J Pharmacol Sci **123**, 9 – 24 (2013)

Journal of Pharmacological Sciences

Survey Review

Effects of Melatonin on Nervous System Aging: Neurogenesis and Neurodegeneration

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Received March 11, 2013; Accepted June 30, 2013

Abstract. Neural aging as a progressive loss of function involves central and peripheral post-mitotic neurons and neural stem cells (NSCs). It promotes neurodegeneration, impairs neurogenesis, and can be considered a cause of cognitive impairment and sensory and motor deficits in the elderly. Age-related morphological atrophic changes and cellular alterations are addressed by neural aging mechanisms. Neurogenesis declines during aging through several mechanisms such as an increase in quiescence state, changes in lineage fate, telomerase dysfunction, the failure of the DNA repair system, increased apoptosis, and the impairment of self-renewal. The self-renewal transcriptional factor Sox2 has been correlated with retrotransposon L1 and certain cell-cycle– and epigenetic-related factors, which are sometimes considered age-related factors in NSC aging. As neurogenesis decreases, non-mitotic neurons undergo neurodegeneration by oxidative stress, sirtuin, insulin signaling and mTOR alteration, mitochondrial dysfunction, and protein misfolding and aggregation. As neurodegeneration and impaired neurogenesis promote the nervous system aging process, the identification of neuronal anti-aging is required to raise life expectancy. The role of melatonin in increasing neurogenesis and protecting against neurodegeneration has been investigated. Here, we review nervous system aging that is correlated with mechanisms of neurodegeneration and the impairment of neurogenesis and evaluate the effects of melatonin on these processes.

Keywords: aging, neurogenesis, neurodegeneration, melatonin, nervous system

1. Introduction

Neural aging is a progressive loss of nervous system function with advancing age. As the nervous system ages, the neurodegeneration of non-dividing neurons and the aging of neural stem cells (NSCs) occur in the central and peripheral nervous systems. Many clinical problems in the elderly are related to nervous system aging, including declines in cognition and memory, impairments to visual acuity and hearing, chronic constipation and some gastrointestinal (GI) tract problems, motor deficits, balance impairment, and falls. Several aging theories related to signaling pathways have emerged, and the most convincing is that of free radicals, which explains the role of antioxidants such as melatonin. Recently, stemness-related aging and epigenetic mechanisms have been linked with many aspects of aging. In this review, we discuss normal nervous system aging mechanisms involved in the processes of neurogenesis and neurodegeneration over the entire life span. Despite the low regenerative potential, neurogenesis occurs in limited areas in the central and peripheral nervous systems and declines with age. Post-mitotic neurons undergo cellular degeneration under normal conditions and in some age-related diseases. These changes can be correlated with various phenotypes of the aging nervous system and with melatonin, whose influence on neurogenesis

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Published online in J-STAGE on August 27, 2013
doi: 10.1254/jphs.13R01SR

Invited article

and neurodegeneration processes may be considered as an anti-aging agent.

2. Nervous system aging

The nervous system undergoes many changes during aging. The most prominent changes include a decrease in brain volume, an increase in ventricular system volume, white matter shrinkage, and gray matter loss in specific areas of the brain, particularly the medial frontal cortical regions (1). Although brain atrophy occurs mostly in the prefrontal cortex (2) and medial temporal lobe (3), the correlation between brain shrinkage and neuron loss or synaptic decline is still under discussion. Substantial evidence suggests that reduced dendrite and synapse volume may be the main cause of brain shrinkage (2). Dendritic branching mostly declines in the pyramidal cells of the prefrontal cortex (4) and in various areas of the hippocampus, but not the molecular layer of the dentate gyrus (5). The number of spines decreases with age in many areas of the macaque brain (6). The density of synapses declines in the hippocampus during aging (7), and postsynaptic density is reduced in some areas, including the hippocampal CA1, leading to electrophysiological silence in some synapses. In this region, long-term potentiation (LTP) decreases and long-term depression (LTD) increases, resulting in impaired plasticity in the hippocampus (8). Postsynaptic LTP is usually NMDAR-dependent, via the calcium/CaMKII pathway, and AMPA receptor trafficking leads to protein synthesis and receptor insertion in a few hours (9). In addition to other changes, LTP deficiency is promoted by increased LTP threshold in the dentate gyrus, the weak summation of EPSPs with high frequency stimulations in CA1, age-related Ca^{2+} dysregulation, and changes in cell interactions and gene expression. These alterations result in impaired plasticity in the hippocampus during aging (5).

Neural plasticity shifts from NMDAR-dependent to NMDAR-independent mechanisms with age. Thus, working memory, short-term recall, and the speed of processing information gradually decline throughout the rat adult life span (10). Age-related cognitive decline is a common complication of aging. Impaired hippocampal neuronal activity produced by aging leads to deficits in learning and memory (11). The dysregulation of calcium homeostasis related to the modulation of L-type voltage gated calcium channels, ryanodine, IP3, and NMDA glutamate receptors by oxidative stress affects LTP and plasticity in aging. Because of the role of calcium in signaling pathways, gene expression is altered by calcium dysregulation (12).

During aging, axons in many parts of the nervous system undergo degeneration. Age-related impairment in axonal transport modulates neuronal homeostasis (13). Many deposits accumulate in the nervous system because of the increase in damaged macromolecules and the impairment of degradation mechanisms. In contrast with the assumed nature of lipofuscin as age-associated waste deposits with lysosomal origin (14), the appearance of oxidatively cross-linked proteins in lipofuscin deposits (15) shows that this accumulation is a significant adaptation process (16). Intracellular F-actin rich hirano bodies, which are found most commonly in aged hippocampal CA1, peripheral neuronal axons, and glia, contain a C-terminal fragment of amyloid-precursor protein intracellular domain (AICD). These inclusions prevent AICD-dependent apoptosis (17) and protect against tau-dependent and tau-independent cell death (18).

Senile plaques contain a core of amyloid as a hallmark of Alzheimer's disease (AD) and some evidence confirms that senile plaques with beta-amyloid deposition represent early stages of AD (19) and are not expressed in normal aging (20). Neurofibrillary tangles (NFTs) consisting of hyper-phosphorylated tau are found in AD, dementia, and normal aging, especially in the subiculum and CA1 of the hippocampus, the amygdala, and laminae II and V of the entorhinal cortex (21, 7).

Neurotransmitters, receptors, and reuptake transporters in these brain areas change upon aging. The altered serotonergic innervation system in the hippocampus, which is associated with decreased density in CA1 and CA3 during aging (22), defines depression and dementia in aged people and makes them susceptible to AD in later life (23). In the suprachiasmatic nucleus (SCN), increased serotonin binding sites ($5\text{-}HT_{1B}$) and decreases in reuptake transporters and neurotransmitters modulate the circadian rhythm (24). The efficacy of the dopaminergic system declines during aging due to the downregulation of the post-synaptic D_1 receptor, pre- and post-synaptic D_2 receptors, and the dopamine transporter, especially in the striatum, and dopaminergic reduction contributes to some age-related cognitive changes (25). The modulation of ionotropic and metabotropic glutamate receptors (mGluRs), especially group 1 mGluRs, affects learning and memory due to changes in LTP and synaptic plasticity in the aged rat hippocampus (26). Aging alters the interaction between neurotransmitters, including changes in the glutamate-dopamine-GABA interaction in the rat nucleus accumbens (27).

3. Melatonin during aging

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone and is produced in vertebrates and invertebrates (28) in pineal and rat extrapineal tissues (29). Its secre-

tion follows light/dark (30) and seasonal rhythms (31). In vertebrates, melatonin acts as a regulator of circadian rhythms (32) and seasonal breeding (31, 33), and it acts as an antioxidant and free radical scavenger, among other biological functions (34).

The melatonin level varies during the lifespan. In the fetal period, the fetus uses maternal melatonin that crosses the placenta (35), and from birth to a peak around puberty, the melatonin level increases (36) and then declines in middle-aged and elderly individuals (37). As nocturnal melatonin declines in adulthood, its functional effects are gradually impaired. Decreased melatonin levels may be considered a mechanism of circadian rhythm and thus there are sleep disturbances and hormonal changes in the elderly and in Alzheimer's patients (38). The effective melatonin level is altered by the age-associated decline in the degraded enzymes and target receptors involved in melatonin synthesis. Melatonin is synthesized from tryptophan via the conversion to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase followed by the synthesis of 5-hydroxytryptamine (serotonin) by the enzyme aromatic L-amino acid decarboxylase. Acetylated serotonin (*N*-acetyl serotonin), produced by arylalkylamine *N*-acetyltransferase (AANAT), is converted to melatonin via the action of hydroxyindole-*o*-methyltransferase (HIOMT) (39). Gene expression of pineal AANAT is driven by the circadian rhythm (40) and controlled by the SCN via stimulation of the β- and α_1-adrenergic receptors and the cAMP/PKA signaling pathway (41). Human melatonin synthesis is reduced by aging (42). It has been documented that serum melatonin concentrations (43) and pineal AANAT activity (44) decrease during aging. Rat pineal and hippocampal AANAT expression decreases in old age (45, 46). In extrapineal tissues (liver, spleen, and heart), it has been shown that the melatonin concentration, enzymatic activity, and the gene expression of HIOMT decrease during aging without specific changes in AANAT or significant alterations in its concentration and enzymatic activity in the rat thymus (47). Exogenous melatonin can cross the blood brain barrier (BBB) and rapidly enter the CNS (48).

The mammalian melatonin receptors MT_1 (Mel_{1a}) and MT_2 (Mel_{1b}) are G protein–coupled receptors (GPCRs) that couple to G proteins (Gαi2/3, Gαq, and G$\beta\gamma$) during activation (49). MT_1 and MT_2 expression decreases with age (50). The potential of melatonin to inhibit CREB phosphorylation by PACAP and via MT_1 in the mouse SCN declines with age (51, 52). The MT_1 and MT_2 mRNA levels in extrapineal tissues (liver, spleen, kidney, and heart) significantly decrease in aging, except in the thymus, where they increase (47). In the hypothalamus, a reduction in MT_2 levels has been documented in age-related neurodegenerative conditions (38).

Melatonin is hydroxylated by hepatic mono-oxygenases (CYP1A1, CYP1A2, and CYP1B1) and is conjugated to the urinary metabolite 6-sulfatoxymelatonin (53) or demethylated to its precursor (by CYP2C19 with contributions from CYP1A2 and CYP1A1) (34). In the central nervous system, melatonin is cleaved to other metabolites, such as N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK) (54), which are well-documented scavengers (55). The secretion of melatonin metabolites, including 6-sulfatoxymelatonin, declines in aged individuals (56). Similar to the melatonin pathway from production to degradation, a significant age-associated functional decline is observed, and this decline may contribute to the reduction of melatonin levels during aging. Mechanisms involved in the normal and pathologic aging of the nervous system and non-nervous systems may also contribute to this reduction.

The mechanisms of gene alteration by melatonin have been found to be linked with epigenetic control of the genome. The epigenetic mechanisms that modulate DNA without altering genomic sequences involve nucleosomes, which consist of DNA and an octamer of histones (H2A, H2B, H3, and H4 monomers), and include many processes: histone modification via acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, isomerization and chromatin remodeling, and DNA methylation. DNA methylation primarily occurs on the cytosine residues of CpG islands by DNA methyltransferases (DNMT1, DNMT2a, DNMT2b in mammals), while histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze histone acetylation and deacetylation. The epigenetic function of melatonin has been documented in tumorigenesis and inflammatory pathways. Melatonin increases the expressions of HDAC3, HDAC5, and HDAC7 and histone H3 acetylation (57). CREB-binding protein (CBP) and P300 are transcriptional activators that have HAT properties (58). The CBP/P300 complex recruits a number of transcriptional co-activators, such as NF-κB, CREB, and nuclear factor erythroid 2–related factor 2 (Nrf-2), for transcription (59). Melatonin suppresses P300 HAT activity (60) and thereby NF-κB acetylation and binding to the promoters of some inflammatory genes (61). The expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) is regulated by NF-κB and inhibited by melatonin (59). In addition to NF-κB suppression, the enzyme COX-2, which converts arachidonic acid to prostaglandin H2 in inflammation and tumorigenesis, is suppressed by melatonin (61). COX-2 is distributed in specific areas of the brain, including the hippocampus, and is involved in learning and memory (62). Thus, by

suppressing CBP/P300 HAT activity, melatonin promotes the anti-inflammatory effect via epigenetic mechanisms and also stimulates the expression of the transcription factor Nrf2, which promotes the expression of some antioxidant genes (59). The anti-inflammatory and antioxidant roles of melatonin can also be explained by the upregulation of Nrf-2, which is involved in both pathways (63) via CBP/P300-mediated acetylation (64).

Melatonin has been suggested to be a resynchronizer of circadian rhythms in age-related malignancies (65). Sirtuin1, a histone deacetylase, interacts with CLOCK/BMAL1 as a promoter of circadian genes by deacetylation of BMAL1, thereby modulating circadian rhythms (66). Thus, it has been supposed that the age-related decline of melatonin may increase sirtuin1 activity and promote cancer development (67).

4. Neurogenesis and melatonin during aging

4.1. Central and peripheral NSCs aging

An aspect of aging is the decline in the regenerative capacity of adult stem cells. Compared with other organs, the brain and spinal cord have a low regenerative potential for homeostasis and repair (68). As non-dividing neurons undergo aging through the accumulation of damaged macromolecules, oxidative degeneration, and other mechanisms of aging, neural stem cells have been assumed to be involved in certain functional stemness-related changes. It has been proposed that during aging, impairments in self-renewal, stem cell senescence (69), increased quiescence, and NSCs fate changes (70) lead to the depletion of the neural stem cell pool or a decreased potential to produce progenitor cells (71).

In the central nervous system, hippocampal neurogenesis occurs in the subgranular zone of the dentate gyrus with different types of progenitor cells. Multipotent type 1 progenitor cells express glial acidic fibrillary protein (GFAP), nestin, brain lipid-binding protein (BLBP), and Sex determining region Y-Box2 (Sox2) (72). Type 2a and 2b progenitor cells with different levels of Sox2 and doublecortin (DCX) expression (72) give rise to type 3 cells, which are transient cells from neuroblasts to post-mitotic immature neurons (73) that express DCX (74) but not Sox2. Newborn neurons migrate and mature, becoming dentate gyrus granule cells. Type 1 hippocampal progenitor cells, which are quiescent neural progenitors and amplifying neural progenitors, decrease with age. This decrease is more marked in quiescent types than in amplifying types, which are active during proliferation, and the rate of depletion of quiescent neural progenitors is significantly lower in old mice compared with young mice (75).

The subventricular zone (SVZ) also contains many types of progenitor cells. Neonatal radial glia, a source of adult neural stem cells, give rise to radial glia-like cells (Type B cells) that express Sox2 (76) and GFAP, exhibit glial properties of both astrocytes and prenatal radial glia (77), and act as neural stem cells in the normal and regenerating brain. Type B1 and B2 are different in their location and structure (78). Type C (transient amplifying) cells are found in the rostral migratory stream as bipotent cells, and they express Olig2 and Ascl1 (79), Dlx2, and Pax2 and give rise to DCX-expressing type A neuroblasts (80). Finally, immature neurons migrate toward the olfactory bulb in rostral migratory chains surrounded by astrocytes and then differentiate into mature neurons. Sox2 is expressed in proliferating Ki67-positive cells in the SVZ (81) and is weakly expressed in PSA-NCAM cells in parts of the rostral migratory stream (76).

In the rat dentate gyrus and SVZ, the process of neurogenesis responds to melatonin, which promotes BrdU-positive (82) and DCX-labeled (83) cell proliferation in the dentate gyrus and increases differentiation in hippocampal neural stem cells (84, 85). Melatonin also stimulates the proliferation and differentiation of neural stem cells in the SVZ (86). During aging, the capacity for neurogenesis mostly declines, although some evidence shows a constant capacity (87). Several mechanisms have been suggested to contribute to the decrease of neurogenesis during aging. BrdU labeling studies show that reduced neurogenesis in the aged dentate gyrus is linked with the decreased proliferative activity of neuronal precursor cells (88 – 90). This reduction also has been shown in the number of DCX-positive newly born neurons (91). In addition to numerical changes and the diminished size of the stem cell pool, neural stem cell niches are both intrinsically and extrinsically involved in functional changes during aging. Analysis of the status of Sox2-positive stem cells shows that reduced neurogenesis can be related to increased quiescence of aged neural stem cells (87). Reduced commitment and increased cell death are considered other causes of NSC aging. An imbalance of symmetric versus asymmetric division or gliogenesis versus neurogenesis leads to changes in cell fate during aging. When neural stem cells convert to astrocytes more than neurons, depletion of the NSC pool occurs over time (70, 75).

Neurogenesis decreases with the occurrence of short telomeres and telomerase deficits in NSCs (92). As neural stem cells divide, TTAGGG repeats of telomeres are lost and decapping at the G-strand combined with telomerase dysfunction and DNA repair system impairment promote the aging process (93). It has been proposed that telomere shortening decreases the mobilization of stem cells from the niche, but its mechanism

is unknown (93). In other proposed models, telomere dysfunction may activate P53-mediated mitochondrial dysfunction or the inhibition of peroxisome proliferation activated receptor γ (PPARγ) co-activator 1α (PGC-1α) and thus influence aging (94). Some evidence shows that melatonin can inhibit telomerase activity and the telomerase reverse transcriptase (TERT) mRNA subunit in the MCF-7 tumor cell line (95), but other studies demonstrate the stimulation of telomerase activity in the retinal pigment epithelium (96) and gastrointestinal mucosal cells in aging (97).

Recently, neural stem cells with the potential for proliferation and differentiation into neurons have been isolated in the peripheral nervous system from postnatal mouse dorsal root ganglia (DRG) (98), rat trigeminal ganglia (99), the rat enteric nervous system (ENS) (100), and guinea pig spiral ganglion (101).

The melatonin receptors MT_1 (102, 86) and MT_2 (103) are expressed in neural stem cells. High concentrations of melatonin in an exposure-timing–dependent manner (104) and at physiologic concentration, particularly through MT_1 receptors in the SVZ, have a modulatory effect on neural stem cell proliferation and differentiation (86). Recent studies have identified functions in neurogenesis for MT_1 and MT_2 agonists, including agomelatine (105, 106), buspirone in combination with melatonin in major depressive disorder (107), and *N*-acetylserotonin, an intermediate of melatonin synthesis (108). Some evidence shows that a combination of melatonin and physical exercise enhances neurogenesis and neural survival (109) and some melatonin-receptor agonists promote proliferation in the dentate gyrus (110). As melatonin enhances dendritogenesis (111), chronic melatonin treatment in the hippocampal dentate gyrus can increase the dendritic maturation of DCX-positive cells and neuronal survival (112). Agomelatine stimulates growth factors, such as brain-derived neurotrophic factor (BDNF), which increase hippocampal neurogenesis (106). The melatonin receptor MT_1 is involved in the effect of melatonin on the production of BDNF (113).

It has been documented that neurogenesis occurs under a circadian rhythmic pattern. BrdU-labeled proliferating cells show a dark/light cycle–dependent pattern (114). M-phase cells increase during the night (115), and constant light exposure decreases neurogenesis in the dentate gyrus (116). Clock genes (Per1, Per2, Cry1, Cry2, Bmal1, and Clock), which are expressed in the dentate gyrus of the hippocampus (117) in neural progenitor cells, can regulate these proliferation and differentiation transcription factors (118).

4.2. NSC self-renewal in aging: transcriptional and epigenetic regulations

Neural stem cells are able to maintain their self-renewal ability throughout life. However, the potential for neurosphere formation and the self-renewal of neural stem cells declines with age. When the balance between self-renewal and commitment of stem cells shifts towards self-renewal, the differentiation potency of stem cells declines, and the aging process is promoted (119). The failure of stem cell self-renewal can lead to a decline in the number of stem cells and the aging-related depletion of the stem cell pool (120). Cell-extrinsic self-renewal factors act through membrane receptors and signaling pathways to affect the transcriptional regulation of pluripotency and multipotency. In the regulation of pluripotency, several factors influence the self-renewal of stem cells, including leukemia inhibitory factor (LIF), mostly via JAK/STAT3 (121); fibroblast growth factors (FGFs) (122); WNT/β-catenin (123); and bone morphogenetic protein (BMP) (124). Insulin-like growth factor 1 (IGF1), mostly via PI3K/Akt and mitogen-activated protein kinase pathways, affects the proliferation and survival of neural stem cells (125) and promotes neurogenesis and synaptogenesis (126). Adult stem cells with low self-renewal potency respond to specific extrinsic factors such as Notch, Shh, BMPs, and Wnts (80).

Activated intracellular signaling pathways stimulate the core self-renewal transcriptional factors Sox2, Oct4, and Nanog to maintain the potential of stem cells via internal negative and positive feedback circuits (127). The HMG-box transcription factor Sox2 is required for neural stem cell multipotency (128) and pluripotency (129), although it has been reported that the overexpression of Sox2 also promotes embryonic stem cell differentiation (130). During self-renewal, Nanog maintains neural stem cells through activation of specific target genes by binding Sox2 and Oct4 to its Octamer/Sox element (131), whereas Nanog is downregulated during differentiation (132). The association of Sox2 with the histone deacetylase HDAC1 represses Sox2 and T-cell factor/lymphoid enhancer factor (TCF/LEF)-binding sites (Sox/LEF) in the promoters of differentiation-specific genes (133). Sox2 is correlated with long interspersed nucleotide element 1 (LINE-1) as a transposable element. The Sox/LEF site in LINE-1 element is influenced by Sox2 (133) and methyl CPG-binding protein 2 (MeCP2) (134). LINE-1 activity is greater in the brain than in other regions of the body, and it is expressed at higher levels in the spinal cord and dentate gyrus than in other parts of the adult nervous system (135). The human LINE-1 retrotransposon creates DNA double-strand breaks (136), point mutations, rearrangements, damaged chromatin, and retrotransposition, which lead to genome

instability (137) and an imbalance between damage and repair during aging (138). It has been proposed that in contrast to the impact of beneficial genetic variation on evolution, LINE-1 activation has a cost in longevity and causes aging (139).

In contrast, P21, a cyclin-dependent kinase inhibitor, suppresses Sox2 expression, and overexpression of Sox2 in the P21 mutant leads to impairment of self-renewal, DNA damage, cell growth arrest, and senescence in a P53-dependent manner (140). Fetal stem cells are more sensitive than ESCs to mitogen-induced activation of CyclinD-CDK4/6 and retinoblastoma (Rb), but in adult stem cells, the emergence of increased cell cycle inhibition leads to the appearance of prolonged G1 and quiescence periods throughout life (129). Epigenetic mechanisms that influence NSC maintenance and differentiation play important roles in the regulation of self-renewal and commitment balance throughout the life span. Although DNA methylation decreases, some promoters undergo hypermethylation during aging (141). Bmi-1, an age-regulator factor in neural stem cells (142, 143), is a member of polycomb repressive complex 1 (PRC1), which binds with the repressive trimethyl lysine 27 of histone H3 (H3K27me3) during the epigenetic control of stem cell self-renewal (142). The overexpression of Bmi-1 increases the self-renewal and proliferation of neural stem cells (144), and Bmi-1 knockdown results in impaired self-renewal (145). Bmi-1 suppresses $P^{16INK4a}$ and P^{19Arf} expression, promotes stem cell self-renewal in the central and peripheral nervous system (146), and affects the aging process via the Ink4a/Arf locus (147). The expression of the Ink4a/Arf locus is considered an aging marker (148), and declined neurogenesis in the SVZ is demonstrated by $P^{16INK4a}$ upregulation during aging (149). During reprograming, Oct4, Klf4, and Sox2 silence this locus, and aging upregulates it. Thus, the Ink4/Arf locus is considered to be responsible for the decreased reprogramming associated with aging (150).

The tumor suppressor protein P53 downregulates E2f target genes and activates cellular senescence via P^{19ARF}, $P^{16INK4a}$, or P^{21} (151) and active hypophosphorylated retinoblastoma (152). Hmga2 is a member of the high mobility group A (HMGA) family that promotes the self-renewal of fetal and adult stem cells and represses $P^{16INK4a}$ and P^{19Arf} expression. The expression of Hmga2 declines with age, and the age-related decrease of Hmga2 that inhibits P^{19ARF} and $P^{16INK4a}$ leads to reduced neural stem cell number and self-renewal in the central and peripheral nervous systems (153). Bmi-1, Ink4a, Hmga2, and miRNA let-7 are considered NSC self-renewal regulators in aging (144).

Extrinsic factors that control chromatin and transcription factors produce reversible changes and can contribute to stem cell rejuvenation (142). Melatonin increases deacetylation via HDAC upregulation and the acetylation of histone H3 in the neural stem cell line (57). Melatonin has a protective effect against degeneration caused by METH administration in premature neurons obtained from the rat hippocampus and prefrontal cortex (154). Exogenous melatonin supplements increase mouse neurogenesis during aging (155), and melatonin enhances proliferation in the ischemic mouse brain (156), especially through MT_2 receptors (157); thus, the pre-ischemic administration of melatonin potentiates hippocampal neurogenesis (158) in elderly populations. Irradiation studies show the possibility of free radical scavenger mechanisms of melatonin in neurogenesis, especially via AFMK, which inhibits the loss of DCX- and Ki67-positive cells in mouse hippocampal dentate gyrus (159). The increased level of free radicals and declined neurogenesis in elderly populations may explain the anti-aging effect of melatonin on neurogenesis. The effect of melatonin on decreasing lipid peroxidation through increases in pCREB has been considered an anti-aging property of melatonin in the mouse dentate gyrus (160).

5. Neurodegeneration and melatonin during aging

Neurodegeneration appears at different levels in normal aging and age-associated degenerative diseases. The brain is a very metabolically active organ with a fixed average energy cost per neuron (161), which makes it more susceptible to aging. Like age-related changes in other organs, several mechanisms have been documented to be involved in normal and pathological neurodegeneration.

The most acceptable theory of aging describes the presence of free radicals and oxidative stress (162). Reactive oxygen species (ROS) are produced mostly by mitochondrial complex I (163) and complex III (164) and promote cellular damage and the development of the aged phenotype. Superoxide anion radical (O_2^-•) is reduced to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and is then converted to H_2O by catalase (CAT), glutathione peroxidase (GP_x), or peroxiredoxin (Prx). Under the Haber-Weiss and Fenton reactions, H_2O_2 is converted to hydroxyl radical (HO•) and damages parts of the cell (165). O_2^-• can also produce peroxynitrite anions ($OONO^-$) by combining with nitric oxide (NO•), leading to cellular damage. Oxidative stress also promotes increased intracellular calcium concentration and cell death (166) because the calcium machinery, especially transporters and channels, is regulated by the redox state of sulfhydryl groups (12). Free radicals in-

duce protein oxidation, DNA damage, and lipid peroxidation, especially in cell membrane long-chain unsaturated fatty acids. However, the accumulation of somatic DNA mutations is considered to be a consequence of aging (167). Free radical generation as a result of metabolism increases with age, but mitochondrial ROS can modulate some enzymes and transcription factors, trigger some repair systems to respond to age-dependent damage, and extend longevity (168). Thus, the role of oxidative damage in limiting the life span is still under discussion. The nervous system, which shows high levels of metabolic activity and calcium trafficking as well as large quantities of iron and copper and phospholipid sheets, is sensitive to oxidative stress (169). Even with antioxidant mechanisms, a chronic state of imbalance between oxidative stress and antioxidants under physiological conditions can damage cellular function and promote aging.

During aging, many regulator proteins are downregulated, and the levels of some enzymes that mediate energy production and oxidative stress increase (170). In the hippocampus, the reduction of most antioxidant enzymes supports the role of oxidative stress in aging (171). Melatonin and its metabolites have an antioxidant role (172, 48). In the CNS, two major metabolites of melatonin, AFMK and AMK, act as free radical scavengers (55). AFMK and AMK, which mostly react with HO• and melatonin, have different relative reactions with free radicals with different scavenging potentials (173), but the potency of AFMK for scavenging O_2^{-}• is similar to that of melatonin (174).

Melatonin can be oxidized to AFMK and recycle nicotinamide adenine dinucleotide (NADH), which has a key role in metabolism and the antioxidant defense system, therefore producing more ATP at the mitochondrial level (175). Melatonin, a free radical scavenger, protects against neurodegeneration and decreases the neurotoxicity of hydrogen peroxide (176). Amphetamine-induced oxidative stress and neurodegeneration models show that melatonin can protect neurons against degeneration (177, 178). Melatonin scavenging of $OONO^{-}$ and inhibition of the nitrosative stress pathway in endothelial cells has a neuroprotective effect in cerebral ischemia via HtrA2/PED (179) and the Kelch protein 1 (Keap1/Nrf2) pathways (180). Thus, inhibition of $OONO^{-}$-mediated nitrosative stress by melatonin could represent a novel vasoprotective approach for stroke treatment. In addition, nitrosative stress resulting in protein disulfide isomerase dysfunction provides a mechanistic link between deficits in molecular chaperones, the accumulation of misfolded protein, and neuronal demise in neurodegenerative disorders. (181 – 183).

Neuroinflammation is involved in the pathology of neurodegenerative disease through the expression of cytotoxic mediators, especially pro-inflammatory cytokines. Melatonin at 1 nM, which approximates the physiological concentration of the pineal hormone at night, significantly inhibited TNF-α, IL-1β, IL-6, and iNOS overexpression induced by methamphetamine (METH) in microglia (184). In addition, the anti-inflammatory effect of melatonin against these METH-induced neuroinflammatory events also occurs in human neuroblastoma dopaminergic cells (185). The anti-neuroinflammatory role of melatonin acted through the inhibition of activated NF-κB and the stimulation of another transcription factor, Nrf2.When testing drugs for the treatment of neurodegeneration due to inflammation, PPARγ was shown to be an important candidate. PPARγ activation can regulate the inflammatory response and decrease the expression of a variety of proinflammatory genes, such as COX-2, iNOS, and various cytokines (186). Interestingly, the combination of a PPARγ agonist and melatonin caused a very significant reduction in cell number and increased apoptosis in breast cancer cells (187). Thus, the combination of melatonin and the activation of PPARγ may result in important therapeutic breakthroughs for neurodegeneration as well as cancer therapy.

The insulin signaling cascade is important in many tissues for extending lifespan. In mammals, insulin, insulin-like growth factor 1 (IGF1), and insulin-like growth factor 2 (IGF2) promote the MAPK/ERK pathway and, via PI(3,4)P2 and PI(3,4,5)P3, activate protein kinase B (AKT), which phosphorylates many proteins and their downstream pathways. AKT promotes many intracellular processes, especially metabolism homeostasis. Brain levels of IGF1 are reduced in aging. An increase has been documented in hippocampal IGF1 receptors in normal aging and in cortical IGF1 receptors in age-related neurodegenerative diseases such as AD (188). Decreased brain insulin receptor substrate-2 (Irs-2) extends the lifespan in mice (189). The insulin/IGF1 signaling (IIS) pathway alters protein aggregation-mediated neurodegeneration and aging in mammals and non-mammals (190). Although increased IIS activity protects against proteotoxicity, such as Aβ aggregation (191), and shows a neuroprotective effect, decreased IIS slows the aging process (190). Thus, a model has been proposed in which IIS must be at an optimal level, and higher or lower levels than the optimal rate shortens the lifespan. Growth hormone resistance and reduced insulin and IGF-1 signaling extend the mouse lifespan (192). An effect of melatonin on the IIS pathway via the MT_1 receptor has been identified in rat pancreatic islet cells (193), and MT_1 downregulation leads to insulin resistance (194). Melatonin can improve insulin resistance via alteration of pancreatic

gene expression in male mice (195). In the rat brain, insulin receptors that are involved in memory respond to melatonin (196).

IIS-related AKT activation can modulate autophagy in mammalian target of rapamycin (mTOR)-dependent and -independent manners (197) and affects the aging process. mTOR is found in two distinct complexes, mTORC1 and mTORC2 (198). mTOR activation can be modulated by several pathways, including IGF/1-AKT, which is a negative regulator of TSC1/TSC2 and affects lysosomal positioning (199), AMP-activated protein kinase (AMPK) (200), and sirtuin1 (201).

The intracellular degradation processes macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) are important in maintaining cellular homeostasis and lifespan (201). Lysosomal autophagy protects cells against oxidative stress and enhances the degradation of dysfunctional mitochondria (202). Melatonin influences aging by regulating autophagy and mitophagy (203). During dietary and caloric restriction, mTOR signaling is downregulated via the inhibitory effect of the TSC1/TSC2 complex. The inhibition of TOR activity increases autophagy and decreases protein translation via the reduction of ribosomal protein S6 kinase phosphorylation (204) and elF4F and extends the lifespan (205). The deficiency of some key autophagy-related proteins promotes neurodegeneration and the aging phenotype. Axonal dystrophy has been shown in autophagy protein 7 (Atg7)-mutant purkinje cells (206). A deficiency in the autophagy-related protein beclin1 modulates amyloid precursor protein accumulation and promotes neurodegeneration (207). Melatonin protects neurons against the Bcl2/Beclin1 autophagic cell death pathway by the activation of the JNK1/Bcl2 cascade (208) and modulates mTOR activity and 4E-BP1 phosphorylation (209).

Caloric restriction modulates hormonal pathways and regulatory protein levels, including PPARγ, PGC-1α, FOXO, sirtuin1, and uncoupling proteins, and it extends the lifespan. Nicotinamide adenine dinucleotide (NAD^+)-dependent sirtuin, a histone deacetylase, enhances metabolic pathways in several tissues, including the liver, adipose tissues, and pancreas. In the nervous system, sirtuin1 activation upregulates Nmnat1 and nuclear NAD biosynthesis and protects axons from degeneration (210). Mammalian brain sirtuin1, which is mostly expressed in the hypothalamus (211), is increased as a result of caloric restriction (212). In addition to mitochondrial and metabolism modulation, sirtuin1 protects against many neurodegenerative diseases via the prevention of protein aggregation by the deacetylation of retinoic acid receptor β (RAR-β) and the increase in α-secretase (ADAM10) to target Aβ and the deacetylation of tau in AD and by the activation of heat shock factor 1 (HSF1) in Parkinson's disease (213). Melatonin increases the deacetylation of sirtuin1 targets, such as NF-κB, ADAM10, p53, FoxO1, and PGC-1α, in neurons (214).

Protein misfolding and aggregation, as well as the impairment of degradation machineries, are the main characteristic features of age-related neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases. Misfolded proteins are degraded in ER-associated degradation or autophagy (215). The homeostasis state between ER stress and unfolded protein responses, such as misfolded protein degradation and the induction of chaperones, is affected during aging. The ER stress response is induced by the activation of inositol requiring element-1 (IRE-1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6), which interact with the binding Ig protein (Bip) chaperone (216). Bip expression in the cerebral cortex and PERK mRNA expression in the hippocampus reduce with age (217). PERK activation leads to eIF2a and Nrf2 phosphorylation and attenuates protein translation and the anti-oxidant response. ATF6 and IRE-1 activate chaperones and stimulate proteasome-mediated protein degradation. Most of these chaperones, including Grp78, Calnexin, and PDI, are sensitive to oxidative stress and decline with age (217, 218).

Reduced protein clearance also leads to excessive protein accumulation. In the ubiquitin–proteasome system (UPS), misfolded and damaged proteins are tagged for proteasome degradation by activated ubiquitin enzyme (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin protein ligases (E3) (219). The effects of UPS decrease in aged brain (220). As a neurodegenerative protein, α-synuclein, which is cleaved by CMA (221) and the proteasomal degradation system (222), accumulates in autophagy and UPS impairment. These alterations have been observed in sporadic Parkinson's disease (223). Elevated α-synuclein levels lead to reductions in dopamine release (224). In amphetamine-induced neurodegeneration models, melatonin decreases the expression of α-synuclein in dopaminergic neurons (178, 225); and in Alzheimer's models, melatonin can increase α-secretase (ADAM10), which could lead to inhibition of the production of Aβ (214, 226). It has been documented that nervous system growth factors such as BDNF support the survival of neurons, reverse age-related gene expression, and influence plaque formation in Alzheimer's and Parkinson's diseases (227). Melatonin receptor MT_1 and MT_2 agonists increase BDNF in the primary cultures of mouse cerebellar granule cells (228) in a translation-dependent manner. Melatonin increased both the amplitude and the frequency of GABAergic miniature inhibitory postsynaptic currents in cultured rat hippocampal neurons, indicating that melatonin

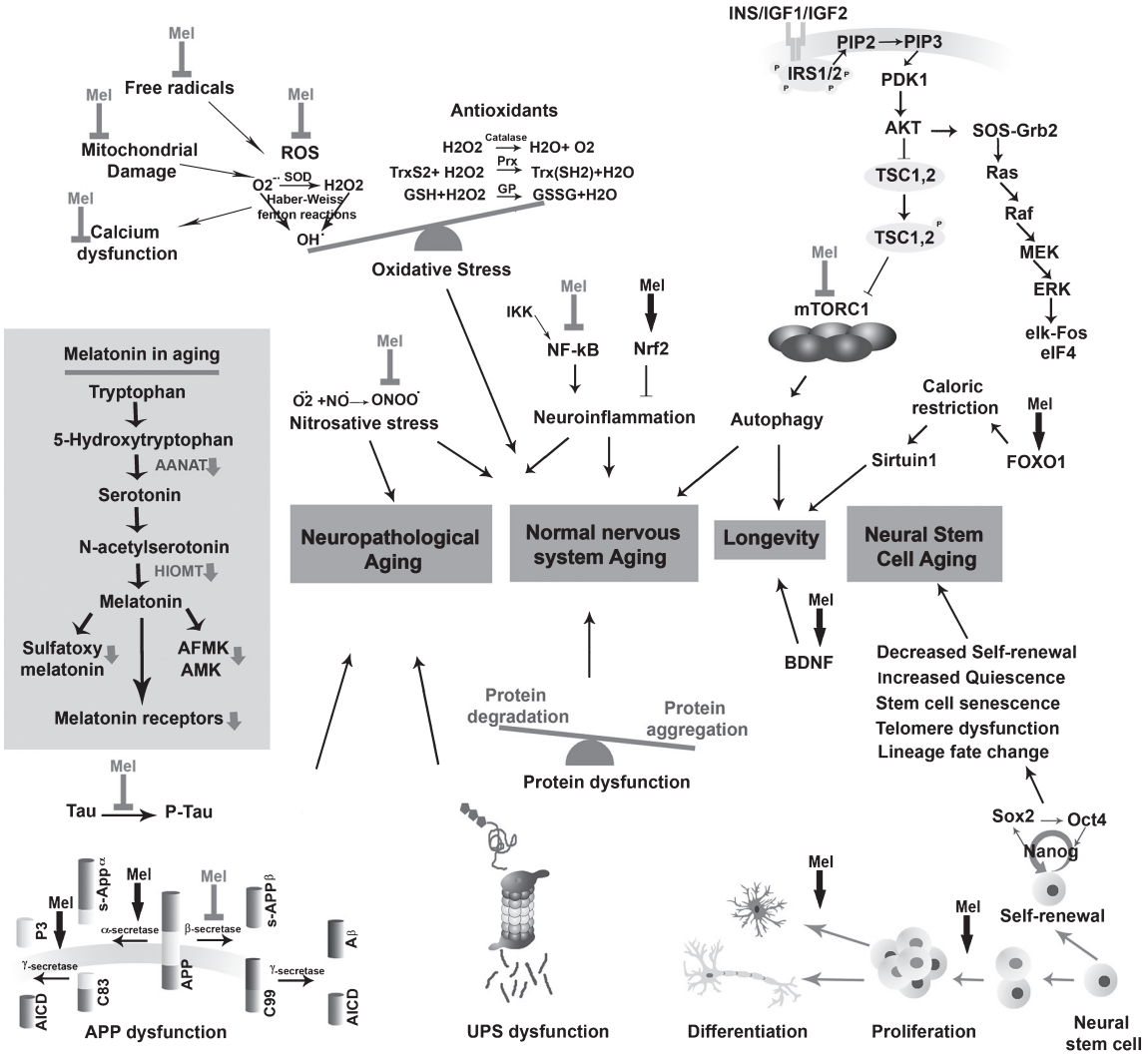

Fig. 1. Mechanisms of nervous system aging and the effects of melatonin. Free radicals and mitochondrial damage promote oxidative stress and nervous system aging when the level of reactive oxygen species (ROS) production is higher than that of antioxidants. Oxidative stress leads to cell damage and calcium dysfunction. Melatonin and its metabolites protect against aging as free radical scavengers. In the nitrosative stress pathway, melatonin has an inhibitory effect during aging. The levels of enzymes, metabolites, and receptors involved in melatonin synthesis decrease during aging, resulting in low levels of melatonin in the elderly. Insulin, insulin-like growth factor 1 (IGF1), and insulin-like growth factor 2 (IGF2) promote the MAPK/ERK pathway and activate PDK1 and protein kinase B (AKT) via PI(3,4)P2 and PI(3,4,5)P3. AKT activates mammalian target of rapamycin (mTOR) and autophagy by inhibition of TSC1 and 2, which promote normal aging and longevity in two ways. Melatonin has an inhibitory effect on autophagy. Caloric restriction promotes longevity via sirtuin1 activation. Melatonin increases the deacetylation of sirtuin1 targets and has a stimulatory effect on the sirtuin1 pathway. In addition to normal aging, protein misfolding, aggregation, and degradation impairment are the main characteristic features of age-related neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Melatonin increases the expression of α- and γ-secretase and decreases β-secretase expression. It also inhibits Tau phosphorylation. In the stem cell niche, depletion of the neural stem cell pool or decreased potential to produce progenitor cells leads to neural stem cell aging via impairments in self-renewal, stem cell senescence, increased quiescence, and neural stem cell (NSC) fate changes. Melatonin increases proliferation and differentiation of NSCs. Mel, melatonin; AANAT, arylalkylamine *N*-acetyltransferase; HIOMT, hydroxyindole-*o*-methyltransferase; P-Tau, phosphorylated Tau; AICD, amyloid-precursor protein intracellular domain; APP, amyloid-precursor protein; UPS, ubiquitin-proteasome system.

enhances GABAergic inhibitory transmission. A loss of hippocampal GABAergic inhibition is believed to underlie the neuronal hyperexcitability in human temporal lobe epilepsy. This suggests a potential pathway for the neuroprotective effects of melatonin (229).

6. Conclusion

Several strategies may be implemented to promote neuronal repair and survival in the neurodegenerative processes. Three main strategies have been proposed by Akwa et al. (230): antagonizing the cytotoxic causal events, stimulating the endogenous protective processes, and promoting the repair of damaged structures. In addition, stimulating neurogenesis via proliferation and/or differentiation also plays an essential role. In this review, we have discussed age-related changes in the nervous system that occur as a result of the mechanisms of impaired neurogenesis and neurodegeneration. Two therapeutic strategies can enhance neurogenesis. The first is transplantation of exogenous NPCs. The second strategy would be to stimulate the proliferation, migration, and differentiation of endogenous NPCs. Neurogenesis is regulated by endogenous factors including chemokines, cytokines, neurotransmitters, and ROS released from damaged neurons, microglia, and astrocytes under neuropathological conditions (231). Understanding the molecular and epigenetic mechanisms of reduced neurogenesis plays an important role in enhancing neural repair in the elderly and helps improve cognitive performance. Although many scientific documents have revealed metabolism-based mechanisms that deteriorate longevity and enhance neurodegeneration, the boundary between normal aging and age-related neurodegenerative diseases remains to be determined. In addition to the required therapeutic advances in geriatric medicine, the prevention of normal age phenotypes and disabilities by anti-aging factors has been investigated. We have used a step-by-step evaluation of investigations into the effects of melatonin on the aging process to demonstrate the proposed anti-aging role of this substance. Its potential role in brain aging is illustrated in Fig. 1. In the future, a greater understanding of the molecular and epigenetic aging and anti-aging mechanisms will be necessary to increase the human lifespan under healthy conditions.

Acknowledgments

This study was supported in part by the Thailand Research Fund (TRF) and a Mahidol University Research Grant to PG.

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