



Review

Melatonin and mitochondrial dysfunction in the central nervous system

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ARTICLE INFO

Available online 25 February 2012

Keywords:

Melatonin
Mitochondria
Free radicals
Oxidative stress
Aging
Parkinson's disease
Alzheimer's disease
Huntington's disease
Melatonin analogs

ABSTRACT

This article is part of a Special Issue "Hormones & Neurotrauma".

Cell death and survival are critical events for neurodegeneration, mitochondria being increasingly seen as important determinants of both. Mitochondrial dysfunction is considered a major causative factor in Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Increased free radical generation, enhanced mitochondrial inducible nitric oxide (NO) synthase activity and NO production, and disrupted electron transport system and mitochondrial permeability transition, have all been involved in impaired mitochondrial function. Melatonin, the major secretory product of the pineal gland, is an antioxidant and an effective protector of mitochondrial bioenergetic function. Both in vitro and in vivo, melatonin was effective to prevent oxidative stress/nitrosative stress-induced mitochondrial dysfunction seen in experimental models of AD, PD and HD. These effects are seen at doses 2–3 orders of magnitude higher than those required to affect sleep and circadian rhythms, both conspicuous targets of melatonin action. Melatonin is selectively taken up by mitochondria, a function not shared by other antioxidants. A limited number of clinical studies indicate that melatonin can improve sleep and circadian rhythm disruption in PD and AD patients. More recently, attention has been focused on the development of potent melatonin analogs with prolonged effects which were employed in clinical trials in sleep-disturbed or depressed patients in doses considerably higher than those employed for melatonin. In view that the relative potencies of the analogs are higher than that of the natural compound, clinical trials employing melatonin in the range of 50–100 mg/day are needed to assess its therapeutic validity in neurodegenerative disorders.

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Contents

Introduction	323
Mitochondrial function and free radical generation	323
Basic physiology of melatonin	324
Melatonin and mitochondrial function	324
Melatonin and mitochondrial dysfunction in AD	325
Melatonin and mitochondrial dysfunction in PD	327
Melatonin and mitochondrial dysfunction in HD	327
Melatonin and its analogs as pharmaceutical tools	327
Conclusions	328
Acknowledgments	328
References	328

Abbreviations: AD, Alzheimer's disease; AFMK, *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine; AMK, *N*¹-acetyl-5-methoxykynuramine; A β , aggregated β -amyloid; c-mtNOS, constitutive mitochondrial nitric oxide synthase; ETC, electron transport chain; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, reduced glutathione; HD, Huntington's disease; i-mtNOS, inducible mitochondrial nitric oxide synthase; iNOS, inducible nitric oxide synthase; MPP⁺, 1-methyl-4-phenylpyridinium; mPT, mitochondrial permeability transition; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MT₁, melatonin receptor 1; MT₂, melatonin receptor 2; mtDNA, mitochondrial DNA; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PD, Parkinson's disease; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase.

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Introduction

Cell death and survival are critical events in neurodegeneration, and mitochondria are increasingly seen as important determinants of both. Abnormalities in mitochondrial functions such as defects in the electron transport chain (ETC)/oxidative phosphorylation (OXPHOS) system and ATP production have been suggested as the primary causative factors in the pathogenesis of neurodegenerative disorders (Beal, 2009; DiMauro and Schon, 2008; Rezin et al., 2009; Rollins et al., 2009; Schon et al., 2010). Mitochondria act as energy suppliers and signaling mediators in the capacity of the cells to produce energy from atmospheric oxygen. Electrons from metabolic substrates are transferred via the ETC to molecular oxygen (O_2), giving rise to a H^+ electrochemical gradient whose energy is used to synthesize ATP (Brand and Nicholls, 2011).

Mitochondrial dysfunction underlies neurodegenerative diseases of different etiologies, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Generally these age-related disorders share mitochondrial dysfunction, oxidative/nitrosative stress and increased apoptosis in different areas of the brain. In the case of AD, decreases in mRNA expression of mitochondrial DNA (mtDNA) encoding cytochrome oxidase subunit II have been reported (Bonilla et al., 1999). Aggregated β -amyloid ($A\beta$) generates reactive oxygen species (ROS) that produce neuronal death by damage of neuronal membrane lipids, proteins and nucleic acids. Protection from $A\beta$ toxicity by melatonin was observed, especially at the mitochondrial level (Dragicevic et al., 2011; Olcese et al., 2009). This is the basis for the use of an antioxidant like melatonin in AD patients (see for ref. Cardinali et al., 2010).

Mitochondrial involvement in PD is suggested by deficiencies in components of the ETC like Complex (C)-I in substantia nigra with a parallel reduction in reduced glutathione (GSH) levels, indicating the existence of oxidative stress (Navarro and Boveris, 2010). In platelets of PD patients C-I is also decreased, and in some cases is accompanied by C-II, C-III and C-IV deficiencies. Studies with cybrids have shown that alterations in C-I is due to a defect in the mtDNA (Navarro and Boveris, 2010). This defect is accompanied by an alteration in the expression of C-IV activity and a reduced H^+ electrochemical gradient, which lowers the apoptotic threshold. Mitochondrial involvement in the pathology of PD has been genetically supported by the finding in early-onset Parkinsonism of mutations in DNA polymerase γ , which is the only DNA polymerase present in mitochondria and necessary for mtDNA synthesis (Luoma et al., 2004).

In the case of HD, deficiencies in the activity of C-II, C-III and C-IV in caudate and to a lesser extent in putamen of patients have been reported (Schapira, 1999). In mitochondria from frontal and temporal lobes of HD patients a mtDNA deletion was found (Horton et al., 1995).

Enhanced production of ROS and possibly accumulation of mtDNA mutations in post mitotic cells are contributory factors in neurodegeneration. Mitochondria not only generate ROS/reactive nitrogen species (RNS) but are also the main target for their actions (Raha and Robinson, 2000). As a result, damage occurs in the mitochondrial respiratory chain, producing further increases in free radical generation and leading ultimately to a vicious cycle (Genova et al., 2004).

During the last decade a number of studies have demonstrated that melatonin plays an effective role in regulating mitochondrial homeostasis (see for ref. Acuña Castroviejo et al., 2011; Srinivasan et al., 2011c). In addition to being a free radical scavenger, melatonin reduces nitric oxide (NO) generation within mitochondria. It maintains the electron flow, the efficiency of OXPHOS, ATP production, the bioenergetic function of the cell and mitochondrial biogenesis by regulating respiratory complex activities, Ca^{2+} influx and the mitochondrial permeability transition (mPT). This article summarizes the several mechanisms through which melatonin can exert neuroprotective actions in neurodegenerative disorders like AD, PD and HD.

Mitochondrial function and free radical generation

The primary function of mitochondria is to generate ATP within the cell through the ETC resulting in OXPHOS. Mitochondrial energy production requires the coordination of several sequential steps tightly interconnected. Even under normal conditions, 1–2% of electron flux is the consequence of the incomplete reduction of O_2 , leading to the production of superoxide anion radical ($O_2^{\bullet-}$) that is subsequently converted into hydrogen peroxide (H_2O_2). Hence, the mitochondria are the main source of ROS production within cells (Beal, 2009; DiMauro and Schon, 2008; Rezin et al., 2009; Rollins et al., 2009; Schon et al., 2010). For a recent, detailed, review of mitochondrial physiology see Acuña Castroviejo et al. (2011).

While a mild increase in ROS can act as a signal to elicit a number of physiological responses, a pervasive augment in free radical production or a burst of oxidative damage risks mitochondrial integrity leading to cell death. For example, $O_2^{\bullet-}$ production is very sensitive to a decrease in H^+ electrochemical gradient. The modulation of H^+ permeability at the mitochondrial inner membrane via uncoupling proteins, mitochondrial ion channels or fatty acids is an important event in the regulation of free radical production by mitochondria (Beal, 2009; DiMauro and Schon, 2008; Rezin et al., 2009; Rollins et al., 2009; Schon et al., 2010).

ETC, which is present in the inner mitochondrial membrane, comprises a series of electron carriers grouped into four enzyme complexes, namely C-I (NADH ubiquinone reductase), C-II (succinate ubiquinone reductase), C-III (ubiquinol cytochrome c-reductase), and C-IV (cytochrome c oxidase) (Lenaz and Genova, 2010). The main function of ETC is to convert redox energy into an electrochemical gradient of protons that subsequently causes ATP formation. The end product of the respiratory chain is water that is generated in a four-electron reduction of O_2 by C-IV. During this process (electron leakage, especially at complexes I and III), a small percentage of O_2 is converted into ROS, such as $O_2^{\bullet-}$ and its secondary products H_2O_2 and reactive hydroxyl radical ($\bullet OH$). Under normal conditions, the iron–sulfur cluster N2 of complex I appears to be the primary source of free radicals in the brain (Lenaz and Genova, 2010).

Melatonin reacts at a high rate with radicals like $\bullet OH$ and with relative low rates with other like $O_2^{\bullet-}$. Melatonin is an efficient inhibitor of lipid peroxidation by scavenging highly reactive species, such as $\bullet OH$, which initiate the degradation process, than by directly trapping peroxyl radicals (Galano et al., 2011).

Mitochondrial NO synthase (mtNOS), localized in the inner mitochondrial membrane, is responsible for generating NO radical ($\bullet NO$) from L-arginine (Esposito and Cuzzocrea, 2010). mtNOS is the neuronal NOS (nNOS) splice variant- α which interacts with C-IV; mtNOS comprises two isoforms, namely constitutive (c-mtNOS) and inducible (i-mtNOS). Because of the easy NO diffusion through the mitochondrial membrane, cytoplasm NOS isoforms are also relevant for generating intramitochondrial $\bullet NO$. High rates of $\bullet NO$ synthesis, which typically occur in the Ca^{2+} -dependent excited state of neurons, contribute to oxidative and nitrosative stress. The availability of $\bullet NO$ determines the rates at which the adduct peroxynitrite ($ONOO^-$) and the other decomposition products are generated.

$\bullet NO$ strongly interferes with components of the respiratory chain, in particular cytochrome c oxidase (Esposito and Cuzzocrea, 2010; Mander and Brown, 2004). Its metabolite $ONOO^-$ and radicals derived from this can damage proteins at the respiratory complexes. Other nitrosation processes, like transnitrosation or reversible nitrosation and nitration as well as irreversible DNA, protein and lipid oxidation, may occur (Stadtman and Levine, 2003). Damage to the mitochondrial ETC can cause a breakdown of the H^+ potential, apoptosis or lead to further generation of free radicals, thus maintaining a vicious cycle that ultimately results in cell death (Lenaz and Genova, 2010).

Melatonin protects ETC and mtDNA from ROS/RNS-induced oxidative damage. It limits the loss of intramitochondrial GSH, reduces mtDNA damage and increases expression and activity of C-IV and the activity of C-I, thus improving mitochondrial respiration and increasing ATP production (Acuña Castroviejo et al., 2011). NO production is also inhibited at the level of NOS gene transcription (Jimenez-Ortega et al., 2009).

Mitochondrial Ca^{2+} is crucial in rate control of energy production and plays an instrumental role in cell apoptosis and necrosis. Mitochondria take up Ca^{2+} through a calcium uniporter, highly dependent on H^+ electrochemical gradient (Oliveira, 2011). In the mitochondrial-mediated cell death pathway, a non-specific increase in the permeability of the inner mitochondrial membrane (mPT) occurs as a consequence of the increase in mitochondrial matrix Ca^{2+} . mPT defines the sudden increase in membrane permeability allowing the transfer of molecules up to 1500 Da in size and causing the dissipation of H^+ electrochemical gradient, uncoupling of OXPHOS and ATP depletion (Lemasters et al., 2009). With Ca^{2+} overload, there is a complete uniporter inhibition, mitochondrial swelling, loss of respiratory control and a release of matrix calcium. Under these conditions, the mitochondria undergo swelling and mitochondrial apoptosis ensues (Toman and Fiskum, 2011). Although the exact molecular nature of mPT remains elusive, its contribution to neurodegeneration is unequivocal and has been extensively reviewed (Gleichmann and Mattson, 2011; Pivovarova and Andrews, 2010). Melatonin effectively prevents induction of mPT in a number of circumstances (Andrabi et al., 2004; Karbownik et al., 2000).

Since mitochondria contain specific mechanisms for activating both the intrinsic apoptotic pathway and necrotic death in a variety of cell types, they can be seen as central regulators of cell fate. The intrinsic apoptotic pathway involves the activation and subsequent translocation to mitochondria of the members of the Bcl2 family (Bax, Bak, and Bid) which after insertion in the mitochondrial outer membrane behave as death channels. Through these channels a number of apoptogenic proteins e.g. cytochrome c, are released from the intermembrane space and are responsible for the activation of the signal cascade to cell death via caspase-9 and -3-dependent proteolysis (Santucci et al., 2010). Melatonin exerts a strong antiapoptotic effect (Sainz et al., 2003).

A number of enzyme mechanisms take part in the control of free radical production. Among these is the action of the enzyme superoxide dismutase (SOD), which is found in the inner side of the inner mitochondrial membrane (Mn-SOD) and that removes $\text{O}_2^{\bullet-}$ (Liochev and Fridovich, 2010). The $\bullet\text{OH}$ generated from H_2O_2 in the presence of reduced transition metals is scavenged by the enzyme glutathione peroxidase (GPx) during the process of GSH oxidation to glutathione disulfide. This is reduced back to GSH by the enzyme glutathione reductase (GRd). These enzymes form part of the endogenous antioxidant defense system and suppress ROS levels within the cell as well as at the mitochondria.

Besides being an antioxidant, melatonin promotes *de novo* synthesis of GSH by stimulating the activity of the enzyme γ -glutamyl-cysteine synthetase (Urata et al., 1999) and also through its effects on gene expression of GPx, GRd, SOD and CAT (Antolin et al., 1996; Jimenez-Ortega et al., 2009; Pablos et al., 1998; Rodriguez et al., 2004). This promotes the recycling of GSH and maintains a high GSH/glutathione disulfide ratio, thus underlining the important role that melatonin plays in mitochondrial physiology.

Basic physiology of melatonin

Melatonin is the major secretory product of the pineal gland released every day at night. In all mammals, circulating melatonin is synthesized primarily in the pineal gland (Claustrat et al., 2005). In addition, melatonin is also locally synthesized in various cells, tissues and organs including lymphocytes, bone marrow, the thymus, the

gastrointestinal tract, skin and the eyes, where it plays either an autocrine or paracrine role (see for ref. Hardeland et al., 2011).

Both in animals and in humans, melatonin participates in diverse physiological functions signaling not only the length of the night (and thus time of the day and season of the year) but also enhancing free radical scavenging and the immune response, showing relevant cytoprotective properties (Hardeland et al., 2011).

As above mentioned, melatonin is a powerful antioxidant that scavenges $\bullet\text{OH}$ radicals as well as other ROS and RNS and that gives rise to a cascade of metabolites that share also antioxidant properties (Galano et al., 2011). Melatonin also acts indirectly to promote gene expression of antioxidant enzymes and to inhibit gene expression of prooxidant enzymes (Antolin et al., 1996; Jimenez-Ortega et al., 2009; Pablos et al., 1998; Rodriguez et al., 2004). Melatonin has significant anti-inflammatory properties presumably by decreasing the synthesis of proinflammatory cytokines like $\text{TNF-}\alpha$ and by suppressing inducible NOS (iNOS) gene expression (Cuzzocrea et al., 1998). It has also a significant antiapoptotic effect (Sainz et al., 2003).

Circulating melatonin binds to albumin (Cardinali et al., 1972) and is metabolized mainly in the liver where it is hydroxylated in the C6 position by cytochrome P_{450} monooxygenases A2 and 1A (Facciola et al., 2001; Hartter et al., 2001). Melatonin is then conjugated with sulfate to form 6-sulfatoxymelatonin, the main melatonin metabolite found in urine. Melatonin is also metabolized in tissues by oxidative pyrrole-ring cleavage into kynuramine derivatives. The primary cleavage product is N^1 -acetyl- N^2 -formyl-5-methoxykynuramine (AFMK), which is deformedylated, either by arylamine formamidase or hemoperoxidase to N^1 -acetyl-5-methoxykynuramine (AMK) (Hardeland et al., 2009). It has been proposed that AFMK is the primitive and primary active metabolite of melatonin (Tan et al., 2007). Melatonin is also converted into cyclic 3-hydroxymelatonin in a process that directly scavenges two hydroxyl radicals (Tan et al., 2007). However, although direct radical scavenging has been effective under numerous experimental conditions, at clearly supraphysiological concentrations, its relevance at physiological levels has been questioned for reasons of stoichiometry. Despite of this, melatonin was shown to protect from oxidotoxicity already at physiological concentrations (Galano et al., 2011; Tan et al., 1994).

Melatonin exerts many physiological actions by acting on membrane and nuclear receptors while other actions are receptor-independent (e.g., scavenging of free radicals or interaction with cytoplasm proteins) (Reiter et al., 2009). The two melatonin receptors cloned so far (MT_1 and MT_2) are membrane receptors that have seven membrane domains and that belong to the superfamily of G-protein coupled receptors (Dubocovich et al., 2010). Melatonin receptor activation induces a variety of responses that are mediated both by pertussis-sensitive and insensitive G_i proteins. In the cytoplasm melatonin interacts with proteins like calmodulin and tubulin (Benitez-King, 2006). Nuclear receptors of retinoic acid receptor superfamily (retinoid Z receptor/ROR, retinoic acid receptor-related orphan receptor α) have been identified in several cells, among them in human lymphocytes and monocytes (Carrillo-Vico et al., 2006).

Melatonin and mitochondrial function

According to Acuña Castroviejo et al. (2011) a role of melatonin in mitochondrial homeostasis seems warranted. Melatonin is a powerful scavenger of ROS and RNS and naturally acts on mitochondria, the site with the highest ROS/RNS production into the cell. Melatonin improves the GSH redox cycling and increases GSH content by stimulating its synthesis in the cytoplasm, mitochondria depending on the GSH uptake from cytoplasm to maintain the GSH redox cycling. Lastly, melatonin exerts important antiapoptotic effects and most of the apoptotic signals originate from the mitochondria.

As above mentioned, data accumulated in the last decade strongly indicate that melatonin plays an important role in antioxidant

defense via the regulation of enzymes involved in the redox pathway and directly through the non-enzymatic, radical scavenger effect that melatonin and some of its metabolites (notably AFMK and AMK) have to scavenge ROS, RNS and organic radicals (Hardeland et al., 2011; Reiter et al., 2009). Hydrogen transfer and electron transfer have been identified as the main mechanisms determining the free radical-scavenging activity of melatonin (Galano et al., 2011). Melatonin reacts at a high rate with radicals like $\bullet\text{OH}$ and with relative low rates with other like $\text{O}_2^{\bullet-}$. In any event, melatonin efficiently inhibits lipid peroxidation by scavenging highly reactive species, such as $\bullet\text{OH}$, which initiate the degradation process, than by directly trapping peroxyl radicals. An alternate concept intends to explain the antioxidant effects of melatonin at the level of radical generation rather than detoxification of radicals already formed (Hardeland, 2005, 2009; Hardeland et al., 2003).

In a recent publication Galano et al. underlined the reasons why melatonin has most of the desirable characteristics of a good antioxidant: (i) it is widely distributed in the body, and is present in adequate concentrations; (ii) it is a broad spectrum antioxidant; (iii) it easily transported across cellular membranes; (iv) it can be regenerated, after radical quenching, and its metabolites still present antioxidant properties; (v) it has minimal toxicity (Galano et al., 2011). These effects of melatonin and its metabolites may be unique and not shared by MT_1/MT_2 melatonergic agonists like ramelteon or agomelatine, in which the indole ring is modified. For example, ramelteon displayed in vitro no relevant antioxidant capacity in a 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) assay compared with melatonin (Mathes et al., 2008).

Safeguarding of respiratory electron flux, reduction of oxidant formation by lowering electron leakage and inhibition of mPT events are among the most important effects on melatonin in mitochondria. Melatonin has been repeatedly shown to reduce, under various conditions, the mitochondrial formation of ROS and RNS, to protect against oxidative, nitrosative or nitrative damage of ETC proteins as well as lipid peroxidation in the inner membrane and, thus, to favor electron flux and energy efficiency. Melatonin (10 mg/kg) was found to increase the activity of C-I and C-IV in mitochondria obtained from rat brain and liver, C-II and C-III being not affected (Martin et al., 2000b). Injections of melatonin counteracted the inhibitory effect of ruthenium red on C-I, C-IV and GPx enzyme (Martin et al., 2000b). In another study, 100 nM melatonin was found to prevent the oxidation of GSH to glutathione disulfide induced by t-butyl hydroperoxide, restoring the normal activity of both GPx and GRd (Martin et al., 2000a). Melatonin increased C-I and C-IV activities in a dose dependent manner, the effect being significant at 1 nM. Melatonin also counteracted cyanide induced inhibition of C-IV, showing that it effectively increases the activity of ETC coupled to OXPHOS and increase ATP synthesis both in normal mitochondria as well as in mitochondria depleted of ATP by cyanide (Martin et al., 2000a). The effect of melatonin in regulating C-I and IV presumably do not reflect its antioxidant role but indicates an interaction with ETC complexes by donating and accepting electrons, an effect not shared by other antioxidants. For a recent, detailed, revision on melatonin effect on mitochondrial function see (Acuña-Castroviejo et al., 2011).

The possible mechanism by which systemically administered melatonin controls mitochondrial respiration in the liver includes depression of Krebs's cycle substrate-induced respiration (Reyes-Toso et al., 2006). In vitro studies in mitochondria from mouse liver cells, 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 effective to maintain the efficiency of OXPHOS and ATP synthesis by increasing the activity of C-I, C-III and C-IV (López et al., 2009).

Interestingly, co-treatment with melatonin prevented the decrease in mitochondrial membrane fluidity caused by δ -aminolevulinic acid,

which disrupts H^+ electrochemical gradient and mPT, in the absence of changes in mitochondrial membrane lipid peroxidation (Karbownik et al., 2000). This effect of melatonin may depend on its capacity to localize in the membrane itself, in a superficial position in lipid bilayers near the polar heads of membrane phospholipids (Ceraulo et al., 1999).

Melatonin's action in preventing t-butyl hydroperoxide induction of mPT was shown in primary skeletal muscle cultures. Melatonin (1–100 μM) desensitized the mPT to Ca^{2+} and prevented t-butyl hydroperoxide-induced mitochondrial swelling and GSH oxidation (Karbownik et al., 2000). Melatonin has also been reported to directly inhibit mPT with a K_i value of 0.8 μM (Andrabi et al., 2004). The high melatonin concentrations needed should be considered in the context of the reported mitochondrial accumulation of melatonin (López et al., 2009; Messner et al., 1998; Venegas et al., 2012). Melatonin has been repeatedly shown to prevent, at pharmacological concentrations, a fatal decline in H^+ electrochemical gradient in various cell types and with high efficacy against different noxa (Hardeland and Pandi-Perumal, 2005). In cardiomyocytes, astrocytes and striatal neurons, it prevented calcium overload (Andrabi et al., 2004; Jou et al., 2004) and counteracted the collapse of the mitochondrial membrane potential induced by H_2O_2 (Jou et al., 2004, 2007), doxorubicin (Xu and Ashraf, 2002) or oxygen/glucose deprivation (Andrabi et al., 2004).

Recently the role of melatonin on cardiolipin and mitochondrial bioenergetics was explored (Paradies et al., 2010; Petrosillo et al., 2008). 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 (Paradies et al., 2010). Melatonin was reported to protect the mitochondria from oxidative damage in part by preventing cardiolipin oxidation (Paradies et al., 2010; Petrosillo et al., 2008) (Fig. 1).

Melatonin can also act on mitochondrial biogenesis via sirtuins. Sirtuins are NAD^+ -dependent protein deacetylases that promote longevity in numerous organisms. The 7 mammalian subforms, SIRT1 to SIRT7, are involved in mitochondrial function. At least SIRT3, SIRT4 and SIRT5 are localized in mitochondria, SIRT3 preventing mitochondrial lysine hyperacetylation (Lombard et al., 2007). SIRT3 interacts with the C-I subunit, the 39-kDa protein NDUFA9, to enhance C-I activity and ATP levels (Ahn et al., 2008). Sirtuins also stimulate mitochondrial biogenesis (Guarente, 2008). Several studies have reported upregulation of SIRT1 by melatonin, e.g., in the brain of senescence-accelerated SAMP8 mice (Gutiérrez-Cuesta et al., 2008) and neuronal primary cultures from neonatal rat cerebellum (Tajes et al., 2009), or the prevention of SIRT1 decrease in the hippocampus of sleep-deprived rats (Chang et al., 2009). In neuronal cultures from cerebellum, melatonin enhanced the deacetylation of various SIRT1 substrates, effects which were largely reversed by the SIRT1 inhibitor sirtinol (Tajes et al., 2009). The melatonin-induced deacetylation of SIRT1 substrates indicates that mitochondrial biogenesis might be stimulated by the methoxyindole in vivo. Indeed, chronic melatonin administration increases the number and size of mitochondria in the pineal and in ependymal epithelium of the choroid plexus (Barratt et al., 1977; Decker and Quay, 1982).

Melatonin and mitochondrial dysfunction in AD

Several recent studies have supported the involvement of mitochondrial ROS and RNS production and abnormal mitochondrial function in the pathophysiology of AD (Bobba et al., 2010; Galindo et al., 2010; Manczak et al., 2010; Massaad et al., 2009; Muller et al., 2010; Santos et al., 2010; Trancikova et al., in press). AD is characterized by extracellular senile plaques of aggregated β -amyloid ($\text{A}\beta$) and intracellular neurofibrillary tangles that contain hyperphosphorylated tau

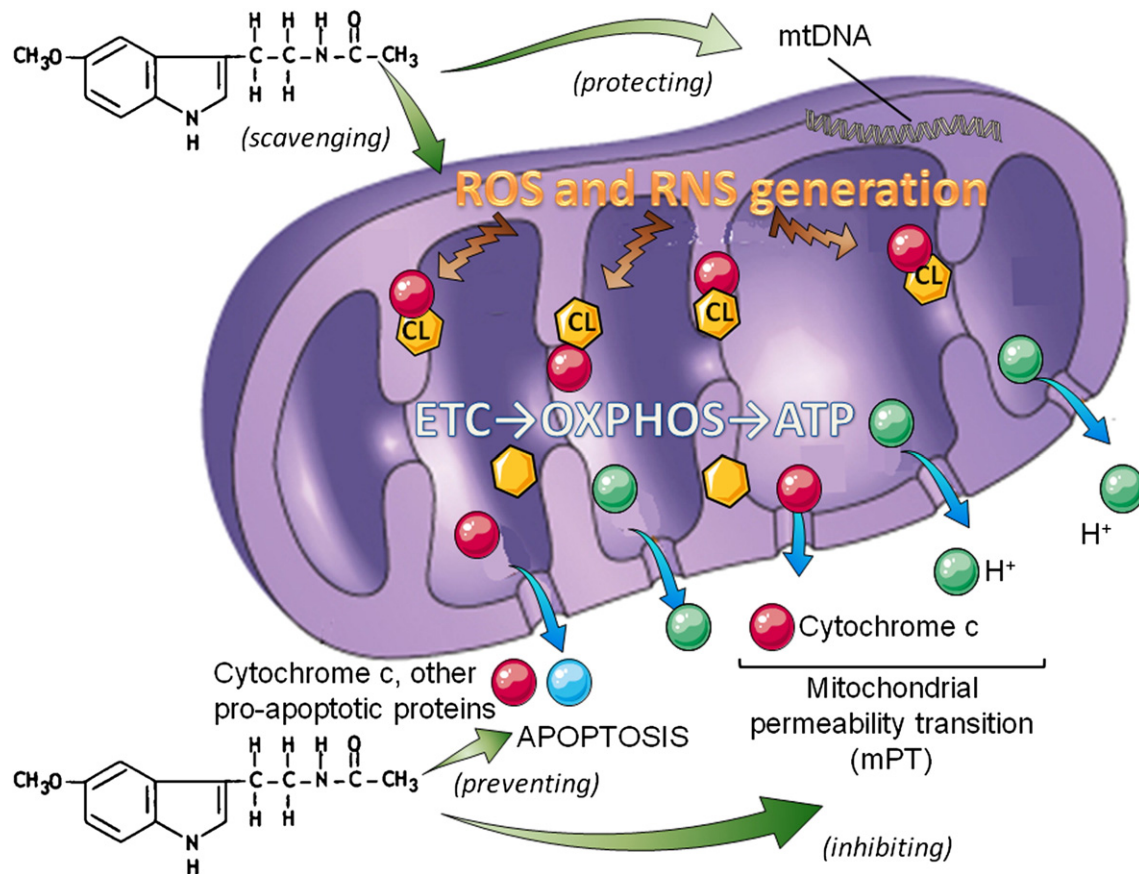


Fig. 1. Melatonin and mitochondrial physiopathology. The mechanisms involved in ETC failure mainly depend on the generation of ROS and RNS in the mitochondria leading to oxidative stress and ATP depletion. Respiratory chain-mediated ROS production, partly via cardiolipin (CL) peroxidation, brings about the detachment of cytochrome c from the inner mitochondrial membrane and changes in mPT that leads to mitochondrial swelling and the release of cytochrome c and other proapoptotic proteins. Apoptosis ensues by activation of the caspase cascade in the cytoplasm leading to cell death. Melatonin and its metabolites (AFMK, AMK) prevent this cascade by acting at multiple sites at the mitochondria. Scavenging of ROS and RNS prevents free radical attack against ETC complexes and mtDNA. Melatonin protects mPT disruption and proapoptotic signal release to the cytoplasm. Melatonin also increases transcriptional activity of the mtDNA, improving mitochondrial physiology.

protein. The resulting clinical effect is a progressive loss of memory and deterioration of cognition.

A β is reported to accumulate in subcellular compartments and to impair neuronal function (Reddy et al., 2010). There is substantial evidence to prove that mitochondrial toxicity is linked to the progressive accumulation of mitochondrial A β (Chen and Yan, 2010). 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 A β protein misfolding into neurotoxic oligomeric aggregates.

Mitochondrial O $_2^{\bullet\bullet}$ production plays a critical role in the pathological events following A β elevation. An increased expression of mitochondrial antioxidant enzyme SOD-2 has been shown to prevent memory deficits and amyloid plaque deposition associated with AD (Massaad et al., 2009). 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.

Several actions of melatonin have been described which antagonize the deleterious effects of A β . The effects of melatonin can be grouped as (i) antioxidant, including influences on mitochondrial metabolism; (ii) antifibrillogenic, blocking A β synthesis; (iii) cytoskeletal, including suppression of tau protein hyperphosphorylation. The antifibrillogenic effects of melatonin were observed not only in vitro but also in vivo in transgenic mouse models (Feng et al., 2004; Matsubara et al., 2003; Olcese et al., 2009).

Protection from A β toxicity by melatonin was observed, especially at the mitochondrial level. In a mutant A β transgenic mouse model of familial AD melatonin can prevent toxic aggregation of A β peptide

and, when taken long term, can protect against cognitive deficits (Olcese et al., 2009). In a recent study APP/PS1 transgenic mice were treated for 1 month with melatonin and the analysis of isolated brain mitochondria indicated that melatonin treatment decreased mitochondrial A β levels to 25–50% of controls in several brain regions (Dragicevic et al., 2011). This was accompanied by a near complete restoration of mitochondrial respiratory rates, membrane potential, and ATP levels of isolated mitochondria from the hippocampus, cortex, or striatum. In APP-expressing neuroblastoma cells in culture, mitochondrial function was restored by melatonin or by its structurally related compounds AFMK or indole-3-propionic acid. This was partially blocked by melatonin receptor antagonists indicating that melatonin receptor signaling is required for the full effect. The authors concluded that treatment stimulating melatonin receptor signaling can be beneficial for restoring mitochondrial function in AD (Dragicevic et al., 2011).

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 β -induced cell death (Jang et al., 2005). 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 (Zhou et al., 2008).

In view of the consequences of excitation-dependent Ca $^{2+}$ overload on mitochondrial membrane potential and mPT sensitivity towards excitotoxins like A β , 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 (Hung et al., 2010). In a recent study a possible melatonin prevention of damage induced by $\text{A}\beta$ was evaluated in young and senescent hippocampal neurons. Rat hippocampal neurons were incubated with $\text{A}\beta_{25-35}$ and cell viability, mitochondrial membrane potential, ATP and the activity of the respiratory chain complexes were measured (Dong et al., 2010). Cells exposed to $\text{A}\beta_{25-35}$ showed decreased mitochondrial membrane potential, inhibited activity of respiratory chain complexes and a depletion of ATP levels. Melatonin attenuated $\text{A}\beta_{25-35}$ -induced mitochondrial damage in senescent hippocampal neurons (Dong et al., 2010). Molecular studies undertaken with mitochondrial preparations suggest that melatonin has a therapeutic value in treating AD through its antiapoptotic activities (Wang et al., 2009).

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 (Quinn et al., 2005). Mild cognitive impairment is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia (Gauthier et al., 2006). Some of these patients develop AD. A small number of controlled trials indicate that melatonin can be useful to treat mild cognitive impairment and to prevent progression to AD (Cardinali et al., 2010).

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 substantia nigra pars compacta and by the formation of Lewy bodies. The initiating factor in PD is still unknown. The possible involvement of an increased release of free radicals has been entertained in view of enhanced signs of oxidative stress found in the brain of PD patients (Gibson et al., 2010).

A reduced C-I activity in the substantia nigra pars compacta and loss of GSH have been reported in PD patients (Navarro and Boveris, 2010). The inhibition of ETC proteins compromises energy availability and leads to apoptosis and death of the dopaminergic cells. Defects in the mtDNA, a reduced H^+ electrochemical gradient and increased apoptosis may underlie specific neuronal death (Luoma et al., 2004; Navarro and Boveris, 2010).

α -Synuclein assembly is a critical step in the development of Lewy body diseases such as PD and dementia with Lewy bodies. Melatonin attenuated kainic acid-induced neurotoxicity (Chang et al., 2011) and arsenite-induced apoptosis (Lin et al., 2007) via inhibition α -synuclein aggregation. Melatonin also decreased the expression of α -synuclein in dopamine containing neuronal regions after amphetamine both in vivo (Sae-Ung et al., 2012) and in vitro (Klongpanichapak et al., 2008). In a recent study melatonin effectively blocked α -synuclein fibril formation and destabilized preformed fibrils. It also inhibited protofibril formation, oligomerization, and secondary structure transitions of α -synuclein as well as reduced α -synuclein cytotoxicity (Ono et al., 2011).

A commonly accepted model of PD is that achieved by the systemic or intracerebral administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Schober, 2004; Terzioglu and Galter, 2008). Its active glial metabolite, 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. By binding to C-I, MPP^+ increases the production of ROS and enhances oxidative stress causing reduction of ATP and death of cells. A neuroprotective effect of melatonin in isolated rat striatal synaptosomes and liver mitochondria treated with MPTP has been demonstrated (Absi et al., 2000). Melatonin prevented the inhibition of mitochondrial respiration by limiting the interaction of MPP^+

with C-I of ETC. In a study conducted in adult male mice, MPTP was administered at a dose of 15 mg/kg in four separate doses (Tapias et al., 2009). The concomitant administration of melatonin or its metabolite AMK (20 mg/kg) significantly reduced the iNOS activity stimulated by MPP^+ .

A small number of controlled trials indicate that melatonin is useful to treat disturbed sleep in PD, particularly rapid eye movement-associated sleep behavior disorder (see for ref. Srinivasan et al., 2011a). Whether melatonin has the potential for treating insomnia in PD patients and, more generally, for arresting the progression of PD merits further investigation.

Melatonin and mitochondrial dysfunction in HD

HD is a neurodegenerative disorder that leads to ataxia, chorea and dementia. It may be produced by a genomic alteration in the DNA encoding huntingtin, a protein of unknown function but associated with increased apoptosis. Lesions in HD include predominantly the γ -aminobutyric acid containing neurons of the caudate nucleus (Schapira, 1999). Mitochondrial dysfunction occurs in HD (Chen, 2011). A mtDNA deletion (mtDNA4977) was found in HD patients particularly in frontal and temporal lobes but its significance is unknown (Horton et al., 1995). There are deficiencies in the activity of C-II, C-III and C-IV in caudate and in a lesser extent in putamen in HD.

A Huntington's chorea animal model was developed by using 3-nitropropionic acid, an inhibitor of mitochondrial C-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 (Tunee et al., 2004). However, although current evidence from genetic models of HD including mutation of the huntingtin gene (mHtt) supports the mitochondrial dysfunction as a major cause of the disease, impairment of ETC appears to be a late secondary event (Oliveira, 2010). 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 (Oliveira, 2010). Evidence is lacking as to whether melatonin's action on mitochondria could affect disease's 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 (Heiser et al., 2000).

Melatonin and its analogs as pharmaceutical tools

As melatonin exhibits both hypnotic and chronobiotic properties, it has been therapeutically used for treatment of age-related insomnia as well as of other primary and secondary insomnia (Leger et al., 2004; Zhdanova et al., 2001). A recent consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia and circadian rhythm sleep disorders concluded that melatonin is the first choice treatment when a hypnotic is indicated in patients over 55 years (Wilson et al., 2010). Since melatonin has a short half life (less than 30 min) its efficacy in promoting and maintaining sleep has not been uniform in the studies undertaken so far. Thus the need for the development of prolonged release preparations of melatonin or of melatonin agonists with a longer duration of action on sleep regulatory structures in the brain arose (Turek and Gillette, 2004). Slow release forms of melatonin (e.g., Circadin®, a 2 mg-preparation developed by Neurim, Tel Aviv, Israel, and approved by the European Medicines Agency in 2007) and the melatonin analogs ramelteon, agomelatine, tasimelteon and TK-301 are examples of this strategy.

Ramelteon (Rozerem®, Takeda Pharmaceuticals, Kyoto, Japan) is a melatonergic hypnotic analog approved by the FDA for treatment of

insomnia in 2005. It is a selective agonist for MT₁/MT₂ receptors without significant affinity for other receptor sites (Kato et al., 2005; Miyamoto, 2009). In vitro binding studies have shown that ramelteon affinity for MT₁ and MT₂ receptors is 3–16 times higher than that of melatonin. Doses of ramelteon vary from 8 to 32 mg/day.

Agomelatine (Valdoxan®, Servier, Neuilly-sur-Seine, France) is a recently introduced melatonergic antidepressant that acts on both MT₁ and MT₂ melatonergic receptors with a similar affinity to that of melatonin; it also acts as an antagonist to serotonin_{2C} receptors at a 3 orders of magnitude greater concentration (Millan et al., 2003). Agomelatine has been licensed by EMEA for treatment of major depressive disorder at doses of 25–50 mg/day.

Tasimelteon, [VES-162] is a MT₁/MT₂ agonist developed by Vanda Pharmaceuticals, Washington DC, USA, that completed phase III trial in 2010. In animal studies, tasimelteon exhibited the circadian phase shifting properties of melatonin (Vachharajani et al., 2003). In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10 to 100 mg/day (Rajaratnam et al., 2009). The FDA granted tasimelteon orphan drug designation status for blind individuals without light perception with non-24-hour sleep–wake disorder in 2010.

TIK-301 (formerly LY-156,735) has been in a phase II clinical trial in the USA since 2002. Originally it was developed by Eli Lilly and Company, Indianapolis, USA, and called LY-156,735. In 2007 Tikvah Pharmaceuticals, Atlanta, USA, took over the development and named it TIK-301. It is a chlorinated derivative of melatonin with MT₁/MT₂ agonist activity and 5HT_{2C} antagonist activity. TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo controlled study using 20 to 100 mg/day doses in healthy volunteers (Mulchahey et al., 2004). The FDA granted TIK-301 orphan drug designation in 2004, to use as a treatment for circadian rhythm sleep disorder in blind individuals without light perception and individuals with tardive dyskinesia.

As shown by the binding affinities, half-life and relative potencies of the different melatonin agonists it is clear that studies using 2–5 mg melatonin/day are unsuitable to give appropriate comparison with the effect of the above mentioned compounds, which in addition to being generally more potent than the native molecule are employed in considerably higher amounts (Cardinali et al., in press). Melatonin has a high safety profile and it is usually remarkably well tolerated. In some studies melatonin has been administered to patients at large doses. Melatonin (300 mg/day) given for up to 3 years decreased oxidative stress in patients with amyotrophic lateral sclerosis (Weishaupt et al., 2006). In children with muscular dystrophy, 70 mg/day of melatonin reduced cytokines and lipid peroxidation (Chahbouni et al., 2010). Doses of 80 mg melatonin hourly for 4 h were given to healthy men with no undesirable effects other than drowsiness (Waldhauser et al., 1984). In healthy women given 300 mg melatonin/day for 4 months there were no side effects (Voordouw et al., 1992). Therefore, further studies employing melatonin doses in the 100 mg/day are needed to clarify its potential therapeutic implications in humans. From animal studies it is clear that a number of preventive effects of melatonin, like those in neurodegenerative disorders, need high doses of melatonin to become apparent (Cardinali et al., 2010; Srinivasan et al., 2011a, b). If one expects melatonin to be an effective neuroprotector, especially in aged people, it is likely that the low doses of melatonin employed so far are not very beneficial.

Conclusions

Abnormal mitochondrial function, decreased respiratory enzyme complex activities, increased electron leakage and mPT, and increased Ca²⁺ entry have all been shown to play a role in the pathophysiology of neurodegenerative disorders. In various neurodegenerative diseases, such as AD, PD or HD, mitochondrial changes are not only

observed at the level of ETC dysfunction, electron leakage and oxidative, nitrosative or nitrative damage, but also in a disturbed balance between mitochondrial fusion and fission, with consequences for intracellular distribution of these organelles (Wang et al., 2009). In the course of disease progression, mitochondrial density decreases preferentially in the cell periphery of neurons. The peripheral mitochondrial depletion is associated with reduced H⁺ electrochemical gradient and ATP production, increases in radical formation and losses of spines at neurites (Wang et al., 2008; Wang et al., 2009).

This key role of mitochondria in neurodegenerative diseases indicates that supporting the integrity and functioning of these organelles should be given a high priority, thereby reducing electron leakage and radical formation. To what extent this is possible with melatonin, perhaps, in conjunction with photic stimulation of the circadian oscillators remains to be thoroughly studied.

Acknowledgments

Studies in authors' laboratories were supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina and the University of Buenos Aires. DPC is a Research Career Awardee from the Argentine Research Council (CONICET) and Professor Emeritus, University of Buenos Aires. ESP and PS are Research Career Awardees from CONICET.

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