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Review Article

Melatonin in Mitochondrial Dysfunction and Related Disorders

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Mitochondrial dysfunction is considered one of the major causative factors in the aging process, ischemia/reperfusion (I/R), septic shock, and neurodegenerative disorders like Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD). Increased free radical generation, enhanced mitochondrial inducible nitric oxide (NO) synthase activity, enhanced NO production, decreased respiratory complex activity, impaired electron transport system, and opening of mitochondrial permeability transition pore all have been suggested as factors responsible for impaired mitochondrial function. Melatonin, the major hormone of the pineal gland, also acts as an antioxidant and as a regulator of mitochondrial bioenergetic function. Both in vitro and in vivo, melatonin was effective for preventing oxidative stress/nitrosative stress-induced mitochondrial dysfunction seen in experimental models of PD, AD, and HD. In addition, melatonin is known to retard aging and to inhibit the lethal effects of septic shock or I/R lesions by maintaining respiratory complex activities, electron transport chain, and ATP production in mitochondria. Melatonin is selectively taken up by mitochondrial membranes, a function not shared by other antioxidants. Melatonin has thus emerged as a major potential therapeutic tool for treating neurodegenerative disorders such as PD or AD, and for preventing the lethal effects of septic shock or I/R.

1. Introduction

Mitochondrial dysfunction is implicated in the etiology of various diseases, such as neurodegenerative diseases, diabetes, cardiovascular disease, various forms of hepatic disorders, skeletal muscle disorders, sepsis, and psychiatric disorders [1–10]. Abnormalities in mitochondrial functions such as defects in the electron transport chain (ETC)/oxidative phosphorylation (OXPHOS) system, Krebs's cycle enzymes, and ATP production have all been suggested as the primary

causative factors in the pathogenesis of neurodegenerative disorders and sepsis.

Impaired mitochondrial dysfunction is regarded as the driving force for the ageing process [11]. Enhanced production of reactive oxygen species (ROS) and possibly accumulation of mitochondrial (mt) DNA mutations in postmitotic cells are considered to be contributory factors to age-related degeneration. Mitochondria not only generate ROS/reactive nitrogen species (RNS) but are also the main target of their actions [12]. As a result of this action, damage occurs in

the mitochondrial respiratory chain, thus producing further increases in free radical generation, ultimately self-inducing a vicious cycle [13].

During the last decade, a number of studies have demonstrated that melatonin plays an effective role in regulating mitochondrial homeostasis. In addition to being a free radical scavenger, melatonin reduces nitric oxide (NO) generation within mitochondria. It maintains the electron flow, efficiency of oxidative phosphorylation, ATP production and bioenergetic function of the cell by regulating respiratory complex activities, Ca^{2+} influx, and mitochondrial permeability transition pore opening [14–18].

In this article, the several mechanisms through which melatonin exerts neuroprotective actions in neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD) and in a number of mitochondrial dysfunction related conditions such as aging, ischemia/reperfusion (I/R), or septic shock, are reviewed. For another recent survey of literature see [19].

2. Mitochondrial Function and Free Radical Generation

Mitochondria contain multiple copies of a circular genome (chromosome) known as mtDNA as it has been characterized in humans [20]. Although the majority of mitochondrial proteins essential for normal bioenergetic function are encoded by nuclear DNA [21], some proteins needed for ETC/OXPHOS are encoded by mtDNA. Human mitochondrial genome encodes for 13 peptides of subunits of complexes I, III, and IV, and ATP synthase complex, 22 transfer RNAs and 2 ribosomal nucleic acids, while nuclear DNA encodes for at least 1000 mitochondrial proteins [22].

The primary function of mitochondria is to generate ATP within the cell through the ETC resulting in OXPHOS. The ETC, which is present in the inner mitochondrial membrane, comprises a series of electron carriers grouped into four enzyme complexes, namely, complex I (NADH ubiquinone reductase), complex II (succinate ubiquinone reductase), complex III (ubiquinol cytochrome-c-reductase), and complex IV (cytochrome c oxidase) [23].

The main function of the ETC is to convert redox energy into an electrochemical gradient of protons that subsequently causes ATP formation from ADP and phosphate by ATP synthase. The end product of the respiratory chain is water that is generated in a four-electron reduction of molecular oxygen (O_2) by complex IV. During this process (electron leakage especially at complex I and III), a small percentage of O_2 is converted into ROS, such as superoxide anion radical ($\text{O}_2^{\bullet-}$) and its secondary products hydrogen peroxide (H_2O_2) and reactive hydroxyl radical ($\bullet\text{OH}$) [23, 24]. Under normal conditions, the iron-sulfur cluster N_2 of complex I appears to be the primary source of free radicals in the brain [23, 25].

Mitochondrial NO synthase (mtNOS) localized in the inner mitochondrial membrane is responsible for generating NO radical ($\bullet\text{NO}$) from L-arginine [26]. Localization of mtNOS refers only to myristoylated nNOS splice variant

alpha, which is also relevant because of its interaction with Complex IV. Because of NO diffusion, cytoplasmic NOS forms are also relevant for generating $\bullet\text{NO}$. High rates of $\bullet\text{NO}$ synthesis, which typically occur in the calcium-dependent excited state of neurons, can contribute to oxidative and nitrosative stress. The availability of $\bullet\text{NO}$ determines the rates at which the adduct peroxynitrite (ONOO^-) and the decomposition products are generated.

The free radical $\bullet\text{NO}$ is produced by several forms of NOS. In the mitochondria, two NOS isoforms, namely constitutive and inducible have been reported (c-mtNOS and i-mtNOS). Since $\bullet\text{NO}$ is an uncharged gaseous compound, it crosses membranes with ease and can enter mitochondria regardless of their neuronal, glial, or vascular origin. $\bullet\text{NO}$ strongly interferes with components of the respiratory chain, in particular cytochrome C oxidase [27–29]. Its metabolite ONOO^- and radicals derived from this can damage proteins of respiratory complexes. Other nitrosation processes, like transnitrosation or reversible nitrosation and nitration as well as irreversible protein and lipid oxidation, can occur.

Upon entering neuronal mitochondria, $\bullet\text{NO}$, in combination with ONOO^- (formed there by combination with $\text{O}_2^{\bullet-}$ from electron leakage), not only interferes with respiratory chain complexes but, when it reaches elevated levels, can trigger free radical-mediated chain reactions that in turn destroy protein, lipid, and DNA molecules [30–32]. As it has been stated above, damage to the mitochondrial respiratory chain can cause a breakdown of the proton potential, apoptosis, or lead to further generation of free radicals, thus maintaining a vicious cycle that ultimately results in cell death [23, 33].

In the mitochondrial-mediated cell death pathway, a nonspecific increase in the permeability of the inner mitochondrial membrane occurs, when mitochondrial matrix calcium is greatly increased [34]. This is known as mitochondrial permeability transition (mtPT), a process associated with the opening of channels in the inner mitochondrial membrane, which in turn causes a flux of molecules of <1500 daltons [35].

Both Ca^{2+} and ROS are major mtPT regulators. The inner mitochondrial membrane possesses a uniporter to transport Ca^{2+} into the matrix. With Ca^{2+} overload, there is complete uniporter inhibition, mitochondrial swelling, loss of respiratory control, and a release of matrix calcium caused by mtPT pore opening [35, 36]. Under these conditions, ATP is hydrolyzed by mitochondria, the mitochondria undergo swelling, and mitochondrial-mediated apoptosis occurs.

Free radicals are continually generated in cells during normal activity. This process is essential for normal cellular physiology [37]. A number of mechanisms take part in the control of ROS/RNS production. Among these is the action of the enzyme superoxide dismutase (SOD), which occurs in the inner side of the inner mitochondrial membrane (Mn-SOD), that remove $\text{O}_2^{\bullet-}$ [38]. $\bullet\text{OH}$ generated from H_2O_2 in the presence of reduced transition metals are scavenged by the enzyme glutathione peroxidase (GPx), during the process of metabolism of reduced glutathione (GSH) to its disulfide (GSSG), which in turn is reduced back to GSH by the enzyme glutathione reductase (GRd) [39].

These enzymes form part of the endogenous antioxidant defense system and suppress ROS levels within the cell as well as within the mitochondria. Antioxidants such as ascorbate, ubiquinone, or α -tocopherol can participate in the mitochondrial antioxidative defense system, but none of them can convert $O_2^{\bullet-}$ to O_2 . It is GSH that participates in scavenging $O_2^{\bullet-}$, as well as in several redox reactions and maintains the mtTP pore closed. The redox cycling in the mitochondria is very active and serves to prevent significant loss of GSH. This is important because the mitochondria contain GPx and GRd activities and depend only on GSH uptake from the cytoplasm to keep adequate GSH levels.

Since melatonin promotes de novo synthesis of GSH by stimulating the activity of the enzyme γ -glutamylcysteine synthetase [40] and also through its effects on gene expression of GPx, GRd, SOD, and CAT [41–45] helping in the recycling of GSH and in maintaining high GSH/GSSG ratio [46], the role it plays in mitochondrial physiology is important [47].

3. Melatonin: Biosynthesis, Metabolism, and Receptors

Melatonin was first isolated and identified by Lerner and coworkers in 1958 [48]. This methoxyindole is synthesized and released to the circulation by the pineal gland. The synthesis of melatonin that occurs in a number of other tissues and cells does not contribute significantly to the circulating melatonin levels but rather exerts an autocrine or paracrine role. Within this category, melatonin synthesis by lymphocytes [49], skin [50], the gastrointestinal tract [51], thymus [52], several parts of the eye [53] or bone marrow [54] should be mentioned.

Tryptophan serves as the precursor for the biosynthesis of melatonin. It is converted into serotonin via 5-hydroxytryptophan. Serotonin is then acetylated to form N-acetylserotonin through the action of arylalkylamine N-acetyltransferase, one of the key enzymes in melatonin synthesis. N-acetylserotonin is then converted to melatonin by hydroxyindole-O-methyltransferase (HIOMT), which has been identified as a rate-limiting enzyme in the biosynthesis of pineal melatonin [55, 56].

Once formed, melatonin is not stored within the pineal gland but diffuses into the capillary blood and cerebrospinal fluid [57]. In a recent study conducted in humans, CSF melatonin levels were found to be higher in the third ventricle compared to the lateral one, thus indicating that melatonin enters the CSF through the pineal recess, even during daytime [58]. The brain has much higher concentrations of melatonin than any other tissue in the body [59].

In the circulation, melatonin is partially bound to albumin [60] and can also bind to hemoglobin [61]. Circulating melatonin is metabolized mainly in the liver where it is first hydroxylated by cytochrome P₄₅₀ mono-oxygenases (isoenzymes CYP1B1, CYP1A2, CYP1A1) and, thereafter, conjugated with sulphate to be excreted as 6-sulfatoxymelatonin. Under certain circumstances, 6-sulfatoxymelatonin may be also synthesized in the brain. Melatonin can be metabolized

nonenzymatically in all cells of the body. It is converted into 3-hydroxymelatonin when it scavenges two \bullet OH [62]. In the brain, melatonin can be metabolized to kynuramine derivatives [63], particularly under inflammatory conditions. These metabolites of melatonin which are formed in the brain, namely, *N*¹-acetyl-*N*²-formyl-5-methoxy kynuramine (AFMK) and *N*¹-acetyl-5-methoxykynuramine (AMK), also share the antioxidant and anti-inflammatory properties of melatonin [64, 65].

Melatonin is involved in the control of various physiological functions of the body such as seasonal control of reproductive processes [66, 67], sleep regulation [68], immune mechanisms [69, 70], and regulation of circadian [71, 72] and sleep-wake rhythms [73, 74]. In addition to the above-mentioned physiological actions, melatonin in pharmacological doses inhibits tumor growth and may have a potential therapeutic value in treating breast cancer, prostate cancer, melanoma, and cancer of GI tract [75, 76]. Melatonin also exerts antinociceptive and antiallodynic actions [77]. As the prototype of the chronobiotic class of drugs [74, 78–80], melatonin regulates the phase and amplitude of circadian rhythmicity by interaction with MT₁ and MT₂ receptors expressed in the hypothalamic suprachiasmatic nuclei (SCN) and other brain areas.

Melatonin exerts many of its actions via membrane receptors, namely, MT₁ and MT₂ receptors that are expressed both singly and together in various tissues of the body [81–83]. A third melatonin binding site that was isolated and purified from hamster kidney has been characterized as quinone reductase type 2 [84]. This enzyme belongs to a group of reductases that participate in the protection against oxidative stress by preventing electron transfer reactions of quinones. Melatonin is also a ligand for retinoid orphan nuclear receptors [85–87].

The melatonin MT₁ receptor is coupled to different G proteins that mediate adenylyl cyclase inhibition and phospholipase C activation [83]. The MT₂ receptor is coupled to a number of signal transduction mechanisms among them phosphoinositide production, inhibition of adenylyl cyclase, and inhibition of guanylyl cyclase [83].

4. Melatonin's Free Radical Scavenging and Antioxidant Actions

Because of its amphiphilic properties, melatonin passes through all biologic barriers with ease. Melatonin gets access freely to all compartments of the cell, and can be especially concentrated in the nucleus and mitochondria [15, 47, 88].

The discovery that melatonin was a remarkably potent scavenger of the particularly reactive, mutagenic, and carcinogenic \bullet OH [89] was the finding that initiated numerous studies on melatonin's role as a protector against free radicals. Melatonin was shown to be much more specific than its structural analogs in undergoing reactions which lead to the termination of the radical reaction chain and in avoiding pro-oxidant, C- or O-centered intermediates [89–91]. Moreover, melatonin scavenged numerous different free radical species and other oxidants, among which the

carbonate radical [92] is important because of its presumed role in mitochondrial damage [93].

Although direct radical scavenging has been effective under numerous experimental conditions at clearly supraphysiological melatonin concentrations, its relevance at physiological levels has been questioned for reasons of stoichiometry. Even though a single melatonin molecule may generate products in a scavenger cascade which may collectively eliminate up to ten free radicals [94], such findings from chemical systems may not be fully applicable to physiological conditions.

In spite of this criticism, melatonin has been shown to protect from oxidotoxicity already at physiological concentrations [95]. A possible indirect action as mediated by upregulation of antioxidant enzymes by melatonin was proposed (reviewed in [96, 97]).

An alternate concept has been put forth to explain the protective effect at the level of radical generation rather than detoxification of already formed radicals [98–100]. If melatonin is capable of decreasing the processes leading to enhanced radical formation, this might be achieved by low, physiological, concentrations of the methoxyindole. Apart from oxidants released by leukocytes, the isoforms of NAD(P)H oxidases (Nox) and mitochondria should be mentioned as main sources of free radicals in the cell. Nox isoenzymes contribute to superoxide formation in a quantitatively substantial manner [101–104]. A recent study showed that melatonin inhibits free radical formation in microglia exposed to amyloid- β_{1-42} by preventing the phosphorylation of the p47 Nox subunit via the PI3K/Akt pathway [105] thus giving support to the hypothesis that melatonin has a protective effect at the level of radical generation.

5. Melatonin and Mitochondrial Function

Melatonin's ability to influence mitochondrial function has been tested both in vivo and in vitro. In initial in vivo studies conducted on rats, ETC complexes from the mitochondria of brain and liver tissues were measured following injections of melatonin (10 mg/kg). Melatonin was found to increase the activity of C-I and C-IV of mitochondrial ETC in a time-dependent manner, C-II and C-III not being affected. The effect of melatonin was observed 30 min after melatonin treatment [15]. Ruthenium red was found to impair mitochondrial metabolism by reducing ETC and ATP synthesis through its cellular oxidative stress action. Inhibition of both C-I and C-IV of the ETC were noted. Injections of melatonin were found to counteract the inhibitory effect of ruthenium red on C-I, C-IV, and GPx enzyme [15].

In an in vitro study, the effect of melatonin on t-butyl hydroperoxide- (t-BHP-) induced mitochondrial oxidative stress was evaluated. t-BHP depletes mitochondrial GSH and inhibits GPx and GRd activities [106]. In mitochondrial preparations, 100 nM melatonin was found to prevent the oxidation of GSH to GSSG induced by t-BHP and also restored the normal activities of both GPx and GRd [14]. Melatonin increased C-I and C-IV in a dose-dependent

manner, the effect being significant at 1 nM. Melatonin also counteracted cyanide-induced inhibition of C-IV showing thereby that melatonin can increase the activity of ETC coupled to OXPHOS and increase ATP synthesis in normal mitochondria as well as in mitochondria depleted of ATP by cyanide [14]. The effects of melatonin in regulating Complexes I and IV presumably do not reflect its antioxidant role but indicates an interaction with ETC complexes by donating and accepting electrons, thereby increasing electron flow, an effect not shared by other antioxidants.

The major consequence of melatonin's action on mitochondria may be avoidance of damage and dysfunction thus contributing to increase ATP production [16, 107]. Melatonin increases the efficiency of ETC thereby limiting electron leakage and free radical generation, and consequently promoting protein synthesis [17, 47].

The possible mechanism by which melatonin controls mitochondrial respiration in the liver was examined in two groups of rats [108]. In one group, melatonin (16 to 50 $\mu\text{g}/\text{mL}$) or vehicle was administered for a period of 45 days. In another study, rats received melatonin in drinking water (50 $\mu\text{g}/\text{mL}$) for 45 days or the same amount for 30 days, followed by a withdrawal period of 15 days. At sacrifice, the liver mitochondrial fraction was prepared and oxygen consumption was measured in the presence of excess concentration DL-3 β -hydroxybutyrate or L-succinate. Melatonin treatment decreased Krebs's cycle substrate-induced respiration significantly at both examined doses. The stimulation of mitochondrial respiration, caused by excess concentration of substrate, recovered after melatonin withdrawal. Basal state 4 respiration was not modified by melatonin. This study shows that melatonin can protect mitochondria from oxidative damage resulting from overstimulation of cellular respiration caused by excess Krebs' cycle substrate [108].

A similar study on melatonin's mechanism of action on mitochondrial respiration was carried out by another group of investigators [18]. In this study, mitochondria from mouse liver cells was incubated in vitro with melatonin at concentrations ranging from 1 nM to 1 mM. Melatonin decreased oxygen consumption, inhibited the increase in oxygen flux in the presence of excess of ADP, reduced membrane potential and inhibited the production of $\text{O}_2^{\bullet-}$ and H_2O_2 . Melatonin was also able to maintain the efficiency of oxidative phosphorylation and ATP synthesis by increasing the activity of the respiratory complexes I, III, and IV. These effects were attributed to the intramitochondrial presence of melatonin, thus showing melatonin's participation in the physiological regulation of mitochondrial homeostasis [18].

Melatonin's action in preventing the opening of the mtPT pore given by oxidative stress caused by t-BHP was shown in another study on primary skeletal muscle cultures [109]. Using isolated mitochondria, melatonin (1–100 μM) fully prevented myotube death induced by t-BHP. Melatonin desensitized the mtPT pore to Ca^{2+} and prevented t-BHP-induced mitochondrial swelling and GSH oxidation. The inhibition of the mtPT pore opening by melatonin was suggested as an explanation for the protective action of melatonin against oxidative stress in myotubes [109].

Recently, the role of melatonin on cardiolipin and mitochondrial bioenergetics was explored [110, 111]. Cardiolipin, a phospholipid located at the level of inner mitochondrial membrane, is required for several mitochondrial bioenergetic processes as well as in mitochondrial-dependent steps of apoptosis. Alterations in cardiolipin structure, content, and acyl chain composition have been associated with mitochondrial dysfunction in various tissues under a variety of pathophysiological conditions [110]. Melatonin was reported to protect the mitochondria from oxidative damage by preventing cardiolipin oxidation and this may explain, at least in part, the beneficial effect of this molecule in mitochondrial physiology [110, 111].

5.1. Melatonin and Mitochondrial Dysfunction in Aging. The enhanced production of ROS and accumulation of mtDNA mutations in mitochondria may be contributory factors to human aging [112]. Many studies have established that the respiratory function of mitochondria declines with age [113–115]. The increased production of free radicals such as $O_2^{\bullet-}$ and H_2O_2 in mitochondria along with advancing age has been demonstrated [116, 117]. Accumulation of mtDNA mutations can cause defective respiratory function resulting in enhanced production of ROS. Many of these mtDNA mutations begin after adults reach their the mid thirties and accumulate with age in postmitotic tissues [115].

Overproliferation of abnormal mitochondria has been shown to occur in the muscle of aged individuals and in patients with mitochondrial myopathies [115, 118]. The presence of these defective mitochondria is one of the factors involved in the decline in respiratory function during the aging process [115].

Enhanced activation of the mtPT pore in the brain and liver of aging mice has also been demonstrated [119]. This in turn causes the release of proapoptotic factors from the intermembrane space of mitochondria. Hence, an increased mitochondrial ROS production, oxidative stress, respiratory functional decline, and susceptibility to apoptosis constitute central events in the aging process [115].

The mechanism of the aging process can be studied in experimental models like the senescence accelerated mouse (SAMP8) and senescence resistant mouse (SAMR1). The SAMP8 is an established murine model of accelerated aging [120]. The accelerated aging seen in this mouse strain is due to oxidative stress, which occurs with greater intensity as compared to the SAMR1 [121]. At an age of 12 months, reductions in the activities of respiratory complexes I and IV have been demonstrated in liver mitochondria of SAMP8 mice, but not in SAMR1 mice [122]. Greater concentrations of lipid peroxidation products in the liver and brain homogenates were found in SAMP8 mice as compared to SAMR1 mice. In contrast to this, the concentration of the antioxidant enzyme GPx from SAMP8 mice at 12 months of age was found to be significantly lower than in SAMR1 mice. These studies support the conclusion that excess free radical generation coupled with less effective defense against the oxidative stress is responsible for alteration of mitochondrial function seen in SAMP 8 mice [122–124].

Since melatonin can readily reach the mitochondria due to its high lipophilicity, it seems feasible that, upon its entry into the cell, it could become concentrated at a superficial position in lipid layers near the polar heads of membrane phospholipids, a key place to function as a free radical scavenger [125]. The effect of melatonin on age-dependent changes in the redox status of mitochondria in the heart and diaphragm was thus evaluated [126]. Melatonin, administered in the drinking water at a dose of 10 mg/kg for 9 months, was shown (i) to counteract the age-dependent increase in lipoperoxidation level, (ii) to increase GSH content in muscle mitochondria of both SAMP8 and SAMR1 mice, (iii) to counteract the reduction of GSH/GSSG ratio in diaphragmatic mitochondria of SAMR1 and SAMP8 mice, (iv) to increase the activity of the antioxidant enzymes GPx and GRd in the mitochondria of SAMP8 mice with no effect on SAMR 1 mice, and (v) to increase the activity of GRd in SAMR1 mice. Therefore, long-term melatonin administration prevented the age-dependent mitochondrial stress in both senescence-accelerated and senescence-resistant mice [126].

As a continuation of the above-mentioned study, the effect of melatonin at earlier stages of the life span was evaluated at the 5th and 10th months of age in SAMP8 and SAMR1 mice [127]. Mitochondrial oxidative stress was determined by measuring the levels of lipid peroxidation, GSH and GSSG, and the activities of GPx and GRd in diaphragmatic mitochondria. Age did not affect diaphragmatic mitochondrial levels of lipid peroxidation in SAMR1 mice but increased them in SAMP8 animals. When melatonin was administered in the drinking water at a daily dose of 10 mg/kg, the level of lipid peroxidation in 10-month-old SAMP8 mice was reduced to that found at 5 months of age. The decrease of GPx seen with age in both strains of mice was counteracted by melatonin administration with a higher effect in SAMP8 mice. As far as GRd, although age caused significant reductions in both strains of mice, treatment with melatonin partially restored GRd activity in SAMR1 mice only. The chronic administration of melatonin significantly increased complex II and complex III activity in SAMR1 and SAMP 8 mice and complex IV activity in SAMP 8 mice [127].

Measurement of ATP levels and ATP/ADP ratio showed that treatment with melatonin counteracted the reduction in ATP levels and ATP/ADP ratio found in SAMP8 mice. As far as longevity, melatonin treatment increased the half-life of SAMP8 mice from 16 to 22 months while longevity increased from 23 to 27 months [127]. Melatonin's beneficial effects on longevity were significantly higher in SAMP8 mice than in SAMR1 mice. The study thus showed that melatonin administration counteracted age-dependent oxidative damage and mitochondrial dysfunction in senescence accelerated mice by improving mitochondrial function as reflected by the increase in ATP production and a prolonged longevity [127].

Another study using rat brain mitochondria was designed to evaluate the beneficial effects of melatonin on age-associated reductions in mitochondrial bioenergetic function [111]. Mitochondria from control and aged rats treated or not with melatonin were obtained, and various

bioenergetic parameters such as complex I activity, rates of state 3 respiration, mitochondrial H_2O_2 production, and membrane potential were evaluated. Melatonin was found to prevent the significant age-related changes that occurred in all of these parameters in untreated animals. The ability to prevent complex I dysfunction and cardiolipin peroxidation was melatonin's principal mechanism of action for achieving its effects [111].

Age-associated impairments in mitochondrial OXPHOS found in the brain of SAMP8 mice did not exhibit any major gender differences [128]. However, a higher reduction in the GSH/GSSG ratio at 10 months of age in female than in male SAMP8 mice has been reported in one study [129]. Chronic melatonin treatment completely prevented age-dependent oxidative stress as assessed by the recovery of the GSH/GSSG in mitochondria of brain samples of both male and female mice [129]. The ability of melatonin to prevent GSH loss with age probably reflects its influence on the activities of the GSH redox cycle enzymes [47].

An impairment of the mitochondrial respiratory chain activity occurs with age. This is not due to reduction of brain mitochondria with aging but has been demonstrated to be due to diminished activities of respiratory complexes I, II, and III [130]. Mitochondrial dysfunction with aging is not an irreversible process as shown by studies using melatonin to prevent age-dependent declines in bioenergetic impairment of brain mitochondria in mice [129].

5.2. Melatonin and Mitochondrial Function in I/R. I/R lesions are seen in many clinical conditions and are triggered by multiple factors including overproduction of ROS [131–133]. For example, ROS produced at the level of complex I and III of the respiratory chain are responsible for injury seen in cardiac I/R [134, 135] as well as in stroke [133].

The available evidence indicates the opening of the mtPT pore is responsible for the cardiomyocyte death occurring during I/R [136]. While these pores remain closed during the ischemic period, at reperfusion the influx of Ca^{2+} into the mitochondria and an associated burst of ROS production caused the opening of mtPT channels. This leads to mitochondrial depolarization, swelling, and rupture of the external mitochondrial membrane, with uncoupling of the respiratory chain and efflux of cytochrome C and other proapoptotic factors, all of which lead to either cell death by either apoptosis or necrosis.

Melatonin has also been shown to be effective in protecting the cardiac musculature against I/R [137–139]. Melatonin's protective effect during I/R has been attributed to its action in inhibiting the mtPT pore [140], and in preserving the content and integrity of cardiolipin molecules [141]. Melatonin treatment resulted in significant reductions in infarct size [142].

The fact that melatonin treatment inhibits both mtPT pore opening and cardiolipin peroxidation following I/R suggests a possible link between these two processes. It has been suggested that that increased levels of peroxidized cardiolipin together with increased Ca^{2+} overload can contribute to the mtPT pore opening during reperfusion [143].

Melatonin, by preventing the oxidative damage of cardiolipin, prevents mtPT channel opening. It must be noted that a significant cytoprotective effect of melatonin was described at a very early phase of a myocardial infarction, when I/R and thus oxidative damage were minimal [144], indicating that not all cardiac protective effects of melatonin are attributable to its antioxidant activity.

5.3. Melatonin and Mitochondrial Dysfunction in Sepsis. Septic shock is a lethal condition caused by a complex chain of pathogen-induced events involving immune cells, the epithelium, the endothelium, and the neuroendocrine system. The lethal effects of septic shock are associated with the production and release of numerous proinflammatory mediators as well as NO and ROS, thus inducing massive apoptosis.

Since many years ago, research interest was focused on the hypothesis that mitochondrial dysfunction plays a pivotal role in septic shock. This hypothesis was indeed confirmed by the finding of decreased respiratory complex I activity and low levels of ATP levels in skeletal muscle biopsies obtained from critically ill patients with septic shock [145]. Increased NO production and decreased levels of GSH were also found in septic shock patients.

The protective effect of melatonin on the lethal effects of bacterial lipopolysaccharide (LPS) on respiratory complex activities I and IV and a mitochondrial subform of iNOS (mt iNOS) activity was examined in liver and lung mitochondria of rats [146]. LPS administered at a dose of 10 mg/kg i.v. was found to increase mt iNOS activity and NO, an effect that was greater in old rats than in young ones. LPS also decreased the activities of respiratory complexes I and IV. Melatonin administration (60 mg/kg, i.p.) prevented LPS toxicity by decreasing mt iNOS activity and NO production. It also counteracted LPS-induced inhibition of the activity of respiratory complexes I and IV. It is interesting to note that the effectiveness of melatonin to prevent the mitochondrial failure that occurs during endotoxemia were more pronounced in older animals than in young ones [146].

Using a long-term (3-day) rat model of sepsis, a number of parameters of mitochondrial dysfunction were investigated. The model comprises a long-term, fluid-resuscitated, fecal peritonitis model utilizing male Wistar rats that closely replicates human physiological, biochemical, and histological findings with a 40% mortality [8]. Compared to sham-operated controls severely septic rats had lower (20–22%) hepatic and muscle complex I activities. Moderate increases in nitrite/nitrate production were seen in both muscle and liver peaking at 24–48 h and returning to sham-operated levels at 72 h. A fall in GSH was associated with lower complex I and increased NO production was also demonstrated [8]. A number of animal model studies and a few clinical observations have now shown that melatonin is beneficial for treating septic shock (see, for a recent review, [147]).

To examine the effect of melatonin on changes in mt iNOS in septic skeletal muscles wild-type (iNOS^{+/+}) and iNOS knockout (iNOS^{-/-}) mice were turned septic by the

cecal ligation and puncture procedure [148]. After sepsis, increases in mt iNOS and NO levels, and decreases in electron chain activity were noted in iNOS^{+/+} mice but not in iNOS^{-/-} mice. In addition, an increase in oxidative stress was also found as indicated by an increase in lipid peroxidation products as well as a reduction in GSH levels and in the activities of GPx and GRd. Melatonin treatment counteracted the changes in mt iNOS activities and oxidative stress, and, further, restored the mitochondrial respiratory chain in iNOS^{+/+} mice [148]. This study confirmed that mtNOS is responsible for the mitochondrial dysfunction seen during sepsis and thus supported the conclusion that melatonin has the ability to protect against mt iNOS-mediated mitochondrial failure. A similar study performed in mitochondria isolated from the diaphragm of septic mice indicated that melatonin administration to iNOS^{+/+} mice counteracted mt iNOS induction and respiratory chain failure, and, finally, normalized the redox state after sepsis [149].

Considering the effects of melatonin and its virtual absence of toxicity, the use of melatonin along with conventional therapy to preserve mitochondrial bioenergetics as well as to limit inflammatory response and oxidative damage should be taken into account as a treatment option [147].

5.4. Melatonin and Mitochondrial Dysfunction in PD. PD is a neurodegenerative disorder with a multifactorial etiology, mainly characterized by the death of dopaminergic neurons in the pars compacta of substantia nigra and by the formation of Lewy bodies. The initiating factor in PD is increased release of free radicals and enhanced signs of oxidative stress as demonstrated in brains of PD patients [150–152].

Although the molecular mechanisms responsible for the pathogenesis of AD are still under intense investigation [128], reduced complex I activity in the substantia nigra [153] and loss of GSH [154] have been reported in PD patients. The selective inhibition of complex I in the ETC compromises energy availability and leads to apoptosis and death of the dopaminergic cells of substantia nigra.

A commonly accepted model of PD is that achieved by the systemic or intracerebral administration of neurotoxins like 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). The loss of dopamine neurons occurring in these animal models causes severe sensory and motor impairment which in turn gives rise to tremor, rigidity and akinesia similar to those seen in PD patients [155, 156]. The active glial metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP⁺), is taken up into the dopaminergic neurons through the dopamine transporter, and then accumulates in the mitochondria of substantia nigra pars compacta [157]. By binding to complex I, MPP⁺ increases the production of ROS and enhances oxidative stress causing reduction of ATP and death of cells in the substantia nigra [157].

In the striatum, the damage caused by MPP⁺ is attributed to increased generation of O₂^{•-} that reacts with NO to generate the highly toxic ONOO⁻. This impairs mitochondrial function as a result of irreversible inhibition of all ETC complexes [158] leading to the death of neuronal cells [159].

A neuroprotective effect of melatonin in isolated rat striatal synaptosomes and liver mitochondria treated with MPP⁺ has been demonstrated [160]. Melatonin prevented the inhibition of mitochondrial respiration by limiting the interaction of MPP⁺ with complex I of ETC.

Although the role of ROS generation has been demonstrated in the etiology of PD, the participation of mtNOS in the mitochondrial dysfunction and nigrostriatal degeneration has only recently been examined [128]. In a study conducted in adult male mice, MPTP was administered at a dose of 15 mg/kg in four separate doses [161]. Animals also received melatonin or its metabolite AMK (20 mg/kg) 1 h prior to MPTP injection. The administration of melatonin or AMK concomitantly with MPTP significantly reduced the iNOS activity stimulated by MPTP.

In the mitochondria, two NOS isoforms, namely, constitutive and inducible, may exist. MPTP administration significantly increased the activity of mt iNOS without affecting mt constitutive NOS activity. Treatment with melatonin or AMK restored the basal activity of i-mtNOS. Interestingly, MPTP administration induced i-mtNOS activity in the mitochondria of substantia nigra whereas i-mtNOS was only slightly induced by MPTP in striatal mitochondria. Treatment with either melatonin or its brain metabolite AMK effectively counteracted i-mtNOS induction, oxidative stress, and mitochondrial dysfunction induced by MPTP [161]. The nitrosative/oxidative stress reduction seen after therapeutic intervention with melatonin or AMK in MPTP treated mice was attributed to an effect in preventing damage to mitochondria. As already mentioned, mitochondria take up melatonin in a concentration- and time-dependent manner [18].

A small number of controlled trials indicate that melatonin is useful to treat disturbed sleep in PD [162, 163], particularly rapid eye movement-associated sleep behavior disorder [164–169]. Whether melatonin or the recently introduced melatonergic agents (ramelteon, agomelatine) have the potential for treating insomnia in PD patients and, more generally, for arresting the progression of PD merits further investigation.

5.5. Melatonin and Mitochondrial Dysfunction in AD. Several recent studies have confirmed the involvement of mitochondrial ROS production and abnormal mitochondrial function in the pathophysiology of AD [170–177]. AD is characterized by extracellular senile plaques of aggregated β -amyloid ($A\beta$) and intracellular neurofibrillary tangles that contain hyperphosphorylated tau protein. The resulting clinical effect is a progressive loss of memory and deterioration of cognition.

$A\beta$ is reported to accumulate in subcellular compartments and to impair neuronal function [178]. There is substantial evidence to prove that mitochondrial toxicity is linked to the progressive accumulation of mitochondrial $A\beta$ [179]. In the early phase of AD, inhibitors of β and γ -secretase can be therapeutically effective to halt AD disease progression by inhibition of the protein misfolding of $A\beta$ into neurotoxic oligomeric aggregates.

TABLE 1: Melatonin activity in bioenergetic functions: evidence from in vitro and in vivo studies.

Animal model studies (in vitro and in vivo)	Pretreatment	Posttreatment with melatonin	Reference(s)
Ruthenium red-effect on ETC system	Inhibition of complex I and IV and impairment of ATP synthesis	Counteracted ruthenium red's inhibitory action complex I and IV	[15]
t-BHP treated mitochondrial preparations	Depletion of mitochondrial GSH; inhibition of GPx and GRd activities	Prevented the oxidation of GSH to GSSG, restored GPx and GRd back to normal	[14]
MPTP effect on isolated striatal synaptosomes and liver mitochondria	Inhibition of ETC complex I	Prevented MPTP-induced inhibition of complex I	[160]
SAMP-8 mice	Elevated lipid peroxidation products	Reduced lipid peroxidation	[122]
LPS-injected rats	Increased mitochondrial NOS, NO decreased ETC complex I and IV	Decreased NO production and counteracted LPS-induced inhibition of complex I and IV	[146]
Ischemia-reperfusion effect on mitochondria	Opens mtPT pores and destroys cardiolipin in mitochondria	Inhibits mtPT pore opening and preserves the structural complex of cardiolipin in mitochondria	[140]
Mitochondria from heart and diaphragm muscle of SAMP-8 mice	Increased LPO; decrease in GSH levels and GPx and GRd activities	Counteracted age-dependent increase in LPO and reduction of GSH, GPx, and GRd	[125]
Skeletal muscle of septic wild-type (iNOS ^{+/+}) and iNOS knockout (iNOS ^{-/-}) mice	Increase of mt iNOS and NO levels, increase of lipid peroxidation products, decrease of GSH levels and of GPx and GRd activities	Decreased mt iNOS and NO levels, counteracted reduction of GSH, GPx and GRd	[148]
Diaphragmatic muscle of septic wild type (iNOS ^{+/+}) and iNOS knockout (iNOS ^{-/-}) mice	Increase of mt iNOS and NO levels, reduction of GSH and of GPx and GRd activities	Decreased mt iNOS and NO levels; counteracted reduction of GSH, GPx, and GRd	[149]
Mitochondria from rat liver	Normal ETC function	Melatonin treatment decreased Krebs's cycle substrate-induced respiration.	[108]
Heart and diaphragmatic muscle of SAMP-8 mice	Decreased levels of GSH and of GPx and GRd activities, increased lipid peroxidation	Increased GSH levels and GPx and GRd activities; decreased lipid peroxidation products	[126]
Diaphragmatic muscle of SAMP-8 mice-effect of aging	Decrease of GSH and GPx, GRd, increase in lipid peroxidation	Counteracted age-dependent decrease in GSH, GPx, and GRd Normalized lipid peroxidation	[127]
Mitochondria from liver of normal mice	Normal function of ETC complexes; opening of mtPT pores after oxidative stress	Increased complex I, III, and IV; closing of the mtPT pores opened by oxidative stress	[18]
t-BHP effect on mitochondria of skeletal muscle	Opening of mtPT and death of myotubules	Prevented t-BHP-induced opening of mtPT pores and swelling of mitochondria	[109]
MPTP effect on mitochondria of neurons in substantia nigra of mice	Increased mt iNOS; increased oxidative stress	Counteracted MPTP-induced increase of iNOS in substantia nigra and reduced the oxidative stress	[161]
Effect of ischemia-reperfusion on mitochondria	Opening of mtPT pores and oxidation of cardiolipin	Inhibited mtPT pores and cardiolipin oxidation	[110, 143]
Effect of A β on hippocampal neurons	Inhibition of ETC; reduced ATP levels	Attenuated A β -induced inhibition of respiratory complexes; restored ATP levels	[187]

Abbreviations used: A β : β amyloid; ETC: electron transport chain; GPx: glutathione peroxidase; GRd: glutathione reductase; GSH: reduced glutathione; GSSG: oxidized glutathione; iNOS: inducible nitric oxide synthase; LPS: lipopolysaccharide; MPTP: 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; mt iNOS: mitochondrial inducible nitric oxide synthase; mtPT: mitochondrial permeability transition; NO: nitric oxide; SAMP-8 mice: senescence accelerated mouse; t-BHP: t-butyl hydroperoxide.

Mitochondrial $O_2^{\bullet-}$ production plays a critical role in the pathological events following $A\beta$ elevation. An increased expression of mitochondrial antioxidant enzyme SOD-2 has been shown to prevent memory deficits and amyloid plaque deposition associated with AD [172]. Although a hypothetical occurrence of mutations in mtDNA could cause increased oxidative stress and energy failure, no causative mutations in mtDNA have been detected in AD so far [180].

Several actions of melatonin have been described which antagonize the deleterious effects of $A\beta$. The effects of melatonin can be grouped as (i) antioxidant, including influences on mitochondrial metabolism; (ii) antifibrillogenic, blocking $A\beta$ synthesis; (iii) cytoskeletal, including suppression of tau protein hyperphosphorylation (for a recent review see [181]). The antifibrillogenic effects of melatonin were observed not only in vitro but also in vivo in transgenic mouse models [182–184]. Protection from $A\beta$ toxicity was observed, especially at the mitochondrial level.

Melatonin also activates the survival signal pathways. One such pathway is the Bcl-2 pathway, which stabilizes mitochondrial function by antiapoptotic Bcl-2 family modulators. Bcl-2-expression was enhanced by melatonin concomitantly with inhibition of $A\beta$ -induced cell death [185]. This in vitro study was mimicked by an in vivo one. Melatonin inhibited free radical formation in microglia exposed to amyloid- β_{1-42} by preventing the phosphorylation of the p47 Nox subunit via the PI3K/Akt pathway [105].

In view of the consequences of excitation-dependent calcium overload on mitochondrial membrane potential and mtPT pore sensitivity towards excitotoxins like $A\beta$, the actions of melatonin at the level of this important cellular compartment deserve particular attention. Modulation of mitochondrial Ca^{2+} handling has been suggested as the potential pharmacological target for AD [186]. In a recent study, a possible melatonin prevention of damage induced by $A\beta$ was evaluated in young and senescent hippocampal neurons. Rat hippocampal neurons were incubated with $A\beta_{25-35}$ and cell viability, mitochondrial membrane potential, ATP, and the activity of the respiratory chain complexes were measured [187]. Cells exposed to $A\beta_{25-35}$ showed decreased mitochondrial membrane potential, inhibited activity of respiratory chain complexes, and a depletion of ATP levels. Melatonin attenuated $A\beta_{25-35}$ -induced mitochondrial damage in senescent hippocampal neurons [187]. Molecular studies undertaken with mitochondrial preparations suggest that melatonin has a therapeutic value in treating AD through its antiapoptotic activities [188].

As outlined, melatonin acts at different levels relevant to the development and manifestation of AD. The antioxidant, mitochondrial, and antiamyloidogenic effects may be seen as a possibility of interfering with the onset of the disease. Therefore, early beginning of treatment may be decisive [189]. Mild cognitive impairment (MCI) is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia [190]. Some of these patients develop AD. A small number of controlled trials indicate that melatonin is useful to treat MCI and to prevent progression to AD [181, 191–194].

5.6. Melatonin and Mitochondrial Dysfunction in HD. A Huntington's chorea animal model was developed by using 3-nitropropionic acid, an inhibitor of mitochondrial complex II. In this model, that replicates the neurochemical, histological, and clinical features of HD, melatonin administration was reported to defer the clinical signs of HD [195]. Current evidence from genetic models of HD including mutation of the huntingtin gene (mHtt), supports the mitochondrial dysfunction as major cause of the disease, with respiratory chain impairment relegated to a late secondary event [196]. Upstream events include defective mitochondrial calcium handling and impaired ATP production. Also, transcription abnormalities affecting mitochondria composition, reduced mitochondria trafficking to synapses, and direct interference with mitochondrial structures enriched in striatal neurons, are possible mechanisms by which mHtt amplifies striatal vulnerability [196]. Evidence is lacking on whether melatonin's action on mitochondria could affect evolution in the genetic model of HD. At least on the accumulation of insoluble protein aggregates in intra- and perinuclear inclusions in HD melatonin had little or no inhibitory effect on huntingtin aggregation [197].

6. Conclusions

Mitochondrial dysfunction is implicated as the major causative factor in a variety of conditions such as the aging process, I/R, and septic shock. In addition, abnormal mitochondrial function, decreased respiratory enzyme complex activities, increased electron leakage, opening of the mtPT pore, and increased Ca^{2+} entry have all been shown to play a role in the pathophysiology of neurodegenerative disorders such as PD, AD, and HD.

In addition to aging as a factor for low melatonin levels, it is well documented that there is a huge interindividual variation in circulating levels of melatonin which is stable within individuals and, which has been hypothesized to be genetic in origin [198–201]. There is now evidence that there are polymorphisms in the gene for HIOMT, the rate limiting enzyme in melatonin synthesis, and that the HIOMT transcript level depends significantly on the genotype distributions [202]. Thus, there may be a genetically determined low melatonin syndrome that causes a predisposition to a variety of diseases.

Among the number of substances involved in maintaining mitochondrial bioenergetics a number of in vivo and in vitro studies in animals (Table 1) indicate that melatonin may emerge as a major therapeutic candidate to preserve the bioenergetic function of mitochondria. Double-blind placebo controlled studies are needed to assess to what extent melatonin has therapeutic value in the treatment of the several disorders associated with mitochondrial dysfunction.

Conflict of Interests

S. R. Pandi-Perumal is a stockholder and the President and Chief Executive Officer of Somnogen Inc., a New York corporation. He declared no competing interests that might

be perceived to influence the content of this paper. All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this paper.

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