Mini-review: CNS Melatonin Receptors and Signaling: Focus on Aging-Related Diseases and Future Perspectives

Jane E. Cavanaugh and Paula A. Witt-Enderby*

Duquesne University School of Pharmacy, Division of Pharmaceutical Sciences, Pittsburgh, PA 15282, USA

Abstract: Melatonin plays important roles in modulating brain function especially in the entrainment of the sleep/wake cycle. This occurs in the suprachiasmatic nucleus of the hypothalamus (master biological clock) through melatonin receptors, MT₁(MT₁R) and MT₂(MT₂R) and the coordinated release of melatonin from the pineal gland that result in peak nocturnal levels of circulating melatonin followed by daytime troughs [1]. Besides sleep, melatonin, acting through melatonin receptors, affects the release of hypothalamic/pituitary hormones involved in reproduction and stress [2-4] and modulates other brain centers involved in pain and blood pressure [5-8]. Though these areas of study are important and affected by age, the focus of this review will be on the role of melatonin and melatonin receptors in brain function greatly impacted by age, with an emphasis on those affecting memory, mood, and movement. The cellular mechanisms underlying melatonin and melatonin receptor action will be discussed in the context of the literature but also will highlight other potential targets to guide future studies in this area.

Keywords: Melatonin receptors, CNS, melatonin, mitogen activated protein kinases, ERK1/2, ERK5.

MELATONIN AND AGING

According to the US Census Bureau, the fastest growing segment of the U.S. population is individuals over the age of 65. The number of people 65 or older is expected to increase to 53.2% by 2050. Similarly, the number of people aged 85 and older is expected to increase from 4.3 million in 2000 to 8.5 million in 2030 and 18.2 million in 2050. One of the most widely accepted hypotheses of aging is the free radical or oxidative stress theory of aging [9]. This hypothesis states that aging occurs due to an accumulation of oxidative insults throughout the lifespan. Adding to this, decreases in defense mechanisms against free radical damage have been reported with age. Therefore, therapies to boost antioxidant mechanisms and scavenge free radicals would, in turn, decrease age-related illnesses and increase the quality of life in the elderly, are needed. One such potential therapy is the natural hormone melatonin, which is reported to be a potent anti-aging compound by many research groups [10-12]. Although somewhat controversial, the increasing evidence for a role of melatonin in neuroprotection during non-diseased and diseased brain aging cannot be ignored.

As mentioned, melatonin levels fluctuate throughout the 24h cycle. With age, circulating melatonin levels (particularly the nocturnal peaks) have been shown to decrease in rodents and humans [13-15], which may further exacerbate free radical damage in the aging brain. In aged female rats, nocturnal melatonin levels are decreased in comparison to young animals [16]. Similarly in humans, the amplitude of the nocturnal peak of melatonin secretion decreases with age until between ages 60 and 70 there is no longer a difference in melatonin levels during the night as compared to the daytime [17]. This decreased peak of melatonin secretion may lead to sleep disturbances, alterations in circadian rhythms, and immune suppression which may, in turn, lead to significant health problems in the elderly [18,19].

Oxidative Stress

Several studies suggest that there is an increase in oxidative damage with aging [20,21] and that oxidative stress plays a role in neurodegeneration [22]. Melatonin has been shown to be a potent antioxidant in many cell types, animal models and humans [23-28]. These antioxidant effects of melatonin are most likely due melatonin's receptor-independent mechanisms of action. Melatonin alone or its brain kynuramine derivatives [29], N¹-acetyl-N²-formyl-5-methoxykynurenamine (AFMK) and N¹-acetyl-5-methoxykynuramine (AMK), may modulate distinct signaling pathways that could be important in brain function [30,31]. Melatonin has been reported to directly scavenge reactive oxygen species (ROS) [32]. Following an interaction with ROS, melatonin is metabolized to its brain kynuramine derivatives, AFMK and AMK, which also act to decrease free radical damage, protect neurons from damage and promote their survival [30,31]. Additionally, melatonin can enhance the expression of antioxidant enzymes to protect the brain from oxidative insults. Using a rat model of aging, Akbulut and colleagues [33] reported that melatonin treatment caused a significant increase in the endogenous antioxidant glutathione (GSH) in the cortex and cerebellum of aged animals as compared to aged animals that were not treated with melatonin. In a mouse model of accelerated aging (senescence accelerated mice; SAMP8) melatonin reduced the levels of oxidative damage and neurodegeneration
via an increase in retinoic acid receptor (ROR-alpha) shown to be important for the control of the neuronal response to oxidative stress [34,35]. In this same model, melatonin treatment leads to a reduction in DNA damage and oxidative damage and cerebral cortical cell loss [36-38]. Moreover, melatonin has been shown to stimulate several antioxidant enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase [32]. Finally, iron-induced oxidative damage of the locus coeruleus, a brain region rich in norepinephrine neurons that is damaged in several neurodegenerative diseases, was decreased by melatonin administration [39].

**Mitochondrial Function**

A significant source of ROS is complex I of the respiratory chain in mitochondria. Impairments in mitochondrial function have been noted with age [40] that may contribute to the age-related increase in oxidative stress. Mitochondria isolated from young and old animals treated with melatonin were healthier compared to mitochondria from untreated young and old animals [41]. Melatonin prevented the elevated production of hydrogen peroxide and complex I activity noted with age. Similarly, melatonin treatment completely inhibited mitochondrial dysfunction in the SAMP8 mouse model [42]. In fact, melatonin not only prevented a decrease in ATP production, which is indicative of mitochondrial respiratory chain function, but it increased ATP production in these mice.

**Immune System and Inflammation**

As one ages, increases in inflammation and decreases in immune defenses occurs. Melatonin has been reported to stimulate the production of granulocyte and macrophage progenitors, natural killer cells and cytokine release as reviewed in [19]. The modulation of melatonin on the gene expression of immune system proteins and inflammatory mediators with age has also been observed using mouse CNS microarray analysis [43]. Overall, melatonin shifted the gene expression pattern in the old mice to that of the young mice and many of the genes altered by melatonin were for proteins that are involved in inflammation and the immune system. Similar results were obtained in mice injected with lipopolysaccharide (LPS) to induce an inflammatory response [44]. Gene expression in old LPS injected mice treated with melatonin was similar to that in young melatonin treated mice exposed to the same LPS challenge. Together these data suggest melatonin may decrease age-related CNS inflammation and boost the immune system with age thereby promoting healthy aging.

**MELATONIN RECEPTORS, AGEING AND DISEASE**

**Receptor Density**

Age-related changes in melatonin receptor density have been reported especially in diseases associated with aging like Alzheimers Disease (AD) [45-48]. Some of these disease states result in differential expression of each of the melatonin receptor subtypes perhaps as a means to compensate for the loss of the other. For example, in the hippocampus of AD patients, MT$_1$R expression increases while MT$_2$R levels decrease [45,46]. Age-dependent decreases in the number of MT$_1$R- expressing neurons are seen without a concomitant decrease in vasopressin or vasoactive intestinal peptide neurons [48]. As the SCN is the central biological clock, a decrease in SCN MT$_2$R expression may lead to and/or exacerbate clinical circadian disorders and sleep disturbances in the elderly. In fact, a loss of SCN sensitivity to melatonin in the aged mice has been reported [49]. However, it should be noted that an earlier study in aged mice, melatonin-induced phase shifts of circadian rhythm were unaltered although an age-related loss of daytime SCN MT$_1$R mRNA expression was reported [50]. In a rodent model, SCN MT$_2$R has been shown to play a role in the phase shift effects of melatonin [51,52]. Perhaps, an imbalance between MT$_1$R and MT$_2$R expression and/or function in the SCN is leading to the modulation of signaling pathways regulating circadian rhythms. Melatonin receptors are expressed in the SCN and circadian rhythm disturbances occur in those afflicted with neurodegenerative disorders [18,53,54].

**Depression**

The incidence of depression has been shown to increase with age and depression is a co-morbidity with many age-related diseases [55]. Interestingly, serum melatonin levels are decreased in patients with major depressive disorders and an increase in melatonin release has been noted following antidepressant treatment that coincides with the clinical effectiveness of these medications [56-58]. Moreover, chronic antidepressant treatment has been shown to modulate melatonin receptor sensitivity and alter the ratio of MT$_1$R to MT$_2$R such that the therapeutic effectiveness of antidepressants is increased with nocturnal release of melatonin [59]. Therefore, with aging and age-related diseases in which melatonin secretion is decreased at night, nocturnal supplementation with melatonin may be a useful therapy and, as such, anti-depressant drugs like agomelatine may be more effective than the ones currently on the market.

**Memory**

Similar to depression, a decrease in memory and processes which support memory, such as long term potentiation (LTP) in the hippocampus, have been noted with aging [60,61]. Several studies have supported a role for melatonin in the reduction of age-related increases in memory deficits [62,63] and these may, in part, occur through melatonin receptors as 2-[125$I$]-iodomelatonin binding sites and both receptor subtypes have been detected in the hippocampus [45,46,64,65]. Prieto-Gomez and colleagues [66] report that melatonin increases the expression of MAP-2, a dendritic marker, in the hippocampus with age. In the SAMP8 model, Cheng and colleagues [67] noted that melatonin treatment increased hippocampal pyramidal cell number and improved performance on the Y-maze and eight-arm radial maze tests for memory. Similarly, it has been shown that melatonin reverses age-induced memory deficits in normal age mice in the elevated plus-maze and passive avoidance behavioral paradigms [68]. Even though the effects of melatonin on LTP are mixed with many studies showing inhibitory actions on LTP [64,69-73] and many showing stimulatory actions on LTP [63,74-76], among the studies that suggest melatonin increases LTP, the consensus is that this effect of melatonin occurs via activation of the MT$_2$R. In MT$_2$R knockout mice,
burst stimulation-induced LTP was decreased as compared to wild-type mice [63]. Furthermore, in the elevated plus maze behavioral test for memory, the MT1R knockout animals did not improve with training as compared to their wild-type counterparts. Together these studies suggest that melatonin via its actions at the MT1R plays a role in hippocampal memory processes and synaptic plasticity. It is important to note that in a zebrafish model, melatonin has been shown to suppress learning and memory in an operant conditioning paradigm [77]. The zebrafish did not perform as well on this behavioral test when they were tested in the nighttime as opposed to the daytime. Furthermore, daytime melatonin supplementation caused a decrease in learning and memory similar to the zebrafish tested at night while pinealectomy or treatment with a melatonin antagonist inhibited the elevation of Ab, increased neuronal survival, improved memory formation during the night. These data underscore the importance of paying mind to the temporal pattern of melatonin secretion in order to determine the best time of day or night for melatonin therapy so as to maximize the beneficial effects and minimize the detrimental effects of this hormone.

Alzheimer’s Disease

In addition to the effects on the brain with normal aging, an increase in oxidative stress has also been shown to play a role in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease [78,79]. Therefore, melatonin has received much interest as a possible therapeutic treatment for these neurodegenerative diseases [80,81]. Alzheimer’s disease (AD) is a debilitating neurodegenerative disorder that alters mood, mental and motor function, leads to cognitive impairments and ultimately results in death. It has been suggested that the incidence of AD will increase approximately 3-fold from 4.5 million persons with AD in the year 2000 to 13.2 million persons with AD by 2050 [82]. Therefore, it is imperative that new discoveries are pursued that may provide treatments for AD patients or even prevent AD. Characteristics of AD include neurofibrillary tangles composed of hyperphosphorylated Tau protein, plaque formation and cell death in the cortex and hippocampus. Accumulation of β-amyloid (Ab) peptides produced from cleavage of the amyloid precursor protein (APP) is also a common feature of sporadic and familial AD. Fibrillar Ab induces apoptosis in neurons and that this cell death is partially responsible for the cognitive decline experienced by AD patients.

Interestingly, melatonin levels are dramatically decreased in AD patients and may contribute to or further exacerbate the neuronal cell death observed in AD and melatonin administration may serve to alleviate the Ab burden of these patients and, in turn, decrease cell death and cognitive decline [56,83-87]. Administration of melatonin protected glial cell cultures from Ab-induced cell death [88]. In animal studies, both long and short forms of Ab that are involved in Ab deposition and plaque formation were found to be reduced in aging mice following dietary melatonin supplementation [89]. Moreover, melatonin treatment inhibited the elevation of Ab, increased neuronal survival, and reversed cognitive impairment of working and spatial memory in a transgenic mouse model (Tg2576) of AD [90,91]. In addition to a reduction in Ab pathology, melatonin has been shown to decrease tau hyperphosphorylation that contributes to the formation of fibrillary tangles [83,92]. However, in one study that utilized the same amyloid plaque-bearing transgenic model as mentioned above, chronic melatonin did not reduce Ab levels [93]. One explanation for this offered by the authors is that melatonin treatment was not given until after plaque formation. Therefore, the results of this study suggest that melatonin may be more useful in early stages or as prophylactic treatment of AD.

Alterations in melatonin receptor expression in the SCN, as seen with non-diseased aging, have been reported to be even more pronounced in AD [18,45-48]. For example, MT1R expressing neurons have been shown to decline in the late, but not early, stage of AD [48]. Therefore, sleep disorders resulting from disturbances in circadian rhythm are even more noticeable in AD patients. In addition to increasing age, family history is a risk factor for AD. Specifically, alterations in the apolipoprotein E4 gene has been shown to increase the deposition of toxic Ab fragments. Melatonin addition to Ab decreased apoE4-induced Ab plaque formation. Furthermore, melatonin treatment blocked Ab and ApoE4 neurotoxicity in vitro [94]. Taken together, the data that support a role for melatonin in neuroprotection in AD, has lead some investigators to propose that the FDA approved drugs for AD, such as memantine and acetylcholinesterase inhibitors may be more effective when given with melatonin supplementation [95].

Parkinson’s Disease

Parkinson’s disease (PD) is the most common age-related neurological disease of motor function, affecting 1% of all individuals above the age of 60 that results largely from the loss of dopamine-containing neurons projecting from the substantia nigra to the striatum. Since the late 1960s the primary treatment for PD has been levodopa (L-dopa). Although L-dopa therapy has been improved since that time, dyskinesias and motor fluctuations, the major side effects of L-dopa, still occur and, in many cases, can be more debilitating than PD itself. Therefore, there is a great need for complementary or alternative therapies for PD patients.

Although the data from few studies suggest a limited benefit of melatonin supplementation in PD [84,96], most studies report a beneficial effect of melatonin in diverse animal models and human and animal cell culture models of PD [97]. For example, melatonin treatment has been shown to protect against rotenone-induced dopamine neuronal loss and dopamine transporter downregulation in vivo [98]. Similarly, melatonin inhibited striatal dopaminergic cell death produced by the dopamine neurotoxin 6-hydroxydopamine [99]. Finally, in vivo and in vitro dopamine neuronal degeneration induced by treatment with the dopamine specific neurotoxin, 1-methyl-4-phenylpyridinium (MPP+), is alleviated by melatonin treatment [100-102].

MELATONIN RECEPTORS AND BRAIN ASSOCIATED SIGNALING MECHANISMS

The role of melatonin on influencing brain processes is complicated by the fact that melatonin may be working through its receptors [64,69] or independent of its receptors to impact on the brain in different ways, for example, by modulating LTP, neuronal excitability, neuronal differen-
tion and neuronal survival. Melatonin can act through MT₁Rs and MT₂Rs that are coupled to G-proteins [103-106] that can link to diverse signaling pathways [107]. More recently, melatonin receptor subtypes, MT₁ or MT₂ can homodimerize, or heterodimerize with each other or with GPR50 [108-110] to influence receptor function and possibly downstream cellular processes. Most important to brain function would be those pathways involved in neuronal excitability, long-term potentiation, survival and differentiation. These, in turn, would impact greatly on diseases like AD and PD that result in cognitive deficits, motor impairment and depression.

Regarding memory, melatonin can impact on memory processes through its actions on LTP and neuronal differentiation and these occur through different signaling pathways. For example, in one study, melatonin is shown to inhibit LTP in the hippocampus through MT₁R coupled to an inhibition of cAMP and PKA [71]. How PKA modulates LTP in the hippocampus is still not clear. PKA may increase LTP by enhancing NMDA receptor (NMDA₉) activity through phosphorylation processes or, perhaps, through its actions on CREB and CRE-containing genes as shown in Fig. (1). Therefore, melatonin-mediated inhibition of PKA and their effects on NMDA₉ activation in the hippocampus should be explored. Also, seeing that kynuramine derivatives could influence NMDA₉s on their own [31], the effect of the melatonin brain derivatives, AFMK or AMK, on hippocampal NMDA₉s and LTP should also be assessed.

Complicating things further are the recent findings that melatonin receptor subtypes and their expression levels can influence melatonin-mediated effects on LTP and downstream signaling processes. For example, mice lacking MT₁Rs show attenuation in LTP induced by melatonin in hippocampal slices when compared to their wild-type counterparts, which also is paralleled by memory deficits [63]. These data suggest that melatonin receptor subtypes play critical roles in modulating brain function and their expression in tissues and cells influence downstream signaling mechanisms. For example, co-expression of both MT₁Rs and MT₂Rs in cerebellar granule cells (CGCs) produces melatonin-mediated decreases in cAMP that are dependent upon Gi proteins. However, removal of one of the receptor subtypes from the cells prevents melanin-mediated decreases in cAMP. The same holds true for ERK1/2 and Akt activation in CGCs. In MT₁/MT₂R-expressing CGCs, melatonin (1nM) attenuates ERK1/2 and Akt activity. However, removal of either receptor from the cells such that only one of the receptor subtypes is being expressed causes these cells to become non-responsive to this same concentration of melatonin [111]. Because recent evidence using recombinant models shows that melatonin receptors, MT₁, MT₂, can heterodimerize [108,109], then, perhaps, heterodimerization between the melatonin receptor subtypes (i.e. MT₁/MT₂R) or between receptor tyrosine kinases (RTKs) [112] (i.e., MT₁R/RTK or MT₂R/RTK) is occurring in CGCs and impacting on signaling cascades involved in memory. In keeping, it is possible that conditions like disease or drug treatments that result in differential modulation of the melatonin receptor subtypes could influence melatonin heterodimerization processes. This could impact on the brain in a positive or negative manner depending on the role of the melatonin receptor subtype being influenced by the condition. For example, in hippocampal regions of those afflicted with AD, differential expression of hippocampal melatonin receptors occurs where levels of MT₁Rs are high and levels of MT₂Rs are low [45,46]. This reduction in MT₁R expression may be contributing to the memory loss associated with AD perhaps due to alterations in MT₁R/MT₂R heterodimerization states in the hippocampus. Attenuation in melatonin-induced LTP and memory occurs in MT₁R knockout mice [63] and changes in MT₁R and MT₂R expression patterns in CGCs influence downstream mechanisms thought to participate in memory processes [111].

By contrast, chronic drug therapy could be used to influence melatonin receptor expression that produces positive effects in the brain, for example, chronic anti-depressant actions on melatonin receptor expression in the hippocampus. It is hypothesized that chronic anti-depressant action may alter the brain ratios of MT₁R and MT₂Rs (i.e., increase MT₁R and decrease MT₂Rs) to enable endogenous melatonin released during the night to further improve the anti-depressant actions in the brain [59]. This is a plausible hypothesis because of the findings that chronic anti-depressant treatment increases MT₁R mRNA levels while decreasing MT₂R mRNA levels in mouse hippocampus [113] and that MT₂R-deficient mice display depressive actions greater than wild-type mice [114]. More studies should be conducted to determine if these changes in melatonin receptor mRNA levels translate to changes in melatonin receptor protein function thus modulating signaling pathways involved in brain function. Studies in the CGCs are a good start and suggest that melatonin acting through melatonin receptors modulates the mitogen activated protein kinases (MAPKs). MAPKs play an important role in neuronal proliferation, differentiation and survival and melatonin’s influence on MAPK activity may underlie melatonin’s effects on memory and plasticity.

Many studies support a role for MAPKs in modulating cellular differentiation induced by melatonin via melatonin receptors [112,115-117]. However, it should be mentioned that melatonin, acting independent of receptors and through PKC and Rho/ROCK signaling cascades increases cellular differentiation [118]. Besides regulating cellular differentiation, the MAPKs extracellular signal-regulated kinases 1 and 2 (ERK1/2), play a role in inhibiting antioxidant-induced cell death and providing neuroprotection in general [119]. Specifically, melatonin has been shown to attenuate cortical cell death induced by ischemic injury via activation of the ERK1/2 cascade and its downstream targets [120]. Therefore, melatonin modulation of MAPK pathways may improve memory by enhancing neuronal differentiation while protecting existing neurons from age-related damage. In a similar model of cell death, Kilic and colleagues [121], noted that ERK1/2 may be more important for the long-term neuroprotective effects of melatonin while other signaling pathways, such as the phosphatidylinositol-3 kinase/Akt cascades, may play a greater role in neuroprotection induced by acute melatonin treatment. These findings underscore the importance of paying close attention to the type of melatonin exposure (i.e., low dose, high dose; acute vs. chronic) and type of melatonin receptor being activated especially when elucidating cellular mechanisms downstream from melatonin receptor activation. For example, in primary cultures of
CGCs expressing both MT1Rs and MT2Rs, an acute (10 min) melatonin exposure decreases ERK1/2 activity in a concentration-dependent manner [111] whereas a longer exposure to melatonin (30 min) attenuates these inhibitory actions on ERK1/2 activity. Perhaps, this is due to melatonin receptor desensitization and internalization processes, mechanisms proposed to underlie cellular differentiation induced by melatonin through scaffolding with β-arrestins [116]. Densensitization and internalization processes are involved in melatonin receptor signaling and these processes, indeed, have been shown to be dependent upon the duration of agonist exposure, the concentration of agonist being used and the melatonin receptor subtype being expressed in the particular model [115,116,122-124].

Besides ERK 1/2, a new MAPK family member, ERK5, may be playing a role in melatonin-induced responses in cells. ERK5 is a particularly interesting protein because like ERK1/2 it is widely expressed in neurons. However, unlike ERK1/2, the role of ERK5 in neuroprotection is just beginning to be elucidated [125,126]. Moreover, many studies have relied on the use of the inhibitors PD98059 and U0126 to implicate ERK1/2 in neuroprotection. Interestingly, PD98059 and U0126 also block the activation of ERK5 [127]. This suggests the interesting possibility that some of the functions attributed to ERK1/2, including melatonin-induced neuroprotection via ERK1/2 activation, may be mediated by ERK5. However, ERK5 activation by melatonin has not yet been explored. Interestingly, however, both melatonin treatment [117] and ERK5 activity [128] been shown to increase neurite outgrowth in separate studies by different research groups. This is an area of great interest to our laboratories and we hypothesize that ERK5 pathway will be activated by melatonin to increase neurite outgrowth and neuronal survival. Furthermore, as a decrease in ERK5 signaling has been observed in the developing brain [125], we hypothesize the ERK5 activity decreases with age and melatonin therapy may be useful in counteracting this age-related decline in ERK5 signaling.

CONCLUSIONS

Overall, melatonin may be a promising therapy for age-related diseases both with non-diseased and diseased aging. Melatonin is a natural hormone that has very limited side effects and can, therefore, be used in an aged population with little worry of toxicity. Importantly, especially in the current economic climate and for the elderly population, melatonin is relatively inexpensive. Melatonin use as an adjuvant therapy to existing therapies targeted at the treatment of
depression or cognitive deficits should be explored. More importantly, melatonin use as a preventative agent against neurodegenerative disorders like AD and PD need to be explored. These types of studies will require assessing the long-term impact of chronic melatonin exposure on the development of AD and PD in rodent models to determine if melatonin can delay or even prevent their development. The hope is to bring melatonin therapies, if shown to be efficacious, to the clinic for use in those at risk for neurodegenerative diseases. Also, since it appears that specific melatonin receptors are involved in specific brain functions, for example hippocampal MT2Rs in memory, then selective agents need to be developed for further elucidation of these roles. This, in turn, will further the development of drug therapies targeted to the individual melatonin receptors.

**ABBREVIATIONS**

CRE = Cyclic AMP response element  
CREB = Cyclic AMP response element binding protein  
LTP = Long-term potentiation

**REFERENCES**


[41] Petroillo G, Fattoretti P, Matera M, Ruggiero FM, Bertoni-Freddari C, Paradies G. Melatonin prevents age-related...


