The Pineal Gland and Pineal Tumours

Updated: January 1, 2011

Authors: Josephine Arendt, PhD, FRCPath, FRSM, Dr.med.h.c.

INTRODUCTION

The pineal gland has variously been described as the ‘Seat of the Soul’ (by Renee Descartes), a good neuroradiological marker, and, in view of its shape in humans, the ‘penis of the brain’. The rhythmic production of its major hormone melatonin (5-methoxy-N-acetyltryptamine) is extensively used as a marker of the phase of the internal clock. Melatonin itself is successfully used as a therapy for certain sleep disorders related to circadian rhythm abnormalities and as a mild hypnotic. It may have more extensive therapeutic applications. In lower vertebrates the pineal is an important determinant of rhythms. In mammals whose seasonal functions are timed by daylength, melatonin production at night provides a (probably) universal time cue for changing daylength. In humans, the evidence to date indicates that it serves to reinforce physiological events associated with darkness, such as sleep and to act as an internal time cue. The profile of its secretion defines “biological night”.

Pineal Structure

The pineal gland (epiphysis cerebri) is a small (100-150mg in humans), unpaired central structure, essentially an appendage of the brain. The mammalian pineal is a secretory organ, whereas in fish and amphibians it is directly photoreceptive (the ‘third eye’) and in reptiles and birds it has a mixed photoreceptor and secretory function. Pinealocytes retain elements of their photoreceptive evolutionary history in both structure and function (1, 2). In humans and some other species the gland usually shows a degree of calcification after puberty (3), and this process may well begin earlier in life. This phenomenon may not be associated with a decline in metabolic activity, except that activity declines in general with aging. The gland is richly vascularized. The principal innervation is sympathetic, arising from the superior cervical ganglion (4) with good evidence for parasympathetic, commissural, and peptidergic innervation (5). Major functional importance has only been shown for sympathetic innervation.

MELATONIN SYNTHESIS AND METABOLISM

Melatonin is synthesized within the pinealocytes- from tryptophan (Fig 1), . Most synthetic activity occurs during the dark phase, with a major increase (7-150 fold) in the activity of serotonin-N-acetyltransferase (arylalkylamine N-acetyl transferase, AA-NAT). AA-NAT is usually rate limiting in melatonin production, but serotonin availability may also play a role. The rhythm of production is endogenous, being generated by interacting networks of clock genes in the suprachiasmatic nuclei (SCN), the major central rhythm-generating system or “clock” in mammals (the pineal itself is a self sustaining “clock” in some if not all lower vertebrates) (6). The main feedback loop involving transcription of a number of genes (Per1, Per2, Per3, Cry1, Cry2, and Reverbα) is activated by heteromeric complexes of CLOCK and BMAL1. This transcription continues into the night until nuclear levels of PER and CRY proteins become sufficiently high to repress CLOCK/BMAL1 activation. Declining levels of PER/CRY in the early morning then allows transcription of the genes again and the cycle continues. Within this cyclical process, the stability of PER and CRY proteins is tightly controlled by casein kinases (CK1d/e) and the F-box protein FBXL3 respectively. Most, if not all peripheral tissues also express this sequence and the SCN is considered to synchronise other clocks such that they are optimally phased (7,8,9) thus establishing links between the central circadian system and virtually all aspects of physiology. The SCN rhythm is synchronized to 24 hours primarily by the light-dark cycle acting via the retina and the retinohypothalamic projection to the SCN. An additional central clock, indepenedent of the SCN, has recently been discovered. It is entrained by food availability and timing. (8).

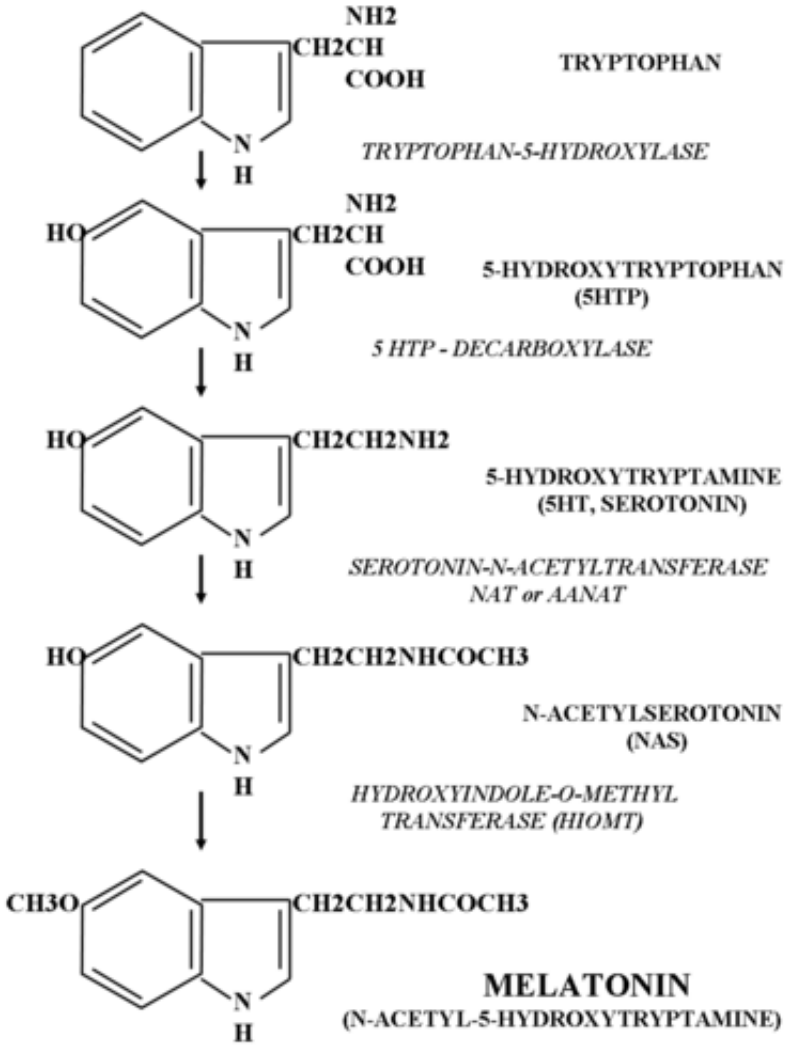


Figure 1.Melatonin synthesis in the pineal gland.

The cDNAs encoding both AA-NAT and the O-methylating enzyme HIOMT have both been cloned (10). There are substantial species differences in regulation of AA-NAT. It is likely that in humans and ovines the enzyme is regulated primarily at a post transcriptional level, whereas in rodents the key event appears to be cyclic AMP-dependent phosporylation of a transcription factor that binds to the AA-NAT promoter. Rapid decline in activity with light treatment at night appears to depend on proteasomal proteolysis (10,11). According to distribution studies of AA-NAT mRNA this enzyme is expressed mainly in the pineal, retina and, to a much lesser extent, some other brain areas, the pituitary and the testis, but apart from the pineal these structures contribute little to circulating concentrations in mammals. There is also evidence that melatonin can be synthesised in numerous other sites where it may have local effects (12,13). In the gastro-intestinal tract it may contribute to gut function (13). Within the rodent retina a self sustaining ‘clock’ maintains rhythmic production of melatonin in vitro as it does in many lower vertebrates (14). Whether or not this is true of humans remains to be seen. In humans and

rodents melatonin is metabolized to 6-sulphatoxymelatonin (aMT6s), primarily within the liver, by 6-hydroxylation, followed by sulfate conjugation. A number of minor metabolites are also formed, including the glucuronide conjugate, which predominates in mice.(15). Exogenous oral or intravenous melatonin has a short metabolic half-life (20 to 60 minutes, depending on author and species), with a large hepatic first-pass effect and a biphasic elimination pattern. In ruminants longer half-lives are seen after oral administration (16,17).

Most effects of pinealectomy can be reversed by melatonin, administered appropriately in physiological concentrations. Hence, it is difficult to consider the numerous other compounds found and/or synthesised within the pineal as major pineal hormones.

Control of Melatonin Synthesis: A Darkness Hormone

Sympathetic denervation of the pineal in mammals abolishes the rhythmic synthesis of melatonin and the light-dark control of its production. Norepinephrine is clearly the major transmitter, acting via beta-1-adrenoceptors with potentiation by alpha-1 stimulation, but the role of neural serotonin is probably not negligible. There is a day-night variation in pineal norepinephrine, with highest values at night, approximately 180 degrees out of phase with the pineal serotonin rhythm. cAMP acts as a second messenger and stimulates AA-NAT activity. Beta-adrenergic receptor binding sites in the rat pineal vary over a 24-hour period, the lowest number being found toward the end of the dark phase, increasing shortly after lights on (16,18). There is evidence for modulation of melatonin synthesis in vitro by other factors, notably neuropeptides (18), but their physiological importance remains obscure. Table 1 summarises factors influencing melatonin secretion and production.

Table 1. Some factors influencing human melatonin secretion. A comprehensive revue addresses mostly animal in vivo and in vitro effects, for references see 18,19. A: antagonist, U: uptake, I: inhibitor, MAO: monoamine oxidase, OC: oral contraceptives, 5HT: 5-hydroxytryptamine, □: increase, □: decrease. Reproduced by permission from (19)

Factor	**Effect(s) on melatonin**	**Comment**
Light	Suppression	>30 lux white 460-480 nm most effective
Light	Phase-shift/ Synchronisation	Short wavelengths most effective
Sleep timing	Phase-shift	Partly secondary to light exposure
Posture	□ standing (night)	
Exercise	□ phase shifts	Hard exercise
ß-adrenoceptor-A	□ synthesis	Anti-hypertensives
5HT UI	□ fluvoxamine	Metabolic effect
NE UI	□ change in timing	Antidepressants
MAOA I	□ may change phase	Antidepressants
α-adrenoceptor-A	□ alpha-1, □ alpha-2	

Benzodiazepines	Variable diazepam, alprazolam	GABA mechanisms
Testosterone		Treatment
OC		
Oestradiol	? Not clear	
Menstrual cycle	Inconsistent	amenorrhea
Smoking	Possible changes ?	
Alcohol		Dose dependent
Caffeine		Delays clearance (exogenous)
Aspirin, Ibuprofen		
Chlorpromazine		Metabolic effect
Benserazide	Possible phase change, Parkinson patients	Aromatic amino-acid decarboxylase-I

Melatonin is synthesized and secreted during the dark phase of the day in virtually all species (16). In most vertebrates the rhythm is endogenous, that is, internally generated. It persists in the absence of time cues, in general assuming a period deviating slightly from 24 hours, and is thus a true circadian rhythm (20,21). Lesions of the SCN lead to a loss of the vast majority of circadian rhythms including melatonin (22). Circadian rhythms are entrained (synchronized) to the 24-hour day primarily by light-dark cycles. Factors (zeitgebers) other than light-dark cycles which are involved in entrainment include behavioral imposition such as forced activity and rest, social and nutritional (rhythmic feeding) cues, temperature variations, knowledge of clock time, certain drugs, possibly electromagnetic fields and melatonin itself (23). There is some recent evidence that in strictly controlled conditions women have slightly higher levels of plasma melatonin than men, at night. (24).

Daylength Dependence of Melatonin Secretion

Melatonin secretion is related to the length of the night: The longer the night, the longer the duration of secretion in most species (16). Ocular (not extra-ocular) light serves to entrain/synchronise the rhythm to 24h and to suppress secretion at the beginning and/or the end of the dark phase (Fig 2). The amount of light required to suppress melatonin secretion during the night varies from species to species, with time of night, and with previous light exposure (25-27). In humans, 2500 lux full spectrum light (domestic light is around 100 to 500 lux) is required to completely suppress melatonin at night (27). However much lower intensities will partially suppress and shift the rhythm in humans (28,29). Image forming vision (rods and cones) is not required for suppression of melatonin, or indeed for synchronising /phase shifting the circadian clock. A novel non-image forming photoreceptor system is implicated, with evidence for a pivotal role of a new opsin: melanopsin (30). In humans maximum suppression for equal numbers of photons is given by blue light (~ 480 nm) with an action spectrum that is distinct from that of scotopic and photopic vision (31-33).

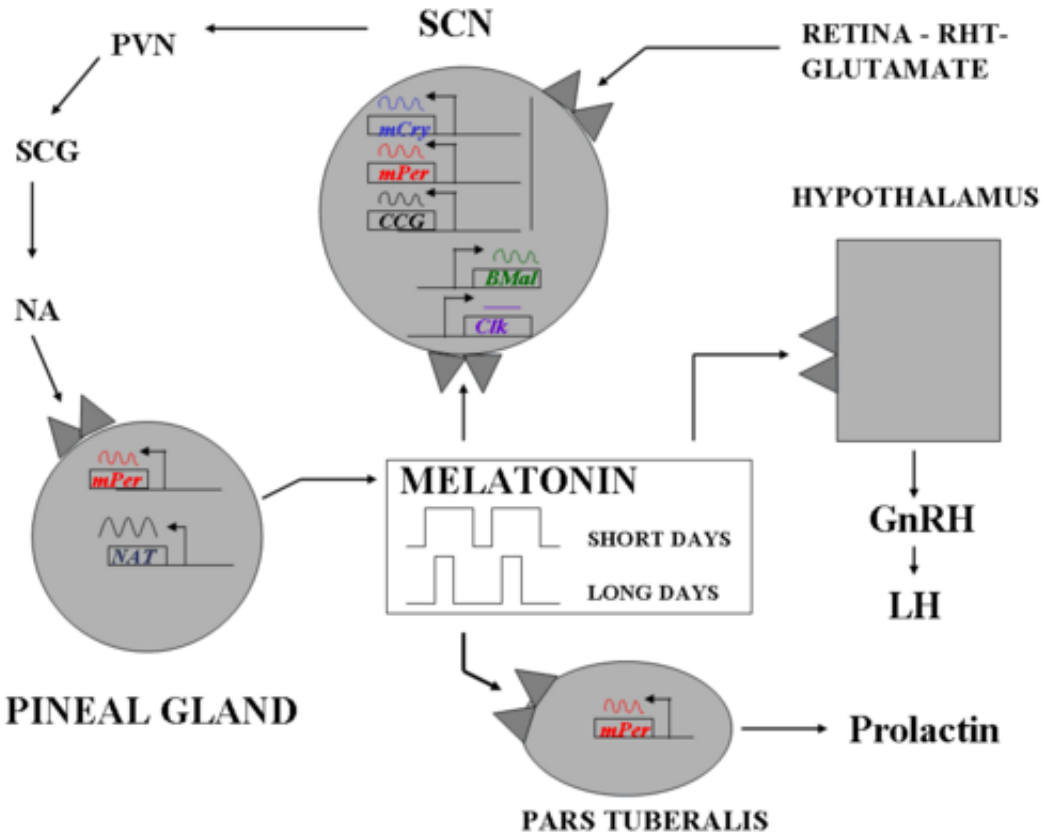


Figure 2.Diagrammatic representation of the control of production and the functions of melatonin, with regard to seasonal and circadian timing mechanisms. RHT- retino-hypothalamic tract, NA ” norepinephrine (noradrenalin), SCN – suprachiasmatic nucleus, PVN – paraventricular nucleus, SCG – superior cervical ganglion. The melatonin rhythm is generated by a closed loop negative feedback of clock gene expression in the SCN, Clock and Bmal, positive stimulatory elements, Per, Cry, negative elements, CCG, clock controlled genes. The SCN via neural pathways drives the pineal melatonin rhythm. Per and NAT mRNA oscillate in the pineal although post transcription control is evident in some species. Melatonin influences SCN activity via one or more receptors. Melatonin conveys photoperiodic information influencing the pattern of per expression in the pars tuberalis for the control of seasonal prolactin variations. Melatonin target sites in the hypothalamus influencing seasonal variations in reproductive hormones have yet to be fully defined, although the premammillary hypothalamus is implicated (34). Based on an original diagram by Dr Elisabeth Maywood, MRC Laboratory of Molecular Biology, Neurobiology Division, Hills Road Cambridge, CB2 2QH, UK.

The initial treatment for winter depression (SAD) with bright light was based on the assumption that melatonin duration was a seasonal signal in humans (35). In order to demonstrate daylength dependence of melatonin secretion in humans it has been necessary to maintain subjects in total darkness for 14h per day for 2 months, when the secretion profile is clearly longer than after 10h total darkness for two months, with accompanying changes in body temperature and sleep (36). This photoperiodic response has sometimes been observed in

polar regions and even in subjects living according to the natural light dark cycle in temperate regions. The most consistent seasonal observation in humans is that melatonin profiles show a phase change from winter to summer, with earlier secretion in summer than in winter e.g. (37).

Synchronisation of the Melatonin Rhythm to the 24h Day

A single daily light pulse of suitable intensity and duration in otherwise constant darkness is sufficient to phase shift and to synchronize the melatonin rhythm to 24 hours in animals (38,39). Phase shifting and entrainment (synchronisation with an appropriate phase) have been demonstrated in humans with suitable intensity, spectral composition and duration of light treatment (40-42). However, the relative contribution of light in a normal environment with numerous other time cues remains to be fully determined. Studies in Antarctica suggest that a structured social routine in a dim light environment suffices to synchronize melatonin to 24 hours in most people (16). Many blind people with no conscious or unconscious light perception living in a normal social environment show ‘free running’ or abnormally synchronised melatonin and other circadian rhythms (39,43,44). Moreover the incidence of circadian desynchrony, with its attendant sleep and other problems, is directly related to the degree of light perception of the individual. The less light the more likely is desynchrony to occur. Some blind subjects retain an intact retino-hypothalamic tract and a melatonin suppression response even in the absence of conscious light perception.(45) . The contribution of the so-called “non-photic” time cues to entrainment has been evaluataed in a small number of blind people (39).

PHYSIOLOGICAL FUNCTIONS OF MELATONIN IN MAMMALS

Melatonin Secretion as a Function of Daylength: a Seasonal Time Cue

When seasonal functions such as reproduction, pelage (coat growth and color), appetite, bodyweight are primarily timed by daylength, species are referred to as photoperiodic . Photoperiod is often critical for the timing of pubertal development (46,47). It is now clear that in photoperiodic mammals and marsupials, an intact innervated pineal gland is essential for the perception of photoperiod change (47-50).

It is possible to administer melatonin by daily infusion or feeding so as to generate at will circulating profiles, with a duration characteristic of particular photoperiods, in the intact or pinealectomized animal (48,49). In this way it has become clear that a particular melatonin duration is the necessary and sufficient condition for the induction of a given seasonal response and is equipotent with a particular photoperiod. Long-duration melatonin is equivalent to short days and short-duration melatonin is equivalent to long days (Fig. 2, see above). The interpretation of the signal, as with daylength, depends on the physiology (for example, long- or short-day breeder) of the species in question. In sheep, melatonin can time the whole seasonal cycle, at least of reproduction, acting as a seasonal zeitgeber for a presumed endogenous circannual rhythm (50). The circannual rhythm of prolactin secretion (synchronised by photoperiod) is dependent on the circadian melatonin signal and is thought to derive from a

pituitary-based timing mechanism whereby melatonin regulated pars tuberalis timer cells serve to coordinate adjacent prolactin-secreting cells which together function as an intrapituitary “pacemaker-slave” timer system. (51)..

Reproduction in domestic ruminants and the winter coat of animals such as mink, arctic foxes, and cashmere goats has commercial significance and can be manipulated by photoperiod and melatonin administration. Implanted melatonin induces short-day effects,and a number of commercial preparations of melatonin have been developed to this end.

Photoperiod via melatonin secretion determines the timing of puberty in some species, provided that a sufficient degree of physical maturity has been reached (46). Interestingly, photoperiod perception by the fetus is present before birth in rodents and ungulates and ensures a rate of development appropriate to environmental conditions (52-54). Melatonin injections to the mother can dictate the timing of postnatal reproductive development. In rats injections of melatonin during the late light phase, during a small window in the late dark phase or even using continuous release implants, specifically during the period of pubertal development, delay reproductive maturity in both males and females (55). Full sexual maturity is eventually achieved; thus the system is not permanently compromised. Moreover in vitro melatonin inhibits gonadotropin-releasing hormone (GnRH)-induced luteinizing hormone (LH) release by cultured rat pituitary glands from prepubertal animals (56). These observations constitute the main evidence for a possible causal role of melatonin in the pubertal development of humans.

Role of the Pineal Gland and Melatonin in Circadian Rhythms

Melatonin is produced rhythmically by both the pineal and the retina in many lower vertebrates and probably serves as the common humoral signal for circadian organization (23). In mammals its role appears to be modulatory with regard to circadian organisation. Pinealectomy of rodents in constant light leads to disruption of the circadian system (57). In rats pinealectomy increases the rate of re-entrainment to forced phase shifts of the light-dark cycle (58). Interestingly, in humans, pharmacological suppression of melatonin by atenolol enhances the magnitude of light-induced phase shifts (59) and melatonin and light can act in concert to effect a phase shift (60). Thus, a possible conclusion might be that the presence of melatonin determines the rate of adaptation to phase shift. A specific melatonin antagonist which can be administered to humans is awaited to resolve these questions. Melatonin is also implicated in circadian thermoregulation (see (61) for a review). Many such effects may involve the thyroid gland.

EFFECTS OF TIMED ADMINISTRATION OF MELATONIN IN MAMMALS

In vivo effects

Daily melatonin administration to rats and some hamster strains, by injection or by infusion, will synchronise free-running activity and temperature rhythms in constant darkness. It is also reported to partially or completely synchronize disrupted activity rhythms in constant light (23).

Circadian phase can be set in fetal hamsters by maternal injections of melatonin at 24-hour intervals at specific circadian times (54). Timed administration to rats hastens adaptation of activity and melatonin production to forced phase shift, and can change the direction of re-entrainment (58,62). A phase response curve (PRC) to single injections of melatonin can be demonstrated with small phase advances of at most one hour during the late subjective day (58).

As the pineal is involved in circadian timing, the presumption must be that it is concerned with the timing of the LH surge and indeed with general estrous timing. There is evidence that in rats, timed melatonin administration can mimic the effects of extending the light-dark cycle on the timing of the LH surge. Observations of the peripheral melatonin rhythm itself show a decreased amplitude during proestrus in rodents but with conflicting reports in other species (see (16,63) for reviews). In the rat, gestation length depends on the ambient light-dark cycle. Small advances or delays of parturition can be induced by daylengths shorter or longer than 24 hours, and the effect can partially be mimicked by timed melatonin administration (64).

A fairly consistent observation in pineal research is the decline in amplitude of the melatonin rhythm in old age (see (16) for references). Pinealectomy accelerates the aging process, and there has been some considerable publicity concerning claims for an anti-aging effect of melatonin (65,66). The most widely published hypothesis is that melatonin acts as a free radical scavenger and anti-oxidant (67,68). Being an easily oxidised molecule melatonin does indeed have some anti-oxidant activity. However the quantities of exogenous melatonin required to generate clinically relevant anti-oxidant activity in vivo remain to be specified.

In Vitro Phase Shifts

Melatonin inhibits 2-deoxyglucose uptake into the SCN in late subjective day, with no effect at other times, and inhibits electrical activity also during late subjective day (23). In this way melatonin may counter a ‘wake’ signal from the SCN. The most convincing evidence for a direct influence on the circadian system is the phase-advancing effect of melatonin on the circadian rhythm of electrical activity in cultured SCN (69). The effect was large, acute, and time-dependent, with shifts of up to several hours being observed.

Retinal Rhythms

Melatonin appears to function as a paracrine signal within the retina. It enhances retinal function in low intensity light by inducing photomechanical changes and regulating the turnover rates of the photoreceptive apparatuses of rods, cones and the surrounding pigment epithelium (70).

The pineal, the retina, and the SCN together form the basic structures perceiving and transducing non-visual effects of light. Melatonin provides a closed loop to this system. It is reasonable to conclude that in adult mammals melatonin serves to modulate circadian phase and strengthen coupling. Since optimal circadian phase is important to health, this is clearly a very significant function. In fetal and neonatal mammals it may help to program the circadian system and to determine the timing of developmental stages, especially puberty.

Miscellaneous

The numerous reports of other effects of melatonin in animals and in vitro are beyond the scope of this article. Many of these concern “protective” effects attributed to anti-oxidant activity (71)(review). Evidence for anti-tumour activity of melatonin is now strong and possible mechanisms have been proposed (72-75). Circadian control of metabolism (9,76,77) means that there is much scope for effects of melatonin in this area. Anti-apoptopic activity of melatonin has been investigated and attributed to mitochondrial mechanisms (75)

HUMAN PINEAL PHYSIOLOGY AND PATHOLOGY

Human Melatonin Production

Pinealectomy in humans removes virtually all plasma melatonin (78). Other consequences of the operation consist of diffuse neurological problems that do not add up to a consistent functional effect as yet and may be more related to non-specific effects of operation. Some preliminary evidence suggests that pinealectomised humans are less “seasonal” than healthy subjects, underlining the predominantly photoperiodic role of melatonin if confirmed (79). There is good evidence that the neural and biochemical pathways known to control pineal function in rats are similar in humans. Pathological or traumatic denervation of the pineal abolishes the plasma melatonin rhythm. Beta-adrenergic antagonists suppress melatonin production, and increased availability of norepinephrine and serotonin are stimulatory (for references see reviews (16,26)). The melatonin content of pineals obtained post-mortem is related to the time of death with, as expected, higher values at night (80).

In a “normal” environment, melatonin is secreted during the night in healthy humans as in all other species. The average maximum levels attained in plasma in adults are of the order of 60 to 70 pg/ml when measured with high-specificity assays. The concentrations in saliva are approximately one third of those in plasma. Minimum concentrations in both fluids are usually below 5 pg/ml. The peak concentrations of melatonin in plasma normally occur between 0200 and 0400 hours. The onset of secretion is usually around 2100 to 2200 hours and the offset at 0700 to 0900 hours in adults in temperate zones. The appearance and peak levels of 6-sulfatoxymelatonin (aMT6s) in plasma are delayed by 1 to 2 hours, and the morning decline by 3 to 4 hours. The mean concentrations of plasma and saliva melatonin together with urinary 6-sulfatoxymelatonin (aMT6s) are shown in Fig. 3a. There are strong correlations between the timing and amplitude of the plasma melatonin and urinary aMT6s rhythms, such that aMT6s is a useful measure of circadian phase in field situations (16, 26, Fig.3b). In urine 50 to 80 per cent of aMT6s appears in the overnight sample (2400 to 0800 hours), and it is low but rarely undetectable in the afternoon and early evening. Possibly the most striking characteristic of the normal human melatonin rhythm is its reproducibility from day to day and from week to week in normal individuals, rather like an hormonal fingerprint. There is however a large variability in amplitude of the rhythm between subjects. A small number of apparently normal individuals have no detectable melatonin in plasma at all times of day (16,26) .

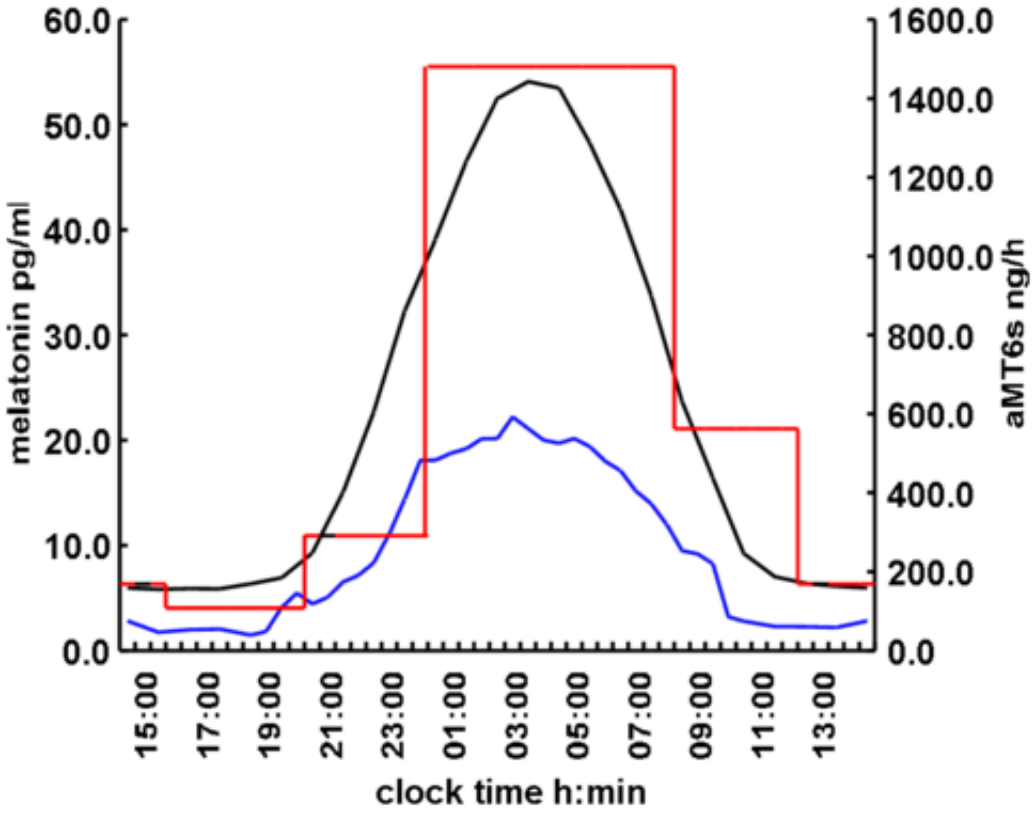


Figure 3a.Average concentrations of melatonin in plasma (black, average N=133), saliva (blue, average N=28) and 6-sulphatoxymelatonin (aMT6s) in urine (red, average N=88), all measurements by radioimmunoassay. Diagrammatic representation of mean normal values (healthy men and women over 18 years old) from the author's laboratory.

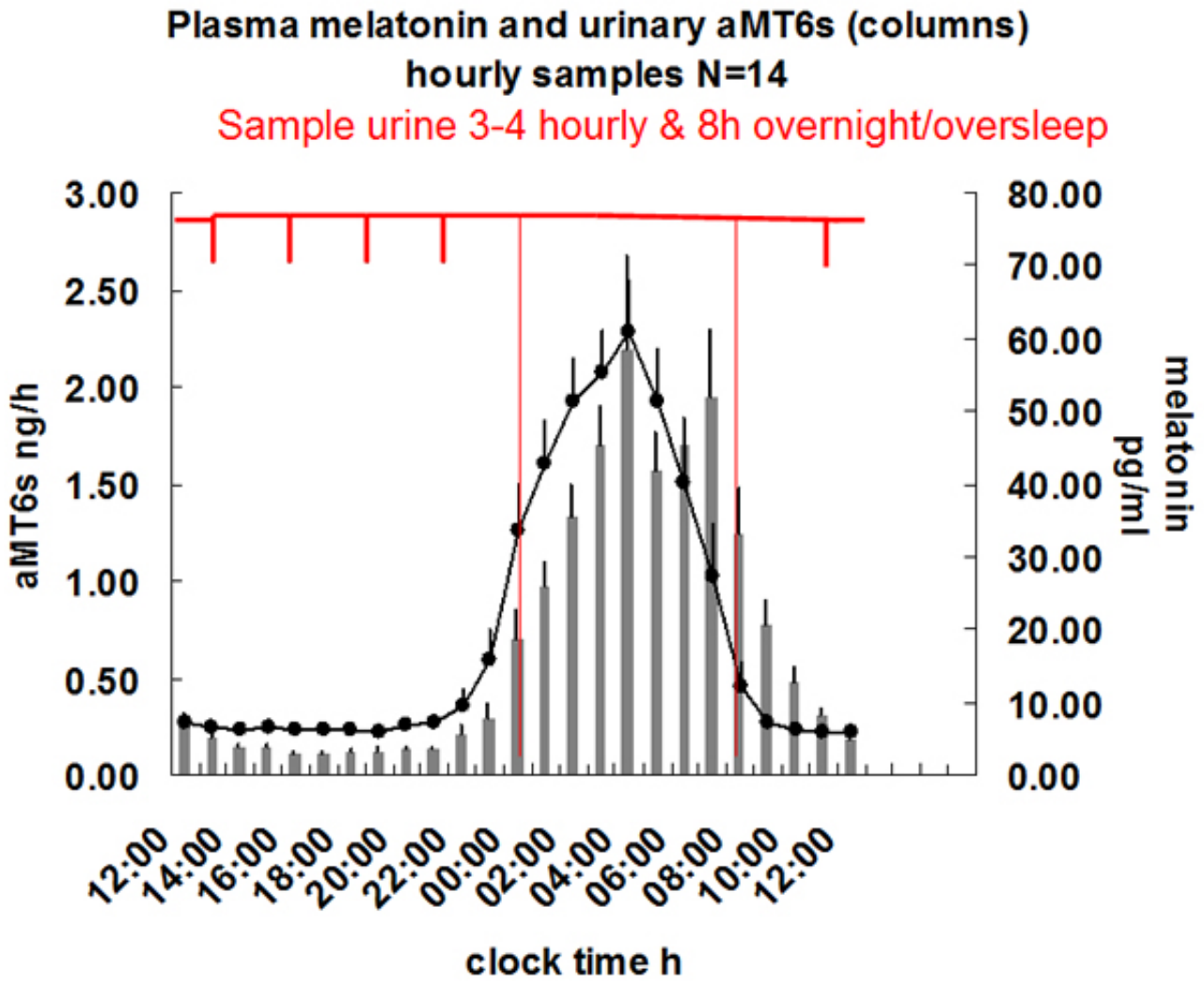


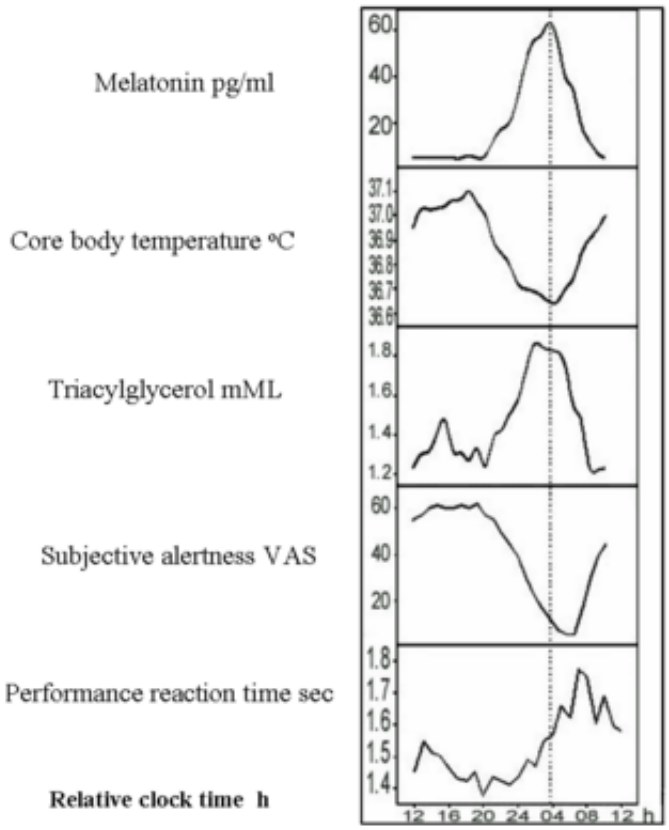
Figure 3b.Plasma melatonin and urin ary aMT6s in hourly samples, mean ” SEM, N=14, to show the delay in the rhythm of aMT6s compared to melatonin. If urine is sampled 3-4 hourly with an over-sleep collection as shown, a close correlation with plasma melatonin amplitude and phase is found. Drawn from data in reference 105.

As stated previously, even domestic intensity light can suppress human melatonin production at night. Exposure to light during ‘biological night’ (LAN) has been perceived as deleterious to health (for example the increased risk of cancer in most epidemiology studies on night shift work) (73,74,81,82). This hypothesis is based on the beneficial effects of melatonin in some situations (see later). For example, the progression or spontaneous appearance of cancer in animals is enhanced by continuous light (82) and in human breast cancer xenografts exogenous melatonin is reported to reverse this effect (83). Whether or not the suppression of endogenous melatonin has undesirable consequences in the long term remains to be evaluated. A more substantive hypothesis considers that LAN disrupts the expression of clock genes with probable deleterious effects on numerous systems. Such disruption is associated

with vulnerability to cancer in animals (73,74,84).

Melatonin and Core Body Temperature

The melatonin peak is closely associated with the nadir in core body temperature, together with maximum tiredness/fatigue, lowest alertness and performance (Fig. 4) (85). Causal links are suggested by a number of observations. For example, bright light at night suppresses melatonin, simultaneously increasing body temperature, alertness and performance, and decreasing sleepiness (86). Exogenous melatonin during the daytime acutely increases sleepiness and decreases core body temperature (87). This latter observation is dependent on posture: subjects must be seated or recumbent (88). The ovulatory rise in temperature during the menstrual cycle is associated with a reported decline in amplitude of melatonin, but the decline in melatonin is not a consistent observation.



Some major circadian rhythms driven by the internal clock

Figure 4.Relationship of plasma melatonin to other major circadian rhythms. Note the close correspondence between the core temperature nadir and the melatonin peak. Sleep propensity closely follows the melatonin rhythm. Reproduced from Rajaratnam SMW and Arendt J. Lancet 358:999-1005, 2001 by permission.

Melatonin and Sleep

Endogenous melatonin production is clearly closely related to the onset and offset of sleep. However sleep deprivation does not abolish the melatonin rhythm and in very dim light does not affect secretion (85). In controlled experimental conditions it is clear that the evening rise of melatonin corresponds closely to the opening of the ‘sleep gate’, following a period of wake maintenance which has been called the ‘forbidden zone for sleep’ (89). Few associations have emerged between melatonin production and sleep stages, with the exception of a relationship between the timing of sleep spindles and certain other EEG characteristics, and the circadian phase of melatonin (90). Possibly the best correlative evidence for a role of melatonin in human sleep is the appearance of daytime naps, in free-running blind subjects when the peak of melatonin (and of course the temperature nadir) occurs during the daytime (91). Another pertinent observation is the poor sleep of patients with Smith-Magenis syndrome, most of whom have an inverted melatonin rhythm with high values during the daytime (92).

The relationships of stress, exercise, and some other non-pharmacological interventions in modification of melatonin production are somewhat unclear and do not appear to play a major physiological role in humans.

Melatonin during Development, Puberty, Menstrual cycle and Aging

Shortly after birth very little melatonin or aMT6s is detectable in body fluids. A robust melatonin rhythm appears around 6 to 8 weeks of life (93). The plasma concentration of melatonin increases rapidly thereafter and reaches a lifetime peak on average at 3 to 5 years old (94). The increment is much greater at night. Subsequently a steady decrease is seen, reaching mean adult concentrations in mid to late teens with the major decline occurring before puberty. Values remain relatively unchanged until 35 to 40 years, and a final decline in amplitude then takes place until (on average) low levels are seen in old age (see (16) for references). Exceptionally healthy elderly may not show this age-related decline. Reports of differences in secretion in adults with gender, height, or body weight are not consistent, although a recent carefully controlled study reports earlier phase and slightly higher levels in women (24). The measured plasma concentrations of melatonin in children are probably related to Tanner stage and possibly body weight (95).

Although lower melatonin has been reported in precocious puberty and higher concentrations in delayed puberty and hypothalamic amenorrhea compared with age-matched controls (96,97), these remain correlative not causal associations, and there is no good evidence for a causal role of melatonin in primate pubertal development. Ovarian suppression with a GnRH analogue in precocious girls is not accompanied by changes in melatonin secretion (98). However, induction of sexual development with estrogen was associated with a very rapid decline of melatonin metabolite excretion in one case report (99). Likewise testosterone treatment of hypogonadal men led to a normalization of previously high circulating melatonin. (100).

Circulating melatonin may or may not vary during the menstrual cycle, the existing data are inconsistent. There is (101) evidence for abnormal melatonin secretion in patients with pre menstrual tension .

Low melatonin is reported to associate (inter alia) with cardiovascular disease and diabetic autonomic neuropathology (102-104). Studies of intensive care unit patients have shown very abnormal rhythms- but the data are confounded by the concomitant medication (105).

Clearly, the importance of the pineal in humans depends on the importance of light in human physiology. It is reasonable to assume that the pineal conveys information concerning light-dark cycles for the organization of seasonal and circadian rhythms in humans as in animals.

PATHOLOGY

Post-Mortem observations: Pineal Hyper- and Hypoplasia

A number of reports of variations in post-mortem pineal weight as a function of cause of death have been summarized by Tapp (106). Of the most interesting, hypoplasia of the pineal in association with retinal disease may be causally interrelated. Tapp has reported that pineals in patients dying of carcinoma of the breast or melanoma are heavier than those from patients with other cancers. Very large pineals (1 g) have been described in a rare genetic syndrome with insulin resistance (107). Sudden infant death syndrome (SIDS) is associated with small pineals and decreased melatonin production (108). SIDS deaths usually occur at night and may be associated with abnormalities of sleep. If melatonin helps to coordinate circadian organization in the developing infant, its underproduction may contribute to the disorder.

Pineal Tumors

Tumors of the pineal region in children are frequently associated with abnormal pubertal development (109). In precocious puberty it was thought that the capacity of the pineal to inhibit sexual development was impaired. Much evidence now suggests that precocity is due to the production of human chorionic gonadotrophin (beta-hCG) by germ cell tumors of the pineal (110,111). Delayed puberty has also been associated with pineal tumors. Pineal tumors are heterogeneous and may arise from germ cells (teratomas, germinomas, choriocarcinomas, endodermal sinus tumors, mixed germ cell tumors), pineal parenchymal cells (pineoblastoma and pineocytoma), and the supporting stroma (gliomas) (112). All are rare (less than 1 per cent of intracranial space-occupying lesions) and tend to occur below 20 years of age with the exception of parenchymal cell tumors, which occur equally in adults and children. Germinomas respond well to radiation therapy, whereas primary surgery is more frequently the treatment of choice in other types. Tumor markers in CSF, alpha-fetoprotein and beta-hCG, together with CSF cytology and imaging (CT or MRI), aid in differential diagnosis. The most common symptoms are secondary to hydrocephalus (headache, vomiting, and drowsiness) together with the triad of visual problems, diabetes insipidus, and reproductive abnormalities (112). Germinomas and teratomas occur predominantly in males. Precocious puberty is more commonly associated with teratoma. As beta-hCG is identical to beta-LH, pubertal development can be directly attributed to ectopic beta-hCG production in many cases. Moreover, the predominance in boys may be explained on the basis that LH alone can stimulate testosterone production, whereas in girls both LH and FSH are required for ovarian follicular development and estrogen production. Reviewing a series of 37 patients, Drummond and Rosenfeld (113)

concluded that there have been significant improvements in outcome over the last 30 years. A 5 year survival of 62% was quoted for germinomas, but only 14% for other malignant tumours.

Classification of pineal parenchymal tumours is complicated by the presence of mixed pineocytoma-pineoblastoma types some with intermediate differentiation. A new classification has been proposed based on histological features which is closely related to patient survival (114).

A new type of pineal tumour was described in 2003 ” the pineal papillary tumour. These tumors of the pineal region are similar to those described for ependymal cells of the subcommissural organ, and may be derived from these specialized ependymocytes (115).

There is no consistent information on overproduction or underproduction of melatonin with specific types of tumor. Some work suggests that melatonin is absent or very low in treated or untreated pineal germinomas, but the consequences remain to be defined (116).

Other Solid Tumors

In tumour-bearing animals both increases and decreases in melatonin production have been reported. In humans low levels may be associated at least with (stage-dependent) breast and prostatic cancer (117) (and some other endocrine-independent tumours) with a negative correlation to tumour size. Remission is associated with normalisation of melatonin levels. In ovarian cancer, on the other hand, elevated melatonin is reported. A number of broad studies that have included various oncological conditions report significant differences, both increases and decreases, in plasma melatonin between types of cancer and control populations. At present these are uninterpretable, and no mechanism has been shown to account for the observed changes. The subject has been extensively reviewed (74, 117). Some data suggests that the growth of human benign prostate epithelial cells depends on both steroids and melatonin (118).

Considerable effort has been expended investigating melatonin timing and production in prospective and retrospective “field” studies of cancer patients and shiftworkers (women shiftworkers have increased risk of breast cancer in most studies) assessed by the urine levels of aMT6s. An increased risk of breast cancer has been attributed to lower melatonin; however, the data are inconsistent and in some cases may be interpreted as a different timing of the rhythm rather than altered production (73,74,119,120,121). Most data are based on aMT6s concentration in morning void urines, where a change in timing of the rhythm can lead to under or over estimation of production. Breast cancer risk is associated with the presence of a clock gene polymorphism (in hper3) (122) which is associated with morning diurnal preference (lark) (123), which in turn is associated with earlier timing of the melatonin rhythm and hence lower morning aMT6s values. When urine collected over 24h is used for aMT6s measurement no associations have been found in 2 prospective studies (121,124). Thus, a causal connection has not been firmly established and many other factors (such as continual disruption of the circadian system in general) may be involved.

The association of both breast cancer and childhood leukemia with environmental exposure to electromagnetic fields (EMF) has also been attributed to melatonin suppression by EMF (81). There is little convincing evidence for this association and most recent data deny any acute suppression of melatonin in humans by EMF.

Psychiatry

Melatonin has been extensively used in psychiatry to assess biological clock status. There is evidence for a decline in amplitude of the melatonin rhythm in depression associated with an increase in cortisol, and possibly an increase in mania, although not all studies are consistent (125). Exceptionally delayed melatonin rhythms in winter were reported in patients with SAD compared with the small delay seen in normals (126,127) and Parry and co-workers have found abnormal melatonin patterns and response to light in premenstrual dysphoric disorder (101). At present there is still no consensus as to what causes SAD. Light treatment for SAD appears to be slightly more efficient when given in the morning (albeit with a large placebo effect) (128), thereby inducing an advance in the melatonin rhythm. However, other mechanisms are also possible. In particular it has been suggested, based on careful timing studies, that a specific phase relationship (phase angle difference) between melatonin and sleep is of primary importance.(129). Many pharmacological antidepressant treatments stimulate melatonin secretion, acting through increased availability of the precursors tryptophan and serotonin and the major pineal neurotransmitter norepinephrine, or by direct action on serotonin and catecholamine receptors . There may be a link between an increase in melatonin production and efficacy of treatment, and this possibility merits exploration. A recently introduced melatonin agonist has been developed for anti-depressant activity through its actions on serotonin-2C (5-HT2C) receptors (130,131).

Melatonin and metabolism

Pinealectomy in rats was reported to induce insulin resistance many years ago (e.g.132). One of the most interesting relationships of this molecule with metabolic problems emerged recently. It was observed that the gene encoding the melatonin receptor 1B (MT2/ *MTNR1B*) possessed variants that were closely associated with fasting glucose and reduced beta-cell function. Moreover the same allele was associated with an increased risk of type 2 diabetes in a meta-analysis of case-control studies totalling 18,236 cases and 64,453 controls (133,134). The risk genotype predicts the future development of type 2 diabetes. Given that this is an ever increasing problem in the developed world, the scope for therapeutic interventions is clearly to be explored.

Miscellaneous

Many clinical attempts have been made to relate circulating melatonin to endocrine and other pathology. The results on the whole are difficult to interpret and inconsistent (see references (16, 26)). Liver disease such as cirrhosis, which impairs metabolic function, leads to higher than normal plasma concentrations of melatonin (135). Drugs that stimulate or suppress hydroxylation and conjugation mechanisms or that compete for metabolic pathways can be

expected to affect circulating melatonin. Surprisingly, little evidence exists for a disturbance of melatonin secretion in major sleep disorders such as narcolepsy and Klein-Levine syndrome. However in delayed sleep phase insomnia delays in the rhythm are usually found and provide a basis for diagnosis (136).

EFFECTS OF MELATONIN IN HUMANS

Sleep and Circadian Rhythms

Circadian rhythm disturbance is associated (among other things) with shift work, jet lag, blindness, delayed and advanced sleep phase syndromes, and old age. The most obvious symptom is poor sleep. A treatment able rapidly to shift the biological clock in all its manifestations would be of substantial benefit to large numbers of people. To date bright light is the only treatment that in suitable intensity and duration is able to do this (but clearly cannot be used in the free running sleep disorder of the blind). Although melatonin has been known to have acute sleep inducing and phase shifting effects for many years, a consensus acknowledging therapeutic benefit has only emerged in the last 10 years (137).

The first evidence dates from 40 years ago when Aaron Lerner, who first isolated the substance, took 100 mg and described sleepiness after the dose . Subsequently a substantial literature, generally using much lower doses (0.3-10mg), has described advance shifts in the timing of sleep after early evening administration, transient sleepiness at several different times of day within 2-4h of the dose, time dependent increases in sleep propensity, effects on the waking EEG comparable to, but not identical with, benzodiazepines, a lengthening of the first rapid eye movement (REM) episode after early evening administration, increases in the fast EEG frequencies after evening naps or night time sleep and ‘beneficial effects’ taken at bedtime. The latter are usually a reduction in wake after sleep onset (WASO) and an increase in total sleep time (TST) evaluated subjectively, by actigraphy and, rarely, by EEG. When melatonin was used to hasten adaptation to a 9h phase advance, TST, sleep efficiency and stage 2 sleep were increased whereas slow wave sleep (SWS) was decreased. The subject has been extensively reviewed (138-141). Convincing evidence supports a primary effect of melatonin on sleep timing, whereby melatonin induced a redistribution of sleep during an imposed sleep opportunity of 16h without an increase in total sleep time (142).

Phase shifting of human circadian rhythms by melatonin was initially described in humans in the early 1980s. Phase advances were seen after 2mg daily at 1700h for one month. There were no significant effects on self-rated mood, or on levels of LH, FSH, testosterone, cortisol, growth hormone, or thyroxine. No deleterious effects were reported by the subjects (143). Advance shifts in sleep, endogenous melatonin, prolactin and core body temperature can be induced by oral administration (0.5- 10mg) in the ‘biological afternoon/evening’ (where biological night is the time of endogenous melatonin secretion). The magnitude of the shift is dose dependent. Delay shifts can be obtained by early ‘biological morning’ administration, and these time-dependent responses have been formalised as a phase response curve (PRC) (144). A simplified PRC diagram is shown in Figure 5. Melatonin given ca. 8-13 hours before core temperature minimum will phase advance, and given ca 1-4h after core temperature minimum will phase delay. More

recently, it has become clear that the rhythms of cortisol and TSH (and no doubt other rhythmic variables) are also shifted by melatonin (145).

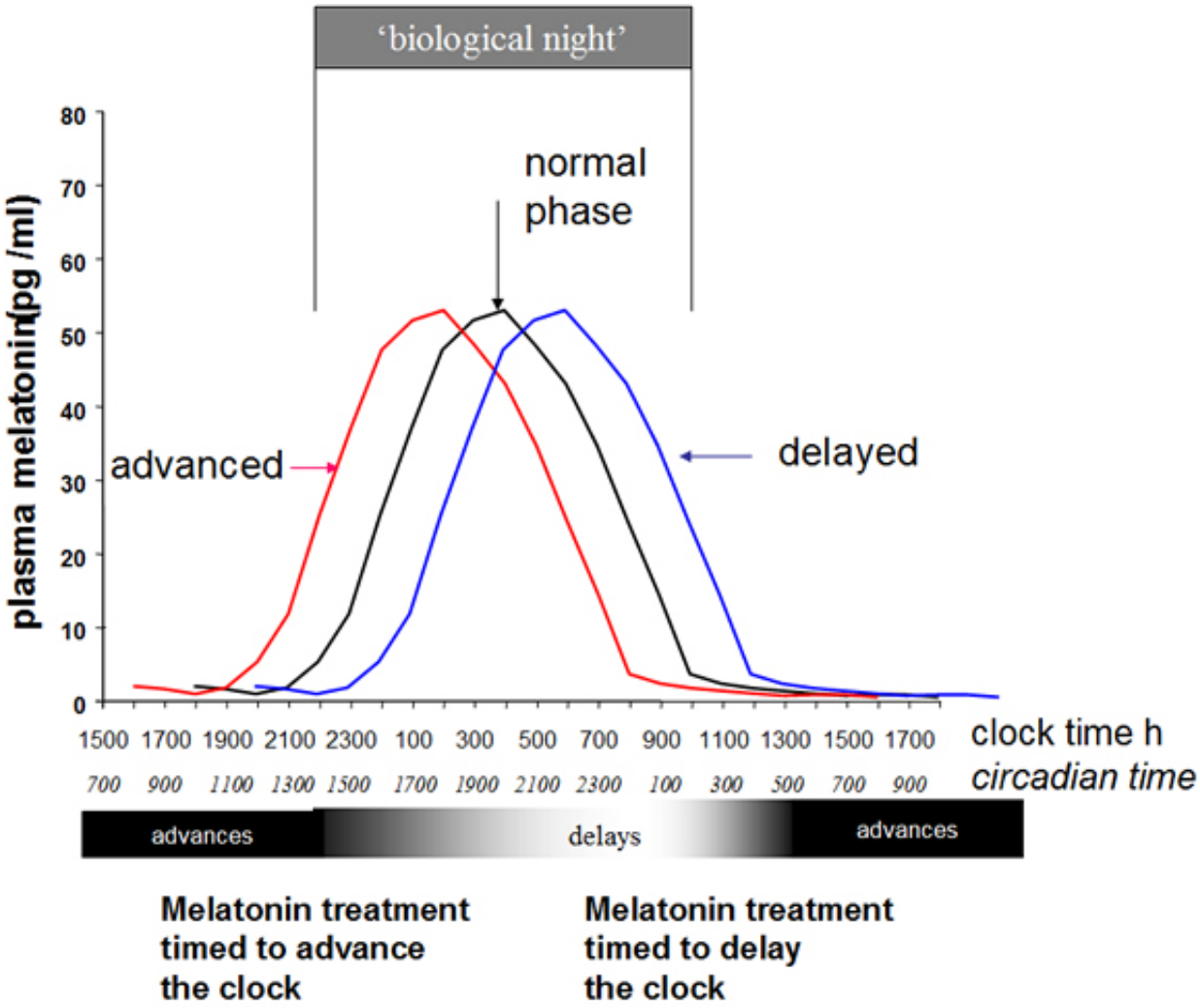


Figure 5.A highly simplified diagram of phase shifts of the circadian system, as evidenced by changes in the melatonin rhythm itself, following oral treatment with fast release melatonin at different times. The maximum advance shift obtainable with a single treatment of 3-5 mg is approximately 1-1.5h. A combination of timed bright light treatment, timed melatonin and timed darkness/sleep applied over several days can produce much larger shifts. “Biological night” is the time of endogenous melatonin secretion and defines “circadian time” or CT which is independent of clock time. Individual timing of treatment according to clock time can vary substantially, as extremely early and extremely late phase people (larks and owls) will have differently timed “biological night”. After time zone travel, or a series of night shifts, circadian phase (or biological night) can be completely reversed with, for example, melatonin production during the daytime.

In addition to these acute effects, melatonin can clearly maintain synchronisation of the circadian clock to 24 hours in sighted subjects living in conditions conducive to free run, and appeared to resynchronise some subjects after a period of free run (146). In the free running blind it has been possible to stabilise the sleep wake cycle to 24 hours with improvement in sleep and mood variables, without synchronising strongly endogenous rhythms such as core body temperature. With suitable dose (0.3-10mg) and timing however, entrainment/synchronisation is possible in most subjects (147-149) (Figure 6). Success was attributed to careful timing either to the advance portion of the PRC or for the treatment to start an hour before preferred bedtime, as the subjects' free running rhythm approached a normal phase. Individual sensitivity to melatonin varies and the pharmacokinetics are very different from one individual to another. The lower dose of 0.3-0.5 mg may be more effective than higher doses in many subjects. Timing at the start of treatment may be critical; however, it is possible that subjects with a very long free running period will not, ever, synchronise to melatonin.

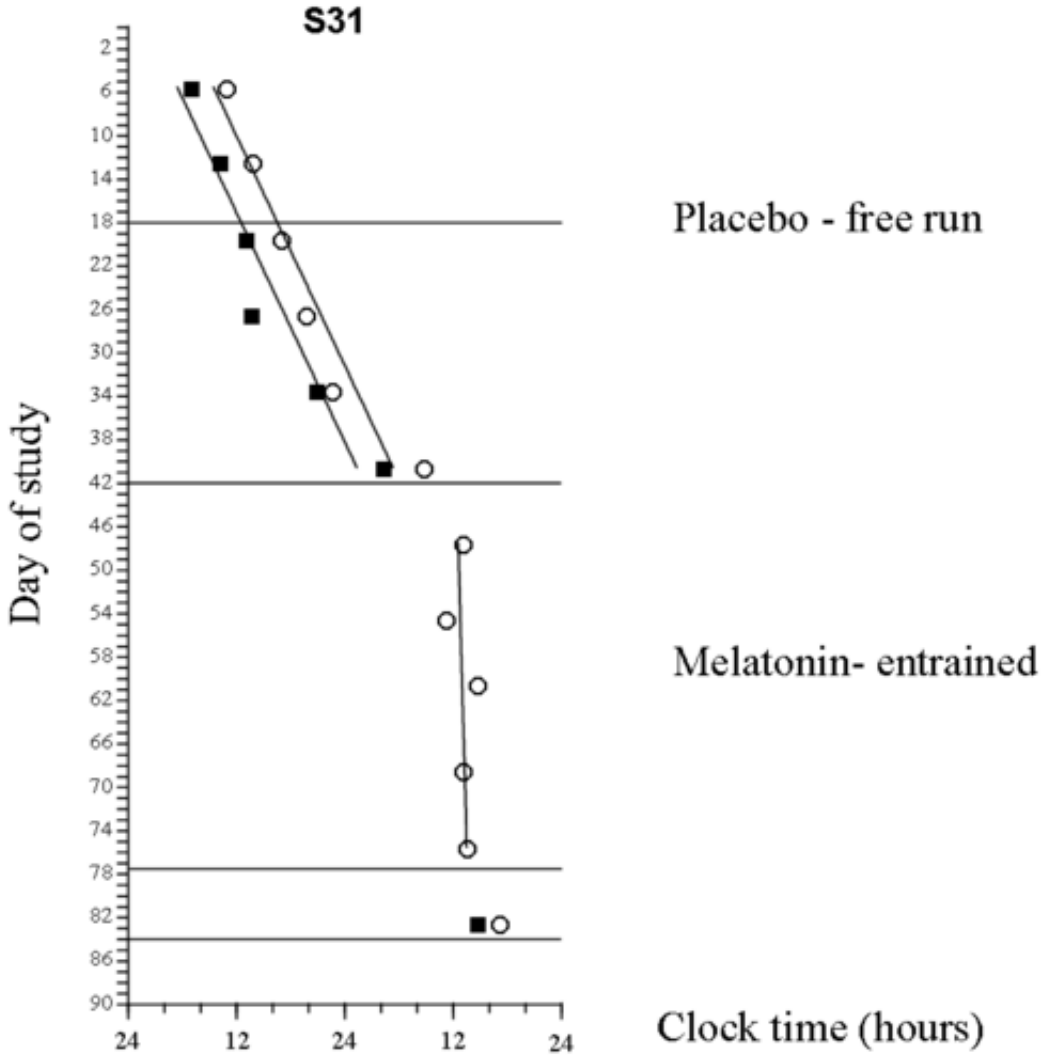


Figure 6.Melatonin can phase shift and, in some cases, synchronise circadian rhythms in some sighted and blind subjects with suitable timing of treatment and dose. Shown are the times of the calculated peaks (acrophases) of urinary 6-sulphatoxymelatonin (squares) and cortisol (circles) of a free-running blind subject with a period of 24.57h treated with placebo or 5mg

melatonin daily, timed to phase advance the internal clock. Note that with melatonin a 24h period is maintained (with beneficial effects on sleep). Redrawn from reference (147).

Even without full circadian synchronisation however, melatonin has generally positive effects on sleep in the blind (150).

Reproductive axis

The effects of melatonin on core body temperature are reported to vary in the course of the menstrual cycle and herein may lie a physiological function (151). LH pulses are amplified in early follicular phase by oral melatonin at 0800 hours (152). Attempts to develop melatonin as a contraceptive pill in combination with a synthetic progestin “minipill” have not been successful (153).

A series of studies in males with and without hypogonadism has reinforced the perception that melatonin is essentially inhibitory to human reproductive function (e.g. (154,155)), and very large doses (100 mg daily) potentiate testosterone-induced LH suppression (156). In the author’s opinion, low, timed doses of melatonin used to reinforce circadian organization are likely to improve fertility in humans. Acute oral doses of melatonin stimulate prolactin secretion (157). Acute effects on other pituitary hormones are somewhat inconsistent, although a relationship between melatonin and and vasopressin secretion has been established (158).

Miscellaneous

Some interesting data suggests that melatonin has anti-hypertensive effects (159). Melatonin, given during the daytime, can impair performance (e.g. 160). The acute pharmacological properties of melatonin in animals include sedation, hypothermia, anxiolysis, muscle hypotonia, decrease in locomotor activity with a rebound increase on increasing the dose, slight analgesia, slight protection against electroconvulsive shock, constriction of cerebral arteries, potentiation of noradrenaline-induced vasoconstriction and very low toxicity (161).

THERAPEUTIC USE OF MELATONIN

Jet Lag and Shift Work

Melatonin treatment timed to induce phase advances and delays has been used in the alleviation of jet lag in numerous real life and simulation conditions of which the vast majority reported beneficial effects. Field studies suggest that self-rated jet lag can be reduced on average by 50 per cent with appropriately timed treatment both westward and eastward (see (140 and 141) for a review and for recommended dose and timing). The improvement appears to be greater with larger numbers of time zones. The subjective impressions are reinforced by

improved latency and quality of sleep, greater daytime alertness, and slightly more rapid resynchronization of melatonin and cortisol rhythms. Neither the dose nor the timing of melatonin administration has been fully optimized although the largest successful study reported, with respect to alleviating sleep problems, that 5 mg was more effective than 0.5mg and a slow release preparation taken at bedtime after flight (162). Three studies have shown no effect”a common factor in two was that the subjects were not adapted to local time before departure with consequent problems for timing the treatment. The third also appeared to use inappropriate timing of treatment. Unpredictable exposure to bright light can theoretically act in opposition to the desired result. A Cochrane review (163) recently concluded that timed melatonin was effective as a jet lag treatment; however, a meta-analysis of the effects of melatonin as a “nutritional supplement” was less enthusiastic (Agency for Healthcare Research and Quality (http://www.ahrq.gov/news/press/pr2004/melatnpr.htm). The American Academy of Sleep Science now recommends the use of melatonin for jet lag, delayed sleep phase syndrome and non-24h sleep wake disorder (mostly seen in blind people) (164).

Some inconsistent work has been published on the use of melatonin in shift work. Preliminary work (165) suggested improved sleep and increased daytime alertness in night shift workers receiving melatonin at the desired bedtime during a night shift week compared with placebo and baseline conditions. A number of recent studies have successfully used melatonin to adapt to simulated or real shift work (reviewed in (166)) although it has to be said that several reports in the literature have shown no beneficial effects. Questions of posture, light environment and timing need to be resolved in field studies. Exposure to bright light sufficient to suppress melatonin secretion during the night is clearly beneficial to alertness and performance on the night shift whilst at the same time being a possible cause of increased cancer risk.. Some recent studies have used glasses (blue blockers) (167) which block the short wavelengths of light known to be most effective at suppressing melatonin, for work on the night shift. In theory these should preserve melatonin whilst enabling the use of bright light. More data is needed before robust recommendations can be made.

Sleep Disorder in the Elderly

Initially encouraging results using melatonin to alleviate sleep disorder in the elderly have proved inconsistent; however, there is no doubt that some subjects will derive benefit. As a result a slow release 2 mg melatonin formulation “Circadin” has been registered for use in insomnia of the over 50s (168). Dose, timing and formulation have not been fully optimized in the author’s opinion. A melatonin agonist, ramelteon, has also been developed for insomnia in general and others are being developed (130). Particularly notable are a series of studies in the demented elderly evaluating the use of increased light environment and/ or melatonin treatment. Positive outcomes were associated with both light and melatonin however the authors noted a tendency to depression with melatonin and advise the use of small (0.5 mg) doses (169).

Delayed Sleep Phase Insomnia

Patients with delayed sleep phase insomnia cannot sleep at the socially acceptable time of night and delay sleep onset until the early hours of the morning, sleeping through much of the

day. This condition has been successfully treated with bright light in the early morning to induce phase advances of the clock. In other trials evening melatonin (0.5-5 mg) has been administered, preferably 5h ahead of endogenous melatonin onset when known, or initial timing to advance the circadian clock, usually late afternoon/early evening. After realignment of sleep time the dose is taken just before normal bedtime . This treatment also advances sleep time significantly (170,171). Both children and adults were patients in one group of studies with successful outcomes (136). Judicious, timed application of both melatonin and bright light as time cues may well be the treatment of choice for rhythm disturbances.

Cancer

There is good evidence for photoperiod dependency and/or melatonin responsiveness of the initiation and evolution of certain cancers, particularly hormone-dependent cancers, in animals. Oncostatic effects are reported on some human cell lines, and in general the pineal and melatonin appear to have anti-tumor activity (172). In dimethylbenzanthracene-induced mammary tumors in rats, pinealectomy greatly increased the incidence of induced tumor growth, and daily melatonin administration in the late light phase greatly decreased incidence (173). Not all reports show positive results, however. A few early reports of positive effects of combination therapy- melatonin and tamoxifen, melatonin and interleukin, require confirmation (174,175). Most recently, survival time and quality of life were significantly enhanced by adjunct melatonin therapy in small cell carcinoma of the lung (176). The World Health Organisation recently concluded in a monograph (IARC) summarized in the Lancet (73,74) that there was good evidence for effects of melatonin on cancer in animals, but insufficient as yet for efficacy in humans.

Melatonin when appropriately administered has generally stimulatory effects on aspects of the immune system, and positive effects on cancer may be a consequence (175). A comprehensive review of the immune system effects of melatonin can be found in (74). The evidence that melatonin also acts as a free-radical scavenger has been discussed previously.

A recent review addresses the general question of the circadian system in relation to cancer: disruption of clock gene function is associated with increased risk of cancer in recent animal studies (9). Perhaps here may lie one aspect of the oncostatic activity of melatonin. By acting as a circadian coupling agent countering desynchrony amongst central and peripheral clocks, and optimising phase with respect to external time cues, cellular and system processes may be optimized and defense systems augmented. These considerations may also apply to risk of other major diseases associated with shift work (heart disease, metabolic syndrome, possible decreased fertility (177,178)).

MECHANISM OF ACTION OF MELATONIN

Target Sites

The actions of melatonin are multiple and many must derive essentially from modification of events in the CNS. However numerous melatonin target sites also exist in the periphery. Any

endogenous free-radical scavenging activity does not require a receptor. Lesions of the SCN and the anterior hypothalamic area can block photoperiodic and/or circadian effects of melatonin in some rodents, but with a degree of disparity between laboratories (179). Implants or infusion of melatonin in the hypothalamus mimic or block photoperiodic responses in several species (49). Melatonin target sites in the hypothalamus influencing seasonal variations in reproductive hormones have yet to be fully defined although there is strong evidence for a role of the premammillary hypothalamus in sheep..A recent revue considers the roles of different hypothalamic targets (180).

In prepubertal rats melatonin inhibits GnRH-induced LH release in pituitary cultures at concentrations comparable to those circulating in the blood (56), and there is evidence that melatonin influences GnRH secretion from the hypothalamus (181).

Using 2-125I iodomelatonin as a ligand, high-affinity (Kd 25 to 175 pM), saturable, specific, and reversible melatonin binding to cell membranes was initially reported in the SCN (151) and the pars tuberalis of the pituitary (183). Subsequently binding has been found in many brain and other areas including cells of the immune system, a number number of cancer cell lines, the gonads, the kidney and, importantly, the cardiovascular system. The SCN shows clear binding in human postmortem tissue (184). Species variation of melatonin-binding sites in the brain is of course apparent. The most consistent (but not universal) binding site between mammalian species is the pars tuberalis. There is good evidence that the pars tuberalis transduces the effects of photoperiod, via melatonin, on seasonal variations in prolactin secretion in ruminants (185). Morgan (186) proposed that pars tuberalis cells secrete an entirely new hormone ‘tuberalin’ that subsequently mediates the physiological effects of melatonin-although to date the structure has not been elucidated. Pars distalis binding is absent in adult rats but persists after birth in the neonate (187). This suggests that binding may indeed underlie function, as melatonin inhibits GnRH induced pituitary LH release in prepuberty but not in adulthood. Moreover, binding is detectable in the brain of neonatal Syrian hamsters whose circadian system responds to melatonin whereas it is lost in adults who do not respond. There are also changes with time of day, with season and as a function of exposure to melatonin (see (180) for references).

Melatonin Receptors

White and co-workers initially demonstrated that melatonin-induced pigment aggregation in amphibian melanophores is a pertussis toxin-sensitive system and that melatonin inhibits forskolin-activated cAMP formation (188). Intensive investigation of the properties of the pars tuberalis binding site has revealed that physiological doses of melatonin inhibit forskolin-activated cAMP production in vitro in a time- and dose-related manner (189,190). Dubocovich and co-workers have demonstrated a functional melatonin receptor, initially in rabbit and chicken retina (inhibition of calcium-dependent dopamine release), which is localized in the inner plexiform layer containing dopamine amacrine cells in rabbits, in the outer and inner segments in mice, and possibly in the pigmented layer in some mammals (191). Nuclear melatonin receptors (RZR/ROR alpha and RZR beta) have been described and may be involved in peripheral melatonin effects (192). Genetic polymorphism has been identified within

melatonin membrane receptors and further investigation of these polymorphisms in relation to photoperioidism, human disease, sensitivity to melatonin etc. is ongoing (193,194). Melatonin membrane receptors have now been cloned and three initial subtypes were named Mel 1a, Mel 1b and Mel 1c (195). The Mel 1a receptor gene has been mapped to human chromosome 4q35.1. Its primary expression is in the pars tuberalis and the SCN but other sites have been described. Of particular interest is the observation that melatonin can alter the expression of clock genes within the pars tuberalis in a manner analogous to photoperiod. Mel 1b has been mapped to chromosome 11q21-22 and its expression is in the retina and the brain. Mel 1c is not found in mammals. Two cloned mammalian receptors (Mel 1a, Mel 1b) have been renamed MT1 and MT2 (191). They are a new family of G protein coupled receptors, have high affinity (Kd 20-160 picomolar) and inhibit forskolin-stimulated cyclic AMP formation. MT1 acts through both pertussis sensitive and insensitive G proteins. The tissue expression in the SCN, the hypothalamus and the PT suggests that the circadian and reproductive effects are mediated through this receptor. Using gene knockout technology and pharmacological manipulations, results have suggested that the phase shifting receptor is MT2, whilst MT1 is associated with acute suppression of SCN electrical activity in addition to its actions within the pars tuberalis. There is also evidence for redundancy in these mechanisms, whereby both receptors can perform similar functions and recent evidence has uncovered co-expression of both MT1 and MT2 in sites associated with seasonal reproductive function in sheep (196). During development, melatonin receptors are transiently expressed in multiple neuroendocrine tissues, suggesting a novel role for melatonin as a neuroendocrine synchroniser in developmental physiology (197).. Numerous other physiological responses have been ascribed to MT1 and MT2 receptors, including (MT1) melatonin-mediated potentiation of adreneregic vasoconstriction and (MT2) modulation of dopamine release in the retina. A third putative mammalian melatonin receptor (MT3) has been identified as the enzyme quinone reductase. Numerous reviews address melatonin receptor pharmacology e.g (191, 198).

Melatonin Antagonists and Agonists

Large numbers of putative and actual melatonin agonists together with some antagonists have now been described. A series of agonists has been developed from napthalene derivatives. They show a range of affinity for the pars tuberalis melatonin receptor, some being of much higher affinity than melatonin. The most interesting have similar effects to melatonin on rhythm physiology in both rodents and humans. Agomelatine is marketed as an anti-depressant in view of its activity at the serotonin-2C (5-HT2C), receptor (130, 198-206)

Probably the first analogues to be synthesised were 6- and 2-halogenated melatonins. Beta-methyl-6-chloromelatonin (LY156735) is being evaluated as a potential treatment for insomnia and for jet lag with efficacy demonstrated in initial trials. Ramelteon is a selective MT1/MT2 agonist and is marketed for long term use in sleep onset insomnia. Another MT1/MT2 agonist, tasimelteon (VEC-162), is under development for the treatment of circadian rhythm disorders (130).

It is likely that SCN receptors mediate the circadian effects of melatonin, those in the mediobasal hypothalamus and pars tuberalis influence photoperiodic seasonal reproduction

with regard to gonadotrophin secretion and prolactin respectively, and those in the retina mediate the retinal processes influenced by melatonin. The physiological functions of the multiplicity of melatonin binding sites in other areas remain to be clarified.

Effects on Clock Genes

Probably the most interesting development in the mechanistic aspects of the effects of melatonin concerns its influence on gene expression in the pars tuberalis. Many clock genes are expressed in the pars tuberalis (Bmal1,Clock, Per1 Per2, Cry1, Cry2) with a 24h rhythmicity different from their expression in the SCN. Per1 is activated at the beginning of the light phase and Cry1 at the beginning of the dark phase. Long or short photoperiod information is encoded within the SCN. Melatonin synthesis, driven by the SCN, conveys this photoperiodic information to the pars tuberalis by virtue of its pattern of secretion. This in turn influences the pattern of expression of the clock genes per1 and cry1 within the pars tuberalis providing a means of translating the melatonin signal for the control of seasonal prolactin variations (207-209). More recently multiple genes, including clock genes, influenced by melatonin have been identified in the pars tuberalis with numerous potential seasonal effects deriving from melatonin (210,211). The immediate early gene EGR1-RE, has been invoked as a contributor to acute melatonin dependent effects in the pars tuberalis (212).

Interestingly maternal melatonin appears to influence the expression of clock genes in the capuchin monkey fetal SCN thus providing a possible mechanism for the timing of post-natal events and the setting of fetal/neo-natal circadian phase (213).

So far melatonin does not appear to influence clock gene expression in the SCN (214,215)). However, it has been proposed that other “calender” cells will be identified in the CNS which regulate seasonal changes other than prolactin and may use the relative phasing of clock gene expression for translating the photoperiodic (melatonin) signal (216).

In rodent pars tuberalis cells rhythmic expression of per1 appears to be dependent on sensitization of adenosine A2b receptors which in turn depend on melatonin activation of MT1 receptors (217). Clearly it is possible that the melatonin signal is a widespread humoral mechanism related to biological timing, acting through modification of clock gene expression . It appears not to be of major importance to rhythm generation in the SCN but it is within the peripheral pars tuberalis system. The effects of melatonin on peripheral, as well as central, clock gene expression is likely to be a rich field of enquiry.

References

1. Vollrath L. The Pineal Organ. Heidelberg: Springer-Verlag; 1981.

2. Collin JP. Differentiation and regression of the cfells of the sensory line in the epiphysis cerebri. In: Wolstenholme GEW, Knight, J., editor. The Pineal Gland. Edinburgh: Churchill Livingstone; 1972. p. 79-125.

3. Welsh MG. Pineal Calcification: Structural and Functional Aspects. Pineal Research Reviews 3:41-68, 1985.

4. Kappers JA. Innervation of the epiphysis cerebri in the albino rat. Anatomical Record 136:220-221, 1960.

5. Moller M. Introduction to mammalian pineal innervation. Microscopy Research Techniques 46:235-287, 1999.

6. Cassone VM, Natesan AK. Time and time again: the phylogeny of melatonin as a transducer of biological time. J Biol Rhythms 12:489-97, 1997.

7. Takahashi JS, Hong HK, Ko CH, McDearmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet 9:764-775, 2008.

8. Fuller PM , Lu J , Saper CB . Differential rescue of light- and food-entrainable circadian rhythms. Science. 320:1074-1077, 2008.

9. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer 3:350-61, 2003.

10. Klein DC, Coon SL, Roseboom PH, et al. The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in the pineal gland. Recent Prog Horm Res 52:307-57; discussion 357-8, 1997.

11. Gastel JA, Roseboom PH, Rinaldi PA, et al. Melatonin production: proteasomal proteolysis in serotonin N-acetyltransferase regulation. Science 279:1358-60, 1998.

12. R-diger Hardeland R, Melatonin: Signaling mechanisms of a pleiotropic agent, BioFactors Volume 35, 183-192, 2009

13. Bubenik GA. Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. Biol Signals Recept 10:350-66, 2001.

14. Tosini G, Menaker M. Circadian rhythms in cultured mammalian retina. Science 272:419-21, 1996.

15. Ma X, Chen C, Kristopher W. Krausz KW, Idle JR, Gonzalez FJ . A Metabolomic Perspective of Melatonin Metabolism in the Mouse. Endocrinology 149: 1869-1879, 2008.

16. Arendt J. Melatonin and the Mammalian Pineal Gland. 1st ed. London: Chapman Hall; 1995.

17. Cavallo A, Ritschel WA. Pharmacokinetics of melatonin in human sexual maturation. J Clin Endocrinol Metab 81:1882-6, 1996.

18. Simonneaux V, Ribelayga C. Generation of the melatonin endocrine message in mammals:

a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. Pharmacol Rev 55:325-95, 2003.

19. Zawilska JB, Skene DJ, Arendt J. Physiology and pharmacology of melatonin in relation to biological rhythms. Pharmacological Reports 61:383-410, 2009.

20 Middleton B, Arendt J, Stone BM. Human circadian rhythms in constant dim light (8 lux) with knowledge of clock time. J Sleep Res 5:69-76, 1996.

21. Czeisler CA, Duffy JF, Shanahan TL, et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284:2177-81, 1999.

22. Moore RY, Klein DC. Visual pathways and the central neural control of a circadian rhythm in pineal serotonin N-acetyltransferase activity. Brain Res 71:17-33, 1974.

23. Cassone VM. Effects of melatonin on vertebrate circadian systems. Trends Neurosci 13:457-64, 1990.

24. Cain SW , Dennison CF , Zeitzer JM , Guzik AM , Khalsa SB , Santhi N , Schoen MW , Czeisler CA , Duffy JF . Sex differences in phase angle of entrainment and melatonin amplitude in humans. J Biol Rhythms 25: 288-296, 2010

25. Reiter RJ. Action spectra, dose-response relationships, and temporal aspects of light's effects on the pineal gland. Ann N Y Acad Sci 453:215-30, 1985.

26. Arendt J. Melatonin: characteristics, concerns and prospects. J Biol Rhythms 20:291-303, 2005.

27. Lewy AJ, Wehr TA, Goodwin FK, et al. Light suppresses melatonin secretion in humans. Science 210:1267-9, 1980.

28. Bojkowski CJ, Aldhous ME, English J, et al. Suppression of nocturnal plasma melatonin and 6-sulphatoxymelatonin by bright and dim light in man. Horm Metab Res 19:437-40, 1987.

29. Boivin DB, Czeisler CA. Resetting of circadian melatonin and cortisol rhythms in humans by ordinary room light. Neuroreport 9:779-82, 1998.

30. Foster RG. Neurobiology: bright blue times. Nature 433:698-9, 2005.

31. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol 535:261-7, 2001.

31a. Brainard GC , Hanifin JP , Greeson JM , Byrne B , Glickman G , Gerner E , Rollag MD . Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. J.N eurosci. 2001 Aug 15;21(16):6405-12

32. Revell VL, Arendt J, Terman M, et al. Short-wavelength sensitivity of the human circadian system to phase-advancing light. J Biol Rhythms 20:270-2, 2005

33. Revell VL, Skene DJ. Light-induced melatonin suppression in humans with polychromatic and monochromatic light. Chronobiol Int 24:1125-1137,2007.

34. Malpaux B, Daveau A, Maurice-Mandon F, Duarte G, ChemineauP. Evidence That Melatonin Acts in the Premammillary Hypothalamic Area to Control Reproduction in the Ewe: Presence of Binding Sites and Stimulation of Luteinizing Hormone Secretion by *in Situ* Microimplant Delivery. Endocrinology 139: 1508-1516, 2009.

35. Rosenthal NE, Sack DA, Gillin JC, et al. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 41:72-80, 1984.

36. Wehr TA. The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). J Clin Endocrinol Metab 73:1276-80, 1991.

37. Broadway J, Arendt J, Folkard S. Bright light phase shifts the human melatonin rhythm during the Antarctic winter. Neurosci Lett 79:185-9, 1987.

38. Illnerova H, Vanecek J. Entrainment of the rat pineal rhythm in melatonin production by light. Reprod Nutr Dev 28:515-26, 1988.

39. Klerman EB , Rimmer DW , Dijk DJ , Kronauer RE , Rizzo JF 3rd , Czeisler CA . Nonphotic entrainment of the human circadian pacemaker **.** A m J Physiol. 274:R991-6, 1998.

40. Wever RA. Light effects on human circadian rhythms: a review of recent Andechs experiments. J Biol Rhythms 4:161-85, 1989.

41. Zeitzer JM, Dijk DJ, Kronauer R, et al. Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 526 Pt 3:695-702, 2000.

42. Revell VL, Arendt, J., Terman, M., Skene, D.J. Short wavelength sensitivity of the human circadian pacemaker to phase advancing light. J Biol Rhythms 20:270-272, 2005.

43. Lewy AJ, Newsome DA. Different types of melatonin circadian secretory rhythms in some blind subjects. J Clin Endocrinol Metab 56:1103-7, 1983.

44. Lockley SW, Skene DJ, Arendt J, et al. Relationship between melatonin rhythms and visual loss in the blind. J Clin Endocrinol Metab 82:3763-70, 1997.

45. Czeisler CA, Shanahan TL, Klerman EB, et al. Suppression of melatonin secretion in some blind patients by exposure to bright light. N Engl J Med 332:6-11, 1995.

46. Ebling FJ, Foster DL. Pineal melatonin rhythms and the timing of puberty in mammals. Experientia 45:946-54, 1989.

47. Arendt J. Role of the pineal gland and melatonin in seasonal reproductive function in mammals. Oxf Rev Reprod Biol 8:266-320, 1986.

48. Goldman BD. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16:283-301, 2001.

49. Malpaux B, Migaud M, Tricoire H, et al. Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. J Biol Rhythms 16:336-47, 2001.

50. Woodfill CJ, Wayne NL, Moenter SM, et al. Photoperiodic synchronization of a circannual reproductive rhythm in sheep: identification of season-specific time cues. Biol Reprod 50:965-76, 1994.

51. Lincoln , GA, Clarke IJ, Hut RA, Hazlerigg DG. Characterizing a Mammalian Circannual Pacemaker. Science 314:1941-1944, 2006.

52. Weaver DR, Reppert SM. Maternal melatonin communicates daylength to the fetus in Djungarian hamsters. Endocrinology 119:2861-3, 1986.

53. Deveson S, Forsyth IA, Arendt J. Retardation of pubertal development by prenatal long days in goat kids born in autumn. J Reprod Fertil 95:629-37, 1992.

54. Davis FC. Melatonin: role in development. J Biol Rhythms 12:498-508, 1997.

55. Sizonenko PC, Lang U, Rivest RW, et al. The pineal and pubertal development. Ciba Found Symp 117:208-30, 1985.

56. Martin JE, Klein DC. Melatonin inhibition of the neonatal pituitary response to luteinizing hormone-releasing factor. Science 191:301-2, 1976.

57. Cassone VM. The pineal gland influences rat circadian activity rhythms in constant light. J Biol Rhythms 7:27-40, 1992.

58. Armstrong SM. Melatonin and circadian control in mammals. Experientia 45:932-8, 1989.

59. Deacon S, English J, Tate J, et al. Atenolol facilitates light-induced phase shifts in humans. Neurosci Lett 242:53-6, 1998.

60. Wirz-Justice A, Krauchi K, Cajochen C, et al. Evening melatonin and bright light administration induce additive phase shifts in dim light melatonin onset. J Pineal Res 36:192-4, 2004.

61. Badia P MB, Murphy P. Melatonin and thermoregulation. In: Reiter RJ YH, editor. Melatonin: Biosynthesis, Physiological Effects, and Clinical Applications. Boca Raton, FL: CRC Press; 1992.

62. Humlova M, Illnerova H. Melatonin entrains the circadian rhythm in the rat pineal N-acetyltransferase activity. Neuroendocrinology 52:196-9, 1990.

63. Reiter RJ. Melatonin and human reproduction. Ann Med 30:103-8, 1998.

64. Bosc MJ. Time of parturition in rats after melatonin administration or change of photoperiod. J Reprod Fertil 80:563-8, 1987.

65. Reppert SM, Weaver DR. Melatonin madness. Cell 83:1059-62, 1995.

66. Arendt J. Melatonin. Bmj 312:1242-3, 1996.

67. Vijayalaxmi, Thomas CR, Jr., Reiter RJ, et al. Melatonin: from basic research to cancer treatment clinics. J Clin Oncol 20:2575-601, 2002.

68. Marshall KA, Reiter RJ, Poeggeler B, et al. Evaluation of the antioxidant activity of melatonin in vitro. Free Radic Biol Med 21:307-15, 1996.

69. Gillette MU, McArthur AJ. Circadian actions of melatonin at the suprachiasmatic nucleus. Behav Brain Res 73:135-9, 1996.

70. Iuvone PM, Tosini G, Pozdeyev N, et al. Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. Prog Retin Eye Res 24:433-56, 2005.

71. Reiter RJ, Tan DX, Gitto E, et al. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol J Pharmacol 56:159-70, 2004.

72. Blask DE, Dauchy RT, Sauer LA, et al. Melatonin uptake and growth prevention in rat hepatoma 7288CTC in response to dietary melatonin: melatonin receptor-mediated inhibition of tumor linoleic acid metabolism to the growth signaling molecule 13-hydroxyoctadecadienoic acid and the potential role of phytomelatonin. Carcinogenesis 25:951-60, 2004.

73. Straif K, Baan R, Grosse Y, Secretan B, Ghissassi FEL, Bou-vard V, et al. Carcinogenicity of shift-work, painting, and fire-fighting. Lancet Oncol. 8:1065-6, 2007.

74. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans volume 98: Firefighting, painting and shift work. Lyon, France: 2-9 October 2007, 2011, in press.

75. Blask DE, Dauchy RT, Sauer LA. Putting cancer to sleep at night: the neuroendocrine/circadian melatonin signal. Endocrine 27:179-88, 2005.

77. Scott EM, Carter AM, Grant PJ. Association between polymorphisms in the Clock gene, obesity and the metabolic syndrome in man Clock polymorphisms and obesity. International Journal of Obesity 32: 658-662, 2008.

78. Neuwelt EA, Lewy AJ. Disappearance of plasma melatonin after removal of a neoplastic

pineal gland. N Engl J Med 308:1132-5, 1983.

79. Macchi MM, Bruce JA, Boulos Z, et al. Sleep, chronotype and seasonality after pineal resection in humans: initial findings. Society for Research on Biological Rhythms Abstracts 9:157, 2002.

80. Skene DJ, Vivien-Roels B, Sparks DL, et al. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. Brain Res 528:170-4, 1990.

81. Stevens RG, Davis S, Thomas DB, et al. Electric power, pineal function, and the risk of breast cancer. Faseb J 6:853-60, 1992.

82. Blask DE, Dauchy RT, Sauer LA, et al. Light during darkness, melatonin suppression and cancer progression. Neuro Endocrinol Lett 23 Suppl 2:52-6, 2002.

83. Blask DE, Brainard GC, Dauchy RT, et al. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. Cancer Res 65:11174-84, 2005.

84. Stevens RG. Circadian Disruption and Breast Cancer: From Melatonin to Clock Genes. Epidemiology 16:254-258, 2005.

85. Akerstedt T, Froberg JE, Friberg Y, et al. Melatonin excretion, body temperature and subjective arousal during 64 hours of sleep deprivation. Psychoneuroendocrinology 4:219-25, 1979.

86. Strassman RJ, Qualls CR, Lisansky EJ, et al. Elevated rectal temperature produced by all-night bright light is reversed by melatonin infusion in men. J Appl Physiol 71:2178-82, 1991.

87. Cagnacci A, Elliott JA, Yen SS. Melatonin: a major regulator of the circadian rhythm of core temperature in humans. J Clin Endocrinol Metab 75:447-52, 1992.

88. Krauchi K, Cajochen C, Wirz-Justice A. A relationship between heat loss and sleepiness: effects of postural change and melatonin administration. J Appl Physiol 83:134-9, 1997.

89. Shochat T, Haimov I, Lavie P. Melatonin–the key to the gate of sleep. Ann Med 30:109-14, 1998.

90. Dijk DJ, Shanahan TL, Duffy JF, et al. Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans. J Physiol 505:851-8, 1997.

91. Lockley SW, Skene DJ, Tabandeh H, et al. Relationship between napping and melatonin in the blind. J Biol Rhythms 12:16-25, 1997.

92. De Leersnyder H . Inverted rhythm of melatonin secretion in Smith-Magenis syndrome: from symptoms to treatment. Trends Endocrinol Metab. 17:291-8. 2006.

93. Kennaway DJ, Stamp GE, Goble FC. Development of melatonin production in infants and the impact of prematurity. J Clin Endocrinol Metab 75:367-9, 1992.

94. Waldhauser F, Weiszenbacher G, Frisch H, et al. Fall in nocturnal serum melatonin during prepuberty and pubescence. Lancet 1:362-5, 1984.

95. Reiter RJ . Melatonin and human reproduction. Ann Med. 30:103-8, 1998.

96. Waldhauser F, Boepple PA, Schemper M, et al. Serum melatonin in central precocious puberty is lower than in age-matched prepubertal children. J Clin Endocrinol Metab 73:793-6, 1991.

97. Berga SL, Mortola JF, Yen SS. Amplification of nocturnal melatonin secretion in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 66:242-4, 1988.

98. Berga SL, Jones KL, Kaufmann S, et al. Nocturnal melatonin levels are unaltered by ovarian suppression in girls with central precocious puberty. Fertil Steril 52:936-41, 1989.

99. Arendt J, Labib MH, Bojkowski C, et al. Rapid decrease in melatonin production during successful treatment of delayed puberty. Lancet 1:1326, 1989.

100. Luboshitzky R, Lavi S, Thuma I, Lavie P. Testosterone treatment alters melatonin concentrations in male patients with gonadotropin-releasing hormone deficiency. J Clin Endocrinol Metab. 81:770-774, 1996

101. Parry BL, Berga SL, Mostofi N, et al. Plasma melatonin circadian rhythms during the menstrual cycle and after light therapy in premenstrual dysphoric disorder and normal control subjects. J Biol Rhythms 12:47-64, 1997.

102. Scheer FA, Kalsbeek A, Buijs RM. Cardiovascular control by the suprachiasmatic nucleus: neural and neuroendocrine mechanisms in human and rat. Biol Chem 384:697-709, 2003.

103. O'Brien IA, Lewin IG, O'Hare JP, et al. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. Clin Endocrinol (Oxf) 24:359-64, 1986.

104. Yaprak M, Altun A, Vardar A, et al. Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. Int J Cardiol 89:103-7, 2003.

105. Naidoo R. Investigation of rhythmic endocrine function in intensive care with emphasis on melatonin. PhD Thesis, University of Surrey, 1999

106. Tapp E. The histology and pathology of the human pineal gland. Progress in Brain Research 52:481-500, 1979.

107. West RJ, Leonard JV. Familial insulin resistance with pineal hyperplasia: metabolic studies and effect of hypophysectomy. Arch Dis Child 55:619-21, 1980.

108. Sturner WQ, Lynch HJ, Deng MH, et al. Melatonin concentrations in the sudden infant death syndrome. Forensic Sci Int 45:171-80, 1990.

109. Axelrod L. Endocrine dysfunction in patients with tumours of the pineal region. In: Schmidek HH, editor. Pineal Tumours. New York: Masson Publishing; 1977. p. 61-77.

110. Wass JA, Jones AE, Rees LH, et al. hCG beta producing pineal choriocarcinoma. Clin Endocrinol (Oxf) 17:423-31, 1982.

111. Cohen AR, Wilson JA, Sadeghi-Nejad A. Gonadotropin-secreting pineal teratoma causing precocious puberty. Neurosurgery 28:597-602; discussion 602-3, 1991.

112. Horowitz MB, Hall WA. Central nervous system germinomas. A review. Arch Neurol 48:652-7, 1991.

113. Drummond KJ, Rosenfeld JV. Pineal region tumours in childhood. A 30-year experience. Childs Nerv Syst 15:119-26; discussion 127, 1999.

114. Jouvet A, Saint-Pierre G, Fauchon F, et al. Pineal parenchymal tumors: a correlation of histological features with prognosis in 66 cases. Brain Pathol 10:49-60, 2000.

115. Jouvet A , Fauchon F , Liberski P , Saint-Pierre G , Didier-Bazes M , Heitzmann A , Delisle MB , Biassette HA , Vincent S , Mikol J , Streichenberger N , Ahboucha S , Brisson C , Belin MF , F-vre-Montange M . Papillary tumor of the pineal region. Am J Surg Pathol. 27:505-12, 2003.

116. Murata J, Sawamura Y, Ikeda J, et al. Twenty-four hour rhythm of melatonin in patients with a history of pineal and/or hypothalamo-neurohypophyseal germinoma. J Pineal Res 25:159-66, 1998.

117. Bartsch C, Bartsch H. Melatonin in cancer patients and in tumor-bearing animals. Adv Exp Med Biol 467:247-64, 1999.

118. Gilad E, Matzkin H, Zisapel N. Interplay between sex steroids and melatonin in regulation of human benign prostate epithelial cell growth. J Clin Endocrinol Metab 82:2535-41, 1997.

119. Megdal SP, Kroenke CH, Laden F, et al. Night work and breast cancer risk: A systematic review and meta-analysis. Eur J Cancer 2005.

120. Schernhammer ES, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst 97:1084-7, 2005.

121. Travis RC, Allen DS, Fentiman IS, et al. Melatonin and breast cancer: a prospective study.

J Natl Cancer Inst 96:475-82, 2004.

122. Zhu Y BH, Zhang Y, Stevens RG, Zheng T. Period3 structural variation: a circadian biomarker associated with breast cancer in young women. Cancer Epidemiol Biomarkers Prev 14:268-270, 2005.

123. Archer SN, Robilliard DL, Skene DJ, et al. A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. Sleep 26:413-5, 2003.

124. Skene DJ, Bojkowski CJ, Currie JE, et al. 6-sulphatoxymelatonin production in breast cancer patients. J Pineal Res 8:269-76, 1990.

125. Arendt J. Melatonin: a new probe in psychiatric investigation? Br J Psychiatry 155:585-90, 1989.

126. Sack RL, Lewy AJ, White DM, et al. Morning vs evening light treatment for winter depression. Evidence that the therapeutic effects of light are mediated by circadian phase shifts. Arch Gen Psychiatry 47:343-51, 1990.

127. Lewy AJ, Sack RL, Miller LS, et al. Antidepressant and circadian phase-shifting effects of light. Science 235:352-4, 1987.

128. Terman M, Terman JS. Light therapy for seasonal and nonseasonal depression: efficacy, protocol, safety, and side effects. CNS Spectr 10:647-63; quiz 672, 2005.

129. Lewy AJ, Lefler BJ, Emens JS, Bauer VK. The circadian basis of winter depression. PNAS 103: 7414-7419, 2006.

130. Arendt J, Rajaratnam SMW. Melatonin and its agonists: an update. The British Journal of Psychiatry 193: 267-269. 2008.

131. Mocaer E, Delalleau B, Boyer PA, et al. [Development of a new antidepressant : agomelatine]. Med Sci (Paris) 21:888-93, 2005.

132. Lima FB , Machado UF , Bartol I , Seraphim PM , Sumida DH , Moraes SM , Hell NS , Okamoto MM , Saad MJ , Carvalho CR , Cipolla-Neto J .Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. Am J Physiol. 275:E934-41, 1998.

134. M-ssig K , Staiger H , Machicao F , H-ring HU , Fritsche A . Genetic variants in MTNR1B affecting insulin secretion. Ann Med. 42:387-93, 2010

135. Iguchi H, Kato KI, Ibayashi H. Melatonin serum levels and metabolic clearance rate in patients with liver cirrhosis. J Clin Endocrinol Metab 54:1025-7, 1982.

136. van Geijlswijk IM , Korzilius HP , Smits MG . The use of exogenous melatonin in delayed sleep phase disorder: a meta-analysis. Sleep. 33:1605-14, 2010.

137. Arendt J. In what circumstances is melatonin a useful sleep therapy? Consensus statement, WFSRS focus group, Dresden, November 1999. J Sleep Res 9:397-8, 2000.

138. Arendt J, Skene D. Melatonin as a chronobiotic. Sleep Medicine Reviews 9:25-39, 2004.

139. Brzezinski A, Vangel MG, Wurtman RJ, et al. Effects of exogenous melatonin on sleep: a meta-analysis. Sleep Med Rev 9:41-50, 2005.

140. Arendt J. Managing jet lag: some of the problems and possible new solutions, Sleep Medicine Reviews, 13: 249-256, 2009.

141. Arendt J , Van Someren EJ , Appleton R , Skene DJ , Akerstedt T . Clinical update: melatonin and sleep disorders. Clin Med. 8:381-3, 2008.

142. Rajaratnam SM, Middleton B, Stone BM, et al. Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol 561:339-51, 2004.

143. Arendt J, Bojkowski C, Folkard S, et al. Some effects of melatonin and the control of its secretion in humans. Ciba Found Symp 117:266-83, 1985.

144. Lewy AJ, Bauer VK, Ahmed S, et al. The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. Chronobiol Int 15:71-83, 1998.

145. Rajaratnam SM, Dijk DJ, Middleton B, et al. Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. J Clin Endocrinol Metab 88:4303-9, 2003.

146. Middleton B, Arendt J, Stone BM. Complex effects of melatonin on human circadian rhythms in constant dim light. J Biol Rhythms 12:467-77, 1997.

147. Lockley SW, Skene DJ, James K, et al. Melatonin administration can entrain the free-running circadian system of blind subjects. J Endocrinol 164:R1-6, 2000.

148. Sack RL, Brandes RW, Kendall AR, et al. Entrainment of free-running circadian rhythms by melatonin in blind people. N Engl J Med 343:1070-7, 2000.

149. Arendt J. Melatonin, circadian rhythms, and sleep. N Engl J Med 343:1114-6, 2000.

150. Arendt J, Skene DJ, Middleton B, et al. Efficacy of melatonin treatment in jet lag, shift work, and blindness. J Biol Rhythms 12:604-17, 1997.

151. Cagnacci A, Krauchi K, Wirz-Justice A, et al. Homeostatic versus circadian effects of

melatonin on core body temperature in humans. J Biol Rhythms 12:509-17, 1997.

152. Cagnacci A, Elliott JA, Yen SS. Amplification of pulsatile LH secretion by exogenous melatonin in women. J Clin Endocrinol Metab 73:210-2, 1991.

153. Voordouw BC, Euser R, Verdonk RE, et al. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. J Clin Endocrinol Metab 74:108-17, 1992.

154. Luboshitzky R, Wagner O, Lavi S, et al. Abnormal melatonin secretion in male patients with hypogonadism. J Mol Neurosci 7:91-8, 1996.

155. Luboshitzky R, Wagner O, Lavi S, et al. Abnormal melatonin secretion in hypogonadal men: the effect of testosterone treatment. Clin Endocrinol (Oxf) 47:463-9, 1997.

156. Anderson RA, Lincoln GA, Wu FC. Melatonin potentiates testosterone-induced suppression of luteinizing hormone secretion in normal men. Hum Reprod 8:1819-22, 1993.

157. Waldhauser F, Lieberman HR, Lynch HJ, et al. A pharmacological dose of melatonin increases PRL levels in males without altering those of GH, LH, FSH, TSH, testosterone or cortisol. Neuroendocrinology 46:125-30, 1987.

158. Forsling ML, Williams AJ. The effect of exogenous melatonin on stimulated neurohypophysial hormone release in man. Clin Endocrinol (Oxf) 57:615-20, 2002.

159. Scheer FA, Van Montfrans GA, van Someren EJ, et al. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 43:192-7, 2004.

160. Arendt J. Safety of melatonin in long-term use (?). J Biol Rhythms 12:673-81, 1997.

161. Guardiola-Lemaitre B. Toxicology of melatonin. J Biol Rhythms 12:697-706, 1997.

162. Suhner A, Schlagenhauf P, Johnson R, et al. Comparative study to determine the optimal melatonin dosage form for the alleviation of jet lag. Chronobiol Int 15:655-66, 1998.

163. Herxheimer A and Petrie KJ. Melatonin for the prevention and treatment of jet lag. Cochrane Database Syst. Rev:CD001520, 2002.

164. Morgenthaler, T.I., Lee-Chiong, T., Alessi, C. et al. Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. Sleep 30:1445-1459, 2007.

165. Folkard S, Arendt J, Clark M. Can melatonin improve shift workers' tolerance of the night shift? Some preliminary findings. Chronobiol Int 10:315-20, 1993.

166. Burgess HJ, Sharkey KM, Eastman CI. Bright light, dark and melatonin can promote circadian adaptation in night shift workers. Sleep Med Rev 6:407-20, 2002.

167. Sasseville A, Paquet N, S-vigny J, H-bert M. Blue blocker glasses impede the capacity of bright light to suppress melatonin production. J Pineal Res 41:73-78, 2006.

168. Lemoine P, Zisapel N . Efficacy and Safety of Circadin- in the Treatment of Primary Insomnia . European Psychiatric Review 1:40-3, 2008.

169. Riemersma-van der Lek R, Swaab DF, Twisk J *et al* . Effect of bright light and melatonin on cognitive and non-cognitive function in elderly residents of group care facilities: a randomized controlled trial. JAMA 299:2642-55, 2008..

170. Arendt J, Skene DJ. Melatonin as a chronobiotic. Sleep Med Rev 9:25-39, 2005.

171. Nagtegaal JE, Kerkhof GA, Smits MG, et al. Delayed sleep phase syndrome: A placebo-controlled cross-over study on the effects of melatonin administered five hours before the individual dim light melatonin onset. J Sleep Res 7:135-43, 1998.

172. Blask DE, Sauer LA, Dauchy RT. Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr Top Med Chem 2:113-32, 2002.

173. Tamarkin L, Cohen M, Roselle D, et al. Melatonin inhibition and pinealectomy enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Cancer Res 41:4432-6, 1981.

174. Lissoni P, Bolis S, Brivio F, et al. A phase II study of neuroimmunotherapy with subcutaneous low-dose IL-2 plus the pineal hormone melatonin in untreatable advanced hematologic malignancies. Anticancer Res 20:2103-5, 2000.

175. Conti A, Maestroni GJ. The clinical neuroimmunotherapeutic role of melatonin in oncology. J Pineal Res 19:103-10, 1995.

176. Lissoni P, Chilelli M, Villa S, et al. Five years survival in metastatic non-small cell lung cancer patients treated with chemotherapy alone or chemotherapy and melatonin: a randomized trial. J Pineal Res 35:12-5, 2003.

177. Morgan L, Hampton, S., Gibbs, M., Arendt, J. Circadian aspects of postprandial metabolism. Chronobiology International 20:795-808, 2003.

178. Knutsson A, Boggild H. Shiftwork and cardiovascular disease: review of disease mechanisms. Rev Environ Health 15:359-72, 2000.

179. Bittman EL. The sites and conseqences of melatonin binding in mammals. American Zoologist 33:200-211, 1993.

180. Revel FG , Masson-P-vet M , P-vet P , Mikkelsen JD , Simonneaux V . Melatonin controls seasonal breeding by a network of hypothalamic targets. Neuroendocrinology.

90:1-14,2009.

181. Vanecek J. Cellular mechanisms of melatonin action. Physiol Rev 78:687-721, 1998.

182. Vanecek J, Pavlik A, Illnerova H. Hypothalamic melatonin receptor sites revealed by autoradiography. Brain Res 435:359-62, 1987.

183. Williams LM, Morgan PJ. Demonstration of melatonin-binding sites on the pars tuberalis of the rat. J Endocrinol 119:R1-3, 1988.

184. Reppert SM, Weaver DR, Godson C. Melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci 17:100-2, 1996.

185. Lincoln GA, Clarke IJ. Photoperiodically-induced cycles in the secretion of prolactin in hypothalamo-pituitary disconnected rams: evidence for translation of the melatonin signal in the pituitary gland. J Neuroendocrinol 6:251-60, 1994.

186. Morgan PJ, King TP, Lawson W, et al. Ultrastructure of melatonin-responsive cells in the ovine pars tuberalis. Cell Tissue Res 263:529-34, 1991.

187. Williams LM, Martinoli MG, Titchener LT, et al. The ontogeny of central melatonin binding sites in the rat. Endocrinology 128:2083-90, 1991.

188. White BH, Sekura RD, Rollag MD. Pertussis toxin blocks melatonin-induced pigment aggregation in Xenopus dermal melanophores. J Comp Physiol [B] 157:153-9, 1987.

189. Barrett P, Morris M, Choi WS, et al. Melatonin receptors and signal transduction mechanisms. Biol Signals Recept 8:6-14, 1999.

190. Hazlerigg DG, Morgan PJ, Messager S. Decoding photoperiodic time and melatonin in mammals: what can we learn from the pars tuberalis? J Biol Rhythms 16:326-35, 2001.

191. Masana MI, Dubocovich ML. Melatonin receptor signaling: finding the path through the dark. Sci STKE 2001:PE39, 2001.

192. Carlberg C. Gene regulation by melatonin. Ann N Y Acad Sci 917:387-96, 2000.

193. Migaud M, Gavet S, Pelletier J. Partial cloning and polymorphism of the melatonin1a (Mel1a) receptor gene in two breeds of goat with different reproductive seasonality. Reproduction 124:59-64, 2002.

194. Ebisawa T, Uchiyama M, Kajimura N, et al. Genetic polymorphisms of human melatonin 1b receptor gene in circadian rhythm sleep disorders and controls. Neurosci Lett 280:29-32, 2000.

195. Reppert SM. Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. J Biol Rhythms 12:528-31, 1997.

196. Cog- F , Guenin SP , Fery I , Migaud M , Devavry S , Slugocki C , Legros C , Ouvry C , Cohen W , Renault N , Nosjean O , Malpaux B , Delagrange P , Boutin JA . The end of a myth: cloning and characterization of the ovine melatonin MT(2) receptor. Br J Pharmacol 158:1248-62, 2009.

197. Johnston JD, Klosen P, Barrett P, Hazlerigg DG. Regulation of MT1 Melatonin Receptor Expression in the Foetal Rat Pituitary. J Neuroendocrinol 18:50-56, 2006

198. Zlotos DP. Recent advances in melatonin receptor ligands. Arch Pharm (Weinheim) 338:229-47, 2005.

199. Dubocovich ML, Masana MI, Iacob S, et al. Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. Naunyn Schmiedebergs Arch Pharmacol 355:365-75, 1997.

200. Zee PC. Sleep promoting role of melatonin receptor agonists. Sleep 28:300-1, 2005.

201. Sumaya IC, Masana MI, Dubocovich ML. The antidepressant-like effect of the melatonin receptor ligand luzindole in mice during forced swimming requires expression of MT2 but not MT1 melatonin receptors. J Pineal Res 39:170-7, 2005.

202. Loiseau F, Le Bihan C, Hamon M, et al. Antidepressant-like effects of agomelatine, melatonin and the NK1 receptor antagonist GR205171 in impulsive-related behaviour in rats. Psychopharmacology (Berl) 182:24-32, 2005.

203. Yous S, Andrieux J, Howell HE, et al. Novel naphthalenic ligands with high affinity for the melatonin receptor. J Med Chem 35:1484-6, 1992.

204. Faust R, Garratt PJ, Jones R, et al. Mapping the melatonin receptor. 6. Melatonin agonists and antagonists derived from 6H-isoindolo[2,1-a]indoles, 5,6-dihydroindolo[2,1-a]isoquinolines, and 6,7-dihydro-5H-benzo[c]azepino[2,1-a]indoles. J Med Chem 43:1050-61, 2000.

205. Redman JR, Francis AJ. Entrainment of rat circadian rhythms by the melatonin agonist S-20098 requires intact suprachiasmatic nuclei but not the pineal. J Biol Rhythms 13:39-51, 1998.

206. Krauchi K, Cajochen C, Mori D, et al. Early evening melatonin and S-20098 advance circadian phase and nocturnal regulation of core body temperature. Am J Physiol 272:R1178-88, 1997.

207. Messager S, Ross AW, Barrett P, et al. Decoding photoperiodic time through Per1 and ICER gene amplitude. Proc Natl Acad Sci U S A 96:9938-43, 1999.

208. Johnston JD, Messager S, Barrett P, et al. Melatonin action in the pituitary: neuroendocrine synchronizer and developmental modulator? J Neuroendocrinol 15:405-8, 2003.

209. Lincoln G, Messager S, Andersson H, et al. Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer. Proc Natl Acad Sci U S A 99:13890-5, 2002.

210. Johnston JD, Tournier BB, Andersson H, Masson-P-vet M, Lincoln GA, Hazlerigg DG. Multiple Effects of Melatonin on Rhythmic Clock Gene Expression in the Mammalian Pars Tuberalis **Endocrinology 147:959-965, 2006.**

211. Dupr- SM, Burt DW, Talbot R, Downing A, Mouzaki D, Waddington D, Malpaux B, Davis JRE, Lincoln GA, Loudon ASI . Identification of Melatonin-Regulated Genes in the Ovine Pituitary Pars Tuberalis, a Target Site for Seasonal Hormone Control. Endocrinology 149: 5527 – 5539, 2008.

212. Fustin JM, Dardente H, Wagner GC, Carter DA, Johnston JD, Lincoln GA, Hazlerigg DG, Egr1 involvement in evening gene regulation by melatonin The FASEB Journal 23: 764-773, 2009.

213 **Torres-Farfan C, Rocco V, Mons- C, Valenzuela FJ, C.** Maternal Melatonin Effects on Clock Gene Expression in a Nonhuman Primate Fetus.Endocrinology 147:4618-4626, 2006.

214. Messager S, Hazlerigg DG, Mercer JG, et al. Photoperiod differentially regulates the expression of Per1 and ICER in the pars tuberalis and the suprachiasmatic nucleus of the Siberian hamster. Eur J Neurosci 12:2865-70, 2000.

215. Poirel VJ, Boggio V, Dardente H, et al. Contrary to other non-photic cues, acute melatonin injection does not induce immediate changes of clock gene mRNA expression in the rat suprachiasmatic nuclei. Neuroscience 120:745-55, 2003.

216. Lincoln GA, Andersson H, Loudon A. Clock genes in calendar cells as the basis of annual timekeeping in mammals–a unifying hypothesis. J Endocrinol 179:1-13, 2003.

217. von Gall C, Garabette ML, Kell CA, et al. Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. Nat Neurosci 5:234-8, 2002.