Melatonin, Neurogenesis, and Aging Brain

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Abstract: Aging is an irreversible process affecting all living organisms. During aging, all organs and tissues of the body reduce their functional capabilities, leading to a progressive loss of physical and cognitive performances. The loss of cells with age is directly related to apoptosis, a mechanism of cell death linked to mitochondrial failure. Attempting to understand the aging process, several theories have been proposed; among them, the mitochondrial free radical theory of aging is the one with more experimental evidence to date. Mitochondria are the source but also the target of these radicals, which produce their age-dependent slow and continuous damage.

It is now recognized that mitochondrial impairment underlies not only the aging process but also many other age-associated pathologies, including neurodegenerative diseases, cancer, metabolic alteractions, etc. These diseases, mainly neurodegeneration, share mitochondrial dysfunction, oxidative/nitrosative stress and apoptosis in particular brain areas as a final common pathway. Consequently, neuronal loss may be associated to mitochondrial dysfunction in those disorders. Although the vast majority of cells in the adult brain are generated during the embryonic and early postnatal period, it is now apparent that some proportion of neurogenesis is also happening during adulthood. The functional significance of the adult neurogenesis is not fully understood and recent data point to a role of mitochondria in the process of stem cell differentiation. Thus, understanding mitochondrial function and regulation should be the first line of research to know how neurogenesis is regulated.

Melatonin is an endogenous indoleamine take up by the mitochondria; once inside, melatonin promotes energy production and reduces free radical generation, thus preventing mitochondrial damage. These functions of melatonin seem to be related to the neurogenesis promoting role of the indoleamine. The physiological and pathophysiological meanings of these functions of melatonin are also revised here.

Keywords: Brain, mitochondria, aging, oxidative stress, inflammation, melatonin therapy, neurogenesis.

REACTIVE SPECIES, MITOCHONDRIA AND AGING

Aging is a progressive and complex procedure during life that include progressive cellular loss, endocrine and metabolic deficits, decreasing defense mechanisms and functional losses that increase the prevalence of diseases and the risk of death. To try to explain and understand this multifactorial process, several theories of aging have been promulgated including the free radical theory of aging postulated by Harman [1], which was refined by the mitochondrial free radical theory of aging postulated by Miquel [2] and complemented by the inflammatory hypothesis of aging postulated by Chung and colleagues [3].

Free radicals are molecules with unpaired electrons, so these molecules are highly reactive and they are able to chemically modify other molecules. A group of free radicals and other highly reactive molecules are the reactive oxygen species (ROS) mainly represented in the cell by superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$). The main source of ROS generation in the cell is mitochondrion, an organelle that plays a central role in the control of O_2 consumption and ATP production through the oxidative phosphorylation (OXPHOS). Mitochondrial respiration coupled to OXPHOS consists in the oxidation of $NADH+H^+$ and $FADH_2$, which are produced by glycolysis, Krebs cycle, and β-oxidation of fatty acids, by the mitochondria respiratory chain, transferring electrons from these precursors to O_2 through the four complexes of mitochondrial respiratory chain. Then, one molecule of O_2 is reduced by four electrons in the mitochondrial complex IV, producing two molecules of H_2O. The intermediate steps of O_2 reduction result in the formation of $O_2^{\cdot-}$, H_2O_2, and $HO^{\cdot}$, corresponding to reduction by one, two, and three electrons, respectively. Under physiological conditions about 1-2% of the consumed O_2 is transformed to ROS [4]. These physiological ROS act as intracellular signaling molecules but also can oxidized DNA, lipids and proteins, provoking an oxidative damage that can be accumulated over time [5]. To avoid oxidative damage, mammalian cells possess a refined antioxidant defense system constituted by molecular antioxidants and various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione

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peroxidase (GPx), and glutathione reductase (GR) [6]. However, ROS generation may increase as a consequence of normal and pathobiological aging and antioxidative defenses may diminish in effectiveness during aging [7, 8]. The result is an increase in ROS in the intracellular environment that may accelerate cell damage and apoptosis, leading to the decline of tissues function [9, 10].

Several evidences have showed the relation between aging, increased oxidative damage and mitochondrial dysfunction (Fig. **1**). Three factors make mtDNA particularly vulnerable to reactive species: the mtDNA is located close to the mitochondrial inner membrane, just near from the generation of ROS; mtDNA is not extensively condensed and protected by histones; the mtDNA repair is limited [11]; and the expression of the entire mtDNA is essential for the maintenance of mitochondrial bioenergetic function, while only about 7% of the nuclear genome is expresses during cell differentiation [12]. Then, mtDNA point mutations and duplications in tRNA, protein-coding genes and D-Loop have been found to accumulate in some post-mitotic tissues during human aging [13-15]. However, it seems that the proportion of mutant mtDNAs is too low to cause a significant impact on mitochondrial function in aging tissues. On the other hand, the distribution of the mutant mtDNA in the cell and tissue is still unknown and the answer of this matter could resolve important questions regarding the importance of mtDNA mutation in aging [16]. Additionally, mitochondria polymerase γ (POLG) deficient mice accumulate high levels of mtDNA mutations resulting in a premature aging phenotype without increase of ROS generation and oxidative damage [17, 18]. Then, these results would support the idea of a direct involvement of mtDNA mutations in aging but would cast doubt on the vicious cycle theories of aging and oxidative stress [19]. However, some explanations have been proposed to account the lack of oxidative stress in POLG deficient mice: aging is the result of alterations in many pathways; alterations in POLG may be downstream from mechanisms that generate ROS; and extensive mtDNA mutations could prevent the generation of ROS [19]. On the contrary of the POLG mutator mice, skin fibroblasts harboring mtDNA point mutation associated to aging show an alteration in the expression profile of antioxidant enzymes [20]. Similarly, transmitochondrial cybrids harboring homoplasmic mutations in mitochondrial tRNA genes showed an increase of ROS production and an increase in the antioxidant enzyme activities when they were grow under glucose-rich medium [21]; cell cybrids harbouring homoplasmic mutations (Leber's hereditary optic neuropathy mutations) in mtDNA encoding subunits of complex I (ND1, ND4 and ND6) showed alterations of the antioxidant defense when the cells were grow under galactose medium (which forces energy production through oxidative phosphorylation [22]), but not under glucose-rich medium [23]; and cell cybrids harbouring mutations in ATP6 (T8993G and T8993C) have showed variable grade of oxidative stress depending on the severity of the mutation, the level of heteroplasmy and the culture conditions [24].

Age-related mtDNA deletions have been also detected in humans with a dissimilar pattern in different tissues [25], and the correlation of the increased of mtDNA deletions and

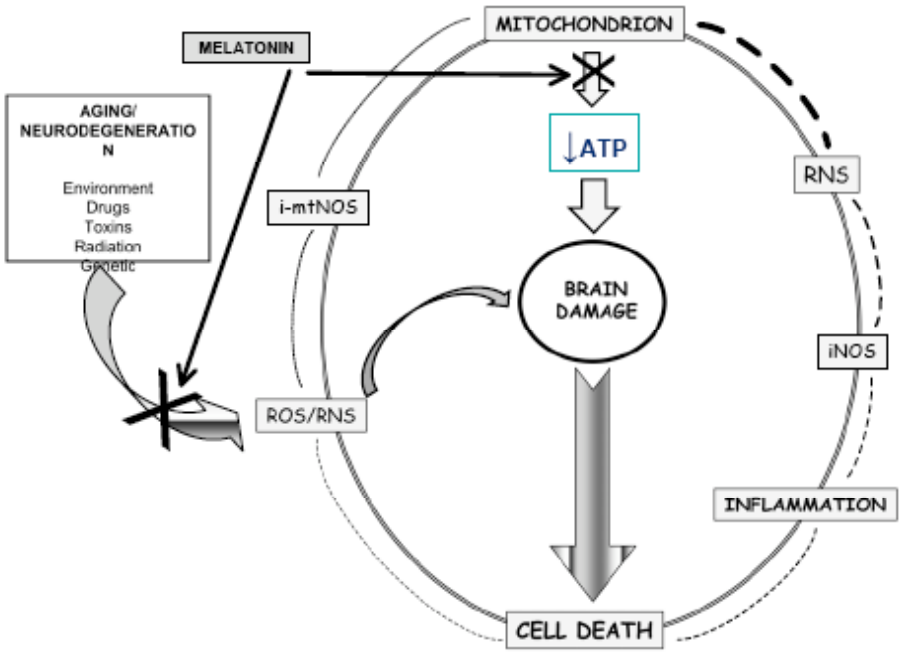

Fig. (1). Brain aging is a vicious cycle in which Reactive oxygen (ROS) and nitrogen (RNS) species generated by different factors produce brain damage and cell death. In turn, cell death may induce an inflammatory reaction and induction of inducible nitric oxide synthase (iNOS), responsible for the production of an excess of nitric oxide (NO). Additionally, the mitochondrial isoform of the inducible mitochondrial NOS (i-mtNOS), is also activated and produces high levels of NO and peroxynitrite, a highly toxic compounds that produce irreversible damage to the mitochondrial respiratory complexes. This leads to an ATP depletion, increase brain damage and cell death. Melatonin is an excellent antioxidant and mitochondrial protector that prevents/counteracts the generation of ROS/RNS and improves mitochondrial function, maintaining the ATP production and cell survival.

mitochondrial respiratory chain malfunction during aging has been amply reported [26-28]. The mechanisms of mtDNA deletions during aging are still controversial but oxidative damage to DNA associated to single or double-stranded breaks has been proposed. This idea has been supported for some studies: the relative amount of mtDNA deletions correlates with the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) [29]; and treatment of human skin fibroblasts with sub-lethal dose of oxidative substances and environmental insults inductors of ROS results in the formation and accumulation of the 4977 bp deletion in mtDNA [30, 31].

In addition to the research related to mtDNA alterations, mitochondrial protein oxidation has been also reported in a variety of organisms during aging [16]. The proteins containing Fe-S cluster seem to be the most susceptible to oxidation [16]. Then, several reports have revealed that oxidation of aconitase, adenine nucleotide translocase and mitochondrial respiratory chain complexes may occur during aging and consequently the activities of these enzymes may diminish during aging [32-34]. Oxidative injury is not limited to mtDNA or proteins but also to mitochondrial membranes. This may lead to a progressive lipid peroxidation (LPO) and cross linking damage, with simultaneous changes in the respiration rate, ATP synthesis, membrane fluidity and permeability, Ca^{2+} homeostasis and apoptosis [35]. Alterations in the expression and activities of the antioxidant enzymes in response to the oxidative ambient in the aging cells has been found in human blood [36-38] and muscle [39, 40], and in a variety of tissues from rats and mice, including skeletal muscles, brain and heart, which are tissues with high energy demand. Additional information about the physiological changes in mammalian aging has been revealed by the studies performed in the senescence-accelerated mouse (SAM) [41]. SAM includes two strains, one prone to accelerated senescence (SAMP) and one resistant to accelerated senescence (SAMR). SAMP8, a sub-strain of SAMP, shows relatively strain specific age-associated phenotypic pathologies such as a shortened life span and early manifestation of senescence (including loss of activity, alopecia, lack of hair glossiness, skin coarseness, periphthalmic lesions, increased lordokyphosis and systemic senile amyloidosis), similar to several geriatric disorders observed in humans [41, 42]. SAMP8 mice show a general hyperoxidative status manifested by increased mitochondrial electron leakage and ROS production, increased LPO and protein carbonyl content, changes in the antioxidant enzymes activities and increase of GSSG:GSH ratio [43-49]. The results are a decrease in the mitochondrial respiratory chain activity, ATP synthesis and energy status of the organism, suggesting that the mechanism of senescence acceleration in SAMP8 mice is related to free radical damage [50, 51]. SAMP8 mice also show an age-dependent increase in IFN-γ and TNF-α, a reduction in IL-2 levels and an increase on nitric oxide (NO•) levels [52], suggesting the existence of an inflammatory process during aging. The increase of NO• levels is particularly relevant since it can react with (O_2^{-}) in mitochondria yielding peroxynitrites [53], which irreversible impairs the mitochondrial respiratory chain and decrease the efficiency of the oxidative phosphorylation, leading to energy depletion and cell death [54, 55].

BRAIN, AGING AND AGED-RELATED DISORDERS: NEURODEGENERATIVE DISEASES

Mammalian brain is a tissue with high energy demand and it posses very active mitochondria metabolism with high oxygen utilization (20% of the total oxygen inspired). Consequently, the reactive oxygen generation in the brain is very remarkable. In addition, brain is very susceptible to free radical damage because of its high concentrations of polyunsaturated fatty acids [56] and transition metals such as iron, which is involved in the generation of the hydroxyl radical [57], and low concentrations of cytosolic antioxidants [8, 58]. Remarkably, it has been shown that the cognitive functions, motor ability, exploratory capacity and neuromuscular coordination in mice are decreased upon aging in parallel to an increase of protein oxidation and a decrease of the mitochondrial complexes activities from the brain of these animals [59, 60]. Oxidative modifications of mtDNA may be of central importance in normal and pathobiological aging. In fact, human brain accumulates the "common" 4977-bp mtDNA deletion [61], especially in the caudate, putamen, and substantia nigra [62], where neurons with high mutation loads can be COX-negative [26, 27].

Brain of SAMP8 mice show an alteration of the nitrosative and oxidative environment resulting in a reduction in mitochondrial respiratory chain activities and decrease of the ATP levels [63]. This mitochondrial malfunction seems to be a consequence of the mitochondrial oxidative damage accumulated during aging [50, 51, 63], as well as the existence of an inflammatory process during aging with the subsequent production of reactive nitrogen species (RNS) [64]. This inflammatory process is associated with an increase of iNOS but a decline in nNOS has been also observed in some brain areas from old rats [65]. Taken together, these changes may impair energy-dependent neurotransmission, contributing to senescence-related decline in memory and other brain functions that are apparent in this mouse [42].

Aged-related disorders including neurodegenerative diseases of different etiologies may share mitochondrial dysfunction, oxidative/nitrosative stress and apoptosis in particular brain areas as a final common pathway. Consequently, neuronal loss may be associated to mitochondrial dysfunction in those disorders. Parkinson's disease (PD) is a mainly sporadic late-onset disorder characterized by bradykinesia, rigidity and tremor. PD is accompanied by the loss of about 60% of dopaminergic neurons of the *substantia nigra pars compacta*. Mitochondrial involvement in PD is suggested by deficiencies of complex I (C-I) in *substantia nigra* [66], with a parallel reduction in GSH levels, suggesting the existence of oxidative stress. In platelets of PD patients C-I is also decreased, and in some cases is accompanied by complex II (C-II), complex III (C-III) and complex IV (C-IV) deficiencies. Studies with cell cybrids have shown that alterations in C-I is due to a defect in the mtDNA [66]. This defect is accompanied by an alteration in the expression of C-IV activity and a reduced mitochondrial potential membrane, which lowers the apoptotic threshold. Mitochondrial involvement in the pathology of PD has been genetically supported by the finding of *POLG* mutations in early-onset Parkinsonism in different families [67, 68]. In some cases, the *POLG* mutations were accompanied by mtDNA deletions, ragged-red and cytochrome c oxidase-

negative fibers and low activities of mitochondrial complexes containing mitochondrial DNA-encoded subunits [67, 68]. On the contrary, a recent review of the evidence for primary mtDNA mutations in PD led to the conclusion that there is no convincing proof for a primary role of mtDNA mutations in this neurodegenerative disorder [69]. However, a series of nuclear genes (*PARK2*, *PARK7*, *PINK1*, *SNCA*, *LRRK2* and *HTRA*2) are recognized to be associated with the familial form of PD and the proteins encoded by these genes interact directly or indirectly with mitochondria and seem to be involved in apoptosis [70]. Moreover, some environmental toxins seem to interact with the products of these genes, which provokes oxidative damage, mitochondrial dysfunction and cell death [71]. These environmental toxins influence PD, as shown by the C-I inhibitory effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone and paraquat. The C-I inhibition is prevented by free radical scavengers indicating oxidative damage to C-I. Moreover, MPTP also stimulates NMDA-dependent nNOS activity thereby increasing NO• production [72], and decreasing the content of mtDNA [73].

Alzheimer's disease (AD) is a predominantly sporadic late-onset disorder characterized by progressive dementia with a relatively long course. Progressive neuronal loss, particularly in the cortex and the hippocampus, is typically observed in the brain of Alzheimer patients. The two main histopathological features of AD are the accumulation of extracellular neuritic plaques, mainly represented by β-amyloid (Aβ), and of neurofibrillary tangles, mainly represented by the hyper-phosphorylated forms of the microtubule-associated protein tau [74]. Additionally, some evidence support the notion that mitochondria are involved in the pathology of AD, including reduction in brain energy metabolism shown by positron emission tomography [75], defects of mitochondrial metabolic enzymes [76, 77], and mitochondrial respiratory chain complexes deficiency [78, 79]). However, a recent review found little evidence in support a role of mtDNA mutations in the development of AD [69]. Furthermore, it has been shown that β-amyloid peptide generates ROS in a metal-catalyzed reaction, which induces neuronal cell death in a ROS-mediated process resulting in damage to neuronal membrane lipids, proteins and nucleic acids. This suggests that the use of antioxidants such as vitamin E, melatonin or estrogens may be beneficial in AD [80, 81].

Amyotrophic lateral sclerosis (ALS) is a late-onset sporadic disorder clinically characterized by progressive muscle weakness, atrophy, spasticity widespread paralysis and premature death. The disease is caused by the degeneration and death of upper and lower motor neurons in the cortex, brainstem, and spinal cord [82]. About 5%–10% of patients have a familial form of ALS (FALS), and about 20% of these harbor mutations in the *SOD1* gene that encodes the Cu,Zn-superoxide dismutase 1 (SOD1) [82]. Mouse models overexpressing mutant SOD1 also develop motor neuron degeneration. Most pathogenic mutations do not impair SOD1 activity, and some studies have proved that a portion of mutant SOD1 is localized in mitochondria, both in FALS patients and in the animal models. Then it has been hypothesized that mutant SOD1 may damage mitochondria by some misunderstood mechanism [82].

Huntington's disease (HD) is a neurodegenerative disorder characterized by ataxia, chorea and dementia. It is known to be caused by an alteration in a gene for nDNA encoding huntingtin, a widely expressed protein of unknown function but associated with inappropriate apoptosis. The pathology of HD involves mainly the GABA-containing neurons of the caudate nucleus and putamen [66]. Excitotoxicity has been suggested to play an important role in this disease. This includes activation of NMDA-dependent neuronal nitric oxide synthase (nNOS) and NO• production. NO• and particularly peroxynitrite mediate the oxidative damage. Increase of mtDNA copy number and multiple mtDNA depletions have been found in HD patients, which are especially common in the frontal and temporal lobes of the cerebral cortex, although its significance is unclear [83, 84]. These mtDNA alterations could be a consequence of the increase of the oxidative damage to DNA reflected by an increase of the levels of 8-hydroxydeoxyguanosine [83]. There are also deficiencies in the activities of C-II, C-III and C-IV in caudate and in a lesser extend in putamen in HD. Others mitochondrial abnormalities have been found in cells and animals models of HD, which include calcium dyshomeostasis and anomalous mitochondrial dynamics [83].

ADULT NEUROGENESIS IN THE AGING BRAIN

The stem cells can be classified in three types: the pluripotent embryonic stem cells (ESC) that have the potential to differentiate into any cell type in the organism; the multipotent cells derived from adult tissue including umbilical cord blood and amniotic fluid, which can differentiate into a limited number of cells types of their own lineage, e.g., mesoderm; and precursor cells, which are adult stem cells committed to differentiation. In the brain, both neural stem cells and neural progenitor cells are responsible to neurogenesis. Neural stem cells are able to produce additional stem cells as well as offspring that go on to differentiate into oligodendrocytes, glial cells and neurons. Neural progenitor cells have a limited replicative potential that are committed to the neuronal lineage [85]. The vast majority of cells in the adult CNS are generated during the embryonic and early postnatal period, but some proportion of neurogenesis is also happening during adulthood. The functional significance of the adult neurogenesis is not fully understood but it has been shown to be involved in several brain function and pathologies [85, 86]. Neurogenesis in the adult brain from mammalian is concentrated in the subventricular zone (SVZ) of the lateral ventricule wall and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus [85]. New cells generated from these regions migrate toward their final destinations, where they differentiate into mature cells and are integrated into the central nervous system (CNS) [85, 87].

Age-related changes have been observed in both SVZ and SGZ, which is associated to a decline of neurogenesis during aging [85, 86]. These changes may affect the different steps on neurogenesis: 1) proliferation of new cells; 2) survival of newly born cells; 3) migration of these cells toward target areas; and 4) differentiation into mature functional cells. Neural stem cell proliferation has been widely studied in rodents by use of cell proliferation markers such as bromo-deoxyuridine (BrdU) and tritiated thymidine [85, 87]. Most results have shown that cell proliferation is

declined by aging in both SVZ and SGZ [85, 87]. However, it is not clear the difference in the proliferation decline between middle age and senescence since some studies have reported significant changes between both ages [88, 89] but others did not [90, 91]. On the contrary, the short-term survival pattern in the newborn cells seems to be unaffected by aging [86]. However, when some brain area is damaged the survival of newborn cells show clearly differences between young and old animals. In one study of stroke simulation, rats subjected to ischemia showed an induction of neural stem cell proliferation in the SGZ. One day after the ischemia induction young adults showed 5.7 fold increase of BrdU labeled cells compared to 10.6 fold increase in old adults. Remarkably, 65.5% of the labeled cells survived after 28 days of the ischemia in young adults, whereas in old adult brains only 15.3% remained [92]. In other study, Zaman and Shetty [93] observed that when hippocampi were first damaged by the action of kianic acid, an specific agonist for kainate receptors with neuroexcitotoxic and epileptogenic properties, the survival of rat fetal hippocampal cells injected into the ventricles of rats were of 30% in middle-aged and old brains compared with the 72 % survival in young brains. Both studies suggested that changes in the environment of the brain areas during aging may be critical for the survival of new cells [92, 93]. Then, the increases of glucocorticoids and the decreases of the levels of Insulin-like growth factor 1 (IGF-1) in aged brain has been associated to a decrease in neurogenesis [94-96]. Interestingly, oxidative stress, which is particularly increased in the rat hippocampus during aging [97], also may play an important role in neural and progenitor stem cell survival. It has been shown that curcumin, and antioxidant and anti-inflammatory agent, can modulate the proliferation of embryonic neural progenitor cells with a biphasic effect in cultured cells. In mouse brain, curcumin administration resulted in a significant increase in the number of newly generated cells in the SGZ of hippocampus [98]. Oxidative stress can disrupt the differentiation of oligodendrocyte precursors and neural progenitor cells into mature oligodendrocyte and neurons [99, 100]. On the contrary, the increase of the antioxidant capacity protects neural progenitor cells *in vitro* and potentiates the formation of cellular networks that provide neuroprotection *in vivo* [100, 101]. An increase in newly generated neural cells in adult brain and an increase in the resistance of neurons to dysfunction and apoptosis have been detected in rodents undergo dietary restriction. Both oxidative stress and dietary restriction are interlinked and related to mitochondrial function. However, few studies have focused in the role of mitochondria in the proliferation, survival and differentiation of neural stem cells, and most of them are related with the mitochondrial apoptotic pathway induced by some toxic agent [102-105]. Interestingly, some studies in embryonic stem cells (ESC) have focused on the activity of mitochondrial genome and of these data could be extensive to neural stem cells. Transmitochondrial embryonic stem cells harboring pathogenic mtDNA mutations have shown to be compromised in neuronal differentiation when the mitochondrial respiratory chain function is severely affected [106]. Thus, some authors have hypothesized that stem cell competence may be verified using functional mitochondrial characteristics [107]. Differentiation of mouse and human ESC results in changes in mitochondrial structure, morphology and pattern of cytoplasmic localization. Mitochondria in stem cells tend to localize perinuclearly [107]. Moreover, ESC have relatively few mitochondria with poorly developed cristae [108, 109], and restricted oxidative capacity. As cells are allowed to differentiate, the number of mtDNA copies increase and these differentiated cells contain increased numbers of mitochondria with distinct cristae, dense matrices and high membrane potentials. These features suggest the initiation of metabolic activity through OXPHOS [110]. Because ESC display low oxygen consumption and thus, poor OXPHOS, an elevation in ATP content per cell may therefore reflect a loss of stemness and the subsequent onset of differentia-tion [107, 109]. Therefore, preservation of immature mitochondria with a perinuclear arrangement, reduced expression of OXPHOS enzymes and low metabolic activity in ESC has led to the suggestion that these mitochondrial properties might be important for the maintenance of pluripotency and should be considered as another ESC marker. Departures from this profile indicate that cells are differentiating or perhaps becoming senescent.

The increase in mitochondrial mass is accompanied by elevated ATP production and, thus, by a greater generation of ROS. Undoubtedly, the intracellular levels of ROS are higher in differentiated than in undifferentiated ESC, due to the increase in OXPHOS metabolism in the former [111]. An increase in ROS levels might have a role in cell signaling and regulation of proliferation and differentiation. Exposure to low levels of ROS has been reported to enhance ESC differentiation whereas continuous exposure to high levels of ROS results in inhibition of differentiation [111]. Therefore, differentiating cells probably activate effective antioxidant systems, including catalase, GPx and others. In summary, successful differentiation of embryonic cells *in vivo* or ESC *in vitro* involves initiation of mtDNA transcription and replication, an increase in the number of mitochondria, and regulation of the enzymes required for aerobic metabolism in order to fulfill the elevated ATP requirements of fully differentiated cells.

The migration and differentiation of neural and progenitor stem cells may also be affected with aging. It has been reported that the capacity of the newly born cells to migrate from SGZ to the granule cell layer is decreased with aging [112]. Several studies have also shown different grades of reduction in the differentiation of neural stem cells into neurons [86] and the reduction in the dendritic maturation during aging [86, 88].

ADULT NEUROGENESIS IN NEURODEGENERATIVE DISEASES

In addition to the alterations in neurogenesis during physiological aging, some insults can also affect dramatically to this process. Neurodegenerative diseases are characterized by loss of neurons and newly generated neurons should appear in the damaged area to repair this injure. However, recent studies have evidenced alterations in neurogenesis in neurodegenerative diseases.

Cell proliferation is significantly repressed in SVZ of PD patients and animals models of PD, resulting in a decrease in the numbers of neural stem cells and neuroblasts.

Importantly, this decrease is more significant in PD patients with cognitive impairments than in those without [113]. AD is characterized by the accumulation of Aβ that suppresses the proliferation of neural stem and progenitor cells and the neuronal differentiation in cell culture [114]. Mouse models of AD with accumulation of Aβ and mice given Aβ intravenously show a defect in neuronal production, survival and differentiation in the SGZ, as well as migration of neuroblasts [115-117]. Other prove of the involvement of neural stem cell in the pathology of AD is the fact that long term administration of cholinesterase inhibitors, which improve cognitive function in AD patients, promotes the survival of newly generated neurons and increases neurogenesis in adult mice [118]. The pathophysiology of HD engrosses the atrophy of the caudate nucleus and putamen, which are adjacent to the SVZ. Related to that, Curtis and colleagues observed an enhanced thickness of the SVZ together with an increase of cell proliferation in the brain of HD patients [119, 120]. Later on, Batista and colleagues reported that the ability of neural stem cells dissociated from the SVZ of the R6/2 mice, a mouse model of HD, to self-renew increases in parallel with the progression of the disease. Likewise, they observed the presence of migrating neuroblasts and newly generated neuron in the striatum of these mice. However, the migration of neuroblasts toward the olfactory bulb was significantly suppressed [121]. On the contrary to the SVZ, the cell proliferation and neurogenesis on the SGZ are decreased in the mouse models of HD [122, 123], while the relation of these findings with the neuropathology of DH has not been clarified.

Independently of the involvement of neurogenesis in the pathology of neurodegenerative diseases, the stem cell research focused in the cell replacement therapy is a promising treatment for neurodegenerative disorders. Different strategies are currently being examined for the treatment of neurodegenerative disorders using neural stem cells, including approaches involving transplantation of exogenous cells or promoting proliferation of endogenous cells. In both cases it is believed that increase of neural stem cells in the brain attenuates anatomic and functional deficits associated with the disease of the CNS via cell replacement, release of specific neurotransmitters and production of neurotrophic factors that protect injured neurons and promote neuronal growth.

In the mouse models of HD, the administration of basic fibroblast growth factor (bFGF) increased the SVZ cell proliferation and increased migration of neuroblasts to the striatum and the regeneration of the striatal projection neurons [124]. The result was an amelioration of the motor dysfunction and the increase of the life-span of these mice. The increase of hippocampal neurogenesis by the enrichment of the mouse environment also delayed the progression of HD symptoms in the mouse model [125]. Recently, mouse neural stem cells were transplanted intraventricularly into R6/2 HD mouse model combined with dietary trehalose, which reduces cellular aggregate formation. The combined treatment resulted in an improvement of motor function, reduction in aggregate formation and increase of the life-span of the animals [126]. However, the information related to the migration and the survival of the grafted neural stem cells was not provided in this study [127]. Previously, it was shown that fetal cell transplantation ameliorated neuronal dysfunction and improved motor function in both HD mouse model and HD patients [128, 129].

Stem cells transplantation has been also experimented in animals models of ALS. Transplantation of neural stem cells isolated from fetal spinal cord or neurons generated from the NT2 human teratrocarcinoma cell line in spinal cord of ALS mice were effective in the functional improvement and in the delay of the progression of the disease [130, 131]. The transgenic SOD1/G93A mouse model of ALS was transplanted with human neural stem cells overexpressing vascular endothelial growth factor (VEGF) resulting in a functional improvement and extended survival. The immunohisto-quemical analysis demonstrated that the transplanted neural stem cells migrated into spinal cord anterior horn and differentiated into motoneurons [132]. Thus, a clinical trial of mesenquimal stem cells transplantation in ALS patients has recently finished the phase I and is currently underway on phase II [133].

Recently, the effect of neural stem cell transplantation has been evaluated in a mouse model of AD (3xTg-AD) that express pathogenic forms of amyloid precursor protein, presenilin, and tau [134]. The results showed that hippocampal neural stem cell transplantation rescues the spatial learning and memory deficits in aged 3xTg-AD mice. However, these improvements were not associated to the alteration of Aß or tau pathology. Interestingly, the mechanism underlying the improved cognition involves an augmentation of hippocampal synaptic density, mediated by brain-derived neurotrophic factor (BDNF) [134]. To further confirm this result, aged 3xTg-AD mice were treated with recombinant BDNF, which was able to mimic the beneficial effects of neural stem cell transplantation. On the contrary, depletion of neural stem cells-derived BDNF failed to improve cognition or restore hippocampal synaptic density [134].

Transplantation of human fetal ventral mesencephalic cells into the striatum of PD patients has been carried out since early 90s in patients with advanced disease [127]. However, the evidences of the poor survival of the transplanted cells into the brain together with the difficulties to obtain enough fetal tissue for transplantation have lead to redesign the stem cell therapeutical strategy in PD [127, 135]. Then, dopaminergic neurons have been generated from embryonic stem cells, mesenquimal stem cells and neural stem cells following different experimental protocols [127]. Dopaminergic neurons generated from monkey embryonic stem cells and human neural stem cells have been transplanted into striatum of monkeys with PD induced by MPTP. The results showed a behavioral improvement of the PD monkeys [136, 137]. Transplantation of immortalized neural stem cells into the striatum of a rat model of PD also induced functional improvement [138]. Other strategies include the transplantation of neural stem cells transfected with specific genes such us hydroxylase (TH) and GTP cyclohydrolase I (GTPCH1) [127].

The use of stem cells for therapeutical purpose is a promising strategy but several issues must be clarified before of the general use in clinical medicine. The concerns include: 1) it must be verified which type of stem cell is most suitable for each purpose; 2) Stem cells that escape differentiation

and selection processes might expand and produce tumor in the graft site following the transplantation; 3) Highly purified populations of neural cell types derived from embryonic or neural stem cells may contain other neuronal or glial cells types that could generated unpredictable interactions among grafted cells or host cells; and 4) Earliest studies have demonstrated that the long-term survival and phenotypic stability of stem cell-derived neurons or glial cells in the graft following transplantation are unsuccessful [127]. Then, it is necessary to establish which factors are involved in the poor survival and stability of the transplanted cell. Among them, the highly toxic oxidative and nitrosative environment in the aged brain and its interaction with mitochondrial function should be taken into account.

MELATONIN, BRAIN AGING AND NEURODEGENERATION

Melatonin is an ancient and highly conserved indoleamine derived from tryptophan, present from one-cell organisms to mammals [139, 140]. In mammals, melatonin is synthesized by the pineal gland in a circadian manner and it is released to blood, where it can concentrate up to 0.5 nM [141]. However, melatonin production in the pineal gland declines progressively with age. Then, old animals and elderly humans the levels of melatonin available to the organism are a proportion of that of young individuals [142]. Melatonin is also produced in most of the tissues and organs of the body and this extrapineal production of melatonin is much more higher than the produced by the pineal [143]. The expression of genes of the key enzymes for melatonin synthesis, N-acetyl-transferase (NAT) and hydroxyindol-O-methyl-transferase (HIOMT), has been confirmed in many the organs [144]. These different sources of melatonin associated to different indoleamine levels in the body are related to the different actions of the indoleamine, i.e. a relationship between melatonin content in different tissues is inversely related to the oxidative damage in the tissue [145]. Interestingly, melatonin is concentrated by subcellular compartments including nucleus and mitochondria, the latter showing 100-200 times more melatonin than cytosol [146, 147]. This means that melatonin would be available at the sites in which free radicals are being maximally generated, thus decreasing the potential damage [148, 149]. Moreover, the high levels of melatonin in some tissues and body fluids support its antioxidant and free radical scavenger ability. Numerous studies have showed that melatonin is able to directly scavenge free radicals, stimulates other antioxidant systems of the cells and inhibits the expression of iNOS with the subsequent reduction in the levels of RNS [6, 35, 150]. Then, the use of melatonin in aging and aged-related disorders has been proposed (Fig. **1**).

Unlike other antioxidants, melatonin is readily available to the brain after oral ingestion [151]. Thus, the effect of melatonin in brain oxidative stress and mitochondrial dysfunction has been tested in the SAMP8 mice [63]. Chronic melatonin administration in the drinking water during 9 months (10 mg/kg b.w.) completely prevented the mitochondrial impairment, maintaining or even increasing ATP production. Likewise, melatonin prevented the increase of mitochondrial LPO and increased GPx and GRd activities normalizing the GSSG/GSH ratio [63]. The immune function in the brain is other important factor in aging. Nitrite levels accurately reflect the nitrosative stress status that is caused by inflammation. Importantly, age-dependent nitrosative status in brain mitochondria was prevented by melatonin administration [63]. The effect of melatonin in the reduction of nitrosative stress has been amply studied in animal models of sepsis. The administration of pharmacological doses of melatonin in rodents with sepsis induced by lipopolysacharide injection or cecal ligation and puncture (CLP) produced a decrease in the expression and activity of iNOS, and consequently nitrite levels, nitrosative/oxidative stress and mitochondrial function were normalized [152-156]. Interestingly, the increase of iNOS expression was more pronounce in aged rats (18 m.o.) than young rats (3 m.o.) but melatonin was able to reduce the expression at the same levels in both groups [152, 157].

The properties of melatonin are also interested for the treatment of neurodegenerative diseases. In mouse models of PD induced by MPTP melatonin administration normalized complex I activity and oxidative status in mitochondria from *substantia nigra* and striatum. Looking for the targets of melatonin action, it was recently shown that melatonin reduced the activity of the mitochondrial iNOS (i-mtNOS), thus decreasing mitochondrial NO• levels, preventing the respiratory inhibition produced by NO• at the level of complex IV [158]. Melatonin also protects against excitotoxicity by reducing the autoxidation of dopamine (DA) which occurs in PD [159]. These effects were demonstrated in MPTP-induced PD in mice [160, 161] in PC12 cells incubated with 6-hydroxydopamine [162]. Melatonin also abrogated cell death induced by cysteamine pretreatment of the PC12 cells; cystamine treatment involves mitochondrial iron sequestration [163]. The age-associated accumulation of redox-active iron in subcortical astrocytes may facilitate the bioactivation of DA to neurotoxic free radical intermediates and thereby predispose the nervous system to PD and other neurodegenerative diseases. In rats injected with kainic acid to produce excitotoxicity-induced apoptotic cell death, melatonin significantly attenuated apoptosis, an effect linked to the reduction in oxidative damage and an increased GSH content [164]. In a spontaneous, age-induced model of apoptosis using cerebellar granule cells, it was shown that melatonin and Ca^{2+}-channel blockers such as amlodipine, inhibited spontaneous apoptosis [165]. This antagonism between melatonin and Ca^{2+}-channels was also demonstrated in electrophysiological and binding experiments [166]. Striatal neurons growing in low density culture in serum-free medium and in the absence of glia die within 3 days by apoptosis. The presence of melatonin rescues striatal neurons from impending cell death, which may have important consequences in neurodegenerative diseases involving nigrostriatal pathway as in PD [167].

In AD patients melatonin levels are decreased in blood and cerebrospinal fluid (CSF) and that reduction seems to go in parallel to the progression of AD neuropathology [168, 169]. Moreover, CSF melatonin levels are already decreased in pre-clinical AD individuals [168]. The administration of melatonin has been tested in order to reduce the neurodegenerative manifestations in AD [170]. When neuroblastoma cells were incubated with Aβ, more than 80% of the neurons died due to apoptosis, but the presence of

melatonin reduced cellular death and DNA damage in a dose-related manner [171]. In human platelets, melatonin also protected against Aβ-induced damage [172, 173]. Recently, melatonin treatment has been tested in the APP + PS1 double transgenic (Tg) mouse, which is considered a mouse model with characteristics of the neuropathology of AD [174]. Melatonin administrated in the drinking water (100 mg/L water) for four months was able to protect the AD mice from cognitive impairment in a variety of tasks of working memory, spatial reference learning/memory, and basic mnemonic function. Immunoreactive Aβ deposition was significantly reduced in hippocampus (43%) and entorhinal cortex (37%) of melatonin-treated AD mice. The levels of tumor necrosis factor (TNF)-alpha were decreased in hippocampus of AD mice treated with melatonin, as well as the cortical mRNA expression of SOD-1, GPx and catalase. Taken the results together, the authors suggested that melatonin's cognitive benefits could involve its anti-Aβ aggregation, anti-inflammatory, and/or antioxidant properties [174]. In AD patients, melatonin has been able to stabilize cognitive function over a 2–3 year period [173, 175]. An additional retrospective study reported that individuals with mild cognitive impairment given melatonin for sleep enhancement also showed significantly better cognitive performance in two widely utilized cognitive assessment tests [176].

A recent investigation have used melatonin in cellular and mouse models of ALS, as well as 31 ALS patients [177]. First, NSC-34 cells, a widely used motoneuron-neuroblastoma fusion line, were exposure to 2 or 10 mM of glutamate for 3 days. A mortality of 29.2% and 52.1% of cells were detected respectively but the treatment with 50 mM melatonin was able to recover the survival by 17.2 % (2 mM glutamate) and 8.5 % (10 mM glutamate). Melatonin reduced the increased ROS production in this cellular model; second, in the $SOD1^{G93A}$-transgenic mouse (an ALS mouse model), melatonin was administrated in the drinking water (0.5 mg/ml water) at 28 days old, resulting in a delayed disease progression and extended survival; third, daily doses of 300 mg melatonin/ were administrated in ALS patients as novel suppositories at bedtime. Melatonin treatment decreased the serum protein carbonyls compared with the elevated levels presented in the serum of the matched untreated ALS patients [177].

Finally, melatonin treatment has been used in a rat model of Huntington disease induced by 3-nitropropionic acid, a mycotoxin that inhibits the mitochondrial succinate dehydrogenase or complex II [178]. The inhibition of complex II by 3-nitropropionic acid was accompanied by an increase of LPO and protein carbonyl content, and a decrease in SOD activity in the brain cortex and striatum. Melatonin administered intraperitoneally (1 mg/kg b.w./day) for 8 days prevented the deleterious effects induced by the acid [178].

MELATONIN, MITOCHONDRIA, AND NEURAL STEM CELLS

Whereas a role of mitochondria in stem cell proliferation and/or differentiation begins to have experimental support, the role of melatonin remains unclear. One can presume that, in view of the specific and significant effects of melatonin on mitochondrial physiology, the indoleamine may also affect mitochondrial physiology in stem cells (Fig. **2**). It was recently reported that melatonin modulates the proliferative and differentiative ability of the neural stem cells from fetal mouse brain in a concentration and exposure-timing dependent manner [179]. Pharmacological concentrations of melatonin (1-100 μM) applied during the proliferation period, diminished the proliferation. Interesting, neural differen-tiation of these cells increased without affecting astroglial differentiation. Other data point towards a net hippocampal neurogenesis in adult mice by melatonin [180]. Interestingly, it was shown that pinealectomy causes loss of pyramidal neurons in rat CA1/3 hippocampal layers, an effect reversed by melatonin administration [181]. Melatonin also promotes neurogenesis and motor recovery after mild focal ischemia or cranial irradiation in mice [182, 183]. New experimental data supports that melatonin is able to induce neurogenesis in dentate gyrus of adult pinealectomized rats [184]. The effects of melatonin on neural proliferation and differentiation might be partly resulting from melatonin´s activity in mitochondria and thus, additional studies are required to uncover underlying melatonin´s actions on neural stem cells.

CONCLUSION

Increasing evidence supports that mitochondria exert a series of roles in the cell that far from those related to energy production. Among these new functions, mitochondria are directly related to cell fate, calcium regulation, energy/heat balance, and control of free radical production. Mitochondria are source and target of free radicals, mitochondrial oxidative damage is directly related with age. Moreover, it is now recognized that, besides aging, mitochondrial dysfunction underlies many of the neurodegenerative diseases. Increasing evidence supports the presence of neurogenesis in the adult brain, a process in which mitochondria seems to be directly involved. Thus, the maintenance of the mitochondrial homeostasis, and the prevention of mitochondrial oxidative damage are of main importance to prevent aging and age-related diseases. Additionally, from a pharmacological point of view, mitochondria should be considered the target for new drugs related to the treatment of neurodegenerative diseases. Finally, a new line of research should be address the way of modulating mitochondrial function to control stem cell proliferation and/or differentiation.

In this regard, melatonin, an endogenous indoleamine with multiple functions in the cell, may be compared as a polydrug. In fact, melatonin exerts important antioxidant and anti-inflammatory functions; regulates de expression of multiple genes involved in inflammatory responses and antioxidant defense, and exerts pro- and anti-apoptotic effects. During the last years a number of papers have revealed the important role of melatonin in the mitochondria. The indoleamine is able to maintain mitochondrial homeostasis, counteracting mitochondrial oxidative stress in aging and neurodegeneration; increases the activity of the respiratory chain and the ATP production; reduces oxygen consumption and the generation of superoxide anion and hydrogen peroxide by mitochondria; maintains the mitochondrial membrane potential and prevents the permeability transition opening, and it is related to the regulation of the mtDNA transcriptional activity. Thus, melatonin acts as a mitochondrial housekeeper. Moreover, recent evidences sug-

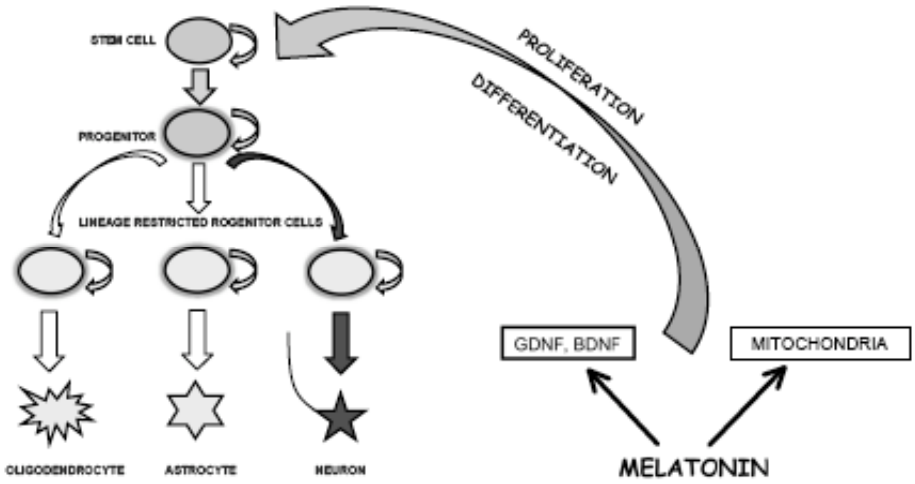

Fig. (2). There is increasing evidence that melatonin can participate in the proliferation, differentiation and/or viability of neuronal stem cells (NSC). Although there are not enough data, at least two mechanisms seem to be involved in the NSC-melatonin interplay: the effect of melatonin to induce the expression of both glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF); and the know effects of the indoleamine on mitochondrial homeostasis.

gest that these effects of melatonin may be involved in its role on neuronal stem cell proliferation and/or differentiation. This is an excitant new line of research on melatonin for the next years.

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