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COMMENTARY

Antioxidant Properties of Melatonin—An Emerging Mystery

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ABSTRACT. Over three centuries ago, the French philosopher René Descartes described the pineal gland as "the seat of the soul." However, it was not until the late 1950s that the chemical identity and biosynthesis of melatonin, the principal hormone secreted by the pineal body, were revealed. Melatonin, named from the Greek *melanos*, meaning black, and *tonos*, meaning color, is a biogenic amine with structural similarities to serotonin. The mechanisms mediating the synthesis of melatonin are transcriptionally regulated by the photoperiodic environment. Once synthesized, the neurohormone is a biologic modulator of mood, sleep, sexual behavior, reproductive alterations, immunologic function, and circadian rhythms. Moreover, melatonin exerts its regulatory roles through high-affinity, pertussis toxin-sensitive, G-protein (or guanine nucleotide binding protein) coupled receptors that reside primarily in the eye, kidney, gastrointestinal tract, blood vessels, and brain. Additional evidence also indicates a role for melatonin in aging and age-related diseases, probably related to its efficient free radical scavenger (or antioxidant) activity. The potential clinical benefit of melatonin as an antioxidant is remarkable, suggesting that it may be of use in the treatment of many pathophysiological disease states including various cancers, hypertension, pulmonary diseases, and a variety of neurodegenerative diseases such as Alzheimer's disease. This review summarizes the biosynthesis of melatonin and its many endocrine and physiological functions, including its therapeutic potential in human disease states. Emphasis is placed on the recent speculations indicating that this pineal hormone serves as an endogenous antioxidant agent with proficient free radical scavenging activity. BIOCHEM PHARMACOL **56**;10:1265–1272, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. melatonin; melatonin receptors; free radicals; antioxidants; aging; neurodegenerative diseases

Yu and Reiter [1] report that greater than three centuries ago, the French philosopher René Descartes described the pineal gland as "the seat of the soul." However, it was not until the late 1950s that studies revealed the chemical identity and biosynthesis of melatonin, the principal substance secreted by the pineal gland [2]. Today, melatonin is known as a stringent biological modulator of mood, sleep, retinal physiology, sexual behavior, seasonal-reproductive physiology and behavior, circadian rhythms, and immunologic function [2–5]. Moreover, there is experimental evidence that reflects the influence of melatonin on aging and age-related processes and disease states [6]; these roles for melatonin appear to be related to its capability as a potent free radical scavenger [7]. This paper reviews the biosynthesis of melatonin and the therapeutic potential of this hormone in human pathophysiology. In particular, the recent speculations that melatonin may serve as an antioxidant agent with efficient free-radical scavenger activity will be investigated.

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PHYSIOLOGY, SYNTHESIS, AND METABOLISM OF MELATONIN

The pineal gland secretes an indoleamide called melatonin, so named because it has the ability in certain fish, reptiles, and amphibians to temporarily turn the skin a dark color (produced by the chemical pigment melanin) [8]. In the human brain, the pineal gland is located on the dorsal surface of the hypothalamus, occupying a central position between the two cerebral hemispheres [1]. The highly vascularized tissue of the pineal gland consists of two types of cells: pinealocytes and neuroglia. In humans, the former predominate and produce both melatonin and peptides such as arginine-vasotocin [2]. The latter is a phylogenetically ancient nonapeptide hormone related to the neurohypophyseal hormones oxytocin and arginine-vasopressin (antidiuretic hormone). This, together with the anatomical presence of the pineal gland in phylogenetically ancient species, indicates a persistent role for the gland throughout vertebrate evolution.

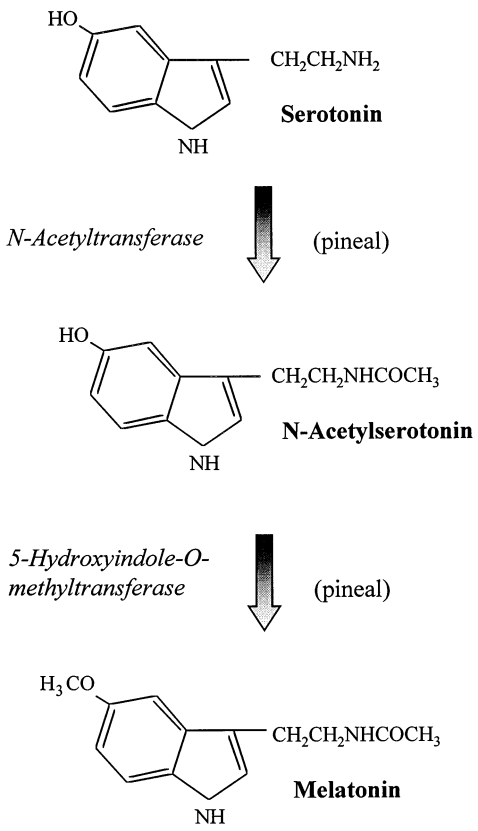

FIG. 1. Biosynthesis of melatonin. Melatonin is synthesized from serotonin, the conversion being regulated by norepinephrine. The primary sites of the greatest synthesis of melatonin in mammals are the pineal gland and the retina [1].

Melatonin, or *N*-acetyl-5-methoxytryptamine, is a biogenic amine with structural similarities to 5-HT.§ Melatonin is synthesized from 5-HT via acetylation reactions catalyzed by NAT and 5-hydroxyindole-*O*-methyltransferase (Fig. 1) [9]. NAT is also known as arylalkylamine *N*-acetyltransferase or serotonin *N*-acetyltransferase. Recent evidence indicates that NAT activity is regulated by specific cAMP-dependent transcription factors, which ultimately regulate the oscillatory synthesis of melatonin [10]. Moreover, the melatonin biosynthetic pathway is regulated by the photoperiodic environment, such that in the presence of light, neuronal impulses originating in the retina are transmitted to the SCN of the hypothalamus and other hypothalamic structures. From the SCN, a projection descends to the intermediolateral cell column in the upper thoracic region of the spinal cord. From the spinal cord, fibers project to the superior (third) cervical ganglion with postganglionic sympathetic fibers terminating at the pineal gland [11]. With the onset of darkness (in humans), these sympathetic fibers release norepinephrine, which binds predominantly to β1-adrenergic receptors (and to a lesser extent to α1-adrenergic receptors) to initiate the intracellular release of stored serotonin and NAT [2]. This cascade of neuronal and intrapineal biochemical signals facilitates melatonin anabolism in humans and is responsible for the circadian synthesis and release of melatonin (Fig. 1).

With the onset of darkness, concentrations of melatonin in the pineal gland increase, and the hormone passively diffuses into the cerebrospinal fluid and the circulation. In humans, the secretion of melatonin peaks in the middle of the night between 2:00 a.m. and 4:00 a.m., and gradually declines thereafter throughout the following 24-hr period [12]. Serum melatonin concentration varies remarkably according to age, with newborns secreting very little. However, concentrations begin to rise very shortly after birth and continue to increase with age. Melatonin levels peak in humans between the ages of 1 and 3 years, after which serum concentrations have been reported to gradually fall, as its secretory pattern becomes circadian during puberty and adolescence [12]. These circadian pulses of melatonin appear to remain consistent until the mid-20s and then slowly begin to decline with age until the late sixth decade of life is reached. Additionally, Humbert and Pevet [13] have shown that human subjects over the age of 60 express significantly lower day and evening levels of melatonin, indicating an association between physiological melatonin concentrations and aging.

Melatonin is rapidly metabolized, primarily in the liver, to 6-hydroxymelatonin. A series of reactions next yields *N*-acetyl-5-methoxy-6-hydroxytryptamine that, depending upon the chemical environment, is conjugated to either sulfate or glucuronide (Fig. 2). In humans, as in the rat, 6-sulfatoxymelatonin (or 6-hydroxymelatonin sulfate) metabolite levels in urine likely represent an important index of pineal function, which can be detected readily by radioimmunoassay [1]. In the retina of non-mammalian species such as *Xenopus laevis*, an alternative metabolism of melatonin exists, initially with deacetylation to 5-methoxytryptamine (Fig. 2). Once synthesized and released, the pineal hormone has been shown to regulate a variety of organ systems in different species. The organ-specific effects of the pineal hormone are mediated via interactions with specific melatonin receptors.

MELATONIN RECEPTORS AND MECHANISMS OF ACTION

Two pharmacologically distinct families of membrane-bound melatonin receptors known as ML1 and ML2 have been identified. Using the potent melatonin agonist 2-[^{125}I]iodomelatonin, the ML1 receptors have been shown to be high-affinity receptors with a K_d of ~75 pM in chick and rabbit retina [14]. In contrast, the ML2 receptors have a lower affinity for the same melatonin receptor agonist

§ *Abbreviations*: 5-HT, serotonin, (5-hydroxytryptamine); ALS, amyotrophic lateral sclerosis; cAMP, cyclic adenosine monophosphate; CAT, catalase; cGMP, cyclic guanosine monophosphate; G-protein, guanine nucleotide binding protein; G_i-protein, inhibitory guanine nucleotide binding protein; IL, interleukin; K_d, equilibrium dissociation constant; LOO·, peroxyl radical; ML1, melatonin receptor family type 1; ML2, melatonin receptor family type 2; NAT, *N*-acetyltransferase; NO·, nitric oxide radical; $O_2^{\cdot-}$, superoxide anion radical; ·OH hydroxyl radical; ONOO·, peroxynitrite radical; PI, phosphoinositide; SCN, suprachiasmatic nuclei; and SOD, superoxide dismutase.

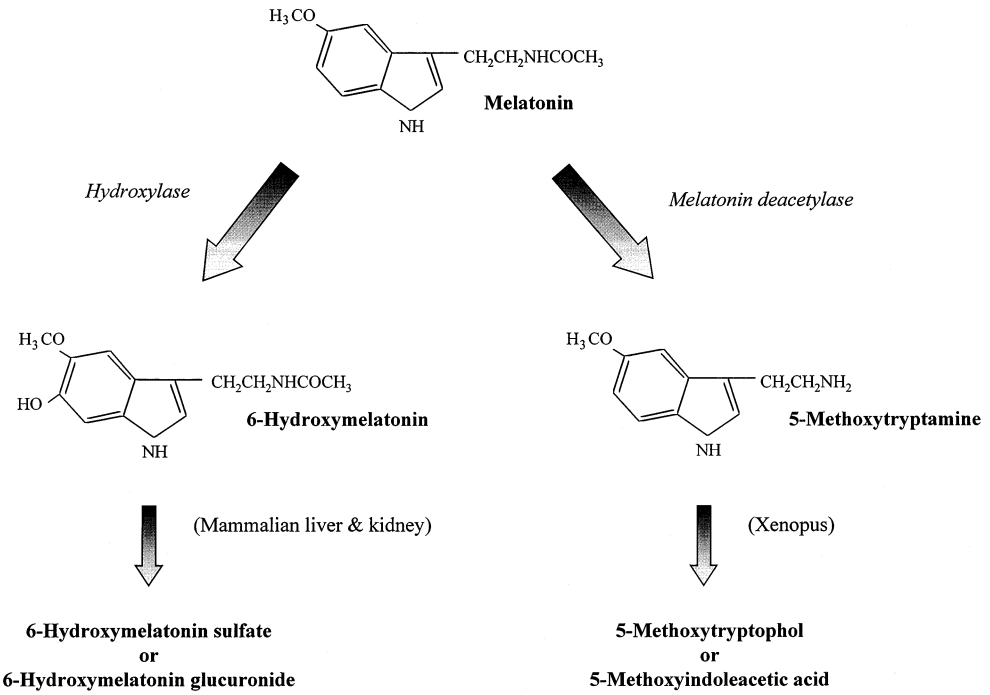

FIG. 2. Metabolism of melatonin. The two major biochemical pathways for inactivation of melatonin are shown. Catabolic inactivation in both mammalian and non-mammalian species occurs in the liver and kidney, with a secondary deacetylation pathway present only in non-mammalian retina.

(K_d > 2 nM in hamster brain; > 1 nM in human brain) [14]. The genes encoding the human melatonin receptors, although on different chromosomes, appear to be quite similar. In particular, both receptor genes exhibit a conserved site that allows DNA encoding of the first cytoplasmic loop, which indicates that other forms of the receptors may exist as consequences of alternative splicing [14].

Polymerase chain reaction experiments have yielded three kinetically distinct subtypes of ML1 receptors, currently known as Mel1a (K_d, 20–40 pM), Mel1b (K_d, 160 pM), and, more recently, Mel1c (K_d, 20–60 pM) [14]. It is believed that the ML1 receptor subtypes are involved in renal function [15], sleep induction, circadian rhythms, reproduction [14], and the contractility of cerebral arteries (via attenuation of Ca^{2+}-dependent, large-conductance K^+ channels located in those vessels) [16]. Furthermore, all ML1 subtypes have also been implicated in the Ca^{2+}-dependent release of dopamine in the mammalian retina, as well as various light-dependent processes such as phagocytosis of retinal photopigment discs [2, 14]. ML1 receptor activation results in the inhibition of adenylyl cyclase, and consequently decreases in 3′,5′-cAMP levels, via its coupling to a G_i-protein [2, 14, 17]. Moreover, only the Mel1a receptor has been shown to induce a parallel signal-transduction pathway that stimulates arachidonate release via a potentiation of phospholipase activation [18]. In addition, the Mel1c receptor has been shown to exert a modulatory influence on intracellular concentrations of 3′,5′-cGMP levels [19], the only melatonin receptor subtype known to affect this particular cyclic nucleotide.

The Mel1a receptor was originally cloned using a cDNA library combination of *Xenopus* dermal melanophore cell lines and a cloning strategy for mammalian cell expression [14]. The human Mel1a receptor expresses a greater than 80% amino acid sequence similarity with other mammalian Mel1a receptors; this may be compared to a 60% sequence similarity with frog melatonin receptors. Currently, it is thought that the inhibitory influence of melatonin on the SCN (which may serve to entrain the "biological clock" of the body [20]) and its circadian and reproductive effects are mediated via the Mel1a receptor [14]. The Mel1b receptor has an amino acid sequence 60% identical to that of the human Mel1a receptor [14]. This subtype of melatonin receptor has been shown, in part, to regulate the melatonin-induced entrainment of mammalian circadian rhythms [20], a clinically important feature of exogenously administered melatonin in the management of jet lag. The third type of melatonin receptor to be cloned, Mel1c, has been found only in chickens, *Xenopus*, and zebra fish [14]. Current data suggest that the Mel1c receptor exerts functional and pharmacological characteristics similar to those of both mammalian Mel1a and Mel1b receptors, but to date a human Mel1c receptor has been identified only in an embryonic kidney (HEK293) cell line [14].

Low-affinity ML2 receptors are believed to be coupled to G-proteins that mediate signal transduction in a fashion similar to that of the ML1 receptors [17]. However, agonist binding studies suggest that ML2 receptors are distinct in that they are linked to PI hydrolysis that may be reversed upon ML2 selective antagonist administration [21]. Interestingly, prazosin, a potent α1-adrenergic antagonist, also has high affinity for ML2 receptors (but not ML1) and

effectively attenuates melatonin-induced PI hydrolysis. However, the nonselective α-adrenergic antagonist phentolamine and the relatively non-selective 5-HT antagonist methysergide have no effect on PI hydrolysis evoked by melatonin [22]. The physiological importance of the ML2 receptors remains undetermined, but, with future molecular approaches, a better understanding of their function and distribution may be reported.

Radioligand binding studies using 2-[^{125}I]iodomelatonin and *in situ* hybridization have shown that the pertussis toxin-sensitive, G-protein-coupled ML1 receptors reside predominately in neuronal regions including the cerebellum and hippocampus [23], as well as in the SCN, hypothalamus, thalamus, preoptic area, the plexiform layer of the retina, and many areas of the cerebral cortex [14]. Non-neuronal ML1 receptors are found in the cerebral and caudal arteries, the hypophyseal pars tuberalis, ovary, kidney, and small intestine [2, 14, 15]. In contrast, ML2 receptors have no well characterized specific distribution in mammalian tissues [2, 17].

Recent reports further indicate that melatonin may readily diffuse into cells to activate intracellular sites through binding to cytosolic calmodulin [2]. Once bound to calmodulin, melatonin can influence calcium signaling through interactions with downstream effector enzymes such as adenylyl cyclase and phosphodiesterases, as well as associations with tubulin and microtubules [2, 7]. Melatonin is also known to interact with a nuclear family of orphan receptors known as retinoid-Z receptors, indicating that melatonin, once in the nucleus, may conceivably regulate gene expression and also function as a free radical scavenger [2, 7]. The diffuse distribution and regulatory functions of the different melatonin receptors provide multiple possibilities for melatonin, endogenous or exogenous, to regulate a wide variety of biochemical and physiological processes.

CLINICAL APPLICATIONS OF MELATONIN

It is quite apparent that the hormonal role for melatonin is functionally related to neuroendocrine physiology, with a strong association also existing between its secretion and the normal occurrence of sleep and (other) circadian rhythms. The onset of normal sleep is characterized by a rapid increase in melatonin concentrations, and it is believed that the overall quality of sleep is contingent upon the natural oscillating pattern of melatonin secretion [13]. In this regard, it is interesting to note that the quality of sleep is profoundly diminished in most elderly subjects, when the circadian phase secretion of melatonin is increasingly blunted. In young adults, exogenous melatonin has been shown to cause a significant increase in sleep propensity and the duration of rapid-eye-movement (REM) sleep [24]. This relationship has contributed to the recently increased public interest in melatonin as a sleeping aid and as a supplement to combat jet lag.

During development, melatonin is also of importance, such that the circadian release of melatonin has been shown to play a role in developing chicken spinal cord. Wan *et al.* [25] have shown a direct action of melatonin on spinal cord function at early stages in chick development. Thus, the emerging circadian pattern of melatonin secretion early on in human life may also indicate a regulatory influence in development. Consequently, the unborn fetus may be exquisitely sensitive to exogenous melatonin during early maturation and development. In further relation to this, melatonin has been found to increase the release of human growth hormone from the pituitary gland [26]. The precise role that melatonin has in regulating growth hormone secretion and early human development is not well understood and requires further investigation.

Circadian rhythms function to associate the internal milieu with the external environment, which is set by the earth's revolution, but is subject also to seasonal variations. Thus, by temporally organizing many physiological and endocrine systems within the circadian, sleep/wake cycle, the body is presumed to generate organized and efficient control mechanisms over a variety of physiological functions. Thus, diseases such as seasonal affective disorder and (circadian-based) sleeping disorders that are intimately associated with these control mechanisms may be amenable to treatment with melatonin, or receptor-specific agonist or antagonist drugs, to reset or regain control in relation to the circadian period. It is early days in our understanding of the role(s) of the various melatonin receptors, but the obvious relationship between melatonin and various aspects of circadian rhythmicity suggests a great deal of hope for the discovery of a variety of therapeutic possibilities.

It is noteworthy that melatonin is chemically closely related to 5-HT, and some melatonin metabolites may exert actions at various 5-HT receptors, including the 5-HT$_2$ receptor family. Although current data do not suggest a direct interaction between melatonin and 5-HT$_{2A}$ receptors [27], biochemical and behavioral evidence does indicate potential heteroreceptor or allosteric interactions between melatonin and 5-HT$_{2A}$ receptors in the mammalian central nervous system [27]. The importance of 5-HT$_{2A/C}$ receptors in the regulation of mood, perception, and other "higher" central nervous system functions suggests that melatonin-like drugs acting at melatonin and/or 5-HT$_{2A/C}$ receptors may, one day, find use in the treatment of psychiatric disorders, such as seasonal affective disorder, depression, and schizophrenia.

Exogenously administered melatonin has a serum half-life of 0.5 to 5.6 min with few or no reported side-effects [2]. In view of its extensive regulatory repertoire, as discussed herein, melatonin administration may be beneficial in treating human diseases ranging from tumors to circadian-based sleeping disorders. However, the short half-life of the parent molecule suggests that more stable (and selective) analogs may be required for the management of such medical conditions. Melatonin is currently under investigation in a wide range of biomedical research areas including, but not limited to, heart disease [28], Alzheimer's

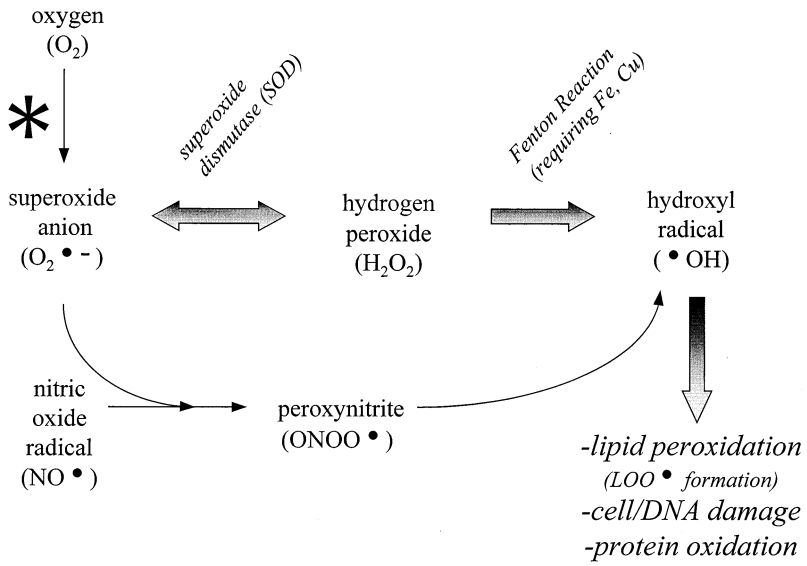

FIG. 3. Generation of oxygen free radicals. Molecular oxygen (O_2) is monovalently reduced to a superoxide radical ($O_2^{\cdot -}$). This occurs when oxygen accepts electrons from various donors including the cytochrome P450 reductase system, xanthine oxidase, semiquinones, and NADPH oxidases found in phagocytic cells (represented by *). Once formed, the superoxide anion radical ($O_2^{\cdot -}$) can react with the nitric oxide radical ($NO^{\cdot}$) to form the peroxynitrite radical ($ONOO^{\cdot}$) and eventually produce a hydroxyl radical ($^{\cdot}OH$) [44]. Alternatively, the superoxide anion radical can be converted to H_2O_2 by an extremely rapid reaction catalyzed by SOD. Cells have an endogenous defense mechanism that detoxifies H_2O_2 to either water or oxygen and water via GSH peroxidase or CAT, respectively. However, when these enzymes are deficient, H_2O_2 undergoes a Fenton reaction to produce the highly toxic $^{\cdot}OH$.

disease [29], AIDS [30], diabetes [31], depression [32], cancer [33], and environmental hazards [34]. Research showing that melatonin protected human white blood cells against radiation damage [35] and prevented cataracts in animals [36] already indicates potential clinically applicable benefits. Furthermore, melatonin shows even greater promise when used in combination with current therapies for many drug-resistant cancers and AIDS [34].

Plasma melatonin concentrations have been shown to increase following hemorrhage in adult mice [37], although it is not known if this is a component of the "stress response" or one specifically related to the alterations in blood volume and/or tissue osmolality. Regardless, melatonin administration has been found to restore the immune dysfunction resulting from soft-tissue trauma and hemorrhagic shock [38]. These positive effects of melatonin on macrophage IL-1 and IL-6 release, as well as splenocyte IL-2 and IL-3 release, may be related to the purported beneficial effects of melatonin adjunct therapy in cancer, AIDS, and other disease states involving altered immunologic competence. It is not known whether the immuno-restorative properties of the hormone are directly related to its free radical scavenging activity, but it is clear that the global antioxidant actions of the hormone are, in themselves, of great importance and interest, and of likely therapeutic utility.

MELATONIN AS AN ANTIOXIDANT

Oxygen comprises 21% of the earth's atmosphere [39] and is essential for the life of aerobic organisms. However, a major paradox is that oxygen, in certain states, can be lethal to the body tissue of organisms that depend on it for life. The majority of oxygen inspired is used to generate energy in the form of ATP [39, 40]. However, a relatively large amount—approximately 5%—of inspired oxygen is converted to free radicals, many of which are extremely toxic [7]. A certain cause of age-related destruction of neuronal tissue is directly attributed to the formation of toxic, free radicals of oxygen.

Radicals are formed from molecules that possess an unpaired electron in their orbitals [7, 39–42]. Oxygen qualifies as a radical because it possesses two unpaired electrons, each in a different orbital but both maintaining a parallel spin [39, 40]. These unpaired electrons allow oxygen to readily accept electrons, thus causing its powerful oxidizing characteristics. However, the electrons that oxygen accepts must maintain a spin in the opposite direction (or antiparallel spin) to the electrons already existing in a given orbital [39, 40]. In maintaining this spin conversion while accepting electrons, oxygen is monovalently reduced to $O_2^{\cdot -}$. The $O_2^{\cdot -}$, in the presence of the enzyme SOD, can be converted to the reactive intermediate H_2O_2. In most cells, particularly those of the central nervous system, H_2O_2 is detoxified by CAT and GSH peroxidase. However, in the presence of transition metal ions such as copper (Cu^{2+}) or iron (Fe^{2+}) (or quinones), H_2O_2 may undergo a "Fenton" reaction to yield the extremely toxic $^{\cdot}OH$ [41, 43]. Alternatively, $O_2^{\cdot -}$ may interact with $NO^{\cdot}$ to form $ONOO^{\cdot}$ that will ultimately yield $^{\cdot}OH$ [44]. These conversion processes are illustrated in Fig. 3 [41, 43, 44].

Free radicals have been shown to act as signal transduction mediators at subtoxic levels [43]; however, their usual tissue destructive actions and DNA damaging characteristics may prove to be life-threatening in most situations. In humans, $^{\cdot}OH$ is potently cytotoxic [43, 45] and has been shown to interact with proteins, phospholipids, nucleic acids, and sugars to produce irreversible damage. Reiter [39] refers to the damage produced by free radicals as oxidative stress. Clinically, oxidative stress is proposed as the cause for tissue damage in many pathophysiological disorders such as hypoxia, inflammation, and tissue ischemia and reperfusion [41, 45]. Other prominent theories regarding free radicals provide for oxidative stress being responsible for

the neurological dysfunction associated with neurodegenerative diseases including idiopathic Parkinson's disease, ALS, Alzheimer's disease, and others [41]. Furthermore, free radicals and oxidative stress have also been implicated in the normal aging process [39, 46–48].

It should not go unrecognized that the body has developed a system to help limit the formation of free radicals, this being known as the antioxidant defense system [7]. Enzymes such as CAT and GSH peroxidase (Fig. 3) help to limit the formation of free radicals, as well as neutralizing them once they are formed. There are also several molecules that directly scavenge free radicals, including many ingested antioxidants such as vitamins C and E as well as β-carotene [7]. Of particular relevance to this review, recent research has revealed that melatonin, both endogenous and exogenous, may help to neutralize free radicals before they can exert their destructive activity [49–59].

Melatonin is highly lipophilic [50] and, when administered exogenously, the hormone can readily pass across the blood–brain barrier to access neurons and glial cells [39]. The ability of melatonin to diffuse into intracellular compartments aids in the capabilities of the hormone as a possible antioxidant, first confirmed by Tan *et al.* [51]. In their initial studies published in 1993, Tan's group utilized high performance liquid chromatography and electron spin resonance spectroscopy to quantify the capacity of melatonin to effectively scavenge the free radical $^{\cdot}OH$. This experiment yielded remarkable evidence, suggesting that melatonin has a 5-fold greater efficiency in neutralizing $^{\cdot}OH$ than the endogenous antioxidant GSH, as well as a 15-fold higher effective scavenger rate than the exogenous scavenger mannitol. These substantial initial findings prompted further research to elucidate the antioxidant properties of melatonin.

In subsequent studies, Pieri *et al.* [50] reported another noteworthy finding adding to melatonin's accumulating reputation as a powerful free-radical scavenger. A fluorescent assay, utilizing β-phycoerythrin as a fluorescent indicator protein, which loses its ability to fluoresce when oxidized by oxygen radicals, was employed to compare the effectiveness of various antioxidants to that of melatonin. Again, the lipid-soluble melatonin was found to have a greater activity than the known potent antioxidants GSH and vitamins C and E. These initial experiments revealed that melatonin is a 2-fold greater $LOO^{\cdot}$ scavenger than vitamin E, a notorious lipid-soluble antioxidant, under *in vivo* conditions [50]. $LOO^{\cdot}$ is formed during the radical-induced breakdown of cellular polyunsaturated fatty acids, a self-propagating process known as lipid peroxidation [39]. In view of the above-discussed role of free radicals in a range of toxic events associated with degenerative disease states, the evidence for melatonin as a scavenger of both $^{\cdot}OH$ and $LOO^{\cdot}$ provides intriguing opportunities for the medical industry.

To quantify the clinical benefits of melatonin as an antioxidant, Tan *et al.* [49, 51] demonstrated that melatonin protected hepatic nuclear DNA from free radical damage induced by the chemical carcinogen safrole. Initially, rats were injected with a high dose of safrole (300 mg/kg) followed by one of two doses of melatonin (0.2 or 0.4 mg/kg). The low dose, at nearly 1500 times less than the administered dose of safrole, produced a decrease in DNA damage of about 50% as measured by thin-layer chromatography. The second, larger dose of melatonin produced a nearly complete abolition of the carcinogenicity of safrole as measured by hepatic DNA damage [51]. Since the doses of melatonin used were only 5-fold higher than "normal" over-the-counter doses of melatonin (0.5 to 5 mg), which yield high physiological concentrations of melatonin in the plasma, the study supports the suggestion that melatonin may represent an endogenous component of the body's natural antioxidant defense mechanisms. Moreover, melatonin may act prophylactically against free radical-induced cancer, neurodegeneration, and aging.

The observations of protection from free radical damage by melatonin reflect the ability of the hormone to efficiently scavenge those radicals that modify the normal DNA configuration and ultimately produce the oxidative stress implicated in numerous pathophysiological disease states. Further evidence of the ability of melatonin to scavenge free radicals was elegantly demonstrated by surgical removal of the pineal gland, which further increased nuclear DNA damage [49]. This important experiment revealed that not only is melatonin an important antioxidant when administered exogenously at pharmacological doses, but also that physiological melatonin concentrations are sufficient to partially reduce the destruction of DNA by free radicals [49]. Furthermore, Pappolla *et al.* [56] found that melatonin is remarkably effective in preventing death of neuroblastoma cells induced by oxidative stress and glutamate excitotoxicity. They report that physiologically relevant doses of melatonin promote cell viability via prevention of oxidative damage caused by both free-radical formation and increased intracellular calcium levels related to glutamate excitotoxicity. Furthermore, the speculation that melatonin is an effective scavenger of H_2O_2, lipid peroxides, and peroxynitrites has been substantiated by several groups [52, 54, 55, 57–59] and suggests that melatonin has a potential physiological role in facilitating the prevention of age-related neurodegeneration, DNA damage, and cell death.

It has been suggested that melatonin, of both endogenous and exogenous origin, exerts its effects via inhibition of the multifunctional oxidase cytochrome P450 [49]. However, this does not appear to be entirely the case. Reiter [7] has shown that melatonin potentiates free radical scavenging by a non-enzymatic process of electron donation. These observations reflect the ability of melatonin itself to act as a free radical, known as the indolyl or melatonyl cation radical [7]. This radical is far less potent than the free radicals it neutralizes *in vivo*. Regardless of how melatonin inhibits the effects of free radicals, the potential clinical applications of this characteristic of the hormone are noteworthy. For example, the formation of DNA adducts

represents a critical step in the initiation of cancer formation [51]. It is known that carcinogens express themselves by directly generating free radicals from oxygen species that cause an immediate destruction or modification of cellular DNA. Thus, the ability of exogenous, and partly endogenous [49], melatonin to suppress the development of carcinogenic processes suggests that melatonin, as a strong component of the antioxidant defense system, helps to prevent carcinogenesis. Furthermore, research indicating that aging is a result of oxidative stress also implies that administration of exogenous melatonin may help to combat the damage incurred due to oxidative stress, thus aiding in the prevention of various negative aspects of the aging process.

CONCLUSION

The biosynthesis of melatonin is largely regulated by the photoperiodic environment. Although the circadian and endocrine effects of melatonin are moderately well understood, the potential role that melatonin plays as a free radical scavenger is only beginning to surface. As our geriatric population continues to grow, so does the incidence of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, the primary dementia in modern society. Furthermore, as people age, they become increasingly susceptible to the mutagenic and carcinogenic properties of free radicals. With no definitive treatment regimens available for such patients, it is potentially of great medical interest to develop pharmacological strategies for preventing, decelerating the progression, or even potentially curing people of these diseases. Melatonin itself appears to be promising as a combatant of such neurodegenerative processes. The age-dependent decreases in melatonin secretion observed in the elderly may play a permissive role in the development of various neurodegenerative and carcinogenic processes. It is evident that the precise antioxidant effects of melatonin need to be fully elucidated; until that time, the full potential therapeutic benefits of melatonin must remain something of a mystery.

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