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PHYSIOLOGICAL REVIEW

The basic physiology and pathophysiology of melatonin

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Summary Melatonin is a methoxyindole synthesized and secreted principally by the pineal gland at night under normal environmental conditions. The endogenous rhythm of secretion is generated by the suprachiasmatic nuclei and entrained to the light/dark cycle. Light is able to either suppress or synchronize melatonin production according to the light schedule. The nycthohemeral rhythm of this hormone can be determined by repeated measurement of plasma or saliva melatonin or urine sulfatoxymelatonin, the main hepatic metabolite.

The primary physiological function of melatonin, whose secretion adjusts to night length, is to convey information concerning the daily cycle of light and darkness to body physiology. This information is used for the organisation of functions, which respond to changes in the photoperiod such as the seasonal rhythms. Seasonal rhythmicity of physiological functions in humans related to possible alteration of the melatonin message remains, however, of limited evidence in temperate areas in field conditions. Also, the daily melatonin secretion, which is a very robust biochemical signal of night, can be used for the organisation of circadian rhythms. Although functions of this hormone in humans are mainly based on correlative observations, there is some evidence that melatonin stabilises and strengthens coupling of circadian rhythms, especially of core temperature and sleep-wake rhythms. The circadian organisation of other physiological functions could depend on the melatonin signal, for instance immune, antioxidative defences, hemostasis and glucose regulation.

Since the regulating system of melatonin secretion is complex, following central and autonomic pathways, there are many pathophysiological situations where the melatonin secretion can be disturbed. The resulting alteration could increase predisposition to disease, add to the severity of symptoms or modify the course and outcome of the disorder.

Introduction

Melatonin was isolated and characterised from the bovine pineal by the dermatologist Aaron Lerner

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as early as 1958.[1] It is the main hormone secreted by the pineal gland. Secondary sources are retina, gut, skin, platelets, bone marrow and probably other structures, whose systemic contribution is insignificant.[2-7] This compound of indole structure (*N*-acetyl-5-methoxytryptamine) is synthesized from serotonin. This aspect and the fact that it lightens the frog skin by contracting melanophores led to the naming of this molecule as Melatonin (i.e. melanophore-contracting hormone; greek: μελαs = black; τουos = tension, in the sense of contraction).

Although melatonin has extensively been detected in the animal kingdom, recently this compound has also been found in different structures of higher plants (leaves, fruits, seeds). The levels are too low, however, to provide a significant melatonin supply. Also, melatonin is present in lower phyla, including bacteria.[8] The ubiquitous molecule melatonin is probably one of the first compounds which appeared on earth to coordinate some basic events of life.

The main physiological functions of melatonin are related to hormonal properties, although it may also exhibit autocrine or paracrine properties, for example in the retina or the gut.[9] The pineal gland was initially shown to be an active neuroendocrine transducer of environmental information in animals, especially in photoperiodic species. For many years, the data had been extrapolated to humans. Today, some understanding of the role of melatonin in human physiology and disease has emerged, but many functions and effects of melatonin remain unresolved. This review will focus on data about melatonin in humans, as an introduction to the following chapters.

Melatonin metabolism

Biosynthesis

Melatonin is synthesized from tryptophan taken up from the circulation and transformed to serotonin; serotonin is converted into melatonin by a two-step process involving the sequential activities of two enzymes, serotonin-*N*-acetyl transferase (NAT), which is the limiting enzyme for the synthesis of melatonin, and hydroxyindole-*O*-methyl transferase (HIOMT).[10] The mRNAs encoding these enzymes are expressed with a day/night rhythm in the pineal (for review, see Ref. 11). The synthesis of melatonin is initiated by the binding of norepinephrine to adrenergic β1 receptors, subsequent activation of pineal adenylate cyclase, increase in cyclic AMP (cAMP) and de novo synthesis of NAT or of its activator. The cAMP-induced gene transcription repressor (ICER), an isoform of the cAMP responsive element modulator (CREM), is activated in conjunction with NAT and represents a mechanism that limits the nocturnal production of melatonin.[12] Also, melatonin synthesis depends upon tryptophan availability because it is reduced after acute tryptophan depletion;[13] other nutritional factors could influence melatonin synthesis, for example folate status[14] and vitamin B6, a coenzyme in tryptophan decarboxylation which is able to stimulate melatonin production in prepubertal children but not in adults.[15,16] Also, fluvoxamine, an inhibitor of serotonin uptake, increases the amplitude and duration of the plasma melatonin peak.[17]

Secretion

Melatonin displays high lipid and water solubility (octanol/water coefficient of partition ≈ 13) which facilitates passage across cell membranes.[18] After release in the circulation, it gains access to various fluids, tissues and cellular compartments (saliva, urine, cerebrospinal fluid, preovulatory follicle, semen, amniotic fluid and milk). As no pineal storage of melatonin is available, the plasma hormone profile faithfully reflects the pineal activity.[19] The secretion occurs at night, with maximum plasma levels around 03:00–04:00 a.m., varying with chronotype, whereas diurnal levels are undetectable, or low in rested subjects. This nycthohemeral rhythm displays the most marked amplitude observed for a hormone, more marked than that of cortisol. If blood sampling is close enough (at least every 10 or 20 min), an episodic secretion is evidenced, with peaks and troughs.[20,21] Whether the short term melatonin secretion is pulsatile has remained a matter of debate for a long time.[22] In addition, no definite relationship between the peaks or troughs and sleep stages has been established. These aspects should be reinvestigated in terms of frequency of blood sampling since plasma melatonin displays a quick turn-over (after I.V. bolus administration, plasma melatonin displays a biexponential decay with a first distribution half-life of 2 min and a second metabolic half-life of 20 min), as well as in regard to sensitivity and reliability of immunoassays and statistical method of detection of peaks. Nocturnal melatonin production rates, as estimated by deconvolution analysis applied to plasma melatonin concentration time series, are between 10–80 μg/night, the lowest values for a hormone secretion.[23]

The plasma melatonin profile displays a great inter-subject heterogeneity. Nonetheless, it is very reproducible from day to day in a same subject and represents one of the most robust circadian rhythms. It provides a good evaluation of the melatonin secretion, in the absence of renal or hepatic abnormality.[24] In some subjects, the nocturnal secretion is extremely low or even absent. The consequences of a low melatonin secretion on vulnerability to rhythmic organisation and morbidity are unknown. At the present time, no polymorphism of enzymes can explain this heterogeneity. Blood melatonin is mainly bound to albumin (70%) and to a lesser extent to orosomucoid.[25] Circulating melatonin can reach all body tissues including brain and is able to cross the blood–brain barrier to modulate brain activity. A PET study showed that the brain radioactivity was maximum 6–8 min after injection of ^{11}C melatonin.[26] In brain, melatonin could also be oxidized into kynurenine, whose function is unknown.[27]

Catabolism

The liver, which clears more than 90% of circulating melatonin, is the primary site for metabolism. Melatonin is first hydroxylated, then excreted in urine as sulphate and, to a lesser extent, as glucuronide conjugates.[28] Urine 6-sulfatoxymelatonin (aMT6S) excretion closely parallels the plasma melatonin profile.[29] About 1% melatonin remains unchanged in the urine. 3-hydroxymelatonin, which is also detected in urine, could represent a biomarker of OH° radical generation. In addition to a lower pineal secretory activity, patients with liver cirrhosis show a decreased melatonin clearance, with consequent delayed rise of plasma melatonin peak and increased daytime levels of this hormone.[30] Also, in patients with chronic renal failure, there are increases of daytime melatonin and aMT6S levels and blunted melatonin rhythmicity.[31]

The regulating system of the melatonin secretion

The melatonin rhythm is generated by an endogenous clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus, like other circadian rhythms in mammals (drinking and feeding, sleep-wake cycle, temperature, cortisol or corticosterone, etc.). Results have been reported in animals, mainly in rodents and monkeys, and extended to humans.[32,33] Pathophysiological observations in patients provide confirmation.[34]

The light/dark cycle is the main Zeitgeber of the regulating system of melatonin secretion. The melatonin rhythm is entrained to the dark period. The photic information is transmitted to the central pacemaker via retino-hypothalamic fibers: during the day, in the presence of light, the output from the retino-hypothalamic tract inhibits melatonin synthesis. Artificial light of sufficient intensity and duration administered at night suppresses melatonin production.[35] Light intensities of 2000–2500 lux for 2 h (02:00–04:00 a.m) completely suppress melatonin secretion, whereas domestic light intensities (50–300 lux) have a modest suppressive effect.[36] In addition, after exposure to light for several consecutive nights, the melatonin secretion escapes the inhibitory effect and progressively shifts (phase-delay) to the morning. Full spectrum bright light is routinely used but the most effective wavelengths are in the range 446–477 nm (blue). Because the action spectrum derived from irradiance response curves does not correspond to either scotopic or photopic action spectra, possible new photoreceptors have been hypothesized.[37,38] Also, there is no significant difference in melatonin suppression between all colour-vision deficients, protanopic, deuteranopic and control subjects.[39] Recently, retinal ganglion cells innervating the SCN were shown to intrinsically respond to light. These melanopsin containing cells are candidate photoreceptors for the photic entrainment of circadian rhythms, because the sensitivity and slow kinetics of the light response are compatible with those of the photic entrainment mechanism.[40] Further, this system appears to send photic information, not only to the endogenous clock in the SCN, but also to other brain areas involved in irradiance detection, such as light activated pupil response.

The suppression of melatonin by exposure to low frequency electromagnetic fields (EMF) has been invoked as a possible mechanism through which exposure to these fields may result in an increased incidence of cancer. The recent data do not report a distinct influence of EMF on the melatonin level.[41]

The neural pathway from the SCN to the pineal gland passes first through the upper part of the cervical spinal cord, where synaptic connections are made with preganglionic cell bodies of the superior cervical ganglia (SCG) of the sympathetic chains (Fig. 1).[42,43] Then, neural cells in the SCG send projections to the pineal gland. The main neurotransmitter regulating the pineal gland is norepinephrine, which is released at night, in response to stimulatory signals originating in the SCN. The data obtained in animals have pharmacological confirmations in humans.[44] β1-adrenergic

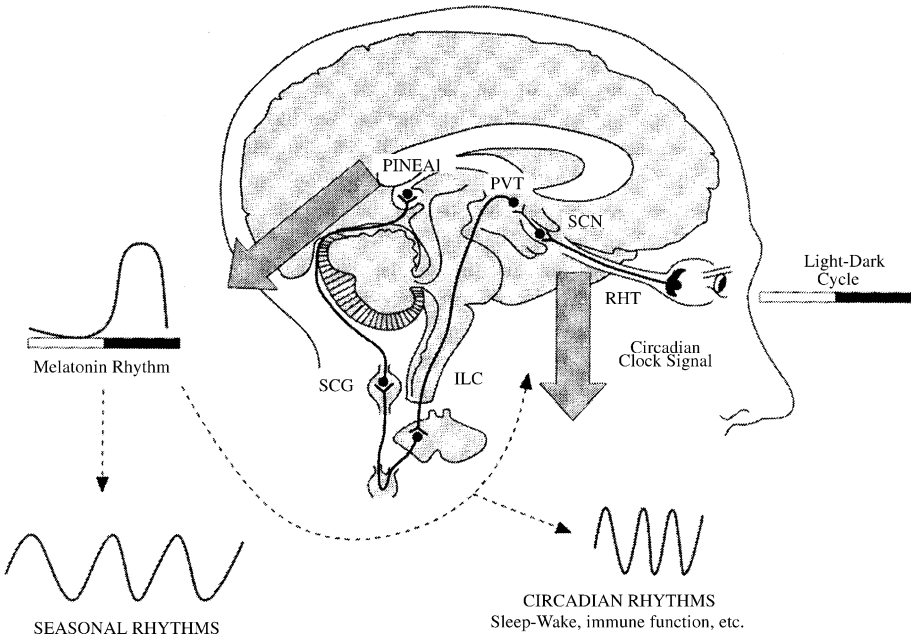

Figure 1 Control of melatonin secretion. Photic information is conveyed to the suprachiasmatic nuclei (SCN), principally through the retino-hypothalamic tract (RHT), where it synchronizes the activity of the circadian oscillator to exactly 24 h. Neuronal efferent pathways from the SCN directly distribute circadian information to different brain areas, including the pineal gland, that generates the melatonin rhythm. The neural route for environmental lighting control of melatonin secretion, after relay in the paraventricular nuclei (PVT), includes the intermediolateral column of the thoracic chord grey (ILC) and the superior cervical ganglion (SCG). The generated melatonin rhythm might be used by the SCN to distribute its rhythmic information. Melatonin can feed back at the level of the SCN, as well as the retina itself. A melatonin-driven circadian rhythm of sensitivity to melatonin may exist in the structure(s) involved in seasonality. Reprinted from *Sleep Medicine Reviews*, Cardinali D, Pevet P, 1998, **2**, 175–190. Basic aspects of melatonin action.

blockers suppress the nocturnal melatonin secretion as well as the $\alpha 2$ blocker clonidine and α-methyl-*para*-tyrosine, which reduces presynaptic catecholamine synthesis. Conversely, melatonin secretion is reinforced by drugs, which increase synaptic catecholamine availability, such as MAO inhibitors or tricyclic antidepressants. In addition to norepinephrine, the sympathetic endings of the SCG release neuropeptide Y. Also, nerve fibers innervating the pineal gland originate in perikarya located in the parasympathetic sphenopalatine and otic ganglia and the trigeminal ganglion which is the sensory ganglion of the fifth cranial nerve.[43] With regard to the parasympathetic innervation, two peptides appear to be important: vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI), whereas substance P (SP), calcitonin gene-related peptide (CGRP), and pituitary adenylate cyclase-activating peptide (PACAP) are present in cell bodies of the trigeminal ganglion.[43] These neurotransmitters involved in the control of the pineal activity are only able to modulate the effect of norepinephrine. In animals, VIP, PACAP and opioids via σ receptors stimulate melatonin secretion, whereas GABA, neuropeptide Y, dopamine and glutamate inhibit melatonin production. Whether the above mechanisms are relevant to melatonin secretion in humans remains to be elucidated. Activation of GABA receptors by benzodiazepines and enhancement of endogenous GABAergic tone by sodium valproate [45] reduce melatonin at night, whereas dopaminergic agonists and antagonists and opioid receptor blocking agents are not capable of any marked modification of melatonin levels. Other drugs such as dihydropyridine calcium antagonists or prostaglandin inhibitors probably alter melatonin secretion. These and many other data strongly warrant an investigation of drug consumption in patients before the evaluation of melatonin secretion.

Functions of melatonin

Melatonin secretion is related to the duration of darkness. The main function of melatonin is to mediate dark signals, with possible implications in the control of circadian rhythmicity and seasonality. The melatonin message, which is generated at night, is differently read in nocturnal animals and humans. In that sense, melatonin does not appear

as the universal hormone of sleep. The role of melatonin for the seasonal changes in physiology and behaviour of various photoperiodic species has been extensively documented. For a long time, humans were claimed to be poorly sensitive to photoperiod variations, as no difference between the summer and winter melatonin duration was found in temperate zones. Studies conducted under appropriate natural or controlled laboratory conditions show that humans also exhibit changes in the daily profile of melatonin. For example, the melatonin rhythm was phase delayed during winter compared with the summer in shift workers living in Antarctica.[46] In temperate latitudes (40–50°N), the data on the influence of the photoperiod are less clear. Wehr showed in laboratory conditions that the melatonin profile duration was enlarged with the lengthening of artificial light and sleep responded to this change in day length.[47] It is proposed that the circadian pacemaker consists of two component oscillators. One is entrained to dusk and controls the onset of melatonin secretion, the other is entrained to dawn and controls the offset. The dusk and dawn entrained components of the circadian pacemaker could be considered to control evening and morning transitions in melatonin secretion and to adjust the timing of these transitions in seasonal changes in day length.[48] However, the response to these seasonal changes is abolished by modern artificial lighting: no summer–winter differences in melatonin, cortisol, thyrotropin and rectal temperature profiles were observed in men exposed to both natural and artificial light in an urban environment.[49]

Clinical observations which meet the classical concept in endocrinology of hormone deficiency and replacement are not available; the pinealectomized patient, whose rhythm of circulating melatonin is abolished, does not provide a pure situation of melatonin suppression, due to the possible side-effects of surgery and/or radiation therapy, especially on adjacent structures. Further, the pineal gland is not essential to life and some effects of melatonin are probably subtle. Chazot et al. were able, however, to put together recurrent symptoms observed after pinealectomy that they called the 'pinealoprive syndrome', mainly consisting of hemicranial headache or unilateral orbital cephalalgia with or without sympathetic abnormality and disturbance of vision.[50] Also, afternoon sleepiness, mood disorders, visual and auditory hallucinations and convulsive seizures were recorded. These observations meet the hypothesis of a stabilizing role for the the pineal gland.

In addition, light given to suppress melatonin secretion should be administered at night, which is incompatible with the study of modulation of sleep by this hormone. Finally, due to its short half-life, melatonin replacement is usually achieved with several mg melatonin given by oral route which, quickly released in the body, do not mimic the endogenous profile, but lead to supraphysiological (pharmacological) levels over a short time, with possibly the occurrence of side-effects rather than physiological effects.

Melatonin, the endogenous synchroniser?

The time of melatonin secretion adjusts to the light/dark cycle. A general opinion is that melatonin, by providing the organism with the night information, could be an endogenous synchronizer able to stabilize circadian rhythms, to reinforce them and to maintain their mutual phase-relationship (Fig. 2).[51,52] Physiological phenomena, which occur at night are mainly involved. The direct effect of melatonin on the temperature rhythm meets this hypothesis: melatonin reinforces the nocturnal decrease of central temperature, an event which facilitates sleep propensity.[53] Since melatonin receptors have been identified in peripheral vasculature, decreased central temperature may be the result of peripheral vasodilation due to melatonin receptor stimulation.[54]

The arguments put forward for the influence of melatonin on cortisol rhythm and sleep–wake cycle are more indirect. Cortisol and melatonin rhythms remain phase-locked, however, after phase-shifting manipulation. In addition, a direct modulatory effect of melatonin on cortisol secretion cannot be excluded, since melatonin receptors have been demonstrated in the primate adrenal gland and physiological doses of melatonin inhibit the in vitro ACTH-stimulated cortisol production.[55] Temperature nadir, sleepiness and melatonin excretion peaks coincide and this temporal relationship remains during a 72-h sleep deprivation.[56] Also, there is a close correlation between melatonin suppression and the enhancement of alertness by light exposure at night.[57] When the melatonin secretion is shifted to the morning after a repeated nocturnal administration of bright light, nocturnal alertness is improved and diurnal sleep, which is synchronous with melatonin secretion, displays a physiological architecture. Further, there is a clear relationship between the durations of sleep and melatonin secretion. Recently, Aeschbach et al.[58] found a longer biological night in long-sleepers than in short-sleepers. The nocturnal periods of high plasma melatonin levels, increasing cortisol levels,

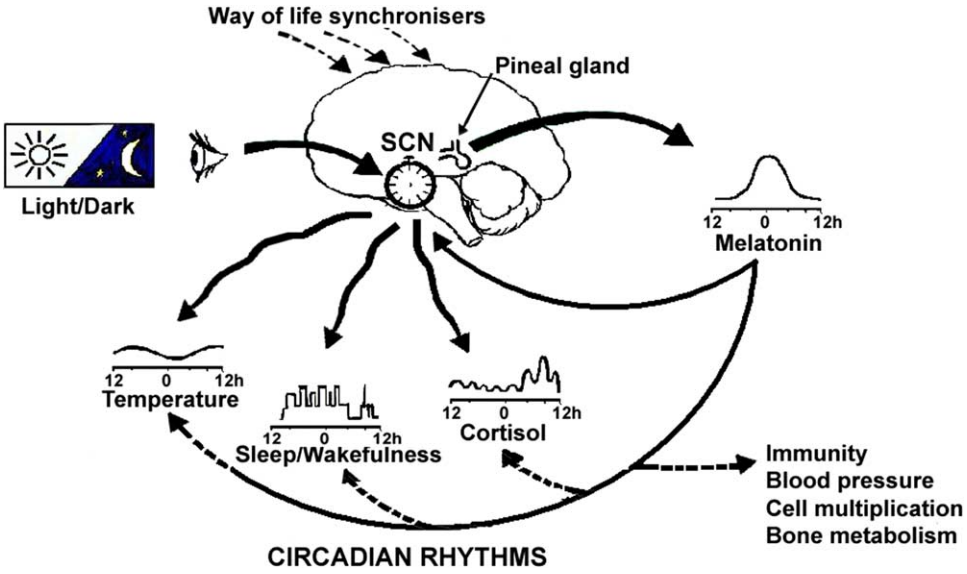

Figure 2 Melatonin acts as an endogenous synchronizer. Reprinted from *Restorative Neurology and Neuroscience* 12. Claustrat B et al. Melatonin, from the hormone to the drug ? 151–157, copyright 1998 with permission from IOS Press.

low body temperature and increasing sleepiness were longer in the former.

Finally, in infancy the imbalance in sleep distribution between night and day becomes progressively more marked with age; around 3–4 months, an age which corresponds to the melatonin rhythm maturation, the infant remains awake during most of the daytime and most of its sleep is concentrated during the night.[59] Taken as a whole, these data support the idea that in physiological conditions melatonin is involved in the sleep–wake cycle regulation.

The existence of a phase-response curve (PRC) of the pineal melatonin secretion to the administration of exogenous melatonin (chronobiotic effect) provides an indirect argument that the melatonin effect on the activity–rest cycle is indirectly mediated via its effect on the phase of the sleep–wake cycle.[60,61] When melatonin is given in the late afternoon or the evening, a phase-advance of the plasma melatonin profile is observed.[62] On the contrary, a phase delay occurs, following the melatonin administration from the early morning to noon. Such a phase-shift has been obtained with a single exogenous hormone signal at the level of a nocturnal physiological melatonin peak.[61] A study by Wirz-Justice et al. involving a different protocol failed, however, to find significant delays in saliva melatonin, core temperature and heart rate rhythms after a morning melatonin administration.[63] There is a possibility that melatonin works via a direct feed-back effect on the clock; melatonin binding sites have been revealed at this level and melatonin can alter the electrical or metabolic activity of the suprachiasmatic nuclei. Consequently, the main rhythms (sleep–wake, temperature, cortisol) controlled by the circadian system can be manipulated.

Melatonin throughout life [64]

Maternal melatonin which crosses the placenta is one of the maternal rhythmic signals capable of synchronizing the fetal biological clock. The pronounced daily melatonin rhythm in the milk could take over in the newborn. After maturation, rhythmic melatonin production reaches the highest levels at the age of 3–6 years. Then the nocturnal peak drops progressively by 80% until adult levels are reached. This alteration is temporally linked with the appearance of sexual maturity and is not simply the consequence of both increasing body size and constant melatonin production due to lack of pineal growth during childhood. Data concerning normal precocious puberty treated by gonadotropin-releasing hormone analog suggest that the reduction of melatonin with normal puberty is not likely to be dependent on pubertal gonadotropin or sex steroid influence.[65] On the contrary, in male primary hypogonadic patients or in patients with gonadotropin-releasing hormone deficiency there is an increase of melatonin secretion; testosterone substitution is followed by a reduction of plasma melatonin levels. During the ovarian cycle, although melatonin may modulate steroidogenesis, especially progesterone production, no clear

consensus has emerged as to the melatonin changes that may occur no preovulatory decrease of melatonin is observed, which could facilitate the LH surge responsible for the ovulation.[66] The transient elevated melatonin secretion during menopause could be related to the dramatic decrease of the estrogen environment.[67] Rather, with aging, the melatonin rhythm progressively dampens, with a tendency to the phase-advance, and can be completely abolished in advanced age.[68] The question whether the impaired melatonin secretion with advancing age is related to an increasing pineal calcification remains open. This decrease of melatonin secretion which was found reinforced in elderly insomniacs was the rationale for treatment with this hormone.[69] These data which are controversial will be discussed in the next chapters.[70]

Do humans display a seasonal rhythmicity via the melatonin message?

The seasonal alterations of the natural photoperiod at high latitudes have a repercussion on melatonin secretion in humans. Is there a response to this seasonal message? In Finland, Kauppila et al. observed a 2 h extension of melatonin secretion in winter, compared with the summer period.[71] A decrease in plasma ovarian steroids ran parallel with the winter increase of the melatonin secretion, in agreement with a variation in conception rate along the year observed at high latitudes, increased fertility being associated with longer days. There is no direct evidence that the changes in duration of melatonin secretion mediate the effect of photoperiod, as occurs in animal models. In temperate areas, the influence of photoperiod on reproduction are less clear. Nutritional and environmental (artificial light and blunting of seasonal changes in temperature) factors could be responsible for the progressive decline in the seasonality of human reproduction.[72]

Seasonal affective disorders (SAD) of winter type are characterised by recurrent depressive episodes during the short photoperiod. Changes in the duration and/or phase of melatonin secretion during this period were initially hypothesised to play a role in the pathogenesis of SAD and prompted its treatment with phototherapy.[73] Although phototherapy is an effective treatment of SAD and could act on some biological rhythms, the beneficial effect of this treatment is not supported by changes in melatonin profiles.[74,75]

Sites and mechanisms of action of melatonin

Melatonin displays pleiotropic physiological functions. Although it is accepted that melatonin mainly acts via specific receptors in cell membranes, the interaction of melatonin with nuclear receptors and intracellular proteins, such as calmodulin or tubulin-associated proteins, as well as its direct or indirect antioxidant effects could explain many general functions of this hormone.[76]

Receptors [77]

Melatonin receptor nomenclature has recently been proposed by the International Union of Pharmacology (IUPHAR). Two subtypes of mammalian receptors have been cloned, the MT1 and MT2 subtypes. Both subtypes are members of the seven-transmembrane G protein-coupled receptor family. MT1 receptor is coupled to different G-proteins that mediate adenylyl cyclase inhibition and phospholipase Cβ activation. The MT2 receptor is also coupled to inhibition of adenylyl cyclase and, additionally, it inhibits the soluble guanylyl cyclase pathway. Studies of 125I-melatonin binding display a large variability among species in the distribution of melatonin receptors. Melatonin availability regulates receptor levels: MT1 mRNA expression and 125I melatonin binding in SCN and pars tuberalis (PT) exhibit daily variations, with elevated levels of both parameters during the daytime, when melatonin levels are low, and also, light exposure during the night increases 125I-melatonin binding. Further, results obtained in the non-photoperiodic laboratory mouse show that MT1 receptors regulate rhythmic PT clock gene expression, namely mPer1. This could be a general mechanism for humoral regulation of the phase of rhythmicity in tissues that are not linked to the SCN by neuronal connections.[78] The MT2 receptor mRNA present in human retina and brain is responsible for entrainment of circadian rhythms in the SCN.[79] MT1 and MT2 polymorphisms have been found in humans and may be associated with sleep disorders.[80]

Some effects of melatonin cannot be explained by membrane receptors or radical scavenging. Due to the small lipophilic structure of melatonin which easily permits its passage across biological membranes, as is observed for steroids, thyroid hormones and retinoids, melatonin appears to be the natural ligand for the orphan nuclear hormone receptor superfamily RZR/ROR. Recent data show

that melatonin nuclear receptors are related to the immunomodulator effect of melatonin.[81]

Antioxidant activity

Melatonin is a potent free radical scavenger, more potent than vitamin E which is the reference in the field.[82] Melatonin directly scavenges the highly toxic hydroxyl radical and other oxygen centered radicals. Also, melatonin displays antioxidative properties: it increases the levels of several antioxidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase. On the other hand, melatonin inhibits the pro-oxidative enzyme nitric oxide synthase. Since considerable experimental evidence supports the idea that oxidative stress is a significant component of specific brain diseases, the ability of melatonin to protect against neurodegeneration was tested in a multitude of models. The first positive results were obtained with pharmacological doses of melatonin. At the present time, there is experimental evidence indicating that the quantity of melatonin endogenously produced is relevant as a physiological antioxidant.[83] Further, the antioxidant defense system displays a daily rhythm which is abolished by pinealectomy in the rat, or by light in humans. Few data on the protective melatonin effect against free radicals are available in humans. Controlled trials are difficult to set up because the life of patients involved in such studies is at stake. In chronic hemodialysis patients, the oxidative stress induced by iron and erythropoietin given for treatment of anemia was prevented by oral administration of melatonin (0.3 mg/kg).[84] Preliminary results in septic newborns showed that high melatonin doses (20 mg per subject) significantly reduced serum levels of lipid peroxidation products and inflammation markers and increased the survival rate and improved the clinical outcome of patients.[85] Similarly, increased blood levels of malondialdehyde and nitrite/nitrate observed in asphyxiated newborns were reduced by melatonin treatment (a total dose of 80 mg per infant). Three of the 10 asphyxiated newborns not given melatonin died within 72 h after birth, whereas none of the 10 who received melatonin died.[86] Despite ethical difficulties, these results of major interest should be replicated in a larger number of patients.

Immunity

Currently accumulated evidence shows that the pineal is able to play an important role in modulating the immune response (for review, see Ref. 87) since functional (constant light condition) and pharmacological inhibition (propranolol administration) of melatonin synthesis in mice is associated with suppressed humoral and cellular immunological responses. Melatonin can interact with specific membrane binding sites in cells from lymphoid organs. The KD value of these binding sites is in the 0.1–1 nM range, similar to the circulating levels.

In addition, interactions between the pineal gland and the immune system are bidirectional since interleukins and cytokines (interferon gamma) affect melatonin synthesis and release.[88] Also, there has been described melatonin scavenging of NO or free radicals in lymphoid cells, which could explain the melatonin-modulated circadian variation in the experimental chronic inflammation. This kind of approach raises new questions regarding the mechanism of chronic inflammation, in disorders like rheumatoid arthritis and nocturnal asthma, diseases that present rhythmic symptoms during a 24 h period.[89,90] It is clear that melatonin provides a functional link between the neuroendocrine and immune-haematopoietic systems.

Cancer

At present, the validity of melatonin as an oncostatic agent seems well established and the antitumor mechanisms of melatonin have been identified: these include its antiproliferative actions, immunostimulatory effects on host anticancer defences and antioxidant activity. Isolated reports of tumor growth stimulation do however exist, especially if melatonin is administered in the morning, indicating a circadian-stage dependency of antitumor action.[91] In the recent past, a limited number of patients with advanced disease has been concerned with open studies. A recent controlled trial shows the possibility to improve chemotherapy in terms of both survival and quality of life by a concomitant administration of melatonin and cisplatinium etoposide in metastatic non-small cell lung cancer.[92]

Melatonin rhythm, a marker of the circadian clock

Melatonin can be considered as a reliable output (the hour-hand) of the endogenous clock. There is a close relationship between the plasma melatonin peak and minimum core temperature, including

entrained conditions and constant routine protocols. In contrast to the temperature rhythm, the melatonin rhythm is not very sensitive to masking effects, except the one exerted by light. Consequently, Lewy and Sack recommend to evaluate the onset of the plasma melatonin profile under dim light (50 lux, 'Dim Light Melatonin Onset' DLMO).[93] Evaluation of the plasma melatonin pattern needs repeated blood sampling. A maximum 1 h interval is necessary to obtain reliable values for onset, offset acrophase and area under curve. The melatonin profile can be simultaneously determined with temperature and sleep recordings and provides an excellent diagnostic element for detecting circadian rhythm sleep disorders. Also, saliva and urine samplings offer a useful alternative for outpatient explorations and laboratory or field studies, but require waking the patients up.[94] Finally, posture should be controlled during investigation.[95]

Pathophysiology of melatonin secretion

Alterations of 24-h melatonin profiles can be associated with a large variety of pathological situations. Some of the changes may have a pathogenetic relationship with a major disease process. Also, since an abnormality at any level of the regulating system unspecifically modifies melatonin secretion, other changes are more a consequence of the existing disorder. In both situations, the resulting alteration of melatonin secretion could favour predisposition to disease, add to the severity of symptoms or modify the course and outcome of the disorder.

Ocular pathology

In complete darkness, the melatonin rhythm generally evolves to free running with a slightly more than 24 h period, in the complete absence of light perception. However, in some totally sightless people who maintain circadian entrainment the visual subsystem that mediates the light-induced suppression of melatonin secretion remains functional.[96] This is not the result of extraocular phototransduction as suggested by conflicting data.[97]

In patients with functional alteration of the retina in relation with uveitis, the plasma melatonin peak is decreased.[98] Abnormal melatonin secretion could accompany other ocular pathologies. Since melatonin is a parameter influencing intra-ocular pressure, altered pineal and/or ocular production may be suspected in glaucoma. Further, sleep disturbance of retinitis pigmentosa could be related to an impaired melatonin profile. In both cases melatonin alteration could be related to abnormal light perception.

Neurological disorders

Pineal region tumors display heterogeneous melatonin profiles, according to the histological type. Germinoma cells infiltrate the pineal gland, resulting in the complete deficit of melatonin secretion, whereas in parenchymal tumors (pinealocytomas or pinealoblastomas) exaggerated melatonin secretion is the exception but rather a qualitative alteration (lost or abnormal rhythmicity) is observed. Premature puberty observed in some cases should be related to chorionic gonadotropin production by the tumor. Taken as a whole, melatonin determination is not a neurodiagnostic tool in pineal region tumors.

Alterations of plasma melatonin profile have been observed in hypothalamic tumors likely including the SCN area. In fatal familial insomnia, the progressive alteration of melatonin levels suggests a role of the thalamus in the modulation of the nycthohemeral rhythm of melatonin.[99] In multiple neurodevelopmental disorders of children, especially of genetic origin (Rett and Angelman syndromes), behavior and sleep disturbances are accompanied by abnormal melatonin secretion (mainly phase delay). In Smith-Magenis syndrome, the melatonin rhythm is completely reversed;[100] the associated sleep disorder can be successfully treated by administration of a β-blocker during the day and controlled-release melatonin preparation at bedtime.

Recent studies in ischaemic stroke patients showed a disruption of nocturnal melatonin rhythm associated with impaired cell-mediated immunity.[101] Also, the nocturnal surge of plasma melatonin was modified in patients with acute cerebral haemorrhage. Patients with lesions in the brainstem or in the third or the lateral ventricles showed the lowest values with absence of nocturnal rise.[102] Decreased melatonin levels have also been reported in patients with some forms of epilepsy and human data suggest that melatonin displays an anticonvulsant action, improving both the frequency of seizures and the EEG tracing; it could be beneficial in combination with other antiepileptic medications. In one case, the association of a very high dose of melatonin (100 mg per day) with phenobarbital led to the stabilization of a severe myoclonic epilepsia unsuccessfully treated with a combination of anticonvulsants.[103] In all the above mentioned clinical situations, the decreased

endogenous antioxidative defense related to impaired melatonin secretion could lead to increased brain vulnerability. In one study, however, melatonin showed pro-convulsant effects in neurologically disabled children.[104] Although a positive effect on sleep disorders was constantly observed, seizure frequency increased after melatonin treatment in four of six children and returned to baseline after melatonin was discontinued.

Several pathological situations point up the role of adrenergic innervation in the control of pineal activity. In preganglionic sympathetic dysfunction (Shy-Drager syndrome), or idiopathic orthostatism hypotension as well as in patients with hyperhidrosis after bilateral T1–T2 ganglionectomy, the nocturnal rise of plasma melatonin or its urinary metabolites is reduced or absent.[105,106] Also, changes have been reported in diabetic patients with autonomic neuropathy.[107] CSF and blood melatonin levels were significantly lower in sudden infant death syndrome (SIDS) compared with controls, which could reflect an abnormal maturation of the sympathetic nervous system. Similar results were observed in patients with sympathetic dysfunction and quadriplegia due to cervical spinal cord transection. In contrast, the maintained cortisol rhythm indicated the integrity of the SCN.[108]

Primary headache (migraine and cluster headache) is, in our opinion, a good model to obtain an insight into the pathophysiology of melatonin.[109] Migraine and cluster headache can be viewed as transient disturbances of the body adaptive response to internal or external environmental changes. Among these factors, light is a major precipitating or aggravating factor of attacks. The reports on migraine and cluster headache melatonin relationship are concordant with a melatonin secretion defect. Several mechanisms, which are not mutually exclusive, might be envisaged: local sympathetic abnormality, hypersensitivity of the retino-hypothalamic pathway, functional disturbance at the level of the suprachiasmatic nucleus.[110,111] Since the pineal gland plays a role in the homeostatic equilibrium of the organism, low melatonin levels could reinforce vulnerability of the rhythmic organization of the central nervous system in migraine and facilitate the cascade of events related to perivascular inflammation in the trigeminovascular system, which also innervates the pineal gland

Psychiatric diseases

Earlier studies showed a reduction of melatonin secretion in depressed patients compared with controls.[112] In addition, Lewy et al. reported higher melatonin levels in bipolar patients when they were manic than when they were depressed and suggested that the amplitude of melatonin production reflects state-dependent changes in noradrenergic function.[113] Recent studies evidence conflicting results (normal melatonin peak, normal or phase-delay rather than phase-advanced peak) which could be explained by methodological differences (size of samples, duration of drug wash-out, selection of patients and comparison of patients with not strictly matched controls) and seniority of the disease.[114] Heterogeneous results were also observed for melatonin profiles in schizophrenia and anorexia nervosa. In most anorectics, however, the melatonin rhythm was unaltered and the nocturnal plasma profile was greater[115]; this could be related in part to abnormal melatonin catabolism. Since all studies reported mean results of patients with ignorance of individual chronotypes, we suggest to further take into consideration this parameter for interpretation of the results.

Sleep disorders, especially circadian rhythm sleep disorders

These aspects will be developed in the next chapters. We should stress, however, that the disturbed sleep–wake cycle observed in Alzheimer disease patients correlates with decreased melatonin levels and a disrupted circadian melatonin rhythm.[116]

Cardiovascular diseases

A preliminary study showed a decreased nocturnal plasma melatonin in coronary heart disease;[117] this finding based on a one-point blood sample was confirmed by further studies. Whether a decreased melatonin level may be a predisposing factor or whether the occurrence of the disease decreases melatonin synthesis remains to be determined. In addition, a similar observation was reported during acute myocardial infarction.[118] The presence of melatonin as an antioxidant could be beneficial to prevent the adverse effects of reactive oxygen species during myocardial ischemia-reperfusion. On the other hand, whether melatonin acts partly as an autonomic regulator is not clearly established.[119] Also, melatonin is probably involved in the control of the circadian rhythm of blood pressure. A preliminary study showed that pinealectomy leads to hypertension in the rat.[120] Nocturnal melatonin secretion is impaired in non-dipper hypertensive patients and daily night-time administration of

melatonin for 3 weeks in patients with essential hypertension reduces blood pressure without alteration of heart rate.[121,122] Such an interesting result should be replicated in a larger group. On the contrary, melatonin impairs efficacy of nifedipine in well-controlled hypertensive patients.[123] This suggests caution in uncontrolled use of melatonin in hypertensive patients.

Concluding remarks

Although melatonin was discovered more than 40 years ago, the data on the physiological role of this hormone in humans are scant. Continuous progress in our knowledge reinforces, however, the idea that melatonin could play the role of a universal endogenous synchronizer, even for physiological functions whose circadian organization does not appear of paramount importance at first sight. The influence of melatonin on hemostasis, glucose homeostasis, phosphocalcic metabolism and blood pressure regulation would deserve further investigation. Also, the development of melatonin antagonists could help to understand the real physiological importance of melatonin in humans.

Because of its complex regulation, melatonin secretion is disturbed in most pathophysiological situations. The evaluation of melatonin secretion should be extended to situations where dramatic disturbances of endogenous rhythms, immune and antioxidative defences are patent, in patients admitted in intensive care units for instance. However, study conditions (light environment, drug intake) should be strictly controlled. We believe that great advances could be achieved by developing these clinical aspects in a collaborative way, generating multicentric trials on the efficacy and long-term toxicity of melatonin.

Practice points

1. Because the anatomical pathways which control melatonin secretion are complex, there are many causes of melatonin disturbance which are not specific to any particular disease. Also, way of life, environment and chronotype influence melatonin secretion.
2. Many drugs can influence melatonin secretion.
3. The evaluation of melatonin secretion in patients requires exploration of the complete 24 h profile, because of a possible shift or reverse secretion. Blood sampling provides a more precise melatonin profile and can be coupled with the temperature profile determination. Study conditions (light environment, posture) should be controlled.

Research agenda

In the future we need to:

1. Re-evaluate the possible relationship between the peaks or troughs detected in the melatonin profile and sleep stages.
2. Investigate the genetic control of melatonin secretion (polymorphism of genes that control enzyme activity) to explain 'low melatonin secretors'.
3. Explore the melatonin secretion in situations where rhythms, immune and antioxidative defences are patent in order to evaluate the efficacy of melatonin in controlled trials.
4. Evaluate the possible side-effects associated with chronic melatonin treatment.

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