Pharmaceutical, chronobiological and clinical aspects of melatonin
Nagtegaal, J.E.

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Part 1

PHYSIOLOGICAL AND PHARMACOLOGICAL ASPECTS OF MELATONIN

- An overview of the physiological and pharmacological effects of melatonin
1.1 AN OVERVIEW OF THE PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF MELATONIN

The pineal hormone melatonin has been highlighted last years for playing its part in important physiological processes in the body. Special attention has been focussed on the effects of melatonin on biological rhythms. Biological rhythms of various periodicity occur in all eukaryotic organisms. The frequency displayed varies from fractions of a second to years. Internally generated rhythms with a period of approximately 24-hour are called circadian rhythms. The general structure of the circadian system has three components: a pacemaker or biological clock, an input pathway for entrainment of the pacemaker and an output pathway for the expression of overt rhythms.

Since endogenous melatonin plays an important role in the regulation of the major biological clock, the use of exogenous melatonin to treat circadian rhythm disorders has become an important field of research. This chapter first reviews the knowledge of endogenous melatonin and its physiological effects. Thereafter the chemical synthesis of exogenous melatonin and the effects of administration of exogenous melatonin in physiological and pharmacological dosages are discussed. The last part of this chapter reviews an underexposed field of exogenous melatonin: the toxicity and side effects.

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1.1.1 Endogenous melatonin

Synthesis of melatonin

Lerner et al have discovered and isolated melatonin from the pineal gland [1,2]. Melatonin is synthesised in the pinealocyte as shown in Figure 1 [3]. As can be seen in this figure the neurotransmitter serotonin plays a role in this synthesis.

Figure 1: Synthesis of melatonin in the pinealocyte

<table>
<thead>
<tr>
<th>Tryptophan</th>
<th>↓</th>
<th>Tryptophan-hydroxylase</th>
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<tbody>
<tr>
<td>5-Hydroxytryptophan (5-HP)</td>
<td>↓</td>
<td>Aromatic amino-acid-decarboxylase</td>
</tr>
<tr>
<td>5-Hydroxytryptamine (serotonin)</td>
<td>↓</td>
<td>N-acetyltransferase (NAT)</td>
</tr>
<tr>
<td>N-Acetylsersotonin</td>
<td>↓</td>
<td>Hydroxyindole-O-methyltransferase (HIOMT)</td>
</tr>
<tr>
<td>Melatonin</td>
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</table>

Melatonin has high lipid and water solubility and gains access to various fluid, tissue, and cellular compartments when released in the circulation [4].

The process of production and release of melatonin is shown in Figure 2. Its 24h rhythmicity is controlled by the endogenous biological clock, which is located in the suprachiasmatic nuclei of the hypothalamus [3]. Suprachiasmatic projections regulate the pineal gland and innervate paraventricular cells of the hypothalamus [5,6] that project through the medial forebrain bundle to intermediolateral cell column of the spinal cord [7].
These nerve projections stimulate preganglionic cells that innervate the superior cervical ganglion. These ganglia are of primary importance to the sympathetic innervation of the pineal gland [8] and mediate all known biochemical and physiological functions of the pineal. Postganglionic noradrenergic cells in the SCG project to the pineal gland via the inferior carotid nerve and the coronary nerve [8].

Figure 2: process of endogenous melatonin production

Sympathetic nerve endings in the pineal release the neurotransmitter noradrenaline (NA) and thus transmit the oscillatory information from the SCN to the pineal. According to the entrained pacemaker's program, the NA release is high at night and low during the day [9]. As illustrated in Figure 3, NA, released at night into the extracellular space interacts with adrenergic receptors. In the rat, stimulation of beta-adrenergic receptors induces the increase in cyclic AMP content [10] in NAT activity [11,12] and in melatonin synthesis [13]. Simultaneous activation of alpha1 receptors potentates the beta-adrenergic-mediated increase in cyclic AMP [14] and in NAT activity [15]. However, in other mammalian species the importance of various adrenergic receptors for induction of melatonin synthesis may be different [16, 17].
1.1.2 Melatonin receptors

Two membrane-bound melatonin-binding sites belonging to pharmacologically and kinetically distinct groups have been identified: ML1 (high-affinity [picomolar]) sites and ML2 (low-affinity nanomolar) sites [18,19]. Activation of ML1 melatonin receptors, which belong to the family of guanosine triphosphate-binding proteins (G protein-coupled receptors) [20], results in the inhibition of adenylate cyclase activity in target cells. These receptors are probably involved in the regulation of retinal function, circadian rhythms and reproduction. The ML2 receptors are coupled to the stimulation of phosphoinositide hydrolysis, but their distribution has not been determined. Two forms of a high affinity melatonin receptor have been designated MEL1a and MEL1b, were cloned from several animals, including humans [21,22]. The MEL1a receptor is expressed in the hypophysial pars tubularis and the suprachiasmatic nucleus (the presumed sites of the reproductive and circadian actions of melatonin, respectively). The MEL1b melatonin receptor is expressed mainly in the retina and, to a lesser extent, in the brain. Autoradiography and radioreceptor assays have demonstrated the presence of melatonin receptors in various
regions of the human brain [23] and in the gut [24], ovaries [25], and blood vessels [26]. Neural receptors (e.g. those in the suprachiasmatic nucleus of the hypothalamus) are likely to regulate circadian rhythms. Non-neural melatonin receptors (such as those located in the pars tubularis of the pituitary) probably regulate reproductive function, especially in seasonally breeding species and receptors located in peripheral tissues (e.g. arteries) may be involved in the regulation of cardiovascular function and body temperature [27].

Figure 4: 24 hour melatonin profile

1.1.3 Kinetics and metabolism of endogenous melatonin

In physiological conditions, melatonin is secreted only during the night with maximum circulating levels of the hormone depending on age from between 142-205 pg/ml in young adults till 76-423 pg/ml in older subjects [28,29,30]. An example of a melatonin plasma profile is presented in Figure 4. The endogenous production of melatonin is lower in
Chapte rr 1.1 8

patients with livercirrhosis [31]. About 70% of circulating plasma melatonin is bound to albumin, presenting its free diffusion across capillary membranes [32]. The primary site for melatonin metabolism is within the liver and secondarily the kidney [3]. In the liver it undergoes 6-hydroxylation, followed by sulphate or glucuronide conjugation, in a classic microsomal phase 1, phase 2 reaction sequence common to the metabolism of steroids and the deactivation and detoxification of many drugs. The relative amounts of sulphate and glucuronide formed probably depend on species. In humans at least 90% of a dose of melatonin may be accounted for by 6-sulfatoxymelatonin in plasma and urine.

1.1.4 Physiological regulation of melatonin in relation to age

Foetal regulation of melatonin

In humans, a 24-h melatonin rhythm is expressed in the blood of pregnant women throughout gestation [33]. The amplitude of the rhythm and total secretion of melatonin appear to be somewhat greater than those of non-pregnant women, especially in the third trimester. The human foetal suprachiasmatic nucleus expresses melatonin binding sites and is, since melatonin crosses the placenta [34], therefore likely to be affected by both maternal and administered melatonin with consequences for the prenatal and postnatal expression and entrainment of circadian rhythms. Caution is warranted, not only concerning the use of exogenous melatonin during pregnancy and lactation but also concerning behaviour that might disrupt the mother's endogenous melatonin rhythm [35]. Melatonin is present in human foetal blood and amniotic fluid as well as in the mother's milk [36]. Although a melatonin rhythm of maternal origin is likely to be present within the human foetus it appears that during the first few weeks after birth the new-born is without a systematic 24-h melatonin rhythm [37]. A 24-h rhythm in maternal melatonin is one of several maternal rhythms to which foetuses normally are exposed. Although it has yet to be established whether circadian rhythms in functions such as heart rate and activity expressed by human foetuses are expressions of an endogenous, entrained pacemaker or are passive responses to maternal rhythms, maternal rhythmicity clearly is a normal feature of the intrauterine environment. Infants born prematurely (30-35 weeks) are deprived of this maternal rhythmicity. When kept in neonatal intensive care units, they may be deprived of other 24-h periodicity as well [35].
In concert with maturation of entrainment pathways a 24-hour melatonin rhythm appears at around 6-8 weeks of life [37].

Regulation of melatonin from childhood to adolescence

Systemic melatonin is high in early childhood and decreases continuously until puberty, especially in relation to body size. In contrast to the pituitary gland that doubles in size, the pineal does not appear to grow between 1 and 15 years of age. The hypothesis for the relationship between changes of melatonin concentrations and start of puberty is that high levels of circulating melatonin during prepubertal development are sufficient to inhibit gonadotropin secretion (for years) and that the eventual fall in melatonin below some critical amount triggers puberty [37].

Regulation of melatonin in the elderly

As stated before, several authors have found a striking decline in the amplitude of melatonin production with age in humans. This has led to speculations that circadian abnormalities present in old age may be secondary to loss of the melatonin rhythm [28]. Opposed to these reports that secretion of melatonin declines with age, Zeitzer and co-authors found no difference in melatonin amount of production and amplitude of the melatonin rhythm in humans [38]. Calcification of the pineal has generally been considered a feature of adults but is probably initiated in early life. There is no evidence that the presence of calcification leads to degeneration of pineal cells and metabolic activity. Melatonin formation is not related to the quantity of calcification [3]. Based on these conflicting results and hypotheses we suppose that circadian abnormalities in the elderly are not simply due to an age-dependent melatonin deficiency. Therefore we recommend assessing endogenous melatonin in individuals before melatonin replacement therapy is started.

1.1.5 Hypnotic effects of endogenous melatonin

The primary physiological function of melatonin is to convey information about the daily cycle of light and darkness to body physiology. By its pattern of secretion during darkness, melatonin indicates the length of the night, thus representing the chemical code of the scotophase [39].
The activities of the pineal enzymes that synthesise melatonin and melatonin itself were shown to be elevated at night [40,41]. The increase in melatonin levels in the evening correlates with the onset of self-reported evening sleepiness [42,43] or with the increase in the evening sleep propensity as reported by Tzischinsky and co-authors [44]. In the study of Tzischinsky et al the relationship between the time of nocturnal onset of urinary 6-sulfatoxymelatonin (aMT6s) secretion, and the timing of the steepest increase in nocturnal sleepiness (‘sleep gate’), as determined by an ultrashort sleep-wake cycle test (7 min sleep, 13 min wake) was investigated in twenty-nine male participants. The results showed that the timing of the sleep gate was significantly correlated with the onset of aMT6s secretion. Since the time courses of aMT6s and melatonin were previously shown to be closely related to each other [45], this indicated a close temporal relation between the secretion of melatonin and nocturnal sleep propensity [44].

Observations in human babies revealed a correlation between the consolidation of nocturnal sleep and the normal onset of rhythmic melatonin secretion, both of which occur when infants are about 3 months old [37]. The declines of melatonin secretion and sleep efficiency with age were postulated to be related phenomena. For example middle aged and elderly insomniacs exhibit lower melatonin production than do good sleepers of the same age [46,47].

In sighted volunteers living in society, the onset of the nocturnal melatonin secretion occurs approximately 2 h before habitual bedtime [44]. In blind people in whom the circadian pacemaker is not entrained [48] and in a sighted subject with non-24-h sleep wake cycle syndrome [49] a tight association between the propensity to initiate sleep and the phase of melatonin secretion has been described.

Although these correlations between endogenous melatonin and sleep seem interesting, it gives no basis to conclude about the direct effect of endogenous melatonin on sleep. There is a need for further studies using physiological doses and delivery systems that generate physiological plasma melatonin profiles to firmly establish the role of the endogenous circadian rhythm of melatonin in the circadian regulation of sleep.

1.1.6 Effects of several agents on endogenous melatonin

The circadian rhythm of melatonin is highly reproducible and generally not easily altered [50]. However, several drugs have been shown to increase or inhibit melatonin secretion or
shift the melatonin curve. In Table 1 and Table 2 these drugs and several hypotheses for the mechanism of action on the melatonin production are summarised.

In humans, beta-blockers (beta-adrenergic antagonists) that are associated with significant increases in sleep disruption depress nocturnal melatonin production [51]. Benzodiazepines, clonidine and dexamethasone may also suppress melatonin production [52,53,54,55].

Parkinson patients under chronic levodopa/decarboxylase inhibitor substitution showed a phase advance of the nocturnal melatonin peak. This phase shift seems to be caused by oral levodopa administration and is more likely to be a central nervous effect than a peripheral one [56].

After administration of the serotonin re-uptake inhibitor fluvoxamine in the early evening, the plasma melatonin level in the morning was significantly increased [57]. More recently it appeared that citalopram, another serotonin reuptake inhibitor does not increase serum melatonin. Since fluvoxamine inhibits cytochrome P450 enzymes in the liver, while citalopram does not, it is hypothesised that fluvoxamine may decrease the metabolism of melatonin resulting in a higher serum melatonin level [58].

Enhancement of melatonin plasma concentrations after intake of desipramine was reported in depressed patients but not in normal subjects [59]. Intake of ethanol between 19:00-19:45 h inhibited the nocturnal melatonin secretion dose-dependently during the first half of the night. [60].
### Table 1: Drugs resulting in lower endogenous melatonin concentration

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hypothesis for mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta blockers</td>
<td>Occupation of beta-receptors on the membrane of the pinealocyte, resulting in less binding sites for NE (see fig 3) [51].</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>'Competition' for GABA [61]</td>
</tr>
<tr>
<td>Clonidine (alpha-2-blocker)</td>
<td>Binding to alpha-2-receptors in the pineal gland and the hypothalamus-pituitary axis results in an inhibitory alpha-2-adrenergic influence on both the pineal gland and the hypothalamus-pituitary axis [55].</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Inhibition by means of mechanisms within the pineal gland [62]</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Inhibition on NE-stimulated melatonin synthesis (see figure 3) [60]</td>
</tr>
<tr>
<td>Non Steroidal Anti Inflammatory Drugs (NSAIDs)</td>
<td>Structural relationship between some NSAIDs and melatonin, less impulse for endogenous melatonin production [own hypothesis]. Body temperature lowering properties of NSAIDs. Since there is reverse relationship between melatonin and body temperature, administration of NSAIDs may lead to less 'impulse' for melatonin production [own hypothesis]</td>
</tr>
</tbody>
</table>

### Table 2: Drugs resulting in higher endogenous melatonin concentration

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hypothesis for mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoralens</td>
<td>1. Sensitise the retina to light thereby increasing the amplitude of circadian rhythms [63]</td>
</tr>
<tr>
<td></td>
<td>2. Inhibition of the metabolism of melatonin [3]</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Inhibition of melatonin metabolism [3]</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>Inhibition of melatonin metabolism [57,58]</td>
</tr>
</tbody>
</table>

### 1.2 LIGHT AND DARK CONTROL OF THE MELATONIN SYNTHESIS

#### 1.2.1 Measurement of melatonin in body fluids

One of the markers of the circadian pacemaker is melatonin. Melatonin is even known as one of the most robust markers, since it is only slightly influenced by external, so called 'masking effects'. The major exception is light that is known to have acute suppressive
effects on melatonin production [3]. Activity during the night may also perturb the melatonin rhythm [50].

In chapter 3.1 a convenient method to measure endogenous melatonin in plasma is described. More recently radioimmunoassays for measurement of melatonin in saliva became available. Because of the greater practicability of frequent saliva sampling over blood sampling, radioimmunoassay (RIA) measurements of melatonin in saliva, were performed. This is described in chapter 2.3. This method appeared to be specific enough to be used as a diagnostic tool in case of suspected circadian rhythm disorders, like for instance in patients diagnosed as suffering from Delayed Sleep Phase Syndrome (DSPS).

1.2.2 Direct suppression of melatonin secretion by light

The mammalian pineal rhythms in serotonin concentration, NAT activity and melatonin content change dramatically following light action at night. Light might either block the stimulatory oscillatory information in the SCN or inhibit its neural transmission to the pineal. The former possibility appears more likely, as light exposure during the dark phase rapidly increases SCN glucose utilisation from low levels to high values comparable to those ordinarily observed during the light phase [64].

There are very substantial individual variations in sensitivity to the amount of light required to suppress melatonin secretion that may be both genetically and environmentally determined. Lewy et al [65] showed that if sufficient intensity of white light (2500 lux for two hours) during the night between 02:00 and 04:00 h was used human melatonin could be suppressed to basal (daytime) levels. Partial suppression, however, can be effected by lower intensity light e.g. 200-300 lux applied for 30 minutes [66].
1.2.3 Phase Response Curves

Light pulses are conventionally used to investigate the control of the circadian pacemaker on circadian rhythms resulting in a Phase Response Curve (PRC). Usually animals are maintained in constant darkness, thus displaying free-running rhythms in activity rest cycles. ‘Circadian time’ is used as a descriptor in these circumstances, where the period (tau) of the free running cycle is divided into 24 circadian hours each lasting tau/24 hours of real time. The phase reference point for the rhythm is the daily onset of (in night active rodents) nocturnal wheel running activity which is designated circadian CT12 and indicates the beginning of subjective night [67]. An example of a phase response curve illustrating the effect of 1 hour light pulses given at various times relative to a hamster’s rhythm of locomotor activity is illustrated by Figure 5.

\[ Figure 5: Phase Response Curve for light [68] \]

Like other circadian rhythms, the melatonin rhythm free-runs under conditions of constant darkness with a period slightly different from 24 hour. The shape of the melatonin curve (see Figure 4) is similar in blind and sighted people. Since the SCN directs the melatonin release, the light-dark cycle is not necessary to either turn on or turn off melatonin production and the melatonin rhythm appears to be regulated by the light-dark cycle in a
way that can be described by a PRC. Unique to melatonin production, light also has an acute suppressant effect: exposure to light during the night immediately and profoundly suppresses melatonin production [69]. Consequently, when using melatonin levels to mark circadian phase position, not only should bright light be avoided throughout the night (to avoid direct melatonin suppression) but also during dusk (to avoid that the entrainment of the pacemaker that initiates melatonin production in the evening is suppressed).

For the determination of melatonin PRCs, the phase reference points that have been used are either the onset of the evening rise in plasma melatonin (this is called the Dim Light Melatonin Onset (DLMO) and can be calculated from interpolation as the first interpolated point above 10 pg/ml that continued to rise), the calculated peak time or the morning and evening onset and offset of N-acetyltransferase. The onset is preferable for several reasons. Once night-time begins, beta-adrenergic receptors in the pineal become subsensitive. Furthermore, during the course of the night, melatonin precursors can become depleted. Both of these phenomena can account for decreasing melatonin levels for reasons other than those related to the timing of melatonin production. Of all of the portions of the melatonin curve, the onset is theoretically least affected by the development of beta-adrenergic subsensitivity and melatonin precursor depletion that might develop during the night [69].

1.2.4 Entrainment of the melatonin rhythm with light

Light-dark cycles are the major environmental factor involved in the entrainment of circadian rhythms in mammals. The importance of light-dark for the entrainment of melatonin was demonstrated in humans where, following an inversion of the light-dark cycle, the urinary melatonin rhythm adapts to the new photoperiod over a period of several days, finally assuming the same phase relationship with the new light-dark cycle as was present with the old light-dark cycle [70]. Recently, Zeitzer et al demonstrated that humans are highly responsive to the phase delaying effects of light during the early biological night [71]. In this study, both the phase-resetting response to light and the acute suppressive effects of light on plasma melatonin have been shown to follow a logistic dose-response curve. Striking was the observation that half of the maximal phase-delaying response achieved in response to a single episode of evening bright light (approximately 9000 lux) can be obtained with just over 1% of this light intensity [71].
1.2.5 Endogenous melatonin and core body temperature

The circadian pacemaker localised in the suprachiasmatic nucleus (SCN) generates the rhythm of the core body temperature and the rhythm of synthesis of melatonin by the pineal. Melatonin on its turn influences the SCN by a feedback mechanism. This relationship appears to be a mammalian modification of an evolutionarily older system. In lower vertebrates, including birds and reptiles, the pineal is a functional circadian oscillator. Thus, interdependence of these two systems in the mammal may have arisen from an older relationship, when both pineal and SCN exerted clock function [72]. Furthermore the regulation of melatonin and body temperature is complicated since melatonin has *acute hypothermic* effects. The mechanisms that mediate this action still are unclear. However, effects on thermoregulatory centres, heat loss, and probably heat production are likely to be involved [73]. In healthy humans, the nocturnal decline of core body temperature is inversely related to the rise of melatonin by a second order function [74].

1.2.6 Photoperiod and seasons

Melatonin secretion in relation to daylength

In normal entrained conditions melatonin is produced during the dark phase. In human and most other species its secretion is related to the length of the night: the longer the night the longer the duration of secretion [67]. From the studies of Carter and Goldman [75] and Karsch et al [76] it has become clear that the duration of melatonin release is the necessary and sufficient condition for the induction of a given seasonal response.

Seasonal variations in mood and melatonin

The changes in duration of nocturnal melatonin secretion, may trigger seasonal changes in mood and behaviour. An example may be the Seasonal Affective Disorder. Seasonal Affective Disorder (SAD) is characterised by recurrent bouts of depression in certain seasons. There is a winter and a summer variant. Recently the prevalence of Seasonal Affective Disorder (SAD) in the Netherlands was assessed by Mersch et al [77]. Three percent of the more than 2500 respondents met the criteria for winter SAD, 0.1% for
summers SAD. The criteria for subsyndromal SAD, a milder form of SAD were met by 8.5%, 0.3% of whom showed a summer pattern.

The winter SAD, also called winterdepression, will be discussed here. The symptoms of winter depression usually begin in November and end in March. Melatonin may play a role in winterdepression. The classic melatonin duration hypothesis of the pathogenesis of winterdepression is based on the fact that seasonal changes in photoperiod induce parallel changes in the duration of melatonin secretion, so that it is longer in winter and shorter in summer. The changes in duration of nocturnal melatonin secretion, in turn, may trigger seasonal changes in mood and behaviour. This hypothesis has been tested in a number of experiments, giving inconsistent results. Supporting the hypothesis are findings that exposure to light, that suppresses the melatonin production, improves winter depression and that exposure of the eyes to light, and not the skin is necessary for this improvement to occur. Also consistent with the hypothesis is the finding that morning treatment with the β-blocker propranolol, which suppresses the terminal portion of nocturnal melatonin secretion and thereby shortens its duration, is associated with improvement of winter depression [78]. Since Rosenthal et al [79] did not find such a antidepressant efficacy of the beta-blocker atenolol in winterdepression Schlager hypothesised that only short acting beta-blockers are useful. His explanation for this is that sufficient daytime must be allowed for drug clearance to avoid further and potentially variable suppression of melatonin onset each evening [78]. In contrast to the antidepressive effects of propranolol which are in favour with the melatonin hypothesis, observations that suppression of melatonin secretion by light is not necessary for improvement to occur during light treatment, however, contradict with the melatonin hypothesis [80, 81].

Another possible explanation for winter depression is the phase shift hypothesis [82]. According to this hypothesis patients become depressed in the winter at least in part because of a circadian delay. Lewy et al [82] have established that bright light scheduled in the morning (which provides a corrective phase advance) is the treatment of choice for this disorder. Other studies have resulted in different as well as comparable findings. Some groups found morning and evening light therapy improving depressive symptoms in patients with SAD independent of their circadian phase or sleep timing, which argues
against a circadian phase delay hypothesis of the pathophysiology of SAD or the necessity of a phase advance by morning light for clinical efficacy [83,84], while others found bright light in the morning was most effective in treatment of winter depression [85,86]. Therefore, it is still not fully clear whether the antidepressant effect of bright light is caused by phase advancing properties or by another mechanism of action [87]. Recently, Terman et al have investigated a possible mechanism of action for the antidepressant response to light-phase advances of the circadian clock measuring the onset of melatonin secretion before and after light treatment in the morning or evening [88]. They found that the antidepressant effect of light is potentated by early morning administration in circadian time, optimally about 8.5 hours after melatonin onset or 2.5 hours after the sleep midpoint. An alternative hypothesis for winter depression relates to the fact that carbohydrate craving, an early and common feature of winter depression, is linked to decreased serotonin levels. Since serotonin is a precursor of melatonin, increased usage of serotonin for the synthesis of melatonin may decrease the storage of serotonin and result in lower basal levels of this neurotransmitter during the winter period [89]. This might lead to a depressive mood.

1.2.7 Blindness and melatonin

Several blind people have abnormal circadian rhythms. In totally blind people, the most commonly observed disruptive circadian pattern is a free running rhythm with a stable non-24-h circadian period (24.2-24.5h) [90-93]. Some blind people, however, are normally entrained. For those individuals, time cues other than light will maintain synchronisation albeit with the weak coupling evident from a delayed phase. Another possibility is that some of those visually blind people have intact retinohypothalamic photic pathways and therefore they still have hypothalamic light perception for the entrainment of circadian rhythms [94,95]. Skene et al have shown that subjects with no conscious light perception have a higher occurrence and more sleep disorders than those with some degree of light perception. A detailed study of 49 blind individuals showed that those with no conscious light perception are likely to have free running circadian rhythms (6-sulfatoxymelatonin, cortisol) including the sleep/wake rhythm [96].
1.3 EFFECTS OF EXOGENOUS MELATONIN

1.3.1 Ratio for administration of exogenous melatonin

There are several Circadian Rhythm Sleep Disorders (CRSD) where rhythm abnormalities are associated with lack of well being and/or poor performance. These CRSD are: shift work, jet lag, delayed and advanced sleep phase syndrome, irregular sleep-wake pattern and non-24-hour sleep-wake disorder [97].

Suitably timed bright light is effective at hastening adaptation to phase shift [98]. However, the use of bright light in some circumstances may be undesirable; in the case of the blind with neither conscious nor hypothalamic light perception, it is clearly inappropriate. The obvious solution to circadian desynchrony problems of this sort is a chronobiotic, a drug that shifts all circadian rhythms in the desired direction and acts as a zeitgeber to maintain stable phase once the latter is obtained. Presumably exogenous melatonin can fulfil this role.

1.3.2 Kinetics of exogenous melatonin

Melatonin can be obtained from pineal glands from bovines and is found in small amounts in several plants [99]. Melatonin can also be synthesised starting with various agents, e.g. 5-hydroxytryptamides, 5-methoxytryptamines or 5-methoxyindoles [100]. Since no monograph was available in the most widely used pharmacopoeias, a product monograph was developed and is described in chapter 2.1 of this thesis. Depending on the route of synthesis impurities with organic agents, arsenic and heavy metals can be expected. Before use of melatonin for pharmaceutical properties limits of these impurities must be tested by the standards of the European Pharmacopoeia. Tablets, capsules, preparations with slow release, a mixture, a nasal spray and intravenous fluids are described in literature [101-107]. Analysis of the chemical substance melatonin is described in the Merck Index [108].

There are great interindividual differences in the pharmacokinetics of melatonin. The clearance of melatonin administered intravenously is biphasic [3] with a mean of 631 ml/min in healthy people [28] and a mean of only 127 ml/min in patients with liver cirrhosis [31]. The half-life times are short: 3 en 45 minutes respectively [3]. The bioavailability for oral formulations differs strongly in the different studies: from 3-6% [109,110,111] to higher values of 23-76% [28]. Following oral administration of 80 mg of
melatonin in gelatine capsules the absorption half-life has been reported as 0.4h, the elimination half-life has been reported as 0.8 h, and the melatonin levels range from 350-10,000 times those occurring physiologically [110]. Comparable results have been found by us by administration of 5 mg of melatonin 5 hours before DLMO in DSPS patients. An example of one of the DSPS patients without and with treatment of 5 mg melatonin is shown in figure 6.

Figure 6: Kinetics of melatonin
- The endogenous melatonin in plasma of a DSPS patient, before treatment with exogenous melatonin.
- The sum of endogenous and exogenous melatonin in plasma after administration of a capsule of 5 mg melatonin at 22:00 h in the same patient.

Zaidan et al [112] administered melatonin in a physiological dose intravenously for 3 hours. There was a remarkable similarity to the original melatonin profile, particularly given the differences in dosing regimens and the difficulties encountered when trying to discern the endogenous melatonin profile from exogenous melatonin levels. Another interesting result of this study was that at least one dose regimen affected the area under the curve (AUC) of the posttreatment endogenous melatonin profile.
When suitably timed most studies indicate that fast release preparations are able to hasten adaptation to phase shift [113]. Sustained release formulations, or multiple dosing regimens, may optimise phase shifting while minimising the total dose. These may be particularly useful when minimising direct soporific 'side effect' of melatonin that appears to be related to the maximum concentration [87].

In a similar vein Dijk et al suggest, that for several indications it seems reasonable to develop delivery systems that can maintain high melatonin levels throughout the sleep episode or even preferentially deliver melatonin in the second half of the sleep episode [114]. We prefer the approach of a study of Bénès et al [115]. These authors have studied which way of administration mimicked endogenous melatonin release most physiologically. In 12 healthy young male volunteers an oral controlled-release capsule, an oral transmucosal form and a transdermal patch were tested. The melatonin concentrations reached by the transdermal patch and the controlled release capsule differed much between the various subjects. The oral transmucosal form was able to mimic the physiological plasma profiles of both melatonin and its metabolite, 6-sulfatoxymelatonin in all subjects.

1.3.3 Effects of exogenous melatonin on core body temperature

*Figure 7: Coherence between melatonin, body temperature and sleep initiation*

Under the entrained conditions of normal daily life, major nocturnal sleep is typically initiated 5-6 hours before the temperature minimum and is terminated shortly after the
minimum. Campbell et al showed that the process of sleep initiation is most likely to occur when body temperature is declining at its maximum rate and it is most successfully accomplished at this phase of the temperature cycle [116]. In Figure 7 the coherence between endogenous melatonin increase, body temperature decrease and sleep initiation is illustrated. To clarify whether the melatonin rise and the core body temperature decline are not only temporally but also causally related, manipulations of nocturnal melatonin levels have been used. Both complete suppression of nocturnal melatonin levels by administration of the β-blocker atenolol [51] and increase of melatonin to pharmacological values by its exogenous administration at night do not immediately modify the phase of the core body temperature nadir [117].

In one study [103] administration of doses below 1 mg (0.3 or 0.1 mg) melatonin, which are claimed to reproduce physiological plasma levels of melatonin, failed to reduce core body temperature. On the basis of this finding, it could be suggested that only levels of melatonin in the pharmacological range, but not in the physiological range, exert an effect on core body temperature. However, reproduction of physiological levels of melatonin in blood may be useful to study the peripheral versus the central effects of the hormone. Indeed, pharmacokinetic studies including primates [118] have suggested that within the ventricular cerebrospinal fluid, levels of melatonin similar to those observed during the endogenous production of the hormone, may be obtained only by increasing its peripheral levels to the pharmacological range. Cerebrospinal fluid is believed to represent the preferential route for melatonin to reach the hypothalamus. Therefore administration of low melatonin doses that maintain physiological levels of the hormone in peripheral plasma for a limited period of time, actually might be insufficient and inadequate to induce its possible central action on thermoregulation.

It is reasonable to assume that following the administration of melatonin, a certain time is required for the body to reduce its heat content. Indeed the maximal effect on core body temperature reduction becomes fully manifested at approximately 4 h after oral administration of pharmacological doses (2.5 mg) of the hormone. At this time, the values of core body temperature are about 0.3 degrees Celsius lower with melatonin than those following the administration of placebo, and this difference is maintained for the entire period throughout which plasma melatonin levels remain elevated [74].
Neurotransmitters can modify core body temperature regulation. Among these, serotonin is believed to decrease and prostaglandines to increase core body temperature [3]. Experimental evidence obtained in animals indicates that in the brain, administration of melatonin increases serotonin levels and serotonergic neurotransmission and is a potent inhibitor of prostaglandin synthesis [118,119]. This will result in lowering of the body temperature.

1.3.4 Effects of exogenous melatonin on circadian rhythms

Phase shift of the endogenous melatonin rhythm by exogenous melatonin

Lewy et al [120] found a relationship between the time of melatonin administration relative to the pre-treatment rise of endogenous melatonin and the resulting phase advance of the melatonin rhythm. This PRC is nearly the opposite in phase with the PRCs for light exposure: melatonin delays circadian rhythms when administered in the morning and advances them when administered in the afternoon or early evening. Figure 8 illustrates the relationship between the timing of exogenous melatonin administration and the measured phase shifts of the endogenous melatonin rhythm.
Figure 8: Phase shifts of the Dim Light Melatonin Onset (DLMO) as a function of circadian time for 9 subjects (a total of 30 trials), providing the first evidence for a human melatonin phase response curve. CT of administration was calculated using the time of the first capsule (free interpretation after Lewy et al [120]). Results of administration of exogenous melatonin at various times with respect to the time of endogenous melatonin production (CT 14 = baseline DLMO for each trial) [120].

Deacon and Arendt [121] described a log-linear relationship between the dose of melatonin and the magnitude of phase shifts in the DLMO for doses of 0.05 mg, 0.5 mg and 5 mg. Melatonin treatment also induced acute, dose-dependent temperature suppression and decrements in alertness and performance efficiency. Earlier sleep onset, offset and better sleep quality were associated with increasing doses of melatonin. The day after melatonin administration in the afternoon, a significant dose-dependent phase advance in the plasma melatonin onset time and temperature nadir was observed with a trend for the alertness rhythm to phase advance.

Czeisler [122] is concerned that some of the reported 'phase shifts' in the melatonin profile may reflect a change in the shape of the endogenous melatonin profile due to endocrine feedback effects from the melatonin administration [87]. This consideration is in agreement with our suggestions based on the results of administration of melatonin 5 hours before the
individual increase of endogenous melatonin in DSPS patients as described in chapter 3.1 of this thesis. The onset of the endogenous melatonin curve could be advanced by about 1.5 hour, while no significant phase advance was observed for offset of the melatonin curve [123]. This advancement of the rising slope while the falling slope did not advance was also reported by Deacon et al [124]. Similar changes of the shape of the curve have been found before and have led to the hypothesis of the two-oscillator model with an oscillator for the onset ('evening' oscillator) and for the offset of melatonin ('morning' oscillator) [125,126]. Cagnacci and colleagues [127] recently published additional experimental evidence that supports the hypothesis that evening onset and morning offset of the human melatonin secretion are regulated by separate circadian processes. They also provide evidence that suggests that these processes exhibit opposite phase responses to the administration of melatonin. They found that morning treatment with melatonin counteracted the phase-advancing effect of morning light on the offset of secretion but potentiated its phase advancing effect on onset of secretion. Thus, when morning light treatments and morning melatonin treatments were combined, the intrinsic duration of melatonin secretion increased.

In Czeisler's view the fact that cortisol does not phase shift together with the endogenous melatonin shifts after melatonin administration, which is in contrast to the shift of both cortisol and melatonin after bright light [47,128,129] seriously undermines the conclusion that the alterations in the endogenous melatonin profiles reported after exogenous melatonin accurately represents shifts of the endogenous circadian pacemaker in humans [122]. However, Deacon and Arendt have shown that body temperature does shift after melatonin administration. An explanation for this finding may be a change of the course of the body temperature induced by a direct effect of exogenous melatonin or may be due to a shift of the circadian pacemaker [121].

Effects on Delayed Sleep Phase Syndrome (DSPS)

The criteria used for the definition and diagnosis of Delayed Sleep Phase Syndrome (DSPS) are given by the International Classification of Sleep Disorders (ICSD) [130]. DSPS is defined as a disorder in which the major sleep episode is delayed in relation to the desired clock time, and therefore results in symptoms of sleep-onset insomnia or difficulty in awakening at the desired time. Individuals suffering from DSPS, despite having
completely normal sleep architecture and sleep duration, experience great difficulty falling asleep before 12 am, if not later, as well as rise at acceptable hours of the morning [131,132]. DSPS is probably the most common of the intrinsic circadian sleep disorders, or at least the most commonly diagnosed. Based on an early survey, it was estimated that approximately 7% of people diagnosed with disorders of initiating and maintaining sleep meet criteria for DSPS [132]. The ICSD gives several markers for diagnosing DSPS. Dagan and Eisenstein [133], have tried to strengthen this definition of DSPS based on data gathered from their own patients. They found that relatively many patients reported early childhood as the age of onset. Almost one fifth of the patients was previously diagnosed as having learning disorders and more than one fifth had personality disorders. Almost 50% of the patients was highly sensitive to light (as opposed to about 20 % of the controls). This suggests light supersensitivity could in some way be involved in th pathophysiology of DSPS. More than the half of the patients had a habit of night eating, especially foods rich of carbohydrates. This is expected to be related to disturbances in other circadian rhythms beside the deviation of the sleep-wake cycle resulting in a shift in the times of feeling hungry. Familial trait existed in almost the half of the population. We have found comparable features in our patients as described in the studies reported in chapters 3.1. 4.1, 4.2, and 4.3.

Two methods to treat DSPS are known in literature: chronotherapy and administration of melatonin. Chronotherapy is a drug-free rescheduling treatment, designed to resynchronise sleep with the patient's biological clock. Since patients with DSPS have inadequate capacity to achieve phase advance shifts of the circadian pacemaker, a phase delay route must be chosen [134]. The original method, as published by Czeisler et al [134], consists of daily 3-hour delays of bedtime and arising time until the patients' sleep schedule is realigned with the desired social schedule. Chronotherapy however, has a great percentage of failure [134].

The first study on the effects of melatonin on DSPS was published by Dahlitz and colleagues [132]. The actions of melatonin on the sleep-wake cycle were investigated by means of a randomised double-blind placebo-controlled trial in 8 subjects with DSPS. Dahlitz et al concluded that melatonin may act as a phase-setter for sleep-wake cycles in subjects with a DSPS, with no influence on the alertness.
A study of Dagan et al [136] describes routine treatment of the administration of melatonin 5mg administered at 22:00h for 6 weeks to 61 subjects diagnosed with DSPS. The efficiency of the melatonin treatment and its possible side effects were investigated by means of a survey questionnaire. Over 95% of the subjects reported melatonin to reduce the complaints with almost no side effects. However, more than 90% reported a relapse to their pre-treatment sleeping patterns within 1 year of the end of treatment. In more than a quarter of them the relapse occurred within 1 week.

In chapter 3.1, the effects of melatonin on DSPS in a placebo-controlled setting are described. The time of administration of the medication was individualised on basis of plasma curves of endogenous melatonin. Lewy and Sack [120], had shown that advancement of the endogenous melatonin curve by exogenous melatonin was largest at CT 9, as illustrated by figure 8. Since CT 14 is the DLMO we administered melatonin 5 hours before the individual DLMO. The number and seriousness of the complaints were decreased and an advance of the rising slope of the melatonin curve was found [123]. All published studies on the effect of melatonin on DSPS were reviewed in 1999 by Campbell et al [137].

Effects on shift work

The ICSD also includes Shift work Maladaptation Syndrome (SMS) as a subtype of Circadian Rhythm Sleep Disorders [130]. The ICSD definition is: symptoms of insomnia or excessive sleepiness that occur as transient phenomena in relation to work schedules. Complaints during the period of night work, such as the poor quality of day sleep, may be reduced by increasing the rate of adaptation of the circadian rhythm to the shifted sleep period. Bright light administration during the early part of the night appears to be effective in facilitating the delay of the temperature rhythm and thus can help to re-establish the association between the temperature trough and the sleep period. In a similar way, melatonin administration in the morning may facilitate a phase delay [138]. Only a few field studies concerning night work have been published [95,139,140]. Several night workers phase shift themselves, without treatment. Because of the variability in phase shifting it may be necessary to focus on subjects who do not shift, or only partially shift to observe a response to melatonin administration. In a placebo controlled laboratory study where shift work situation was simulated Dawson et al [138], compared adaptation to
night shift in three groups of subjects. The first treatment group received timed exposure to bright light, the second treatment group received 2 mg of exogenous melatonin and the placebo group received either dim red light at less than 50 lux or a placebo capsule. Using the DLMO as a circadian marker, the bright-light group had the largest shift (an average delay of 8.8 h), whereas there was no significant difference in phase shift between the placebo and the melatonin groups (a delay of 4.2 h and 4.7 h respectively). The failure of melatonin treatment to induce greater phase shifts than placebo might be related to the divided dose regimen (4 mg in three divided doses across the day sleep period) that fell on both the advance and delay portion of the melatonin response curve [120].

Sack et al [141] found that the timing of melatonin production was distinctly different in a group of nine permanent night-workers compared to a group of day-active controls. This indicates a major adaptation of the circadian pacemaker to the atypical schedule for activity, sleep and light exposure. However, there is a suggestion that adaptation remains incomplete (and perhaps unstable) because the timing of sleep appears to be at an earlier circadian phase than is typical for day active subjects. Until now, no studies have been done that conform the existence of a (relative) desynchronisation by longitudinal measurements of melatonin phase together with precise measurements of sleep.

Five years later than the study of Sack et al as mentioned above [141], Sack and Lewy [95] performed a randomised placebo-controlled double blind cross-over study in 24 subjects, with objective sleep data and with a rotating schedule. The subjects had taken melatonin 0.5 mg or placebo during two weeks. The authors found an impressive variability in the magnitude and direction of phase shifting. Also other authors found variable responses in sleep shifting effects under consistent work schedules [142,143].

It can be expected that an acceleration of the adaptation to night work will cause a worsening of the problems of re-adaptation following night work, and thus a worsening of the chronic sleep disturbance and waking fatigue. These symptoms may occur most severely at the transitions from the day shift to the night shift, and vice versa.

Because the SMS symptoms mainly present themselves as after-effects, i.e. in the period following shift work, our study, described in chapter 3.2 focused upon the days directly after a period of night work. The goal of this study was to assess if melatonin administered in the early evening during the days after a period of night work may act as a countermeasure, by facilitating the recovery from the effects of night work. Effects on
sleep related parameters were measured during the two periods of administration of study medication, while body temperature and daily performance were measured directly after the two periods of administration of study medication.

Jet lag

According to the definition of the ICSD, jet lag syndrome consists of varying degrees of difficulties in initiating or maintaining sleep, excessive sleepiness, decrements in subjective daytime alertness and performance, and somatic symptoms (largely related to gastrointestinal function) following rapid travel across multiple time zones [130]. Melatonin is thought to accelerate re-entrainment and therefore reduce jet lag. Several studies confirmed this [reviewed in 104][144]. The first published placebo-controlled study on the effects of melatonin on jet lag with improvement of subjective and objective parameters is a study of Arendt et al [145]. In this study melatonin 5 mg or placebo were taken at 18:00h, three days before the flight over 8 timezones from London to San Francisco, and the administration was continued after arrival during 4 evenings at 23:00h local time.

Other studies with positive results of melatonin administration for treatment of jet lag are described by Petrie et al [146] and Claustrat et al [147]. Petrie and co-authors performed a study with a comparable treatment scheme and found that melatonin could alleviate jet lag and tiredness after long haul flights [146]. Claustrat et al described a simplified treatment protocol, where no melatonin had to be taken before the departure. From this study, where subjects took melatonin or placebo on the flight from North America to France, melatonin showed significant efficacy on global treatment efficacy, morning fatigue and evening sleepiness [147].

Improvement of jet lag was also found in a placebo-controlled study with fifty-two employees of an airline company, flying from Auckland to Los Angeles and then to London. However in this study the subjects complained about sleepiness during the use of melatonin before the departure [148].

In a study of Spitzer et al, however, no effect of melatonin was found [149]. In this large study 249 people were included, divided over 4 treatment groups with different dosages and different times of intake. People travelled from Oslo to New York, and returned after 4 days to Oslo. At the return they were treated. No differences were found between the
various groups. The comments upon this study design are that the baseline of the biological clocks of the participants at the start was not known and 4 days for synchronising is relatively short [150].

Despite these studies, the mechanism of action of melatonin is not clear. It is still a matter of debate if it works by acceleration of the adaptation of the circadian clock or indirectly by its soporific properties [151].

Effects on circadian rhythms in blind people

Melatonin has been administered to blind people in an attempt to strengthen the entrainment of circadian rhythms. There have been a few reports of satisfactory entrainment in blind people by melatonin [152], but these were not proven conclusively [153-155]. Although there is clear evidence of phase shifting, entrainment of totally blind with free running rhythms is not easily achieved. Particularly in subjects whose free-running periods are quite long (e.g. >24.5h) melatonin may not be sufficiently potent to achieve the necessary phase shifts. Thereby some people will not entrain since they lack a phase shifting response to melatonin for unknown reasons [95]. Sack et al [156] have recently hypothesised that melatonin may promote sleep by counteracting the daytime alerting process generated by the circadian system. This model postulates that both the phase-shifting and sleep-promoting effects of melatonin are mediated by receptors in the suprachiasmatic nuclei [157]. Normally, the circadian alerting signal opposes the expression of sleep drive that accumulates during the day. This build-up in sleep drive is proportional to the duration of prior wakefulness. At night (in normally entrained individuals) the circadian alerting signal wanes and the accumulated sleep drive is expressed until it is dissipated and the circadian pacemaker begins to generate an alerting signal the following morning [158]. However, in blind free runners, when rhythms are desynchronised, during certain periods the alerting process occurs during the night and sleep is disrupted. In this sense melatonin may not produce sleepiness; rather it permits or releases sleep propensity that otherwise would be opposed by the circadian system. From systematic melatonin trials in blind free runners it may be possible to estimate the relative impact on sleep of phase-shifting versus a direct hypnotic action of melatonin. If melatonin works mainly by circadian mechanisms, then it may be important for blind patients to take
it at the same time of the day, every day, so that it can function as a consistent circadian time cue (zeitgeber). On the other hand, if melatonin works mainly by counteracting the circadian alerting signal, then it need only be taken on the days that the patients are symptomatic. In this case the timing of administration is of less importance. Obviously, both mechanisms could underlie its therapeutic effects [95].

1.3.5 Hypnotic effects of exogenous melatonin

Zhdanov and Wurtman have reviewed the numerous published studies on the acute effects of melatonin on human sleepiness and sleep [30]. Except a few negative or inconclusive results, the majority of these studies have shown that a substantial increase of circulating melatonin levels was associated with sedation, fatigue, decreased alertness, significantly increased reaction time, shortening of latency to sleep, increased sleep efficiency and total sleep time, or increased sleep propensity [30]. An hypnotic effect by exogenous melatonin in humans was established with oral doses of 1-6 mg [159] to 100 mg [160] or intravenously administered doses of 50 mg [161]. When melatonin doses under 1 mg were tested, the dose dependency was revealed [103]. All the doses tested augmented subjective sleepiness or shortened latency to sleep onset. Zhdanova compared the effects of 0.3 mg and 1 mg melatonin and confirmed that increasing circulating melatonin levels to within the physiological range promotes polysomnographically detected sleep onset of afternoon naps [162] and of overnight sleep [43] in young healthy volunteers. This effect of melatonin treatment occurs independently of the time of administration [162,163]. Since melatonin induced shifts in circadian rhythmicity are limited to 20-60 min per day after administration of a single dose of the hormone at a favourable time point [112,120] the observation of time independence is a strong argument against interpreting the acute sleep-promoting effect of melatonin as a part of its phase shifting activity. On the other hand Mendelson, believes [164] that there is not yet convincing body of evidence that melatonin improves sleep in insomniacs with noncircadian sleep disturbances. So, in his view the sleep promoting effects are strongly connected to the circadian effects.

Lavie [165] showed that all studies that have investigated daytime administrations of melatonin reported increased sleepiness even at doses that do not increase plasma levels of melatonin beyond its physiological level. By contrast, night-time increase in sleepiness was
achieved only after administration of high doses. Based on these findings and on the precise coupling between the endogenous nocturnal increase in melatonin secretion and the opening of ‘the sleep gate’, an abrupt transition from a period of low sleep propensity to a period of high sleep propensity that persists during the night period, Lavie et al suggested that melatonin participates in the regulation of the sleep-wake cycle by inhibiting the central nervous system wakefulness generating system [165]. Clinical findings on decreased levels of nocturnal melatonin in chronic insomniacs and on the efficacy of exogenous melatonin in improving sleep in melatonin deficient insomniacs, are congruent with this hypothesis [165].

The consensus is that the circadian drive for sleep is lowest as the circadian temperature reaches its crest. Constant routine studies carried out immediately on release from entrainment have demonstrated that in young subjects the body temperature crest is located in the evening between 17-19h [166]. After this nadir in sleep propensity there is a sudden and rapid increase in the ability to fall asleep [158,167]. This has been referred to as the opening of the sleep gate or the dissipation of the circadian drive for wakefulness. In some protocols, an increase in the ability to fall asleep has also been observed approximately 10 to 14 h after the temperature minimum [168]. However, the magnitude of the mid-afternoon increase in the ability to fall asleep is much smaller than the nocturnal increase in sleep propensity.

Melatonin exerts some effects on the main characteristics of human sleep, that is a shorter latency to sleep onset, better sleep consolidation and tendencies of decrease in the duration of stage 4 sleep and increase in the duration of stage 2 sleep. Some studies suggest that higher doses of melatonin can increase REM sleep [169], especially during nocturnal sleep, although other studies do not reveal significant changes in REM sleep [161]. Changes in REM sleep often are interpreted as reflecting changes in the circadian regulation of this sleep state. It should be pointed out, however, that minor shifts of the circadian pacemaker (1-3h) are not associated with changes in REM sleep. The effects of melatonin on REM sleep indicate either that there is a very large shift of the circadian pacemaker or that the effects of melatonin on REM sleep are mediated by other mechanisms such as the lowering of core body temperature.

Dijk et al [170] and Nave et al [171] both pointed out that the effects of melatonin are, to some extent similar to the changes induced by benzodiazepine hypnotics. This may lead to
the suggestion that melatonin's hypnotic effects are exerted through the same mechanism. Reports that melatonin modifies GABA-ergic neural transmission also support this assertion [172]. Interestingly the effects of melatonin on EEG spectra could not be blocked by flumazenil, which may indicate that the effects are not mediated by GABAa benzodiazepine receptor complex [173] or that a unique subtype of the GABAa -benzodiazepine receptor complex is involved in mediating melatonin effects [165].

Based on these data we conclude that melatonin has sleep-inducing properties indeed. However, since there is a lack of long-term safety data and there is only little information on the use of melatonin in concomitant medication, we agree with the consensus statement about the circumstances in which melatonin can be used as sleep therapy, which was recently published [174]. The consensus justifies the administration of melatonin for the combination of sleep-inducing and phase shifting effects, that make it potentially useful to shift the timing of sleep. The group also state that there appears to be no point in addressing sleep disorders of unknown origin with melatonin treatment [174].

1.4 SIDE EFFECTS OF MELATONIN

1.4.1 Toxicity

For a drug which is used so widespread there is a great lack in knowledge of toxicity data [175].

Assessment of melatonin and melatonin analogues using the Ames test indicates that melatonin, and 2-iodomelatonin are devoid of mutagenic activity [176,177]. In rats and mice, oral doses of melatonin in excess of 1000 mg/kg are needed to induce death; the estimated doses required to cause death in 50% of the animals treated (LD50 values) are 1250 and 3200 mg/kg in mice and rats respectively [178]. 2-Iodomelatonin, which is at least 10-fold more potent than melatonin in affecting biological responses, caused death in a minority of animals, even at the highest doses tested (800 mg/kg orally, 600 mg/kg by intraperitoneal injection) [177]. These doses are so far above the doses recommended for human consumption as to be nearly irrelevant (maximal intake in humans is approximately 5 mg/kg in women taking 300 mg/day).

Several sources cite that 6 g melatonin has been taken with no or minimal toxicity. However the basis for this citation involves an observational study of 11 subjects, in which
one subject took a maximum daily dose of 6.6 g for 35 days and another took 5.4 g for 34 days. These 2 subjects reported somnolence during the day, as did 4 other subjects taking lower doses. All patients were started at 50 mg three times a day and progressively increased to reach an individualised maximum dose. Nine of the subjects took 3 g to 4 g daily for 15 to 31 days, with a few isolated but definite episodes of cutaneous flushing, abdominal cramps, diarrhoea, scotoma lucidum and headaches typical of migraine [179].

A recent study on the toxicology of 10 mg melatonin during 28 days was done in 40 volunteers. Many laboratory parameters characteristic for several organ functions were screened. Except a statistical reduction of stage 1 sleep no differences between placebo and melatonin were found. [180].

1.4.2 Suspected drug reactions of melatonin in general

Although melatonin is a physiological substance, the patterning, timing and levels of melatonin by 'therapeutic' ingestion of the hormone often bear little resemblance to the characteristics of the endogenous melatonin rhythm [27]. Therefore serious research for suspected adverse drug reactions is necessary. In chapter 2.2 of this thesis all Suspected Adverse Drug Reactions (SADRs) of the first 97 treated patients are described.

In subjects taking melatonin, no deaths or serious accidents have been reported until now [181]. However, secondary effects have been reported such as gastrointestinal disorders, hypotension, headaches, fever, hyperkinesia, dizziness, haemorrhages, pigmentation, ankle oedema, flushing, diplopia, hepatic pain, thrombosis, hyperglycaemia and nightmares (in a patient with diabetes type 1 on insulin treatment). These secondary effects could be linked to pharmacological activity or pharmacodynamics and metabolism of melatonin [27,181,182,183].

Two serious cases of toxicological effects after ingestion of relatively high doses of melatonin are reported. Force reported a case of an elderly woman who developed an acute psychotic episode after reportedly ingesting a large dose of melatonin (30 mg) in combination with her daily medication of 10 mg fluoxetine [184]. Holliman and Chyka [185] reported a case of a 66 year old man who became lethargic and disoriented after taking 24 mg melatonin to aid relaxation and sleep the evening before prostate surgery. The melatonin was taken in combination with several prescription sedative drugs: diazepoxide and amitriptyline [185].
In a study with daily intake of an anticonceptive pill with 75 mg melatonin and 0.5 mg norethindrone three other suspected drug reactions were reported more than once: abnormal bleeding, breast complaints and neurosensory problems, however, in our view these side effects might as well be due to the norethindrone [186].

1.4.3 Hypnotic suspected drug reactions of melatonin

Zhdanova et al report disruption of the sleep pattern after repeated melatonin administration (3 mg), combined with increased motor activity that is significantly higher than after a physiological dose of 0.3 mg or with placebo. Thereby the daytime alertness was perceived as less than usual [182]. In some of their subjects, repeated administration of pharmacological doses of melatonin (7 days) have been associated with reports of daytime fatigue [182]. Fragmented sleep patterns caused by exogenous melatonin were reported too by Middleton et al. [187] They stress the importance of giving melatonin at correct times and warn against indiscriminate use of melatonin to avoid these undesirable effects.

1.4.4 Hormonal suspected drug reactions of melatonin

Women taking melatonin as a contraceptive agent, based on a substantially increase of the prolactin secretion during the hours following intake of large amounts of melatonin (80 mg up to 300 mg), indicated that no toxic effects were noted in the 4-month treatment period [188]. Alterations in hormone concentrations noted in this study are viewed as evidence of melatonin's efficacy rather than as an indication of toxicity. However, when high doses of melatonin are used for other indications hyperprolactinemia may be a potential problem because it is associated with infertility in both men and women [189,190,191]. This may result in delayed timing of puberty. When taken during pregnancy and lactation melatonin intake may effect the circadian status of the foetus and neonate and the future development of the child's circadian system [175].

Considering the evidence for interaction of melatonin with oestrogen receptor systems, chronic melatonin treatment might interfere with oestrogen action in bone, resulting in promoting osteoporosis [27]. Conversely could the interference with steroid hormone systems by melatonin reduce the rate of occurrence of hormone-dependent cancers of the
breast and the prostate [192]. The effect of melatonin on tumours is variable for the
different cancers: it has been shown in animals that tumour growth was accelerated by
pinealectomy and that restoration of normal melatonin levels in pinealectomized animals
was able to inhibit this tumour growth. These effects of melatonin probably implicate its
immunoregulatory role. Activation of melatonin receptors enhances the release of T-helper
cell cytokines such as interferon and interleukin-2 as well as opioid cytokines. These
mediators may counteract secondary immunodeficiencies, synergize with interleukin-2 in
cancer patients, and affect hematopoiesis [181].

1.4.5 Retinal suspected drug reactions of melatonin

Bright light induces retinal damage in rats, and this is enhanced by large doses of
melatonin [193]. Retinal function is, in general, an important problem in the use of
melatonin, given the presence of a clock in the mammalian eye together with
physiologically relevant melatonin receptors [194,195]. To date, there has been one report
of retinal damage in a woman who had taken sertraline, an antidepressant drug that blocks
the reuptake of serotonin at the neural synapse, for 4 years in combination with a high
protein diet with melatonin supplementation during 2 weeks. Visual acuity and colour
vision improved within 2 months after melatonin and the high-protein diet were
discontinued. The authors' hypothesis is a melatonin/dopamine imbalance in the retina,
manifesting as a topic optic neuropathy [196].

It is supposed that other comparable interactions may exist and that inappropriate
melatonin administration or high doses of melatonin could induce form deprivation
myopia, intraocular pressure glaucoma, or an increase of the phagocytosis of retinal
pigment epithelium inducing age related maculopathy [197,198].

1.4.6 Suspected drug reactions of precursors and metabolites of melatonin

The pharmacological and the pharmacodynamic effects of melatonin and/or its
metabolites need to be taken into account for assessment of the safety of melatonin,
particularly when huge doses of melatonin have been absorbed.

N-acetyl-5-methoxy-kynurenamine (AMK), one of the main metabolites of melatonin is a
potent inhibitor of prostaglandin synthesis and is able to inhibit diazepam binding from
brain synaptosomes. AMK and N-acetyl-2-formyl-5-methoxy-kynurenamines (AFMK),
another metabolite, have been shown to inhibit sexual development in a protein-restricted prepubertal rat model [199].

Since L-tryptophan is one of the precursors of melatonin, it is of interest to consider its metabolization. Among several metabolites, L-kynurenine has been reported to have a convulsing effect and quinolinic acid to be a neurotoxin with neuroexcitatory activity at the level of the N-methyl-D-aspartate receptor [200,201]. Since there is a great analogy between serotonin (a precursor of melatonin) and melatonin, these two agents have several pharmacological targets in common. Important are the vasoconstrictory effects at physiological concentrations of melatonin (nanomolar) and vasodilatory effects in higher concentrations (micromolar or millimolar) suggest biphasic pharmacology of melatonin [202,203]. Other tools which have these agents in common are: gastrointestinal effects; melatonin shows a worsening of gastric ulceration induced by nonsteroid anti-inflammatory drugs [181] and effects on the glucose metabolism, there are indications for antihyperglycemic effects, at least in rats [204].

1.5 FURTHER READING IN THIS THESIS

This overview of the physiological and pharmacological effects of melatonin serves as an introduction for further reading. The studies described in this thesis are divided into three parts: in part 2 the pharmaceutical aspects, in part 3 the chronobiological aspects and in part 4 the clinical aspects of melatonin are presented.

In part 2 a product monograph for melatonin, an article about the Suspected Adverse Drug Reactions (SADRs) found in our studies and a comparison of the methods and outcomes of radioimmunoassays for measuring melatonin in saliva, and the radioimmunoassays for measuring melatonin in plasma are described.

Part 3 about the chronobiological aspects of melatonin is based on two placebo-controlled studies on the effect of melatonin on two different circadian rhythm sleep disorders: Delayed Sleep Phase Syndrome (DSPS) and Shift Maladaptation Syndrome (SMS).

Part 4 about the clinical aspects of melatonin starts with a publication about the quality of life of patients with DSPS before and after treatment with melatonin, compared to a random Dutch sample and groups of patients with other chronic diseases. Chapter 4.2 deals with the relation between headache and DSPS and the effect of treatment with
Chapter 1.6

melatonin. At least an interesting case of a patient who had developed a prominent DSPS following traumatic brain injury is described.

1.6 FUTURE STUDY ON ENDOGENOUS AND EXOGENOUS MELATONIN

Several circadian rhythm disorders frequently start during early childhood (e.g. DSPS) or at old age. Therefore, further study on the physiological regulation of endogenous melatonin in relation to age may lead to better understanding of the pathology of several circadian rhythm disorders. When the physiology and the function of endogenous melatonin has become more clear, this may lead to a better understanding of the optimal schemes of treatment with exogenous melatonin.

Although a lot of information has become available about the effects of several drugs on endogenous melatonin, more research must be carried out especially on this field. The outcome of this kind of studies may be used to get more insight in the mechanism of action of endogenous and exogenous melatonin.

It is known that there is a relationship between lowering of body-temperature, increase of melatonin level and initiation of sleep, however, which parameters is the primary cause is still unclear.

There are still a lot of unanswered questions about the effects of exogenous melatonin on circadian rhythms. The optimal dose and time for phase shifting the endogenous curve must be worked out further for healthy subjects, but also for patients suffering from different circadian rhythm disorders. The hypnotic effects of exogenous melatonin need to be studied as well, e.g. polysomnographic studies in patients with different sleep problems, comparison of melatonin to standard hypnotics, efficacy of melatonin when taken chronically and the effect of stopping melatonin medication [205]. Finally the still existing question must be solved if effects on the circadian pacemaker or its circadian rhythms automatically enhances sleep quality [205].

There are no published long-term safety data on the daily use of melatonin for longer than 6 months, except two specified cases [168]. Side effects, which can be expected, can also be averted to functions of tissues that contain detectable levels of melatonin receptors. Recently however, it has become apparent that a low density of melatonin receptors may be present in many tissues [206] in addition to site specific patterns of melatonin receptor
expresses in brain. Thus many tissues may contain low levels of melatonin that could be important from a physiological viewpoint.

Literature


88. Terman JS, Terman M, Lo ES, Cooper TB. Circadian time of morning light administration and therapeutic response in winter depression. Arch Gen Psychiatry 2001; 58(1):69-75.


