MELATONIN SIGNALING AS A LINK BETWEEN SLEEP AND CIRCADIAN BIOLOGY: PRACTICAL IMPLICATIONS

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ABSTRACT

Normal circadian rhythms are synchro- nized to a regular 24 h environmental light-dark cycle. Both the suprachiasmatic nucleus (SCN) and melatonin are essen- tial for this adaptation. Melatonin exerts its chronophysiological action in part by act- ing through specific membrane receptors (MT_1, MT_2), which have been identified in SCN cells as well as in several neural and non-neural tissues. Both receptors have been cloned and share general features with other G protein linked receptors. Melatonin also exerts direct effects on in- tracellular proteins, such as calmodulin or tubulin, has strong free radical scavenger properties, which are non-receptor mediat- ed, is an effective mitochondrial protector and may interact with proteasome to affect intracellular physiology. Within the SCN, melatonin reduces neuronal activity in a time-dependent manner. The disruption of these circadian mechanisms causes a num- ber of sleep disorders known as circadian rhythm sleep disorders (CRSDs). CRSDs include delayed or advanced sleep phase syndromes; non-24 h sleep-wake rhythm disorder, time zone change syndrome ("jet lag") and shift work sleep disorder. Distur- bances in the circadian phase position of plasma melatonin levels have been found in all these disorders. In addition, comor- bidity of severe circadian alterations with neurodegenerative diseases like Alzheim- er's disease (AD) has been documented. Currently there is sufficient evidence to implicate endogenous melatonin as an im

portant mediator in CRSD pathophysiolo- gy. The documented efficacy of melatonin to reduce chronic benzodiazepine/Z drug use in insomnia patients is also discussed.

7.1. INTRODUCTION

The objectives of this chapter are to discuss the manner, in which the circadian system regulates melatonin and the sleep–wake cycle, the linkages between melatonin, the circadian system and the sleep–wake cycle and studies on the use of melatonin to treat Circadian Rhythm Sleep disorders (CSRDs), the circadian alterations seen in Alzheimer's disease (AD), and benzodiaz- epine (BZD)/Z drug abuse.

CRSDs have become a major focus of attention in recent years.[1,2] Major in- dustrial, air, and train accidents have been generally attributed to inefficient handling of situations by individuals suffering from fatigue due to a malfunctioning circadian time keeping system. Also contributing to in-job accidents is the scheduling of the work itself.[3] There is evidence that work performance is negatively impacted by night shift work, especially when the hours of work include the period when the hor- mone melatonin is normally at its peak of production (the "circadian trough"). The resulting decrements in alertness and per- formance are further exacerbated by poor quality sleep, another condition, which often afflicts night shift workers. Similarly, affected are long distance truck drivers and others who must do extended highway driving. It has been reported that sleep- related motor vehicle accidents are about 20 times greater at 0600 h than at 1000 h.[3] Synchronization of the sleep-wake rhythm and the rest-activity cycles with the light-dark (LD) cycle of the external environment is essential for maintaining man's normal mental and physical health. Circulating melatonin, which is produced mainly in the pineal gland, is essential for this physiological adaptation. This is par- ticularly apparent in pathological condi- tions, such as CRSDs, some of which are known to result from disturbances in the rhythm of melatonin secretion.[1,2,4,5] The melatonin secretion cycle represents a con- venient means for observing the body's cir- cadian time keeping system. Since a disrup- tion in the rhythm of melatonin secretion is a central feature of CRSDs, an increasing amount of evidence now shows that the strategic application exogenous melatonin itself can be of benefit in resynchronizing the altered circadian pattern.

CRSDs include delayed sleep phase syndrome (DSPS), advanced sleep phase syndrome (ASPS), non-24 h sleep-wake rhythm disorder, time zone change syn- drome ("jet lag"), and shift work sleep disorder.[2,6] Disturbances in the circadian phase position of plasma melatonin lev- els have been found in all these disorders. CRSDs respond better to chronobiological manipulations involving, for example, the use of phototherapy or melatonin, rather than to conventional hypnotic therapy.[2,6]

In AD patients melatonin secretion decreases and exogenous melatonin administration improves sleep efficiency, sundowning and, to some extent, cognitive function (see recent review).[7] This effect can be particularly important in mild cognitive impairment (MCI), an etiologically heterogeneous syndrome characterized by cognitive impairment preceding dementia. Approximately, 12% of MCI patients convert to AD or other dementia disorders every year. Recent studies indicate that melatonin can be a useful add-on drug for treating MCI in a clinical setting.[7]

The ultimate goal of anti-insomnia therapy is symptomatic and functional recovery that helps a return to everyday life. However, a large proportion of patients under BZD/Z drug treatment (the most common antiinsomnia drugs prescribed) fail to achieve a complete and sustained re- covery and are left with residual symptoms produced by the treatment itself, like toler- ance or dependency, that make relapse or recurrence more likely, and poorer quality of life a reality. Thus BZD/Z drug abuse has become a public health issue and has led to multiple campaigns to reduce both prescription and consumption of BZD/Z- drugs. Melatonin has been promoted as a drug to improve sleep in patients with in- somnia mainly because it does not cause hangover or show any addictive potential.[8]

7.2. MOLECULAR AND NEURAL BASES OF THE MAMMALIAN CIRCADIAN TIMING SYSTEM

Circadian timing provides temporal orga- nization of most biochemical, physiologi- cal, and neurobehavioral events in a man- ner beneficial to the organism. This is the basis of a predictive homeostasis that al- lows the organism to anticipate events for an optimal adaptation. For example, every day prior to waking, plasma cortisol, sympathetic tone, and body temperature rise, anticipatory to increased activity, and postural change.

The circadian timing system com- prises a hierarchy of pacemakers with the hypothalamic suprachiasmatic nucleus (SCN) as the master pacemaker. The SCN includes a small neuronal group that co- ordinates timing of the sleep–wake cycle as well as coordinating it with circadian rhythms in other parts of the brain and peripheral tissues.[9] The SCN consists of a set of individual oscillators that are coupled to form a pacemaker. Anatomically, the mam- malian SCN comprises two major subdivisions, a core and a shell. The core lies ad- jacent to the optic chiasm, predominantly comprises neurons producing vasoactive intestinal polypeptide or gastrin-releasing peptide co-localized with γ-aminobutyric acid (GABA) and receives dense visual inputs from the retino-hypothalamic tract (RHT), and geniculo-thalamic tract (GHT) as well as midbrain raphe afferents. It contains a population of non-rhythmic cells that

are responsive to light.[9,10] In con- trast the shell surrounds the core, contains a large population of arginine vasopressin- producing neurons in its dorsomedial por- tion, and a smaller population of calretinin- producing neurons dorsally and laterally, co-localized with GABA, and largely re- ceives input from non-visual hypotha- lamic, brainstem, and medial forebrain re- gions. There is overlap in cell populations and functions between these anatomical regions.[9–11]

At a molecular level, circadian clocks are based on clock genes, some of which encode proteins able to feedback their own transcription (Fig. 7.1).[12–14] The mammalian circadian oscillator is composed of two interlocking transcription/translation feedback loops: that is, core and auxiliary loops. The positive drive to the daily clock is constituted by helix-loop-helix, PAS-domain containing transcription factor genes, called *Bmal1* and *Clock* (or its paralog *Npas2*). The protein products of these genes form heterodimeric complexes that control the transcription of other clock genes, notably three period (*Per1*/ *Per2*/*Per3*) genes and two cryptochrome (*Cry1*/*Cry2*) genes, which in turn provide the negative feedback signal that shuts down the *Clock*/*Bmal1* drive to complete the circadian cycle. Accumulated PER and CRY proteins intensively repress E-box-mediated transcription until their levels sufficiently decrease once again. Additionally, CLOCK and BMAL1 also control the transcription of the nuclear receptors RORα and REV-ERBα, which modulate the BMAL1 mRNA levels by competitive actions on the rev responsive element (RRE) residing in the *Bmal1* promoter. Collectively, the cycling of clock components also determines the levels of clock-controlled genes by transcription via the E-box and/or RRE, thus achieving an oscillating pattern and generating rhythmic physiological outputs. In addition, a number of signaling molecules, including kinases, fine-tune these molecular clock loops. For example, Casein kinase Ie (CKIe) and CKId form a complex with PERs and CRYs, phosphorylate PERs and then promote proteasome-dependent degradation of these negative regulators. AMPK, an AMP-dependent kinase, phosphorylates CRYs, and promotes their degradation so as to terminate the suppressive effects of CRYs on the CLOCK/BMAL1 heterodimer. Thus clock gene expression oscillates because of the delay in the feedback loops, regulated in part by phosphorylation of the clock proteins that control their stability, nuclear re-entry, and transcription complex formation.[12–14]

In murine SCN the transcription of *Per* and *Cry* occurs during the light phase of daily photoperiod, while translation into their respective proteins occurs several hours later.[15] Another important observation was that *Bmal1* transcription occurs during most of the scotophase with a rhythm about 180 deg out of phase with that of *Per* and *Cry*.[15–17] Circadian expression of over 300 SCN transcripts was documented.[18] In mice at least 120 SCN proteins are expressed in a circadian manner, 23

of them being up-regulated or down regulated by light.[19]

In the absence of periodic environmen- tal synchronizers, the circadian pacemaker is free running with a period very near to 24 h in mammals. In humans, the interindividual differences are small. However, a large scale epidemiologic study showed that differences in sleep–wake times follow a near Gaussian distribution with extreme cases at each end; extreme early cases woke as extreme late ones fell asleep.[20] The rhythm is adjusted to 24 h by the action of light, the main (but not the unique) Zeitgeber in humans. Brief exposures to light are sufficient to entrain the SCN clockwork to solar time, adjusting the oscillator to a precise 24 h cycle.

Genetic screening has shown that poly- morphisms in human clock genes are correlated with alterations in sleep or diurnal preferences.[21] For example, in DSPS a correlation with certain polymorphisms in the clock gene hPer3 has been demonstrated[22] while a mutation in the hPer2 gene is as- sociated with familiar ASPS.[23]

7.3. SYNTHESIS AND MECHANISM OF ACTION OF MELATONIN

Melatonin is the primary hormonal output of the circadian system. Its synthesis as well as other circadian rhythms is controlled by a circadian signal from the SCN. The photoperiod is the major entraining influence on the SCN with inputs arriving from the retina via the RHT and the GHT.

Partially originating from a subset of directly photosensitive retinal ganglion cells that contain melanopsin as a phot- opigment, the neurotransmitters partici- pating in the RHT are glutamate and the pituitary adenylyl cyclase-activating pep- tide, both released at the SCN level.[24] The action spectrum for melatonin suppression in man is in keeping with a shortwave non- rod, non-cone photopigment.[25] Moreover, it has been shown that alerting effects of light are most pronounced at very short (420–460 nm) wavelengths.[26] Retinal pro- jections to the intergeniculate leaflet of the lateral geniculate complex subsequently project to the SCN via the GHT.[9] Light acts via these tracts to entrain the rhythm in the SCN as well as acting downstream of the SCN clock to block the activity of the pineal gland.

The SCN acts on the pineal gland via a complex multisynaptic pathway. It projects to the autonomic subdivision of the hypothalamic paraventricular nucleus, which in turn projects directly to the upper thoracic intermediolateral cell column. Preganglionic sympathetic noradrenergic fibers project to the superior cervical ganglion, which sends postganglionic sympathetic noradrenergic fibers to the pineal gland to stimulate synthesis of melatonin.27 Norepinephrine released from the sympathetic fibers acts on the pineal via a dual receptor mechanism. It activates adenylyl cyclase via β1 -adrenergic receptors28 and protein kinase C activity via α1B –adrenergic receptors,29 which

potentiate β1 –adrenergic receptor activation of adenylyl cyclase. There is therefore a very rapid, large increase in cyclic 3', 5'-adenosine monophosphate, which leads to phosphorylation of the enzyme arylalkylamine N-acetyltransferase (AANAT). When phosphorylated, AANAT is activated by formation of a reversible regulatory complex with 14-3-3 proteins.30

AANAT, the enzyme that converts serotonin to N-acetylserotonin, has a pivotal role in the timing of melatonin synthesis. It increases speedily with a doubling time of about 15 min in response to darkness onset and in response to light it shows an even more rapid half-life of degradation of 3.5 min.30 Since melatonin itself has a halflife in the circulation of about 30 min in man, its levels change rapidly in response to circadian signals and light.31 Hydroxyindole-O-methyltransferase (HIOMT), the enzyme that catalyzes production of melatonin from N-acetylserotonin, is responsible for the amplitude of the nocturnal peak of melatonin.32,33 By using a combination of molecular approaches together with a sensitive in-vivo measurement of pineal indoles it was shown that N-acetylserotonin is present in vast excess during the night allowing the conclusion that although AANAT is the rhythm-generating enzyme it is not rate limiting for nocturnal production.34

Once formed, melatonin is not stored in the pineal gland but is immediately secreted into the bloodstream. In plasma melatonin binds mostly to albumin.35 It then passes through the choroid plexus to the cerebrospinal fluid.36 In a recent study, melatonin concentration was measured in CSF sampled during neurosurgery in both lateral and third ventricles in patients and compared with their plasma levels.37 A significant difference in melatonin concentration was observed between lateral and third ventricles, with the highest levels in the third ventricle. Melatonin levels were significantly higher in third ventricle than in the plasma, suggesting that melatonin may enter directly the CSF through the pineal recess in humans.37

eal recess in humans.37 Endogenous melatonin, whether measured in saliva or in urine, is often referred to as a "hormonal finger print", having a profile, which is both unique and yet consistently predictable (on a daily and weekly basis) within the individual. This differs from the high interindividual variability of circulating melatonin levels, presumably of a genetic origin.38 In humans, plasma melatonin begins to increase steadily after 1900–2300 h and reaches its peak value between 0200 and 0400 h. The levels then decline, reaching their lowest values during daytime hours. The rhythm is well preserved from childhood to adulthood but after approximately the age of 55 the nocturnal peak of melatonin production begins to decline, a possible contributing factor to insomnia, which is often seen among the elderly.

The first attempts to identify brain melatonin receptors employed 3 H-

melatonin as a radioligand.39,40 This was followed by the discovery of the first functional melatonin receptor in a neuronal mammalian tissue, the rabbit retina.41 In 1984, Vakkuri et al.42 introduced the radioligand 2- 125I-iodomelatonin as a tracer for use in melatonin radioimmunoassay. This molecule turned out to be the silver bullet of melatonin receptor research as its selectivity and high specific activity allowed the field to move forward. By using this ligand, binding sites for melatonin were identified in a wide variety of central and peripheral tissues.43 Molecular cloning of the first high affinity melatonin receptor (MT1) was accomplished using a cDNA library constructed from a dermal cell line of melanophores,44 the first tissue in which melatonin's action had been demonstrated. This initial finding led to the discovery of another Gi -protein coupled melatonin receptor in humans (MT2),45 which is 60% identical in amino acid sequence to the MT1 receptor. Yet a third receptor, now called GPR50, shares 45% of the amino acid sequence with MT1 and MT2 but does not bind melatonin.46,47 It is unusual in that it lacks N-linked glycosylation sites and that it has a C-terminal that is over 300 amino acids long. Many G protein-coupled receptors (GPCR), including the MT1 and MT2 receptors, exist in living cells as dimers. The relative propensity of the MT1 homodimer and MT1 /MT2 heterodimer formation are similar, whereas that of the MT2 homodimer is 3-4 fold lower.48 It is of considerable interest that the GPR50 receptor, though lacking the ability to bind melatonin, abolishes high affinity binding of the MT1 receptor through heterodimerization.49,50 Thus the GPR50 receptor may have a role in melatonin function by altering binding to the MT1 receptor.51

A fourth 2- 125I-iodomelatonin binding site was also identified in mammals (MT3 , initially called ML-2).52 Unlike the picomolar membrane receptors it is a nanomolar binding site with a specific pharmacologic profile and fast kinetics of association/ dissociation.53 It has now been purified from hamster kidney and characterized as the analog of quinone reductase type 2.54

A combination of reagents derived from the molecular clones and pharmacologic tools have revealed a considerable amount of information about the MT1 and MT2 receptors.55 For example, it has been shown that the MT1 receptor inhibits firing acutely in SCN slices, and that both MT1 and MT2 may contribute to phase shifting in these slices. MT1 and MT2 have also been shown to differentially regulate GABAA receptor function in SCN.56 Mapping of the MT1 and MT2 receptors in brain though not yet complete has revealed much information. As expected, MT1 and MT2 receptors are present in the SCN.57 They are also found in several other brain areas and in the periphery. The MT1 receptor is extremely widely distributed in the hypothalamus of particular note, it is co-localized with corticotrophin in the PVN and with oxytocin and vasopressin in the PVN and supraoptic

nucleus.57 MT1 receptors are found in the cerebellum>= occipital cortex>= parietal cortex> temporal cortex> thalamus> frontal cortex >= hippocampus.58 MT2 receptors have been identified in the hippocampus,59 MT1 plus MT2 in the occipital cortex60 and MT1 in the dopaminergic system.61

Melatonin also binds to transcription factors belonging to the retinoic acid receptor superfamily, in particular, splice variants of RORα and the product of another gene, RZRβ (for ref. see).62 Although these nuclear binding proteins have for quite some time been a matter of debate and although their affinity to melatonin is lower, compared to MT membranes receptors, their classification as nuclear receptors now seems to be justified. RORα subforms are ubiquitously expressed in all mammalian tissues tested to date.63 RORα expression levels frequently depend on the differentiation state of cells or may vary within the cell cycle.63–65

Melatonin is the prototype of a class of drugs that influence the circadian apparatus and are referred to as chronobiotics.66–69 The response to melatonin follows a phase response curve (PRC), so that morning administration causes a delay, while evening administration causes an advance in circadian rhythms.70 This PRC is about 12 h out of phase with the PRC to light which causes a phase advance in the morning and a phase delay in the evening.71 A detailed PRC for melatonin (3 mg) established that the maximum advance portion peaked about 5 h before DMLO in the afternoon, the maximum delay portion was about 11 h after DMLO shortly after habitual awakening and a dead zone was in the first half of usual sleep.72 Maximum advance and delay shifts were 1.8 and 1.5 h, respectively.

In a study of one hour sleep schedule advance with early morning light it was shown that the addition of afternoon melatonin treatment (0.5 or 3 mg) caused a significantly greater phase advance of 2.5 h, again with minimal side effects.73 There was no difference between the two melatonin doses. Thus effects of morning light and evening melatonin are additive and can be used to cause pre-adaptation prior to eastward flight.

Current recommendations for light treatment require the patients to sit in front of a bright light box for at least 1 h daily, thus limiting their willingness to comply. Light applied through closed eyelids during sleep might not only be efficacious for changing circadian phase but also lead to better compliance because patients would receive light treatment while sleeping. Two studies investigated the impact of a train of 480 nm (blue) light pulses presented to the retina through closed eyelids on melatonin suppression and on delaying circadian phase.74 Both studies employed a sleep mask that provided narrow-band blue light pulses of 2 sec duration every 30 sec. The results demonstrated that blue

light pulses given early in the sleep episode significantly delayed circadian phase in older adults therefore indicating the efficacy and practicality of light treatment by a sleep mask aimed at adjusting circadian phase in a home setting.74 In a same vein, the effects of filtering short wavelengths (< 480 nm) during night shifts on sleep and performance in shift-work nurses were assessed indicating that this manipulation can be an approach to reduce sleep disruption and improve performance in rotatingshift workers.75

7.4. MELATONIN AND THE REGULATION OF THE SLEEP–WAKE CYCLE

Melatonin phase-shifts circadian rhythms in the SCN by acting on MT1 and MT2 melatonin receptors expressed by SCN neurons, thus creating a reciprocal interaction between the SCN and the pineal gland. Melatonin's phase-altering effect is caused by its direct influence on the electrical and metabolic activity of the SCN. The effect of melatonin on SCN clock genes is seen after a lag period of about 24 h suggesting that clock gene transcription was not the immediate target in situations in melatonin-induced phase advances of circadian rhythms mediated by MT1 and MT2 receptors.76 Thus the involvement of nuclear RORα receptors should be consid ered. This may result in the inhibition of the ubiquitin–proteasome system recently attributed to melatonin.77 Melatonin inhibition of the proteasome would tend to stabilize clock proteins in the SCN (such as BMAL1) transcribed during the scotophase. Additionally, melatonin-mediated phosphorylation of proteins through classical receptor-mediated pathways could indirectly influence the susceptibility of proteins to degradation by the proteasome.77

The circadian rhythm in the secretion of melatonin has been shown to be responsible for the timing of the sleep rhythm in both normal and blind subjects (i.e. in the absence of the synchronizing effect of light).78 The daily sleep-wake cycle is influenced by two separate processes: (1) the endogenous biological clock that drives the circadian rhythm of sleep-wake cycle (process C, for "circadian") and (2) a homeostatic component (process S, for "sleep") that influences "sleep propensity", a state which is determined by the immediate history of sleep and wakefulness and additionally the duration of previous sleep episodes.79,80 These two processes, which interact continuously, determine the consolidated bout of sleep at night and the consolidated bout of wakefulness during daytime. Observations of subjects whose circadian rhythms had been experimentally desynchronized have supported the inference that homeostatic processes drive slow sleep while rapid eye movement (REM) sleep is driven by the circadian

component.81

The role of SCN in the regulation of sleep-wake cycle is relevant, interacting with both sleep regulatory mechanisms,82 as first studied in the squirrel monkey. In this primate species lesions made in the SCN caused either loss of sleep or prolonged sleep.83 It is suggested that circadian signals emanating from the SCN promote wakefulness during the day. Additionally, SCN activity may facilitate sleep during the subjective night. It is interesting to note that the neural pathways from the SCN that promote wakefulness are complemented by those that are involved in the promotion of sleep.82

Studies in humans under constant routine conditions have defined the so-called ''biological night'' that corresponds to the period during, which melatonin is produced and secreted into the bloodstream. The beginning of the biological night is characterized by onset of the melatonin surge, an accompanying increase in sleep propensity as well as a decrease in core body temperature; the opposite occurs as the biological night and sleep end.84 Due to its neuronal suppressive actions on MT1 receptors85 and as a result of its phase shifting activity, mediated via its actions on MT1 and MT2 receptors,86 melatonin modulates the electrical activity of the SCN. The firing rate of the SCN decreases during the transition from non-REM to REM sleep.82 Crucial for optimal treatment of CRSD, melatonin and other treatments should be administered at a time related to individual circadian timing (typically assessed using the dim light melatonin onset (DLMO).87 If not administered according to the individual patient's circadian timing, melatonin and other treatments may not only be ineffective, they may even result in contrary effects. It has been proposed that knowing the patient's individual circadian timing by assessing DLMO improves diagnosis and treatment of CRSD with melatonin as well as other therapies such as light or chronotherapy.88

7.5. MELATONIN AND DELAYED SLEEP PHASE SYNDROME

DSPS is mainly encountered in young individuals. A common sleep-wake disorder that accounts for 10% of insomniacs who are diagnosed in sleep laboratory, DSPS is due to altered physiological timing in the biological clock.6 In this condition, the timing of sleep onset and wake time are delayed. The onset of sleep is delayed in some cases to 0200 – 0600 h. Neither sleep architecture nor the maintenance of sleep is affected.6 However, persons suffering from this disorder experience chronic sleep onset insomnia so that forced early awakening results in daytime sleepiness. It has been shown that the peak melatonin secretion occurs between 0800 h and 1500 h in some DSPS patients demonstrating the abnormal phase

position of melatonin in this sleep disorder.89,90 DSPS is the most frequently occurring CRSD. Dagan and Eisenstein91 found that 83.5% of 322 CRSD patients were of the DSPS type. The prevalence of DSPS in adolescence is more than 7% .89 Among those with DSPS onset of symptoms, which occurred in early childhood was reported by 64.3% of the sample, in the beginning of puberty by 25.3%, and during adulthood by 10.4%.91 Even a minor brain injury or head trauma can act as a trigger for the development of DSPS.92 DSPS can also follow whiplash injury.93 Frequently occurring jet lag or frequently occurring shift-work are also risk factors for the development of DSPS.94 A great proportion of patients have a prior history of depression.95 DSPS persists even after remission of the depression thus suggesting that DSPS may be a cause rather than a consequence of depression.96

Dahlitz and co-workers were the first to report a placebo-controlled study that demonstrated the efficacy of melatonin in the treatment of DSPS patients.97,98 A 5 mg dose of melatonin was administered orally at 2200 h to patients suffering from DSPS for a period of four weeks. In those studies, it was noted that melatonin significantly advanced the sleep onset time by an average of 82 min, with a range of 19 to 124 min. The mean wakefulness time also was advanced by 117 min.97,98 Though the total duration of sleep remained unaltered (mean about 8 h) after melatonin treatment, there was a significant improvement in sleep quality. Several other confirming reports followed.99–102

For example, Wilhelmsen-Langeland et al.103 reported that a combined bright light and melatonin treatment improved subjective daytime sleepiness, fatigue, and cognitive function in a three month study of DSPS patients. Although an alternative strategy, gradual advancement of rise times produced positive effects on subjective sleepiness, fatigue, and cognitive performance during short-term treatment of patients, the benefits from gradually advanced rise times wear off, indicating that the continuation of bright light and melatonin treatment is needed to maintain positive effects over time.103 Remarkably, a recent consensus on the roles of melatonin in children and on treatment guidelines concluded that the best evidence for melatonin efficacy in children is in DSPS.104 Melatonin is most effective when administered 3–5 h before DLMO. It was also concluded that there was no evidence that extended-release melatonin confers advantage over immediate release preparations.104 For maximum treatment effectiveness the timing of administration is just as critical for melatonin administration as it is for the application of bright light therapy.105 No adverse effects of melatonin were noted. Melatonin was also shown in placebo-controlled studies to be effective for treating children with idiopathic chronic sleep onset insomnia, which is related to

child onset DSPS.106,107

7.6.MELATONIN AND ADVANCED SLEEP PHASE SYNDROME

The changes in sleep patterns, which occur with advancing age can, in part, be attributed to changes in the functioning of the circadian oscillator.108 The characteristic pattern of ASPS includes complaints of persistent early evening sleep onset and early morning awakenings.6 Typically in ASPS, sleep onset occurs at around 2000 h and wakefulness occurs at around 0300 h. The quality of sleep is progressively impaired by increased awakenings occurring during the night. It has been suggested that this impairment is due to an attenuation of amplitude of the rhythm in melatonin secretion, which in turn may disrupt the phase relationships of the sleep-wake cycle as well as other circadian rhythms. Leger et al.109 in studies undertaken in 517 human subjects aged 55 years and above noted a significant decline in the secretion of 6-sulfatoxymelatonin, the principal urinary melatonin metabolite, in subjects suffering from insomnia. Melatonin replacement therapy was administered in dosages of 2 mg of controlled release tablets and was found to improve significantly the sleep quality of patients in the insomnia group [109]. Also affected were mea sures of alertness and behavioral integrity, which also showed improvements in these subjects. These findings were interpreted to support the conclusion that decrements in sleep quality, which are often seen among the elderly, are largely attributable to a decline in the production of melatonin, which also occurs with advancing age.8 The evidence was also taken to support the conclusion that melatonin promotes sleep possibly through circadian entraining effects as well as by a sleep-regulating effect. Genetic testing has provided support for the conclusion that familial ASPS is an inherited sleep–wake rhythm disorder with an autosomal dominant mode of inheritance.21 Indeed, alteration in the function of clock genes has been documented as one of the major causes of CRSDs.110–112

7.7 MELATONIN AND TIME ZONE CHANGE SYNDROME (JET LAG)

Rapid transmeridian flight across several time zones results in a temporary mismatch between the endogenous circadian rhythms and the new environmental LD cycle.113,114 As a result endogenous rhythms shift in the direction of the flight; an eastbound flight will result in a phase advance of rhythms while a westbound flight will produce a phase delay. The re-establishment of normal phase relationships differs from one rhythm to another.

Because of this phenomenon a transient desynchronization of circadian rhythms occurs, giving rise to a cluster of symptoms. These symptoms typically include transiently altered sleep patterns (e.g. disturbed night time sleep, impaired daytime alertness, and performance), mood and cognitive performance (e.g. irritability and distress), appetite (e.g. anorexia), along with other physical symptoms such as disorientation, fatigue, gastrointestinal disturbances and light-headedness that are collectively referred to as “jet lag”. Several studies have reported an improved quality and duration of sleep or accelerated resynchronization of rhythms by giving melatonin (for ref. see).113,114 Doses used in these studies ranged from 0.5 to 10 mg, with more soporific effect with doses of 5 or 10 mg and little difference in phase response between doses. Melatonin at bedtime after eastbound flight provides benefits from both the soporific and phase resetting effects. Studies using melatonin on westbound flights have been few and have revealed less benefit than after eastbound flight. An exception is a study115 using a combination of 3 mg bed time melatonin with exercise and light exposure in 2–3 h time blocks (08:00 to 11:00 and 13:00 to 16:00) for six days at the destination after 12 h westward flight in athletes. Although this study lacked a placebo control, resynchronization was reported after 2.13 days, much more rapidly than would be expected. Use of light in this manner is theorized to cause blunting or masking of the endogenous circadian signal and sensitization to Zeitgebers.116 In a follow-up study that again used 3 mg melatonin at bedtime in combination with only 30 min or more of outdoor exercise in the same two time blocks it was reported that after westward travel of 11 h resynchronization occurred in 2.54 days while after 13 h eastward travel it occurred in 2.27 days.68 Although there was no placebo control, adaptation to the new environment occurred much more rapidly than would be expected. Recommendations for using melatonin and light therapy in jet lag from the American Academy of Sleep Medicine indicate that for an individual flying eastward, in whom advancing the sleep phase is desired, 0.5–5 mg melatonin administered following flight at the desired time of bed along with early morning bright light exposure will assist with circadian shift . 106 In contrast, for westward travel, which is considered easier to adapt to because of endogenous circadian periodicity of 24 h, only bright light in the evening is recommended.

7.8.MELATONIN AND SHIFT WORK SLEEP DISORDER

It has been estimated that in our modern 24/7 society at least one fifth of total work force is engaged in rotating shift work117. These individuals are forced to

forego their nocturnal sleep while they are on a nightshift, and sleep during the day.118 This inversion of the sleep-wake rhythm with work at night at the low phase of the circadian temperature rhythm and sleep at the time of peak body temperature has given rise to insomnia-like sleep disturbances. Sleep loss impairs the individual's alertness and performance that affects not only work productivity but also has been found to be a major cause for industrial and sleep-related motor vehicle crashes.117 Sleep-related crashes occur most commonly in the early morning hours (0200–0600 h).3 Sleep deprivation and the associated desynchronization of circadian rhythms are common in shift-work sleep disorder.119–121 Many treatment procedures have been advocated. Czeisler and co-workers administered bright light for improving the physiological adaptation of the circadian rhythms of night-shift workers to their inverted sleep-wake schedules.122 In their study bright light was found effective for resynchronizing alertness, cognition, performance, and body temperature to the new work schedules. Following the successful application of bright light, melatonin has been used in shift workers to accelerate adaptation of their circadian rhythms and sleep-wake rhythms to the new work schedules (see, e.g.).84,123 A phase delay in plasma melatonin was noted in shift workers, when melatonin was administered at the morning bedtime following the night shift. The shift in melatonin secretion has been associated with increase in work performance as well.124 Correctly timed administration of melatonin is advocated for hastening adaptation of circadian rhythms in shift-workers, inasmuch as melatonin administration in the evening (1600 h) does not affect daytime sleepiness and mood.125 Melatonin (1.5 mg at 1600 h) was found to advance the timing of both endogenous melatonin and cortisol rhythms without causing any deleterious effects on endocrine function or daytime mood and sleepiness. There is thus evidence that combination of both bright light and melatonin can be an effective and reliable strategy for treating shift work disorder. Recently, a Cochrane meta-analysis to evaluate the effects of pharmacological interventions to reduce sleepiness or to improve alertness at work and decrease sleep disturbances whilst off work was published.126 Primary outcomes were sleep length and sleep quality while off work, alertness, and sleepiness, or fatigue at work of 15 randomized placebo-controlled trials including 718 participants. Nine trials evaluated the effect of melatonin and two the effect of hypnotics for improving sleep problems. One trial assessed the effect of modafinil, two of armodafinil and one examined caffeine plus naps to decrease sleepiness or to increase alertness. Melatonin (1–10 mg) after the night shift increased sleep length during daytime sleep as compared to placebo. Both modafinil and armodafinil increased alertness

and reduce sleepiness to some extent in employees who suffer from shift work sleep disorder but they were associated with adverse events. Caffeine plus naps reduced sleepiness during the night shift, but the quality of evidence was assessed as low.126

7.9. MELATONIN AND NON 24 HOUR SLEEP-WAKE DISORDER

As mentioned above, the neuroendocrine effects of light are mediated primarily via specialized non-rod, non-cone ganglion cell photoreceptors. Consequently, most blind people with no perception of light have either non-entrained or abnormally phased circadian rhythms due to this inability to detect light. Conversely, most visually impaired individuals with some degree of light perception should exhibit normal entrainment, emphasizing the functional separation of visual and “non-visual” photoreception. This was re-inforced in a recent study on the prevalence of circadian disorders in blind women.127 The circadian type of 127 individuals was determined from the timing and time course of the melatonin rhythm as measured by urinary 6-sulfatoxymelatonin. The results indicated that 37% of participants with no perception of light were classified as normally entrained (bilateral enucleation, retinopathy of prematurity) while 69% of participants with some degree of light perception (retinitis pigmentosa. age-related macular degeneration) were normally entrained.127 Patients with non-24 h sleep-wake disorder suffer from recurrent insomnia and daytime sleepiness. The circadian rhythm of sleepiness has shifted out of phase with the desired time for sleeping. Melatonin has been employed to correct right abnormal sleep-wake rhythms in blind human subjects128 and the Food & Drug Administration approved the melatonin analog tasimelteon to be used with this aim in 2013. In a recent observational study of 21 patients with non-24 h sleep-wake disorder who kept a sleep-wake schedule of their choosing the circadian phase was determined using the melatonin onset from plasma or saliva samples and actigraphy.129 A total of 469 assessments were conducted over 5,536 days of study. Although subjects lacked entrainment such that circadian phase drifted an average of 0.39 h later per day there was notable intersubject and intrasubject variability in the rate of drift including relative coordination and periods of transient entrainment, during which there was little to no drift in the circadian phase. Hence the relative coordination and transient entrainment may result in misdiagnosis and responsiveness to environmental time cues and influence treatment success with oral melatonin.129 The authors recommended the use of actigraphy to adjust the timing of melatonin in this

situation. The prevalence of non-24 h sleep-wake rhythm disorder among sighted patients is unknown. Most of the reported cases belong to the Japanese population and very few cases have been documented outside of Japan and these have been predominantly male and associated with avoidant or schizoid personalities (see for ref.).2,6 Thus, non-24 h sleep-wake disorder is a rare sleep disorder among sighted patients in Western populations. In the Japanese population, it has been estimated that non-24 h sleep-wake rhythm disorder comprises 23% of all CRSDs.130 The prevalence of circadian rhythm disorders in this population (0.13–0.4%) is consistent with that observed in other populations.131 It is likely that this sleep disorder is rare in Western populations because it is underdiagnosed. Diagnosis is complicated by the fact that at times non-24 h sleep-wake rhythm disorder can resemble both ASPS and DSPS and in fact exhibit the same polysomnographic features. Under certain circumstances, irregular CRSD may arise. For example, treatment with psychotropic drugs such as haloperidol132 can trigger a CRSD of an irregular type. In addition, prolactin secreting microadenomas,133 or occupational inadequate exposure to bright light can be related to the development of irregular CRSD.134 Minor head trauma can cause irregular CRSD as well as DSPS.92 Most of reports of this type of CRSD refer it to the influence of environmental and medical conditions.

7.10. MELATONIN AND CIRCADIAN RHYTHM ABNORMALITIES IN ALZHEIMER´S DISEASE

AD is an age-associated neurodegenerative disease that is characterized by a progressive loss of cognitive function, loss of memory, and several neurobehavioral manifestations.135 Concomitantly, melatonin levels are lower in AD patients compared to age-matched control subjects. Decreased CSF melatonin levels observed in AD patients reflect a decrease in pineal melatonin production rather than a diluting effect of CSF. CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I–II), suggesting that the reduction in CSF melatonin may be an early marker for the first stages of AD.136 AD patients with disturbed sleep– wake rhythms did not only exhibit reduced amounts of melatonin secreted, but also a higher degree of irregularities in the melatonin pattern, such as variations in phasing of the peak.137 Therefore, the melatonin rhythm has not only lost signal strength in clock resetting, but also reliability as an internal synchronizing time cue. Loss or damage of neurons in the hypothalamic SCN and other parts of the circadian timing system may account for the circadian rhythm abnormalities seen

in demented patients, especially as the number of neurons in the SCN of AD patients is reduced. In both elderly subjects and in patients suffering from dementias the administration of exogenous melatonin has been found to not only to enhance sleep quality but also to improve the sleep-wake rhythm in clinical setting studies.138–142 In a doubleblind study, the major findings with regard to melatonin effects on sleep–wake rhythmicity, cognitive and non-cognitive functions were confirmed.143 In a multicenter, randomized, placebo-controlled clinical trial144 the increase in nocturnal total sleep time and decreased wake after sleep onset, as determined on an actigraphic basis, were only apparent as trends in the melatonin treated groups. On subjective measures, however, caregiver ratings of sleep quality showed significant improvement in the 2.5 mg sustained-release melatonin group relative to placebo. Large interindividual differences between patients suffering from a neurodegenerative disease are not uncommon. To test whether the addition of melatonin to bright-light therapy enhances the efficacy in treating rest-activity (circadian) disruption in institutionalized patients with AD 50 subjects were examined in a randomized, controlled trial.145 Light treatment alone did not improve nighttime sleep, daytime wake, or rest-activity rhythm while light treatment plus melatonin (5 mg/day) increased daytime wake time and activity levels and strengthened the rest-activity rhythm. In another randomized controlled trial on the effect of combined bright light and melatonin on cognitive and non-cognitive function in 189 elderly residents of group care facilities bright light had a benefit in improving cognitive and non-cognitive symptoms of dementia, which was amplified by the conjoint administration of melatonin.146 Melatonin alone had a slight adverse effect on mood. It must be noted that in another double-blind randomized placebo-controlled trial of 24 institutionalized patients with AD melatonin failed to improve sleep or agitation.147 Since the circadian oscillator system is obviously affected in AD patients showing severe sleep disturbances, the efficacy of melatonin should be expected to depend heavily on disease progression. One cannot expect a profound inhibition of disease progression once a patient is already in an advanced demented state, notwithstanding a very few case reports with anecdotal evidence of slight mental improvements. Therefore, the use of melatonin in the early stages of AD should be important, like MCI. MCI is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia. Approximately, 12% of MCI convert to AD or other dementia disorders every year.148 Since MCI may represent prodromal AD it should be adequately diagnosed and treated. The degenerative process in AD brain starts 20–30 years

before the clinical onset of the disease. During this phase, plaques and tangles loads increase and at a certain threshold the first symptom appears. As already mentioned, CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I–II),136 suggesting that the reduction in CSF melatonin may be an early trigger and marker for AD. In a recent study, 30 patients with MCI and 28 age-matched controls underwent psychiatric, medical, and neuropsychological assessment, followed by overnight polysomnography and dim light melatonin onset assessment.149 Participants also performed an episodic memory task while in the laboratory. Patients with MCI had advanced timing of their melatonin secretion onset relative to controls, but the levels of melatonin secreted did not differ between groups. The MCI group also had greater wake after sleep onset and increased rapid eye movement sleep latency. There were differential associations between dim light melatonin onset and cognition between the two groups, with earlier dim light mela tonin onset being associated with poorer memory performance in MCI patients. The authors concluded that circadian misalignment and sleep disruption is evident in patients with MCI, and is consistent with changes observed in Alzheimer's disease.149 We previously reported a retrospective analysis, in which daily 3–9 mg of a fast-release melatonin preparation p.o. at bedtime for up to three years significantly improved cognitive and emotional performance and daily sleep-wake cycle in 25 MCI patients.150 Another series of 96 MCI outpatients, 61 of who had received daily 3–24 mg of a fast-release melatonin preparation p.o. at bedtime for 15–60 months in comparison to a similar group of 35 MCI patients who did not receive it, was published.151 Patients treated with melatonin exhibited significantly better performance in mini– mental state examination and the cognitive subscale of the AD assessment scale. After application of a neuropsychological battery comprising a Mattis´ test, digit-symbol test, Trail A and B tasks and the Rey´s verbal test, better performance was found in melatonin-treated patients for every parameter tested. Abnormally, high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with the improvement in the quality of sleep and wakefulness.151 These results further supported that melatonin is a useful add-on drug for treating MCI in a clinic environment. Thus, an early initiation of melatonin treatment can be decisive for therapeutic success. In recent evaluation of the available data in the literature,7 six double blind, randomized placebo-controlled trials and two open-label retrospective studies (N = 782), consistently showed that the administration of daily evening melatonin improves sleep quality and cognitive performance in MCI patients indicating that

melatonin treatment could be effective at early stages of the neurodegenerative disease. There are two reasons why it is convenient to use melatonin in MCI patients. In the course of the neurodegenerative process, the age-related deterioration in circadian organization becomes significantly exacerbated and is responsible for behavioral problems like sundowning. Age-related cognitive decline in healthy older adults can be predicted by the fragmentation of the circadian rhythm in locomotor behavior. Hence, replacement of the low melatonin levels occurring in brain can be highly convenient in MCI patients. Moreover, the bulk of information on the neuroprotective properties of melatonin derived from experimental studies (see for ref.)7 indicates that it may be highly desirable to employ pharmacological doses in MCI patients with the aim of arresting or slowing disease´s progression. The mechanisms accounting for the therapeutic effect of melatonin in MCI patients remain to be defined. Melatonin treatment mainly promotes slow wave sleep in the elderly152 and can therefore be beneficial in MCI by augmenting the restorative phases of sleep, including the augmentation of the secretion of GH, and neurotrophins. Additionally, the antioxidant, mitochondrial, and antiamyloidogenic effects of melatonin can be seen as potentially interfering with the onset of the disease.7 Therefore, when melatonin treatment begins can be decisive for final response. One important aspect to be considered is the melatonin dose employed, which may be unnecessarily low when one takes into consideration the binding affinities, half-life and relative potencies of the different melatonin agonists on the market. In addition to being generally more potent than the native molecule, melatonin analogs are employed in considerably higher amounts.153 Indeed, melatonin has a high safety profile, it is usually remarkably well tolerated and, in some studies, it has been administered to patients at very large doses. Moreover, some of the melatonin agonists would not be expected to have antioxidant effects.

7.11. MELATONIN AND BZD/Z DRUG ABUSE

In aging individuals a combination of altered sleep and sleep pathologies increases the risk of drug-induced insomnia or excessive diurnal somnolence.154–156 Many old adult patients are treated for longer periods or with higher dosages of hypnotic drugs than are recommended, generally with a lack of individual dosage titration. An ideal hypnotic drug should not produce undesired side effects, such as impairment of memory, cognition, next psychomotor retardation, and day hangover effects, or potentiality of abuse. Melatonin as a chronobiotic fulfills many of these requirements8 with recent meta-analysis supporting the

efficacy of melatonin in primary sleep disorders.157 The chronic and extensive use of BZD/Z drugs has become a public health issue and has led to multiple campaigns to reduce both their prescription and consumption. Since melatonin and BZD shared some neurochemical (i.e. interaction with GABA-mediated mechanisms in brain) 158 and behavioral properties (e.g. a similar day-dependent anxiolytic activity),159 melatonin therapy was postulated as an effective tool to decrease the dose of BZD needed in patients. Two early observations pointed to the possible beneficial effect of melatonin in this respect. One of us reported in an open label study that eight out of 13 insomnia patients either discontinued or reduced BZD use by 50–75% after receiving a 3 mg dose of fast release melatonin.160 Dagan et al. published a case report on the efficacy of 1 mg of controlled release melatonin to completely cease any BZD use in a 43 year old woman who had suffered from insomnia for the past 11 years.161 A double-blind, placebo controlled, study followed by a single blind period enlisted 34 primary insomnia outpatients aged 40–90 years who took BZD and had low urinary 6-sulphatoxy melatonin levels, 14 out of 18 subjects who had received prolonged-release melatonin (2 mg), but only four out of 16 in the placebo group, discontinued BZD therapy.162 An open label study further supported the efficacy of fast release melatonin to decrease BZD use, that is, 13 out of 20 insomnia patients taking BZD together with melatonin (3 mg) could stop BZD use while another four patients decreased BZD dose to 25–66% of initial doses.163 In the retrospective analysis of 96 MCI outpatients mentioned above the comparison of the medication profile indicated that 9.8% in the melatonin group received BZD verses 62.8% in the non-melatonin group thus supporting decrease BZD use.151 A retrospective analysis of a German prescription database identified 512 patients who had initiated treatment with prolonged release melatonin (2 mg) over a 10 month period.164 From 112 patients in this group who had previously used BZD, 31% discontinued treatment with BZD three months after beginning melatonin treatment. Therefore, melatonin helps to facilitate BZD discontinuation in older insomniacs. In a study aimed to analyze and evaluate the impact of anti-BZD/Z-drugs campaigns and the availability of alternative pharmacotherapy (melatonin) on the consumption of BZD and Z-drugs in several European countries it was found that campaigns failed when they were not associated with the availability of melatonin in the market.165 In this pharmacoepidemiological study, the reimbursement of melatonin supports better penetration rates and a higher reduction in sales for BZD/Zdrugs. A post marketing surveillance study of prolonged release melatonin (2 mg) was performed in Germany. It examined the effect of three weeks of treatment on sleep in 597 patients. Most of the patients

(77%) who used traditional hypnotics before melatonin treatment had stopped using them and only 5.6% of naïve patients started such drugs after melatonin discontinuation.166 A major advantage of melatonin use as a chronobiotic is that it has a very safe profile, it is usually remarkably well tolerated and, in some studies, it has been administered to patients at very large doses and for long periods of time without any potentiality of abuse.

7.12. CONCLUSIONS

The evidence at present suggests that dysfunctionality in melatonin secretion may play a central role in the pathophysiology of CRSDs. Melatonin has been found useful in treating the disturbed sleep-wake rhythms seen in DSPS, non-24 h sleepwake rhythm disorder and irregular type of CRSD, as well as in shift-work sleep disorder and jet lag. A number of melatonin analogs are now in the market that shares the potential activity to treat CRSDs.167 Ramelteon (Rozerem™) is a MT1 /MT2 melatonin receptor agonist, synthesized by Takeda Chemical Industries with a half-life much longer (1–2 h) than that of melatonin. Ramelteon acts on both MT1 and MT2 melatonergic receptors present in the SCN (for ref. see).168

Agomelatine (Valdoxan™, Servier) is a novel antidepressant drug, which acts as both a melatonin MT1 and MT2 receptor agonist and as a 5-HT2c antagonist (for ref see).169 Animal studies indicate that agomelatine accelerates re-entrainment of wheel running activity and a study performed in healthy older men indicated that agomelatine phase-shifts 24 h rhythms of hormonal release and body temperature.170 The melatonin MT1 /MT2 agonist tasimelteon (Hetlioz™, Vanda) was recently introduced in the market to treat non 24 h sleep-wake disorders in blind people. A randomized controlled trial of for transient insomnia after sleep time shift was published.171 After an abrupt advance in sleep time, tasimelteon improved sleep initiation, 138 Synopsis of Sleep Medicine and maintenance concurrently with a shift in endogenous circadian rhythms indicating that it may have therapeutic potential for transient insomnia in CRSDs.171 Several studies underline the efficacy of melatonin to treat circadian and cognitive symptoms in AD. In this case, however, the neuroprotective activity of melatonin seems not to be mediated by melatonin membrane receptors but rather by the strong antioxidative activity that melatonin and its endogenous metabolites have. So far none of melatonin analogs has been shown to display such a neuroprotective activity.

ACKNOWLEDGMENTS

DPC is a Research Career Awardee from the Argentine Research Council (CONI- CET). Studies in DPC's Laboratory were supported by a grant from the Agencia Nacional de Promoción Científica y Tec- nológica, Argentina (PICT 2012, 0984).

KEY WORDS

- **melatonin**
- **circadian rhythm sleep disorders**
- **suprachiasmatic nucleus**
- **Alzheimer´s disease**
- **Benzodiazepine/Z drug abuse**

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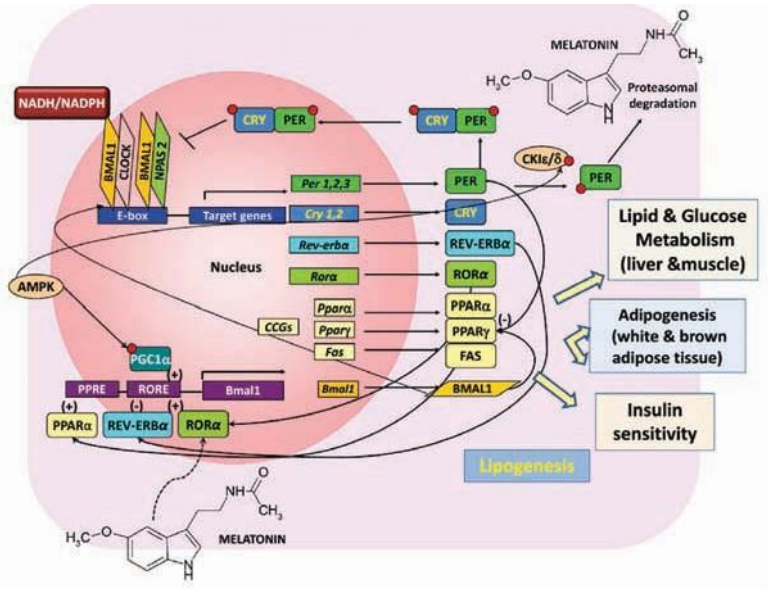

Figure 7.1. A network of transcription-translation feedback loops constitutes the mammalian circadian clock. The transcription factor BMAL1 forms heterodimers with CLOCK. These heterodimers drive the transcription of three mammalian period genes *Per1*, *Per2*, and *Per3*, two Cryptochrome genes *Cry1* and *Cry2*, and *Rev–Erb-α* genes by binding to their respective CACGTG E-Box elements present in the promoters. Rhythmic output of the clock is achieved through E-box elements in clock-controlled genes (CCGs), which can impact a range of cell processes and physiology. In the figure 7.1 a number of genes involved in lipid and glucose metabolism are shown. The BMAL1–CLOCK heterodimer can also inhibit BMAL1 transcription. After transcription and translation, the Rev–Erb-α protein enters the nucleus to suppress the transcription of *Bmal1* and *Cry* genes. A second cluster of genes are driven by RORA (RAR-Related Orphan Receptor-A), which are sensitive to negative regulation by Rev–Erb-α and so are expressed in phase with BMAL1. Rev-Erb-α/RORA is involved in gene expression during circadian night, which is in phase with BMAL1 and in antiphase to Per2 oscillations. As the Per proteins, such as PER2, accumulate in the cytoplasm, they become phosphorylated by CSNK-I-Epsilon/Delta (Casein Kinase-I-Epsilon/Delta). The phosphorylated forms of PER are unstable and are degraded by ubiquitylation and proteasomal degradation. Late in the subjective day, however, CRY accumulates in the cytoplasm, promoting the formation of stable CSNK-I-Epsilon/Per/Cry complexes, which enter the nucleus at the beginning of a subjective night. Once in the nucleus, Cry1 disrupts the CLOCK/BMAL1-associated transcriptional complex, resulting in the inhibition of *Cry*, *Per*, and Rev–Erb-α transcription and derepression of BMAL1 transcription. The interacting positive and negative feedback loops of circadian genes ensure low levels of Per and Cry, and a high level of BMAL1 at the beginning of a new circadian day. In the SCN neurons, the intracellular levels of CLOCK remain steady throughout the 24 h period, whereas BMAL1 expression levels are high at the beginning of a subjective day and low at the beginning of a subjective night. In addition, a number of signaling molecules derived from cellular metabolism fine-tune these molecular clock loops (e.g. AMPK, an AMP-dependent kinase; NADP/NADPH via sirtuins). The effect of melatonin on SCN clock genes is seen after a lag period of about 24 h suggesting that clock gene transcription was not the immediate target in situations in melatonin-induced phase advances of circadian rhythms mediated by MT_1 and MT_2 receptors. Thus the involvement of nuclear RORα receptors and the inhibition of the ubiquitin–proteasome system deserve to be considered.