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Melatonin: clinical relevance

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This chapter reviews the neural connections between the retinas and the pineal gland and summarizes the role of the light:dark cycle and the biological clock, i.e. the suprachiasmatic nuclei, in regulating pineal melatonin synthesis and secretion. The cellular mechanisms governing the nocturnal production of melatonin are described together with the way in which the misuse of light interferes with the circadian melatonin cycle and the total quantity of the indole generated. The chapter describes the nature of the membrane melatonin receptors and their signal transduction mechanisms in peripheral organs. The clinical implications and potential uses of melatonin in terms of influencing the biological clock (e.g. sleep and jet lag), immune function, and cancer initiation and growth are noted. Additionally, the chapter includes a description of the newly discovered free radical scavenging and antioxidant activities of melatonin; it also includes a list of clinical situations in which melatonin has been used with beneficial effects.

Key words: melatonin; pineal gland; oxidative stress; sleep; jet lag; circadian rhythms; cancer; light:dark cycle.

Melatonin (*N*-acetyl-5-methoxytryptamine) was characterized after its isolation from bovine pineal tissue by Lerner et al[1,2] roughly 50 years ago; this indole is now known to be the major secretory product of the pineal gland in all mammals, including man. The impetus for the characterization of melatonin was the fact that earlier studies in tadpoles had shown that it is a potent skin-lightening agent, i.e. it inhibits the dispersion of melanin in epidermal melanocytes. Shortly after its discovery, melatonin was tested for this property in humans and found to be ineffective in this regard.

In the intervening years since its structural identification, melatonin has been functionally linked in various species to the regulation of circadian[3] and seasonal[4] rhythms, immune function[5], retinal physiology[6], tumour inhibition[7] and, most recently, it has been found to be a free radical scavenger and antioxidant.[8,9] The review of the literature that follows discusses the regulation of melatonin synthesis, its mechanisms of action at the peripheral level and the clinical implications of these findings.

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REGULATION OF PINEAL FUNCTION

Unlike classical endocrine organs, the pineal gland in general, and melatonin synthesis in particular, is not markedly influenced by hormones from other ductless glands or cells. Rather, the major regulator of melatonin production is the prevailing light:dark environment. In this regard, the pineal gland is an end organ of the visual system not unlike the visual cortex.[10] Only during darkness at night does the pineal gland produce melatonin in abundance. What this means, of course, is that information about light perception at the level of the retinas must be transferred to the pineal gland. This is accomplished by a series of neurons that originate in the retinas and eventually end in the pineal gland.

The classical photoreceptor cells, i.e. the rods and cones, seem not to be involved in light perception that modulates pineal melatonin production. Rather, there are specialized neurons which contain a unique photopigment in the retina that respond to light.[11,12] This information is transduced into a neural message which is transferred to the anterior hypothalamus via axons of retinal ganglion cells in the optic nerve; this is part of the so-called retino-hypothalamic tract. In the hypothalamus, the axons from the retina terminate in the suprachiasmatic nuclei (SCN), a type of nucleus whose neurons exhibit inherent circadian electrical rhythms; these nuclei constitute the biological clock or the central circadian pacemaker.[13] Between the SCN and the pineal gland, the neural pathways, at least centrally, are somewhat less defined but are believed to be as follows: SCN, paraventricular nuclei, intermediolated cell columns of the upper thoracic cord (preganglionic sympathetic neurons), superior cervical ganglia (postganglionic sympathetic neurons), pineal gland (Figure 1). This circuitous pathway conveys information about the light:dark environment to the pineal gland and thereby determines the melatonin synthesis cycle.

The regulation of melatonin production in the pineal gland has been defined in significant detail.[14,15] The primary neurotransmitter released from the postganglionic sympathetic terminals that terminate in the pineal gland is norepinephrine (NE)

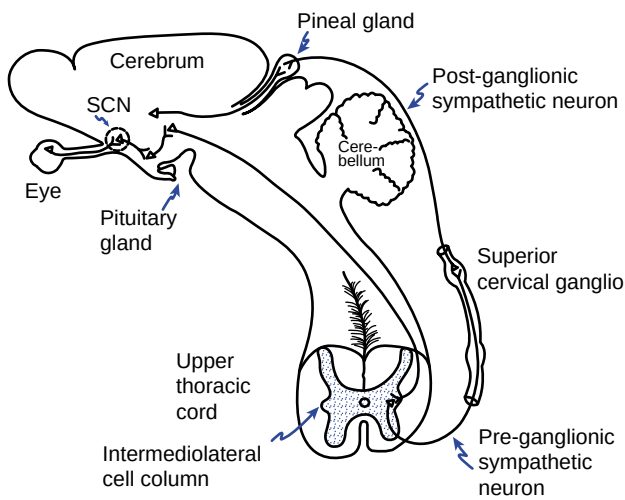

Figure 1. The circuitous neural connections between the eyes and pineal gland involve neurons in both the central and peripheral sympathetic nervous system. Interruption of the innervation to the pineal gland, for example, by superior cervical ganglionectomy, renders the pineal non-functional.

(noradrenalin); during darkness at night, NE is discharged onto the pinealocytes, the endocrine cells of the gland, where it couples especially to beta-adrenergic receptors. This leads to a marked rise in intracellular cAMP levels, to de novo protein synthesis and eventually to the stimulation of the rate-limiting enzyme in melatonin production, arylalkylamine-*N*-acetyltransferase (AA-NAT). AA-NAT N-acetylates serotonin to *N*-acetylserotonin (NAS), the immediate precursor of melatonin (Figure 2). Once generated, NAS is quickly *O*-methylated, a step catalyzed by hydroxyindole-*O*-methyltransferase (HIOMT); this reaction requires the transfer of a methyl group from 5-adenosylmethionine to the 5-hydroxy group of NAS. The dramatic rise in AA-NAT drives melatonin synthesis and, consequently, the melatonin content of the pineal increases substantially at night.

Unlike other endocrine organs, the pineal does not store melatonin for later release after it is synthesized. Rather, melatonin quickly diffuses out of the pinealocytes into the rich capillary bed[16] within the gland and possibly directly into the cerebrospinal fluid (CSF) of the third ventricle.[17] As a result, blood and CSF levels rise at night and the concentration of melatonin in these fluids is generally accepted as an index of its concurrent synthesis within the pineal gland; circulating nocturnal levels of melatonin are commonly 10–20 times higher than concentrations measured during the day.

The amount of melatonin produced in the pineal is genetically determined. Among individuals of the same age, the night-time rises in blood melatonin concentrations vary widely. Thus, while some individuals exhibit what is considered to be a robust nocturnal increase in blood nocturnal melatonin concentrations, in others the amplitude of the peak may be severely attenuated. Given that the amplitude of the melatonin rhythm is repeated with great fidelity from night to night, clearly some individuals over the course of their lifetime produce much more melatonin than others. The significance of these marked differences in the lifetime quantities of melatonin generated by the pineal gland remain unknown.

The introduction of artificial light has significantly compromised the quantity of melatonin the human pineal gland produces inasmuch as light is used indiscriminately during the normal periods of darkness. Light at night prevents the SCN from signalling the pineal gland to activate the molecular machinery to produce melatonin. For example, while the light:dark cycle at the equinoxes is 12:12 (in h), the actual duration of darkness humans witness is usually significantly less. As a result, the use of artificial light (sometimes referred to as the misuse of light) truncates the period of melatonin synthesis to an interval shorter than it would normally be, thereby limiting the total amount of melatonin produced.[18] In this case, light acts a 'drug' to reduce melatonin levels.[19] Light exposure has two basic functions on the melatonin synthesis cycle: acute light exposure at night (even of very short durations) inhibits melatonin production while alternating periods of light (and darkness) serve to synchronize the melatonin rhythm to 24 h. When these regularly alternating periods of light:dark are disturbed, so too is the rhythm of the biological clock, i.e. the SCN, and the melatonin synthesis cycle. The degree of inhibition of melatonin synthesis by mistimed light depends on its wavelength, intensity and the circadian phase at which the exposure occurs.

A second major factor that influences the quantity of melatonin synthesized in the pineal gland is age.[20] While the melatonin rhythm described above is typical of young to middle-aged individuals, in elderly humans, the amplitude of the nocturnal melatonin increase can be severely attenuated although there seem to be significant variations among individuals in the rate at which melatonin is lost.[21] The gradual waining of the synthetic capability of the pineal gland probably relates to several factors, including a reduction of the number of beta-adrenergic receptors on the pinealocyte membranes,

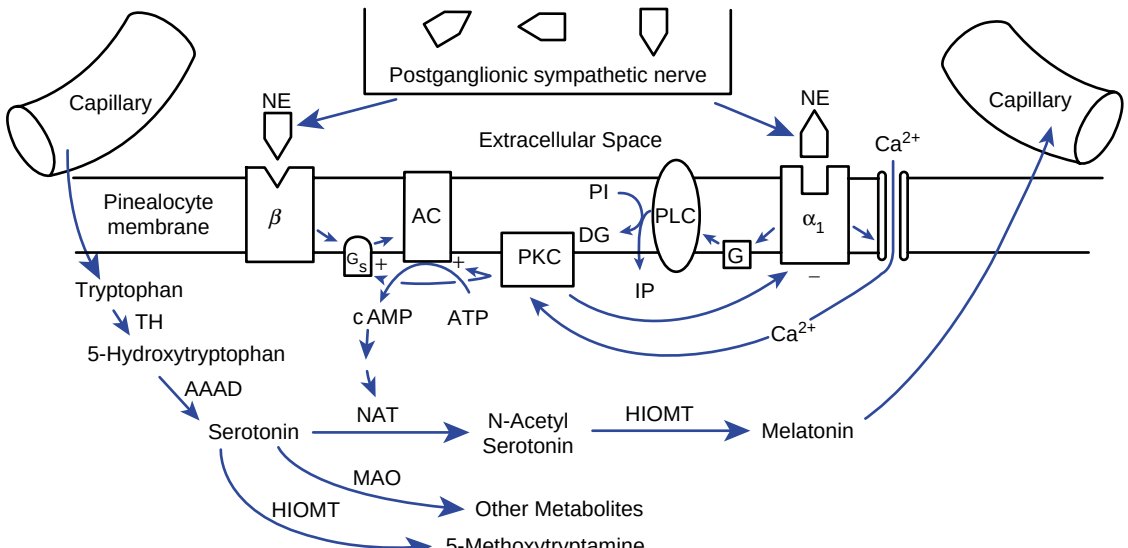

Figure 2. Interactions of NE (noradrenalin) released from postganglionic sympathetic fibres with beta-adrenergic receptors in the pinealocyte membrane. This interaction initiates a series of intracellular events which culminate in a large rise in the acetylation of serotonin to *N*-acetylserotonin by the enzyme *N*-acetyltransferase (NAT). Once produced, melatonin is quickly discharged into the capillary bed in the pineal gland and possibly directly into the CSF of the third ventricle.

deterioration of the melatonin synthetic machinery within the pineal gland and a progressively weakening signal from the SCN.

MELATONIN METABOLISM IN PERIPHERAL TISSUES

In the liver, melatonin undergoes 6-hydroxylation followed by conjugation, primarily to sulphate.[14] The resulting product, 6-hydroxymelatonin sulphate, is excreted in the urine as a major melatonin metabolite. The quantity of this product in the urine is greater at night than during the day, reflecting the pineal melatonin synthesis and secretion cycle.

Melatonin is also converted, presumably in all cells, to cyclic 3-hydroxymelatonin.[22] This metabolite is formed when melatonin directly scavenges two hydroxyl radicals and it can likewise be measured in the urine where the quantity is proportional to the degree of oxidative stress an individual has experienced.[20]

SITES OF MELATONIN ACTION

Melatonin has a variety of means by which it influences the physiology of the organism; some of these actions are receptor-mediated while others are receptor-independent. Besides its classical endocrine effects, melatonin has autocrine and paracrine actions and also functions as a direct scavenger of free radicals.[23]

Membrane melatonin receptors were initially defined on the basis of their pharmacological and kinetic characteristics. The known melatonin receptors have seven transmembrane domains and they are in the superfamily of G-protein-coupled receptors.[24] Two receptor subtypes with high affinity for melatonin have been cloned and characterized in a variety of animals/tissues, including in the human SCN; according to the IUPHAR classification, these receptors are identified as MT1 and MT2.[25] A third high-affinity membrane melatonin receptor has been cloned but it is yet to be found in mammals. The likelihood is great that other receptors and/or isoforms that bind melatonin will be uncovered.

The distribution of the known melatonin receptors is remarkably widespread in mammalian tissues, although less is known of their localization in humans. Those mammalian tissues in which melatonin receptors are most consistently found include the SCN[26] and the pars tuberalis[27] of the adenohypophysis but, in reality, as data continue to accumulate, it seems that few tissues are devoid of membrane receptors, for melatonin.

The signal transduction pathways for the known melatonin receptors have been described. The cloning of melatonin receptor cDNA resulted in the development of cell lines in which MT1 or MT2 recombinant receptors were expressed. When the human melatonin receptors (hMT1 and hMT2) were cloned and expressed in a variety of cells, they were found to inhibit forskolin-stimulated cAMP. Activation of recombinant hMT1 receptors induces a variety of cellular responses that are mediated by both pertussis (PTX)-sensitive and PTX-insensitive G-proteins. The recombinant hMT2 receptor is also coupled to inhibition of adenylyl cyclase activity via a PTX-sensitive G-protein. The significant pharmacology of the membrane melatonin receptors is under intense investigation.

Given melatonin's high lipophilicity and the ease with which it enters cells, investigations into potential intracellular binding sites have also been initiated.

Melatonin has been shown to bind calmodulin in the cytosol,[28] and nuclear binding sites or receptors have been pharmacologically characterized in white blood cells of humans.[29] The role of these binding sites in determining the day-to-day actions of melatonin remains to be fully defined. Additionally, within cells, melatonin functions as a direct scavenger of free radicals, and as an indirect antioxidant.[30,31] The former of these activities is a receptor-independent function of the indole and the direct scavenging effects of melatonin, which do not require receptor mediation, have been shown to exist in cell membranes, in the cytosol and in the nucleus.

CLINICAL ASPECTS OF MELATONIN

Chronobiotic effects

Melatonin acts at the level of the SCN to modulate its activity and influence circadian rhythms. Properly timed melatonin administration shifts circadian rhythms, facilitates re-entrainment to a novel light:dark cycle and alters the metabolic activity of the central circadian pacemaker, i.e. the SCN. The direction in which melatonin phase-shifts the circadian system depends on its time of administration. When given late in the subjective day (at dusk), melatonin phase advances the clock while its administration early in the subjective day (at dawn) phase delays circadian rhythms. Likewise, the ability of melatonin to re-entrain rhythms after a phase advance of the light:dark cycle, for example, travelling from the USA to Europe, is also time-of-day dependent; thus, taking melatonin at the time of the former dark onset (in the USA) reduces the rate of re-entrainment, while melatonin administration at the new dark onset (in Europe) hastens the rate of re-entrainment.[32] The chronobiotic effects of melatonin explain its benefit in reducing the severity and/or duration of jet lag[33] and in promoting restful sleep.[34]

Melatonin's influence on sleep processes has been widely investigated and the relationships seem to be highly complex. There is a common misconception that pineal melatonin synthesis at night requires sleep; this is not the case. The only requirement for increased melatonin production is darkness at night. Conversely, the night-time rises in circulating levels of melatonin seem to promote sleep onset and maintain restful sleep in some individuals. Currently, there is general agreement that melatonin is probably not a direct soporific or hypnotic. Rather, the most commonly proposed mechanisms for melatonin to induce sleepiness relates to its effects on the circadian clock, i.e. it 'opens the sleep gate'[35] and also it slightly reduces body temperature[36] which promotes sleep. Melatonin has these effects over a wide range of doses from physiological (250 μg) to pharmacological (1–10 mg) levels. When used to improve sleep, i.e. to decrease sleep latency and/or cause more prolonged sleep, it is taken roughly 30 min prior to bedtime. Melatonin has been successfully used with various degrees of effectiveness to enhance sleep processes in elderly individuals with insomnia and in individuals with restless leg syndrome, REM sleep disorder behaviour, delayed sleep phase syndrome, manic patients with insomnia and in patients with fibromyalgia.[37]

Oncostatic effects

A considerable amount of evidence has been amassed which documents the efficacy of melatonin in reducing tumour growth. While the bulk of these data have accurred from studies on experimental animals[7,38], trials in humans[39] with a wide variety of different cancers are also suggestive of the oncostatic actions of melatonin.

As with the sleep-promoting function of melatonin, the concentrations of the indole that reduce cancer cell proliferation, tumour growth and the incidence of metastases vary from physiological to pharmacological. If, in fact, physiological levels of melatonin normally restrain tumour growth, the age-associated reduction in melatonin production may be contributory to the increased frequency of cancer in the elderly. There is also some evidence to indicate that the efficacy of melatonin in limiting tumour cell proliferation depends on time of day of its administration, with melatonin given late in the light phase being more effective.[38] In humans, the use of melatonin in some cases reduced tumour growth and prolonged survival of cancer patients compared with individuals given conventional cancer therapy.[39] Importantly, melatonin administration, when combined with standard chemotherapies, often improve the quality of life. This probably relates to melatonin's ability to reduce the toxicity of chemotherapeutic agents.[8] The findings in humans are made more remarkable by the fact that melatonin was used as a cancer treatment only after all other therapies were found to be essentially ineffective.

Mechanistically, how melatonin inhibits tumour cell proliferation has been, in part, defined, and it apparently involves a number of mechanisms. In the case of experimental hepatomas and human breast cancer xenografts, melatonin acts on specific membrane receptors to limit the transport of linoleic acid (LA), a growth factor, into tumour cells.[7] With decreased LA uptake, intracellular 13-hydroxyoctadecadienoic acid (13-HODE) levels drop; 13-HODE is a mitogenically active metabolite that normally increases tumour cell proliferation via MAPK.

There are, however, a variety of other actions that have been implicated to explain melatonin's oncostatic effects. In oestrogen-receptor-positive human breast cancer cells, melatonin is thought to modulate oestrogen receptor expression and transactivation.[40] Still other potential mechanisms include melatonin's ability to reduce angiogenesis in tumours, to delay the G_1 to S phase transition in the cell cycle, to improve cellular communication between normal and cancer cells, and to alter the intracellular redox state.

Besides inhibiting established tumours, melatonin may also decrease their initiation. As an antioxidant (see below), melatonin has been found to be particularly effective in reducing free-radical-mediated damage to DNA.[41] Damaged DNA, if it goes unrepaired, may mutate and initiate a tumour. As a significant portion of the cancer humans acquire is believed initially to involve DNA damage as a consequence of toxic oxygen and nitrogen by-products, antioxidants that protect DNA from such mutilation would be expected to reduce cancer incidence; the evidence is strong that melatonin protects DNA from such damage more effectively than other classic antioxidants.[41]

Effects on the immune system

Interactions between melatonin and the immune system have been known for roughly 30 years, and in virtually all cases, melatonin has been proven to have immunoenhancing effects. The number of publications on this subject are extensive and the findings are summarized in several reviews.[42,43] In humans, daily oral melatonin administration increases natural killer (NK) cell activity.[5,42] Additionally, melatonin reportedly regulates gene expression of several immunomodulatory cytokines including tumour necrosis factor-α (TNFα), transforming growth factor beta (TGFβ) and stem cell factor (SCF) by peritoneal macrophages as well as the levels of interleukin-1beta (IL-1β), interferon gamma (INFγ), TNFα and SCF by splenocytes.[43] The rise in blood melatonin levels in humans at night stimulates associated rises in the thymic production of peptides including thymosin 1α and thymulin.[44] Finally, melatonin is a potent inhibitor of apoptosis in immune cells.[45]

The actions of melatonin on immunocompetent cells seem to be mediated by both membrane and nuclear binding sites. On both human lymphocytes and monocytes, receptor sites for melatonin have been characterized; binding sites with similar pharmacological characteristics have been identified in a variety of immune cells from animals. Additionally, however, in both animal and human immune cells, nuclear binding sites for melatonin have been documented.[5] The binding of melatonin to these sites is displaced by a specific ligand of the putative nuclear melatonin receptor RZR/ROR.[46] Thus, immune cells apparently possess two reasonably well-characterized receptors, i.e. membrane and nuclear. The interactions of melatonin with the immune system are summarized in the hypothetical scheme shown in Figure 3.

Given that melatonin is generally considered to be immunostimulatory, the question as to whether it should be taken by individuals with an autoimmune disease has been raised. To date, the information is meager regarding this issue, although in one case of Crohn's disease, a condition of excessive immune reactivity of the gut wall, melatonin did aggravate the condition.[47] Whether this will be a general finding in autoimmune diseases, however, remains to be established.

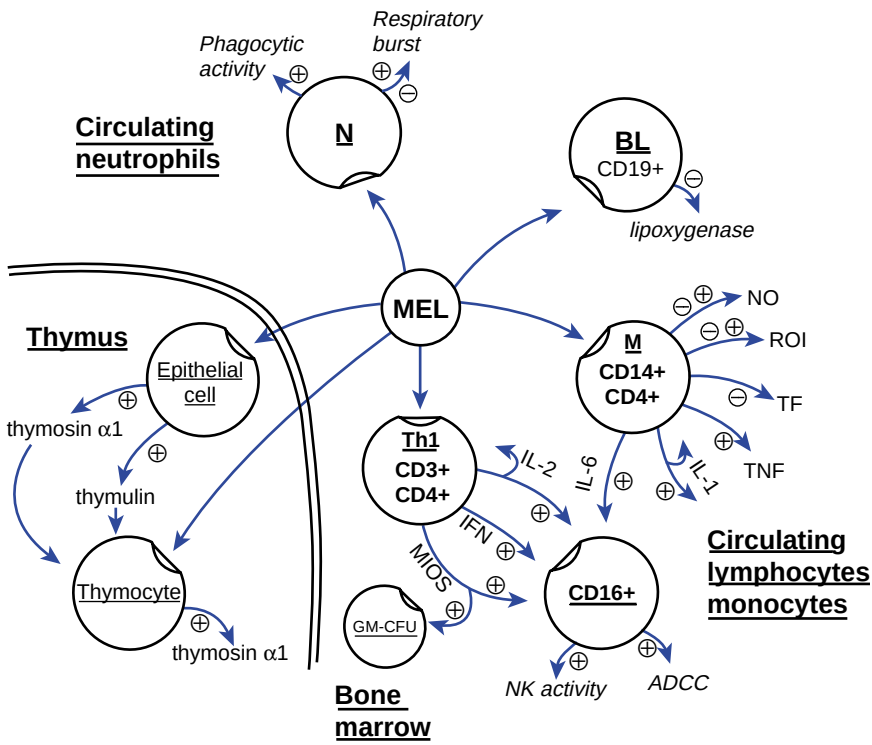

Figure 3. A summary of the presumptive actions of melatonin on immunocompetent cells. By influencing the activity of the monocyte ($CD14^+/CD4^+$ cells) and T cell, melatonin increases NK cell activity, as well as antibody-dependent cellular cytotoxicity (ADCC) and stimulates the production of progenitor cells for granulocytes and macrophages (GM-CFU). Furthermore, melatonin inhibits 5-lipoxygenase activity by B cells and increases thymosin 1α and thymulin by thymic epithelial cells. MIOS, melatonin-induced opioid system; ROI, reactive oxygen intermediates; NO, nitric oxide; TF, tissue factor.

Antioxidant effects

Slightly over a decade ago, melatonin was found to be a highly effective scavenger of free radicals and general antioxidant.[48] In the intervening years, these effects of melatonin have been documented in hundreds of published reports and it has been found to reduce oxidative damage in numerous models where free radicals contribute to the aetiology of a particular condition.[8,9,30,31,49]

Melatonin directly neutralizes a number of toxic oxygen- and nitrogen-based reactants, including the hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$) and the peroxynitrite anion ($ONOO^-$) or peroxynitrous acid (OHOOH).[9,31] Furthermore, melatonin has indirect antioxidative actions, including stimulating the synthesis of another important intracellular antioxidant, i.e. glutathione (GSH)[50], as well as promoting its enzymatic recycling in cells to ensure it remains primarily in its reduced form.[30] Finally, melatonin preserves the functional integrity of other antioxidative enzymes, including the superoxide dismutases and catalase. Melatonin may also reduce free radical generation in mitochondria by improving oxidative phosphorylation, thereby lowering electron leakage, and increasing ATP generation (Figure 4).[51]

Free radical damage has been implicated in a wide variety of diseases and in experimental models of a diverse range of these conditions; melatonin has been shown in all of these cases to be protective.[52,53] Examples of situations in which melatonin has been found to lower induced oxidative damage include ischaemia/reperfusion injury (in the brain, heart, gut, liver, lung, urinary bladder), toxic drug exposure, bacterial toxin exposure, schistosomias, heavy metal toxicity, amyloid β (Aβ) protein exposure (as a model of Alzheimer's disease)[54], 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure (as a model of Parkinsonism)[55], etc. The finding that melatonin reduces Aβ damage to neurons[54] prompted its use in Alzheimer's patients where it improved the status of these individuals.[56–58] Melatonin has also been successfully used as an adjuvant treatment in neonates with sepsis (a high free radical condition)[59] and with transient ischaemia/reperfusion.[60] Other conditions in humans where melatonin has been shown to be beneficial include the following: skin erythema due to exposure to ultraviolet radiation[61], iron and erythropoietin administration[62] and tardive dyskinesia.[63] Each of these is believed to involve, as part of the destructive processes, free radical damage to essential macromolecules. Given the virtual absence of toxicity of melatonin[8], reports of its use in humans to combat free radical damage will probably continue to appear.

CONCLUDING REMARKS

In such a brief summary, it is not possible to mention all the data which document the clinically relevant aspects of melatonin. Metatonin has been administered in both physiological and pharmacological amounts to humans and animals, and there is widespread agreement that it is a non-toxic molecule. Furthermore, melatonin limits tissue damage induced by drugs whose toxicity is a consequence of free radical generation.[8]

The actions of melatonin summarized herein suggest that it is generally a highly beneficial molecule for reducing tissue and cellular deterioration and possibly for lowering the incidence of some diseases. Many of the molecular mechanisms by which

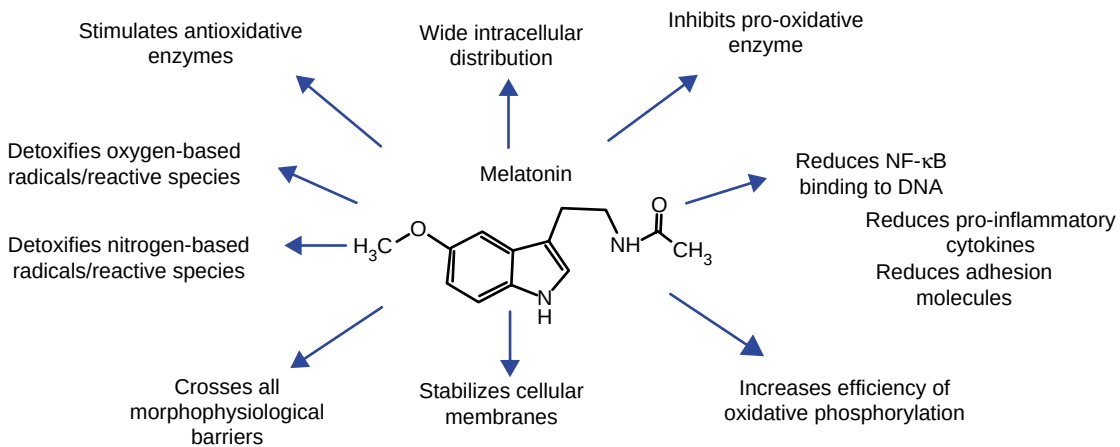

Figure 4. This figure summarizes the multiple actions whereby melatonin protects cells from oxidative damage. Both receptor independent processes, for example, direct scavenging of free radicals, and receptor-mediated actions, for example, stimulation of antioxidative enzymes, presumably account for the highly protective effects of this molecule in vivo.

melatonin achieves these changes require further definition. While the pharmacological use of melatonin is well established, how the gradual reduction in endogenous melatonin levels due to ageing—or its suppression by excessive light exposure—effects humans remains unknown.

Finally, whereas melatonin is generally classified as a hormone, it is in fact a molecule with paracrine, autocrine and antioxidant actions.[23] In reference to its antioxidative actions in humans, physiological blood levels of melatonin positively correlate with the total antioxidant capacity of that fluid.[64] Considering its diverse actions via both receptor and receptor-independent actions, to classify melatonin exclusively as a hormone seems inappropriate.

Practise points

- do not overlook the importance of a regular light: dark cycle in good health
- bright light exposure after darkness onset at night should be avoided since it disrupts the melatonin rhythm and the circadian clock are altered as a consequence
- when used for night-time sleep promotion, melatonin is taken 30 min in advance of desired sleep onset
- melatonin is a highly effective antioxidant

Research agenda

- the role of excessive light exposure (decreased dark exposure) at night should be examined in terms of its impact on human health
- trials are needed regarding the oncostatic effects of melatonin
- the efficacy of melatonin in reducing the toxicity of a variety of prescription drugs requires examination
- trials on the benefits of melatonin in the aged are warranted, particularly in reference to neurodegenerative disease

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