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Melatonin in the Context of Circadian Oscillator Genes and Signaling Polymorphisms

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Abstract

Melatonin is a highly pleiotropic regulator molecule that acts almost everywhere in a mammalian body. Because of this diversity of actions, decreases in its level by aging or diseases have numerous health implications. Melatonin has synchronizing properties in the circadian system, at the hypothalamic master clock and, presumably, other oscillators, too. Different forms of circadian dysfunction exist that can be caused by mutations in circadian oscillator genes, epigenetic changes in their expression or neurodegenerative processes. Circadian malfunction has been observed in circadian rhythms sleep disorders as well as in mood disorders with circadian etiology, such as bipolar disorder, seasonal affective disorder and sub forms of major depression. In these cases, re-entrainment of rhythms by melatonin is an option of treatment. Melatonin also influences the expression of sirtuin 1. In aging tissues, it upregulates sirtuin 1, which is known to enhance circadian amplitudes, an effect that should improve the functioning of the circadian system. However, in profoundly dysregulated oscillators of tumor cells, melatonin suppresses sirtuin 1 expression and inhibits proliferation. Polymorphisms have been detected in genes of melatonin synthesis and melatonin receptors. These may lead to forms of melatonergic dysfunction. An allele of the melatonin receptor 2 has been shown to act in a prodiabetic way by becoming overexpressed in midlife, with the consequence of suppressing insulin secretion in pancreatic β cells.

Keywords: Circadian; Core oscillator; Melatonin; Melatonin receptors; Sirtuin

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Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a highly pleiotropic regulator molecule produced in numerous organs [1]. Although its quantities present in extrapineal organs exceed those formed in the pineal gland by orders of magnitude, its synthesis in the pineal gland is privileged in chronobiological terms, since the fraction of pineal origin participates in the regulation of the circadian system, whereas most of the extrapineal melatonin is retained at the sites of production. From the pineal gland, melatonin is released to the circulation and also, via the pineal recess, into the third ventricle of the brain [2]. The chronobiological importance of melatonin entering the CSF of the third ventricle is increasingly perceived, because this fraction gets an immediate access to the hypothalamic circadian master clock, the suprachiasmatic nucleus (SCN), which is located adjacent to this ventricle [3,4].

In mammals, the relationship between the SCN and the pineal gland is a mutual one, because melatonin acts as both an input and an output factor of the SCN [5]. The pineal gland is under the control of the SCN, which transmits the light/dark information via a neuronal pathway and, thereby, largely determines the high-amplitude rhythmicity of pineal melatonin synthesis and secretion, along with some minor contributions by other input pathways [6]. On the other hand, melatonin feeds back to the SCN, thereby readjusting the circadian phase in terms of its capacity as a chronobiotic, but, additionally, it also influences numerous peripheral circadian oscillators, which depend on the SCN to a variable degree and can be semi-autonomous or almost autonomous [7,8].

The multiplicity of melatonin's actions has numerous implications for human health. These include aspects of circadian function, also with regard to circadian gene polymorphisms, variant alleles

of genes involved in melatonin synthesis and signaling and also changes observed in the course of aging, which includes both deteriorations of the circadian system and reductions of melatonin secretion [7-11].

Deviations in Circadian Oscillators and Options of Melatonergic Treatment

Circadian rhythms can deviate from normal because of genetic, epigenetic or neurodegenerative reasons. Numerous cases have been reported in which mutations in human core oscillator genes, including non-translated regulatory regions, are associated with various diseases and disorders. This includes mutations of Per1 in attention deficit hyperactivity disorder (ADHD) [12], Per2 in winter depression [13,14] and obesity [15], Per3 in bipolar disorder [16,17] and schizophrenia [18,19], Cry1 in major depression [20], Cry2 in bipolar disorder [21] and winter depression [22], Bmal1 (=Arntl) in type 2 diabetes and hypertension [23], bipolar disorder [17,24] and winter depression [13], Bmal2 (=Arntl2) in bipolar disorder [24], Clock in ADHD [25] and bipolar disorder [24] and Npas2 in major depression [20], winter depression [13] and autism [26]. In other words, these examples concerning core oscillator genes underline the important role of the circadian system in the maintenance of a healthy state. Additional literature, including findings on accessory oscillator genes, has been summarized elsewhere [7]. Moreover, a large body of evidence exists for corresponding findings in animal models and for circadian dysfunction in cancer [7]. The role of oscillator genes in cancer will be discussed in the next section.

Although many associations of clock mutants and pathologies have been demonstrated, the resulting deviations in the circadian system concerning period length of the spontaneous oscillation, amplitudes and changed alignments of rhythms have been rarely evaluated. One of the exceptions is a variable tandem repeat (VNTR) polymorphism in the Per3 gene, for which alleles with either 4 or 5 repeats exist. The 5-repeat variant has been shown to be typical for morning types, whereas the 4-repeat allele was more frequent in evening types [27]. These findings also indicate a corresponding difference in the spontaneous periods of the two groups of allele carriers. However, this difference became less pronounced in the course of aging [27], which is not that much surprising, because the circadian period length shortens by age, with the consequence that previous evening types change into the direction of a morning type [28,29]. Apart from the demonstrable deviation of phasing and period length in carriers of the 5-repeat Per3 allele, this variant was reported to be responsible for an advanced melatonin peak [30], for deviations in sleep parameters [31] and for an earlier onset of bipolar disorder in the course of life [32].

With regard to the complexity of the circadian multioscillator system [7,8], the numerous pathology-related changes in circadian clock genes are indicative of a necessary alignment of rhythms with external periodicities as well as within the body. Internal desynchronization, which has been shown to be possible in humans, has been discussed as a cause of illness [33]. However, deviations that lead to circadian dysfunction should not only be seen as a consequence of mutations, because other alterations may lead to similar consequences. Especially in the course of aging, the circadian system reveals differential changes in the various tissue-specific oscillators [34]. In rats, it was nicely shown that some clocks lose their amplitude, whereas others do not. In further cases, phase positions of maxima change. Some of the more or less arrhythmic oscillators have been found to be re-activatable. These findings indicate a role of epigenetic mechanisms that may cause circadian dysfunction during aging. In fact, tissue-specific changes in DNA methylation were observed in oscillator genes of aging mice [35]. Generally, numerous epigenetic mechanisms have been discovered in the circadian system [36]. An additional cause of circadian dysfunction can be neurodegeneration, especially if the SCN or its neuronal connections are concerned. Whatever the precise causes of dysfunction are, the consequences may be insomnia, mood disorders, nocturia and decreased fitness because of external and/or internal misalignment.

With regard to melatonin, correction of circadian dysfunction should be possible in many cases in which synchronization by external time cues is impaired, e.g. by strongly deviating period lengths outside the normal range of entrainment, or in which rhythms have been flattened, e.g. because of an age-related reduction of melatonin secretion and, thus, insufficient melatonergic stimulation. As far as the main task is strengthening the entrainment of rhythms, melatonin treatment may represent an option. The pineal hormone displays the important property of acting as a chronobiotic, i.e. an agent that can phase shift and synchronize rhythms. It shows effects on the master clock, SCN, as well as on other central and peripheral oscillators [7]. However, the objective of improving synchronization requires adherence to circadian rules. As recently outlined [37-39], the phases in which melatonin pulses are effective in delaying or advancing rhythms have to be considered. Moreover, high levels of melatonin that is less effective than lower doses should be avoided. It is also important to distinguish between sleep induction and phase shifting, because the effective phases are not identical [39]. In principle, synthetic melatonergic drugs may also be suitable for entraining circadian systems, but there is no obvious advantage for those compounds, because their recommended doses are considerably higher than that for melatonin and because even lower doses than present in approved melatonin pills are already effective [9,39].

Melatonergic treatment for correcting circadian deviations is promising for improving entrainment in circadian rhythm sleep disorders, such as delayed sleep phase syndrome or familial advanced sleep phase syndrome, but also in mood disorders with a circadian etiology, in particular, bipolar disorder, seasonal affective disorder and, perhaps, some sub forms of major depression [37,39].

The Sirtuin Connection – Unexpected Fundamental Differences in the Actions of Melatonin

In a normally functioning organism that is devoid of substantial genetic deviations, circadian oscillators exist in variants. These

are largely cell type-specific, but multiple in parallel acting oscillators can be even found in the same tissue. Several reasons are responsible for the differences. Orthologs and, sometimes, paralogs of core oscillator genes can substitute for each other. Apart from orthologs such as Per1, Per2 and Per 3 or Cry1 and Cry2 or Bmal1 and Bmal2, the gene clock can be substituted in neurons by its paralog, Npas2. Additionally, core oscillators are embedded in a host of accessory oscillator components, which are either widely expressed or rather tissue-specific. These accessory components strongly influence the properties of the respective cellular oscillator. Sirtuins, previously discovered as aging suppressor agents, have been shown to be intimately involved in circadian function. In particular, this holds true for the sirtuins 1 and 6 (SIRT1, SIRT6) [40]. SIRT1 has been shown to be involved in the regulation of genes containing an E-box in their promoters, among them Nampt (nicotinamide phosphoribosyltransferase), the enzyme controlling the NAD+ salvage pathway [41-43]. This results in a circadian cycle in the concentration of the SIRT1 substrate NAD+ and, thus, SIRT1 activity. SIRT1 has first become known as a deacetylase that acts on histones and other proteins and has additional regulatory properties via protein-protein interactions. The NAD+-dependent activity of SIRT1 has been shown to be more important than SIRT1 expression [44]. Moreover, the chromatin-associated SIRT6 is also rhythmically driven by the NAD+ cycle [40].

Importantly, the role of SIRT1 includes enhancements of circadian amplitudes. Two different mechanisms have been suggested to be involved in this amplitude-promoting activity, (1) modulation of E-box activation [41,43] and (2) activation of RORα by deacetylated PGC-1α [45]. The amplitude-enhancing properties of SIRT1 are of particular importance to aging. The assumed role of SIRT1 as an anti-aging factor seems to indicate that the expression of this protein may decline during senescence and, thereby, contribute to the reduction of circadian amplitudes. Moreover, melatonin secretion is also declining during aging, at least, in the majority of individuals [8-10]. Several recently summarized reports [8] show that melatonin increases SIRT1 expression in various aging tissues, including parts of the CNS. These findings should be considered as being highly relevant for understanding the declining amplitudes in the course of lifetime. It will be important to further study directly whether melatonin administration will, via SIRT1 upregulation, increase circadian amplitudes in tissues that have gradually lost their rhythmicity.

These findings of SIRT1 upregulation strongly contrasted with other results of melatonin on SIRT1 expression in tumor cells [8,36,46-48]. In most cancer cells studied, melatonin strongly suppressed SIRT1 expression. These results may have appeared contradictory to many researchers, but, in fact, they are not. First, one has to consider the expression levels of SIRT1 and, second, their connection to other components of the core oscillator. In the tumor cells investigated, SIRT1 was typically strongly upregulated and, where studied, this was associated with corresponding upregulations of clock, whereas other core oscillator components were suppressed, in particular, Per2. This indicates that circadian oscillators of these tumor cells are strongly dysregulated [8,36]. For understanding this dysregulation more properly, it is helpful to consider the different roles of core oscillator components with regard to cell division. In a normal circadian cycle, high levels of CLOCK are found in phases that allow the initiation of cell proliferation, which is under circadian control in terms of the so-called gating. Usually, this gate that allows entrance into the cell cycle is soon closed again. The roles of other clock components are entirely different. The Per2 gene was first described as a tumor suppressor, whose dysfunction causes mice to become cancer-prone [49]. Thereafter, other clock genes were also found to possess tumor suppressor properties [7,8,50]. Therefore, a transformed cell can only reach and maintain the state of a tumor cell, if it downregulates those clock components that have antitumor properties and upregulates genes that favor proliferation, such as Clock. In fact, hypermethylation or altered DNA methylation patterns have been detected in the promoters of various oscillator genes, such as Per1, Per2, Per3, Cry1, Cry2 and Bmal1 [7,35,36]. In tumor cells expressing high levels of Clock and SIRT1, Per2 was typically suppressed [8,51].

In the light of these profound differences between tumor and non-tumor cells, the opposite effects of melatonin on SIRT1 and Clock expression have to be interpreted on the basis of the oscillator. An oscillator weakened by age may profit from a melatonin-induced increase in SIRT1, which may enhance the amplitude. On the other hand, a completely dysregulated oscillator that may exist in a non-oscillatory or poorly oscillatory state is pushed by melatonin to reduce Sirt1 and Clock gene expression, an effect that inhibits cell proliferation as observed [47]. Notably, this is not the only divergence by which melatonin acts differently in tumor and non-tumor cells. While melatonin is known for anti-apoptotic effects in numerous non-tumor cells, it has been shown to be pro-apoptotic in various tumor cell lines [52-54]. This capability of melatonin to distinguish between tumor and non-tumor cells has given rise to call melatonin a “smart killer” [55].

Decreased Melatonin Levels Because of Impaired Synthesis

Apart from aging and diseases, another cause of impaired melatonergic function can be a reduction of melatonin formation because of variants of its biosynthetic enzymes with strongly reduced activity. For instance, several mutations of ASMT (N-acetylserotonin-O-methyltransferase), the last enzyme of melatonin formation, are known that have only 4-8% of wild type activity and cause strongly reduced melatonin levels [56]. These were detected in patients with ADHD, autism spectrum disorders, bipolar disorder and intellectual disability, but in some cases also in the general population. This indicates that the variant alleles are mostly not more than risk factors. From a fundamental point of view, circadian oscillators can be functional in the absence of melatonin, as known from numerous strains of melatonin-deficient mice, which have been subject to numerous chronobiological studies. However, this statement does not seem to be valid for all circadian oscillators in a body. For instance, the semi-autonomous oscillator in the adrenal cortex of melatonin-deficient C57BL mice displayed only very weak and poorly

phased rhythms in PER1 and BMAL1 protein expression, contrary to the well-pronounced rhythms in melatonin-proficient C3H mice [57]. Deficiency of the melatonin receptors, MT1 and MT2, can lead to similar forms of dysfunction, comparable to a lack of melatonin, however, with a certain degree of tissue specificity. Striatal neurons from MT1 receptor knockout mice were shown to have lost the responsiveness of oscillator gene expression to melatonin [58]. Even in the rat SCN, which is anyway composed of differently acting subsets of oscillatory neurons [7,59], a loss of melatonin by pinealectomy caused changes in the phase relationships between Per1 and Per2 mRNAs that were reversed by exogenous melatonin [60]. Therefore, it seems likely that strong reductions of melatonin or melatonin deficiency will cause disturbances within the circadian multioscillator system, including flattened rhythms in some tissues and unfavorable phase relationships between poorly coordinated oscillators, even though the entire circadian machinery remains far from breaking down. Nevertheless, such a situation may bear some health risks. Treatment with melatonin in suitable circadian phases may partially correct the deficits. As it is still impossible to appropriately mimic the natural pattern of the pineal hormone by exogenous melatonin, it may be recommendable to select phases of treatment that allow entrainment [38].

Loss-of-function and Gain-of-function Mutations in Melatonin Receptor Genes

Any attempt of treating circadian deviations by melatonin or synthetic melatonergic agonists can only be successful, if melatonin receptors and their downstream factors are properly working. Studies on polymorphisms of the melatonin receptor genes, MTNR1A and MTNR1B, which encode the receptor proteins MT1 and MT2, respectively, have revealed deviations of different types. Some mutants of the MTNR1A and MTNR1B genes can be totally or largely dysfunctional. The stop mutant Y170X in MT1 has been detected in ADHD [61]. Various amino acid replacements in either MT1 or MT2 cause either reduced signaling, imbalances between the two signaling pathways of cAMP reduction and ERK1/2 activation or lack of membrane localization [62]. For instance, the replacement I212T in MT1 prevents expression at the membrane and melatonin binding. Surprisingly, this variant had still retained some ERK activation capacity, which appears to be constitutive [62]. All the alleles mentioned in this context were loss-of-function mutations.

However, in the context of searching for risk factors of type 2 diabetes, a prodiabetic mutation of the MTNR1B gene was discovered, which contains the SNP rs10830963 and which is frequently referred to in the literature as the G allele. This property has been amply documented and extensively reviewed elsewhere [7,9]. On the one hand, melatonin typically decreases in type 2 diabetes and, sometimes, already in prediabetic states, a reason for why melatonin reductions at midlife have been considered as a risk factor for this disease. On the other hand, the G allele had been shown to represent, surprisingly, a gain-of-function mutation. Especially in homozygosity, the G allele becomes strongly overexpressed in the pancreatic β cells [63]. This effect is only tendentially present in young individuals, but gains relevance around midlife. Homozygous subjects above 45 years exhibit manifold higher expression levels than carriers of the wild type allele [63]. The reason for this late increase is not entirely clarified, but may include a compensatory response to previous melatonin reductions and may have a circadian aspect [8]. The prodiabetic consequences of MTNR1B overexpression have been more recently interpreted in terms of the common MT2 signaling pathway of cAMP reduction via Gi and αi-dependent inhibition of adenylyl cyclase [64]. As insulin secretion is cAMP-dependent, the profound decrease of this second messenger by overexpressed MT2 leads to a blockade of insulin release. In fact, this interpretation can explain an impaired glucose tolerance. However, it does not yet convincingly explain another feature of type 2 diabetes, namely, insulin tolerance. As has been recently discussed [8], this gap, along with the fact that other, loss-of-function mutations in melatonin receptors are also associated with type 2 diabetes, would require further detailed studies on the role of melatonin in this disease.

The risk of promoting diabetes means that carriers of the G allele should not be treated with melatonin, as long as it has not been clarified whether the decrease of melatonin during midlife is related to the overexpression. In carriers of loss-of-function mutations, melatonin may not be helpful, as long as the hormone is normally secreted. However, MT1 and MT2 receptors can partially substitute for each other [1]. Therefore, in these cases, melatonin may still be of value at advanced age, when the endogenous level has substantially decreased.

Conclusion

Melatonin is multiply intertwined with the circadian multioscillator system. Its synthesis in the pineal gland and its secretion into the blood and the SCF of the third ventricle depends on the SCN, to which melatonin also feeds back. An additional role of melatonin concerns peripheral oscillators. Their sensitivity to melatonin is worth further clarification, although many details are indicative of a substantial contribution of melatonin to the maintenance of high-amplitude peripheral rhythms and favorable phase relationships between them. The capability of melatonin to induce SIRT1 in various tissues should be expected to enhance circadian amplitudes at the respective sites.

Circadian dysfunction can be caused by various factors. These include mutations in oscillator genes as well as their epigenetic downregulation. Reduced melatonin levels seem to be also involved in impaired rhythmicity, especially in aging or as a consequence of diseases. Melatonin has the potential of correcting circadian dysfunction by re-entraining rhythms, if it is applied in phases that allow phase shifts, as described by the so-called phase response curve [38,65]. To what extent melatonin is also capable of reversing epigenetic dysregulation, e.g. by erasing DNA methylation in CpG islands [66,67], remains to be directly demonstrated. Effects of melatonin on dysregulated oscillators in tumor cells may be taken as a hint for this possibility.

Generally, melatonin treatment seems to be an option in cases

circadian rhythm sleep disorders and in those forms of mood disorders that have an etiology of circadian malfunction. In cases of mutations in melatonin receptors, the usefulness of melatonin is questionable or, at least, limited. This is especially the case in the G allele of MTRN1B, which is overexpressed around midlife. From that point on, melatonin should be strictly avoided.

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