytes, gut, bone marrow, skin, and gonads where it acts in a paracrine or an autocrine manner. The presence of melatonin in the GI tract suggests that it has a protective role in this organ system. Melatonin reduces the systolic, diastolic and mean blood pressure of hypertensive patients. Melatonin has significant bone-protecting properties and plays a role in energy expenditure and body mass regulation. Melatonin has been demonstrated as an efficient antioxidant under both *in vivo* and *in vitro* conditions. Not only melatonin, but also the kynuric pathway of melatonin, provides a series of radical scavengers. Melatonin up-regulates antioxidative enzymes, such as glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase. At the mitochondria, melatonin reduces radical formation and increases complex I and complex IV activities, thereby maintaining the proton potential and enhancing mitochondrial respiration and ATP synthesis. The complex pattern of protective actions may turn out to be of major clinical significance, for example in retarding the progression of neurodegenerative diseases such as AD or Parkinson's disease. The antitumor effects of melatonin seem to be exerted at multiple levels, from modulation of the glutathione system to interference with lipid mediators and receptors of other hormones. The immunoenhancing actions of melatonin, in conjunction with its antioxidant properties, suggest a therapeutic value in a variety of diseases, including bacterial and viral infections.

In comparison with other signaling molecules, the numerous actions that have been attributed to melatonin are exceptional. This should be taken as an expression of its overall importance as a modulator at various levels of hierarchy. The practical applicability of melatonin, however, remains unconfirmed inasmuch as most of the effects described have not been demonstrated at clinically relevant concentrations. Moreover, a pleiotropic agent may have side-effects, which, to date, have still not been investigated in detail. For instance, an immunoenhancing substance may not be beneficial in patients afflicted by an autoimmune disease. On the other hand, pure preparations of melatonin have usually been remarkably well tolerated. It will be an important matter of future research to investigate the clinical efficacy and safety of melatonin in detail, under different pathological situations.

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