Arch Toxicol
DOI 10.1007/s00204-015-1475-z

REVIEW ARTICLE

Protective role of melatonin in mitochondrial dysfunction and related disorders

Giuseppe Paradies · Valeria Paradies · Francesca M. Ruggiero · Giuseppe Petrosillo

Received: 14 January 2015 / Accepted: 9 February 2015

Abstract Mitochondria are the powerhouse of the eukaryotic cell through their use of oxidative phosphorylation to generate ATP. Mitochondrial dysfunction is considered an important contributing factor in a variety of physiopathological situations such as aging, heart ischemia/reperfusion injury, diabetes and several neurodegenerative and cardiovascular diseases, as well as in cell death. Increased formation of reactive oxygen species, altered respiratory chain complexes activity and opening of the mitochondrial permeability transition pore have been suggested as possible factors responsible for impaired mitochondrial function. Therefore, preventing mitochondrial dysfunction could be an effective therapeutic strategy against cellular degenerative processes. Cardiolipin is a unique phospholipid located at the level of inner mitochondrial membrane where it plays an important role in mitochondrial bioenergetics, as well as in cell death. Cardiolipin abnormalities have been associated with mitochondrial dysfunction in a variety of pathological conditions and aging. Melatonin, the major secretory product of the pineal gland, is a well-known antioxidant agent and thus an effective protector of mitochondrial bioenergetic function. Melatonin was reported to prevent mitochondrial dysfunction from oxidative damage by preserving cardiolipin integrity, and this may explain, at least in part, the beneficial effect of this compound in mitochondrial physiopathology. In this article, mechanisms through which melatonin exerts its protective role in mitochondrial dysfunction and related disorders are reviewed.

Keywords Melatonin · Mitochondria bioenergetics · Cardiolipin · Oxidative stress · Physiopathology

G. Paradies (✉) · V. Paradies · F. M. Ruggiero
Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy
e-mail: g.paradies@biologia.uniba.it

G. Petrosillo (✉)
Institute of Biomembranes and Bioenergetics, National Research Council, Bari, Italy
e-mail: g.petrosillo@ibbe.cnr.it

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a highly conserved molecule derived from tryptophan that is found in all organisms from unicells to vertebrates (Hardeland and Fuhrberg 1996; Hardeland et al. 2006; Tan et al. 2007). Melatonin and its metabolites can function as endogenous free radical scavengers and broad-spectrum antioxidants (Tan et al. 2002; Reiter et al. 2008). Due to its small size and amphiphilic nature, melatonin can reach numerous cellular and subcellular compartments, particularly mitochondria (Menendez-Pelaez and Reiter 1993), raising the possibility of functional significance for this targeting with involvement in situ in mitochondrial bioenergetic processes (Leon et al. 2004; Paradies et al. 2010a). Most of the beneficial consequences resulting from melatonin administration may depend on its effect on mitochondrial physiology (Acuña-Castroviejo et al. 2007, 2011).

Mitochondrial dysfunction is considered an important contributing factor in a variety of physiopathological situations such as neurodegenerative and cardiovascular diseases, diabetes, various forms of hepatic disorders, skeletal muscle disorders, as well as in aging (Beal 1998; Wallace 1999; Schon et al. 2010). Alterations in mitochondrial function such as defects in the electron transport chain (ETC) activity and oxidative phosphorylation (OXPHOS) have all been suggested as the primary causative factors in the pathogenesis of these disorders.

Published online: 18 February 2015

Mitochondria are considered the main intracellular source of reactive oxygen species (ROS), as well as the major target of free radical attach. ROS are generated at very low levels during mitochondrial respiration under normal physiological conditions, while the level of these oxidants increases in several pathological conditions and aging. ROS produced by the mitochondrial ETC attach mitochondrial constituents including proteins, lipids and mitochondrial DNA (mtDNA). ROS-induced alterations to mitochondrial membrane constituents may lead to a decline of the mitochondrial bioenergetic function, and this may contribute to the etiology of a variety of pathological conditions, including heart ischemia/reperfusion (Chen and Zweier 2014), aging and age-related cardiovascular and neurodegenerative diseases (Boveris and Navarro 2008; DiMauro and Schon 2003; Raha and Robinson 2000).

Recent studies have demonstrated that melatonin plays an effective role in preserving mitochondrial homeostasis (Martín et al. 2002; López et al. 2009; Paradies et al. 2010a; Acuña-Castroviejo et al. 2011; Navarro-Alarcón et al. 2014), which may explain the protective effect of this molecule in several physiopathological conditions including neurological (Patki and Lau 2011; Pandi-Perumal et al. 2013; Cardinali et al. 2013) and cardiovascular disorders (Dominguez-Rodriguez and Abreu-Gonzalez 2010; Yang et al. 2014) as well as aging (Bondy et al. 2004; Dong et al. 2010), all of which are associated with mitochondrial dysfunction. This protective effect of melatonin may be explained, at least in part, on its antioxidant and free radical scavenging properties, thus preserving the stability, integrity and function of mitochondrial membranes (García et al. 2014; Reiter et al. 2014).

Cardiolipin (CL), a unique phospholipid located at the level of the inner mitochondrial membrane (IMM), has been shown to play a central role in the mitochondrial function (Hoch 1992; Houtkooper and Vaz 2008; Ren et al. 2014; Paradies et al. 2014a). Abnormalities in CL not only alter fluidity and folding of the IMM, but can profoundly alter the organization and function of the respiratory chain complexes and/or their organization in supramolecular structure, such as supercomplexes (Musatov and Robinson 2012; Mileykovskaya and Dowhan 2014). In particular, oxidation and depletion of CL have been associated with mitochondrial dysfunction in several metabolic and degenerative diseases (Chicco and Sparagna 2007; Paradies et al. 2009, b). Recently, melatonin was reported to preserve mitochondrial CL from oxidative damage, and this may explain, at least in part, the beneficial effect of this molecule in mitochondrial dysfunction and associated disorders (Petrosillo et al. 2009c; Paradies et al. 2010a). In this review, we discuss the several mechanisms through which melatonin can exert its protective role in mitochondrial dysfunction and related disorders.

Mitochondrial function and ROS generation

Mitochondria contain multiple copies of circular genome known as mtDNA as it has been characterized in humans (Anderson et al. 1981). The majority of mitochondrial proteins needed for normal bioenergetic processes are encoded by nuclear DNA, while some proteins essential for ETC and OXPHOS are encoded by mtDNA. Human mtDNA encodes for 13 polypeptides of subunits of complexes I, III and IV and ATP synthase, 22 tRNA and two ribosomal nucleic acids.

Mitochondria play a central role in energy-generating processes within the cell through the ETC, the primary function of which is ATP synthesis through the OXPHOS process. The ETC, which is located in the IMM, comprises a series of electron carriers grouped into four enzyme complexes, namely complex I (CI) (NADH-ubiquinone reductase), complex II (CII) (succinate-ubiquinone reductase), complex III (CIII) (ubiquinol-cytochrome c reductase) and complex IV(CIV) (cytochrome c oxidase) (Lenaz and Genova 2010). The electrons are transferred to molecular oxygen via the electron transport complexes, resulting in the reduction of oxygen to water at complex IV. During this process, protons (H^+) are pumped by CI, CIII and CIV into the intermembrane space to form an electrochemical gradient, which is the major contributor to the mitochondrial inner membrane potential. Complex V (CV) (ATP synthase) utilizes the stored energy of this proton gradient to drive the formation of ATP from ADP and inorganic phosphate. ATP formed is then transferred by the ADP/ATP carrier (ANT) to the intermembrane space in exchange with ADP.

It is estimated that around 0.2–2 % of the oxygen taken up by the cell is converted by mitochondria to ROS, mainly through the production of $O_2^{\cdot-}$ (Boveris and Chance 1973). OXPHOS, however, comes with an additional cost, the production of potentially harmful ROS. Mitochondrial ETC is considered the main source of ROS production. The primary ROS generated into the mitochondria is superoxide anion ($O_2^{\cdot-}$), which is then converted to hydrogen peroxide (H_2O_2) by spontaneous dismutation or by superoxide dismutase (SOD). Hydrogen peroxide in turn is broken down into water by glutathione peroxidase or catalase; otherwise, H_2O_2 can undergo Fenton's reaction in the presence of divalent cations such as iron to produce hydroxyl radical (·OH), which can be even more harmful to the mitochondrial biomolecules. The sites of superoxide anion production along the ETC have been the subject of many studies (for a recent review, see Murphy 2009). The two major sites of $O_2^{\cdot-}$ production are complex I and complex III. Mitochondria can produce $O_2^{\cdot-}$, predominantly from complex I, when the matrix NADH/NAD+ ratio is high, leading to a reduced FMN site on complex I, and when they have a

high proton-motive force and a reduced coenzyme Q pool, leading to reverse electron transport. The site of superoxide production at complex III is probably the unstable ubisemiquinone molecules (Turrens et al. 1985) or cytochrome b (Nohl and Stolze1992). The production of $O_2^{\cdot-}$ at complex I is believed to occur at the matrix site of the IMM. At complex III, $O_2^{\cdot-}$ is released to both the matrix and the cytosolic sides of the IMM. The relative contributions of complex I and III to ROS production appear also to be dependent on types of tissues, species and experimental conditions. The rate of ROS production is also affected by mitochondrial metabolic conditions. $O_2^{\cdot-}$ production is highest under state 4 respiration; when oxygen consumption is low, the proton-motive force is high and ETC complexes are in reduced state (Skulachev 1996; Korshunov et al. 1997). ROS are also produced to a lesser extent outside of the mitochondrion. Examples of extra-mitochondrial ROS producing reactions include xanthine oxidase, D-amino oxidase, the P-450 cytochromes and proline and lysine hydroxylase.

Mitochondria can also produce nitric oxide (NO) from mitochondrial nitric oxide synthase (Ghafourifar and Richter 1997; Giulivi et al. 1998), located in the IMM, from L-arginine. NO can be then converted to various reactive nitrogen species (RNS) such as nitroxyl anion (NO^-) or the toxic peroxynitrite ($ONOO^-$). The oxidizing reactivity of $ONOO^-$ is generally considered equivalent to that of $^{\cdot}OH$. NO strongly interferes with components of the ETC, in particular with cytochrome c oxidase (Mander and Brown 2004). NO in combination with $ONOO^-$ not only interferes with respiratory complexes, but can also trigger free radical-mediated chain reactions that in turn damage proteins, lipids and DNA molecules (Rubbo et al. 1994; Levine and Stadtman 2001). Damage to the mitochondrial respiratory chain can cause a collapse of membrane potential with further generation of free radicals, triggering a vicious cycle that ultimately leads to cell death.

Mitochondrial antioxidant defense systems

Mitochondria are equipped with an intricate array of enzymatic and nonenzymatic antioxidant defense systems poised to detoxify the ROS/RNS production. Nonenzymatic components of the system include hydrophilic and lipophilic radical scavengers, such as cytochrome c, alpha-tocopherol, ascorbate, ubiquinone, glutathione and melatonin. Another specific mitochondrial defense mechanism is the mild uncoupling that prevents marked increase in membrane potential and hence $O_2^{\cdot-}$ formation. Enzymatic components of the antioxidant systems include manganese-superoxide dismutase (Mn-SOD), catalase, glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase. Within the mitochondrial matrix, Mn-SOD converts $O_2^{\cdot-}$ to H_2O_2, which can be further metabolized by glutathione peroxidase (Gpx I) and peroxiredoxine (Prx III) or diffuse from the mitochondria into the cytosol. Part of the $O_2^{\cdot-}$ produced by the mitochondrial ETC can be released into the inner membrane space where it can be converted to H_2O_2 by Cu–Zn SOD. The $O_2^{\cdot-}$ present in the intermembrane space could be scavenged by the oxidized form of the cytochrome c or diffuse into the cytosol through the voltage-dependent anion channel (VDAC; Madesh and Hajnoczky 2001). $O_2^{\cdot-}$ may also react with nitric oxide (NO) to form highly reactive $ONOO^-$. Glutathione (GSH) and multiple GSH-linked antioxidant enzymes exert also an important mitochondrial antioxidant protection. Among GSH-linked enzymes involved in mitochondrial antioxidant defense are Gpx 1 located predominantly in the cytosol and Gpx 4, also known as phospholipid hydroperoxide glutathione peroxidase, which is associated with contact sites of the two mitochondrial membranes. These enzymes catalyze the reduction of H_2O_2 and of lipid hydroperoxides. Gpx 4 reduces hydroperoxide groups on phospholipids, lipoproteins and cholesteryl esters. Gpx 4 is considered to be the primary enzymatic defense mechanism against oxidative damage to cellular membranes.

The redox cycling in the mitochondria is very active and serves to prevent significant loss of GSH. Melatonin promotes de novo synthesis of GSH by stimulating the activity of the enzyme γ-glutamyl-cisteine synthase (Urata et al. 1999) and also through its effect on gene expression of Gpx, SOD and catalase (Antolín et al. 1996; Reiter et al. 2000), thus favoring the recycling of GSH and maintaining high GSH/GSSG ratio. These effects of melatonin may have important implications in mitochondrial physiology (Escames et al. 2010).

Melatonin and mitochondrial oxidative stress

Mitochondria are the most powerful intracellular source of ROS and also the primary target for their damaging effects. The interaction of ROS with mitochondrial components impairs the function of these organelles and directly affects cell viability and triggers cell death. ROS-induced structural and functional modification of proteins is one of the hallmarks of aging and several pathological disorders in biological systems (Stadtman 2002). One important target of ROS is mtDNA, which encodes polypeptides that are essential for ETC and ATP generation by OXPHOS. MtDNA is particularly susceptible to attack by ROS because of its proximity to the ETC and lack of protective histones. MtDNA therefore represents a critical cellular target for oxidative damage that could lead to lethal cell injury through the disruption of electron transport, mitochondrial membrane potential and ATP generation. ROS-induced

mtDNA damage is probably a major source of mitochondrial genomic instability responsible for the mitochondrial dysfunction. The instability of mtDNA is thought to be one of the most important factors in aging (Wei and Lee 2002).

In addition to mtDNA and proteins, mitochondrial membrane lipids are highly susceptible to oxidative damage. Phospholipids are the most abundant lipid components of the cellular and subcellular membranes, including mitochondria. Phospholipids are essential structural components of the mitochondrial membranes where they play multiple roles. They define the essential membrane permeability barrier and modulate the proper membrane fluidity, which is required for the optimal functional activities of proteins and enzymes. Polyunsaturated fatty acids (PUFAs) are essential components of mitochondrial phospholipids. The presence of a methylene bridge between two double bonds renders the PUFAs particularly sensitive to ROS attack, enabling them to participate in long free radical chain reactions generating hydroperoxides as well as endoperoxides. These lipid peroxidation products can undergo fragmentation to produce a broad range of reactive intermediates, among them are malondialdehyde (MDA) and the most reactive, 4-hydroxy-trans-2-nonenal (HNE). Oxidation of membrane phospholipids is considered one of the major causes of mitochondrial dysfunction in a variety of physiopathological situations and aging. In fact, lipid peroxidation alters the structural and functional organization of the lipid bilayer, changing membrane fluidity and permeability, thereby affecting respiration and OXPHOS process, maintenance of mitochondrial membrane potential and mitochondrial Ca^{2+} buffer capacity (Pamplona 2008).

Data accumulated in the last decade indicate that melatonin plays an important role in antioxidant defense preserving mitochondrial homeostasis, reducing free radical generation and stimulating ETC complex activity (Martín et al. 2002; López et al. 2009; Paradies et al. 2010a; Acuña-Castroviejo et al. 2011; Navarro-Alarcón et al. 2014). The earliest evidence of the antioxidant capacities of melatonin was reported in 1993 (Tan et al. 1993). Melatonin scavenges two molecules of $^{\cdot}OH$, and in the process, it is converted to cyclic 3-hydroxymelatonin (Tan et al. 1998). This later compound was detected by mass spectra analysis and carbon and proton–nuclear magnetic resonance in the urine of human and rats under oxidative stress conditions and treated with melatonin (Tan et al. 1998). It was reported that melatonin does not directly scavenge H_2O_2 in vitro, while a direct interaction of melatonin with H_2O_2 occurs only in the presence of traces of the transition metal ions (Fowler et al. 2003), and this may have important implications in vivo under stress condition. Besides its effects on ROS, melatonin is also a powerful scavenger of RNS. Nitric oxide is produced by several forms of NOS. In the mitochondria, two NOS isoforms, namely constitutive (c-mtNOS) and inducible (i-mtNOS), have been reported (Ghafourifar and Richter 1997). NO strongly interferes with components of the respiratory chain in particular cytochrome c oxidase (Mander and Brown 2004). NO in combination with $ONOO^-$ not only interferes with respiratory chain complexes, but can trigger free radical-mediated chain reactions that in turn damage proteins, lipids and DNA molecules (Rubbo et al. 1994; Stadtman and Levine 2003).

Polyunsaturated fatty acids, the main constituents of phospholipids (PLs), are particularly sensitive to peroxidation. This process is considered to proceed via a sequence of steps, including the abstraction of a hydrogen atom from unsaturated fatty acids, forming an alkyl radical ($PL^{\cdot}$), followed by a rapid addition of oxygen to form the peroxyl radical ($PLOO^{\cdot}$), and then formation of a hydroperoxide (PLOOH) via abstraction of a hydrogen from another acyl chain; as a consequence, the reaction is repeated and the whole process continues in a free radical chain reaction. Due to this auto-oxidative chain reaction, a single initiation event could theoretically lead to the oxidation of all lipids in a cellular organelle or in a cell. Other reactive species which initiate lipid peroxidation include $ONOO^-$ and singlet oxygen $^1O_2$. Because of the highly destructive structural and functional nature of lipid peroxidation, there is great interest in identifying molecules which reduce the initiation and/or progression of the denaturation of PUFAs. It has been well documented that melatonin and its derivatives exert a protective effect against lipid peroxidation induced by oxidative stress in mitochondrial membranes (Reiter et al. 2014). The ability of melatonin to protect against lipid peroxidation has been repeatedly documented either in animal or in plant tissues under various oxidizing conditions such as ionizing radiation, heavy metal toxicity, and drug metabolism. (García et al. 2014). The precise mechanism by which melatonin and its derivatives affect lipid peroxidation is not yet established. Melatonin has been shown to scavenge the peroxyl radical $PLOO^{\cdot}$ (Pieri et al. 1994; Livrea et al. 1997), which is produced during lipid peroxidation and being sufficiently reactive to propagate the chain reactions. The efficacy of melatonin to function as a $PLOO^{\cdot}$ scavenger was evaluated by measuring inhibition of metal ion-, radiation- or human macrophage-mediated oxidation of human low-density lipoprotein (Abuja et al. 1997). Melatonin was shown to be more effective than vitamin E in neutralizing $PLOO^{\cdot}$ and inhibiting lipid peroxidation (Pieri et al. 1994, 1996). Another major contributor to lipid peroxidation is $ONOO^-$, which is a powerful initiator of lipid breakdown. Due to the ability of melatonin to neutralize $ONOO^-$, this is another means whereby melatonin may protect membrane lipids (Cuzzocrea et al. 1997). It is presumed that melatonin inhibits lipid peroxidation by interfering with the radicals that initiate this process, especially

the $^{\cdot}OH$ and $ONOO^{-}$, and by positioning itself in a superficial location in membrane lipids layers near the polar heads of these molecules (Ceraulo et al. 1999). Its small molecular size and its amphiphilic properties facilitate melatonin's penetration into subcellular compartments. In vitro assays showed that melatonin inhibits lipid peroxidation in rat brain homogenates, brain and liver microsomes and mitochondria treated with an ascorbate-Fe^{2+} system (Teixeira et al. 2003). Melatonin has been found to protect against lipid peroxidation in many experimental models (Maharaj et al. 2006; Parlakpinar et al. 2002).

Mitochondrial membranes, which are rich in phospholipids containing PUFAs, are fluid structures, and optimal membrane fluidity is required for their proper function. When PUFAs are oxidized, mitochondrial membranes become more rigid; thus, their protection from oxidation is essential for optimal function of these organelles. Since the degree of lipid breakdown in mitochondrial membranes generally correlates with the fluidity of these organelles, it could be predicted that melatonin, by preventing lipid peroxidation, could preserve the proper fluidity and function of the membranes. Aging is characteristically associated with elevated cell membrane rigidity. Depressed level of melatonin naturally occurring with aging leads to elevated levels of lipid peroxidation and to more rigid cellular membranes (Reiter et al. 1999; Hardeland 2013). Likewise, treatment of senescence-accelerated prone mice with melatonin preserves mitochondrial membranes in a more fluidity state (García et al. 2014).

Melatonin and mitochondrial function

Melatonin is highly lipophilic molecule that crosses cell membranes to easily reach cellular compartments including mitochondria, where it seems to accumulate in high concentration (Acuña-Castroviejo et al. 2003). Several studies have shown that melatonin can influence mitochondrial homeostasis by stabilizing mitochondrial inner membrane, thereby improving ETC activity and mitochondrial function (López et al. 2009; Martín et al. 2002). Melatonin was reported to increase the activity of C I and C IV in a time-dependent manner in mitochondria isolated from rat brain and liver tissues, while having no effect on C II and C III (Martín et al. 2002). Melatonin administration also prevented the inhibitory effect of ruthenium red on C I and C IV activities as well as on GPx enzyme in rats (Martín et al. 2000). Melatonin (1 nM) significantly increases the C I and C IV activities in rat liver mitochondria, while higher concentrations of this compound are required to stimulate the activity of these complexes in rat brain mitochondria. The effects on C I were also studied using BN-PAGE histochemical procedure to measure change in its activity induced by melatonin. This study documented an increase in C I activity following melatonin treatment. Melatonin also counteracted cyanide-induced inhibition of C IV, as shown by the increase in the ETC activity coupled to OXPHOS and ATP synthesis, both either in normal or in rat brain and liver mitochondria depleted of ATP by cyanide treatment (Martín et al. 2002). The stimulatory effect of melatonin on the C I and C IV activities does not only rely on the antioxidant role of this indoleamine. Because of its high redox potential (0.94 V; Tan et al. 2000), melatonin may interact with the ETC complexes by donating and accepting electrons, thereby increasing electron flux, an effect not shared by other antioxidants.

Recently, another effect of melatonin on mitochondrial bioenergetic parameters was described (López et al. 2009). Experiments carried out in vitro with normal mitochondria demonstrate that this indoleamine protected the mitochondrial function from oxidative damage, decreasing oxygen consumption in the presence of ADP in a concentration-dependent manner and reducing the membrane potential, thereby inhibiting the production of $O_2^{\cdot-}$ and H_2O_2 (López et al. 2009). In addition, melatonin maintained the respiratory control ratio, the efficiency of the OXPHOS and ATP synthesis, while enhancing the activity of ETC complexes (mainly C I, C III and C IV). These effects of melatonin probably depend on a direct interaction of this indoleamine with mitochondria, as shown by the fact that mitochondria take up melatonin in a time- and concentration-dependent manner, and thus, the effects of melatonin were due to its presence within the mitochondria. The ability of mitochondria to accumulate melatonin is of great pharmacological interest because it means that, following exogenous administration in vivo, melatonin enters the mitochondria and exerts its beneficial action on mitochondrial function.

Cardiolipin and mitochondrial function

Cardiolipin is commonly referred to as the signature phospholipid of mitochondria. This phospholipid is associated with membranes designed to generate an electrochemical gradient that is used to produce ATP, such as bacterial plasma membrane and the IMM (Ren et al. 2014). This association between CL and energy transducing membranes suggests an important role for CL in bioenergetic processes (Schlame et al. 2000; Ren et al. 2014). In fact, CL has been shown to interact with a number of IMM proteins including, among others, the ETC complexes involved in OXPHOS (Musatov and Robinson 2012; Schlame et al. 2000; Houtkooper and Vaz 2008; Schlame and Ren 2009) and the anionic substrates carriers (Klingenberg 2009). Indeed, CL is required for optimal activity of C I (Sharpley et al. 2006), C III (Fry and Green 1981), C IV (Robinson

1993) and C V (Eble et al. 1990). Crystallographic studies have shown the presence of a few tightly bound CL molecules in each of the crystal structures of the C III, C IV and ADP/ATP carrier (Lange et al. 2001; Ozawa et al. 1982; Eble et al. 1990). These results suggest that CL is an integral component of these proteins, the presence of which is critical for their proper folding. CL seems to facilitate the association and stabilization of respiratory chain complexes into supercomplexes (Zhang et al. 2002; Schagger 2002). Such supercomplexes formation is thought to improve the efficiency of OXPHOS by eliminating the need for diffusion of substrates and products between individual ETC component (Genova and Lenaz 2014). CL is also required for the interaction between ADP/ATP carrier proteins and respiratory supercomplexes (Claypool 2009).

Another important role of CL is its participation in the process of apoptosis in animal cells through the interaction with a variety of death-inducing proteins, including cytochrome c (Gonzalvez and Gottlieb 2007; Ott et al. 2007a, b). This hemoprotein is believed to acts as peroxidase, which reacts quite specifically with CL, causing oxidation and then hydrolysis of the product CL hydroperoxide (Kagan et al. 2005). The consequence is that the cytochrome c is released into the intermembrane space, while the oxidized CL is translocated to the outer mitochondrial membrane and participates in the opening of the mitochondrial permeability transition pore (Petrosillo et al. 2006a). The opening of this pore facilitates the release of several proapoptotic factors, including cytochrome c, from mitochondria into the cytosol where they trigger apoptosis. CL appears to play an important role in mitochondrial morphology and dynamics including fusion and fission processes (DeVay et al. 2009; Ban et al. 2010), as well as in the protein insertion and assembly into the mitochondria (Marom et al. 2009). Given the role played by CL in mitochondrial bioenergetic processes as well as in apoptosis and in mitochondrial membrane stability and dynamics, it is conceivable that abnormalities in CL structure, content and acyl chains composition may have important implications in mitochondrial dysfunction associated with several physiopathological states and diseases (Chicco and Sparagna 2007; Paradies et al. 2014b, c). Alterations in mitochondrial CL profile may occur mainly as a consequence of: (1) loss of the CL content due to the changes in the CL synthase activity; (2) changes in acyl chain composition due to altered CL remodeling and (3) CL oxidation due to ROS attack.

Melatonin and cardiolipin oxidation

Oxygen free radicals lead to primary reaction and damage in the immediate surroundings of where they are generated, due to their high chemical reactivity. Therefore, the effect of these reactive species should be greatest at the level of mitochondrial membrane components such as phospholipid molecules particularly rich in PUFAs. Among phospholipids, CL molecules are particularly sensitive to oxidation, either because of their location in IMM near to the site of ROS production, or because of their high content of PUFAs. In fact, CL molecules are rich in unsaturated fatty acyl chains, particularly linoleic acid in heart and liver, or docosahexanoic and arachidonic acids in brain tissue mitochondria. In addition, CL molecules are located in the mitochondrial membrane near to the site of ROS production, mainly represented by the respiratory chain complexes I and III to which CL molecules are associated.

As described above, melatonin and its derivatives exert a protective effect on lipid peroxidation in mitochondrial membranes (Catala´ 2007; García et al. 2014; Reiter et al. 2014). Recently, we have studied the ability of melatonin to inhibit CL oxidation in isolated mitochondria (Petrosillo et al. 2009c). CL oxidation in mitochondria was induced by treating these organelles with t-butylhydroperoxide (t-BuOOH), a lipid-soluble hydroperoxide that closely resembles endogenous lipid hydroperoxides generated during oxidative stress. Treatment of rat heart and brain mitochondria with t-BuOOH in the presence of micromolar concentrations of copper ions resulted in a loss of CL content and in an increase in the level of oxidized CL, the latter being detected by a normal phase HPLC technique with UV detection at 235 nm, indicative of conjugated dienes. Melatonin, at micromolar concentration, was able to prevent the oxidation/depletion of CL. This inhibitory effect of melatonin on CL oxidation in mitochondria can be reasonably explained on the ability of this indoleamine to inhibit the peroxidation of linoleic acyl chains, which are the main constituents of CL molecules. In fact, previous results have demonstrated the antioxidant effect of melatonin on linoleate oxidation initiated by $HO^{\cdot}$ free radical generated by water gamma radiolysis (Mekhloufi et al. 2005). Using linoleate micelles as lipid model, two index of peroxidation have been measured, i.e., conjugated dienes and hydroperoxides. The results obtained in this study demonstrate that melatonin displays strong in vitro lipid peroxyl radicals ($LOO^{\cdot}$) scavenging properties, as shown by its inhibitory effect on the radiation-induced peroxidation of linoleate.

Emerging insights have linked CL oxidation/depletion to mitochondrial dysfunction associated with a variety of diseases and physiopathological settings including heart ischemia/reperfusion, diabetes, aging and age-associated disorders (Chicco and Sparagna 2007; Paradies et al. 2009, 2014b, c). CL oxidation is also emerging as a key player in the regulation of several of the mitochondrial steps of cell death and in mitochondrial dynamics (Ott et al. 2007a, b; Paradies et al. 2014b). Therefore, the ability of melatonin

to prevent CL oxidation in mitochondria may have important implications in mitochondrial dysfunction and related disorders.

Cardiolipin and MPTP

Mitochondrial permeability transition pore (MPTP) is defined as the sudden increase in IMM permeability to low molecular weight metabolites (˂1.5 kDa) in response to many stimuli, including high levels of Ca^{2+} and oxidant stress (Crompton 1999; Leung and Halestrap. 2008). Opening of the MPTP promoted by elevated matrix Ca^{2+} levels, associated with high phosphate, low adenine nucleotide concentrations and oxidative stress, induces the collapse of transmembrane ion gradients, resulting in membrane depolarization and uncoupling of OXPHOS. This causes irreversible damage to mitochondria, resulting in cell death predominantly through necrosis. A number of molecules were accepted as key structural components of the MPTP, including, Cyp-D in the matrix, ANT and phosphate carriers in the IMM and VDAC (also known as porin) in the outer membrane (Halestrap 2009). More recently, dimers of the F_0F_1 ATP synthase were suggested to be new putative components of the MPTP. In fact, reconstituted dimers of F_0F_1 ATP synthase, incorporated into lipid bilayers, form Ca^{2+}-activated channels with properties similar to those of the mitochondrial mega-channel, the electrophysiological equivalent of the MPTP (Giorgio et al. 2013).

Release of cytochrome c from mitochondria into cytosol is considered a central event in the induction of apoptotic cascade that ultimately leads to cell death (Ott et al. 2007b). Cytochrome c is normally bound to the outer surface of the IMM primarily to CL molecules (Rytomaa et al. 1992). Oxidation of CL promotes the detachment of cytochrome c from mitochondrial membrane and its release into the extramitochondrial space (Petrosillo et al. 2003b; Ott et al. 2007a). It has been proposed that the release of cytochrome c from the mitochondria takes place by a two-step process involving, first, the dissociation of this hemoprotein from the IMM, followed by permeabilization of the outer membrane probably through its association with Bcl2 family proteins, such as Bax and Bid, and/or through the MPTP opening (Ott et al. 2007b). CL oxidation may be involved in the permeabilization of the outer mitochondrial membrane, probably through its association with Bcl2 family proteins such as Bax and Bid (Kagan et al. 2004; Ott et al. 2007a; Jiang et al. 2008).

We have shown that exogenously added oxidized CL to mitochondria sensitizes these organelles to Ca^{2+}-induced MPTP opening (Petrosillo et al. 2006a). This synergistic effect of Ca^{2+} and oxidized CL on the induction of MPTP opening suggests that both these compounds could play a coordinated role in this process by interacting with components of the MPTP, probably with adenine nucleotide carrier and/or with ATP synthase dimers. The involvement of oxidized CL in MPTP opening is further demonstrated by our recent study, showing that oxidation of intramitochondrial CL molecules results in MPTP induction (Petrosillo et al. 2009c). Interestingly, the induction of MPTP opening by oxidized CL and Ca^{2+} is associated with the release of cytochrome c from mitochondria.

Melatonin and MPTP

Studies carried out in our and other laboratories have demonstrated a role of melatonin in the modulation of MPTP opening (Andrabi et al. 2004; Hibaoui et al. 2009; Petrosillo et al. 2009c; Jou 2011). A direct MPTP inhibition by melatonin has been reported (Andrabi et al. 2004). Melatonin diminished MPTP current with an IC_{50} of 0.8 μM, a concentration which would require accumulation of melatonin within mitochondria. Indeed, it has been demonstrated that melatonin due to its amphiphilic nature can be actively accumulated by mitochondria (Messner et al. 1998; López et al. 2009). The direct MPTP inhibition by melatonin should be interpreted on the basis of a low affinity binding site. This effect may contribute to the anti-apoptotic properties of melatonin.

Our studies have shown that melatonin, at micromolar concentrations, inhibited both the Ca^{2+}/t-BuOOH-induced CL peroxidation and MPTP opening, as indicated by the protective effect this indoleamine on matrix swelling, $\Delta\Psi$ collapse and release of preaccumulated Ca^{2+} Petrosillo et al. 2009c). These results suggest that melatonin, by preventing endogenous CL peroxidation, inhibits MPTP opening. In addition, our results demonstrate that the release of cytochrome c from mitochondria associated with the MPTP opening induced by oxidative stress is almost completely prevented by melatonin. This effect of melatonin can be explained on its ability to inhibit CL peroxidation, thereby preventing both cytochrome c detachment from the IMM and MPTP opening. The ability of melatonin to prevent MPTP opening may have important implications in those physiopathological situations characterized by alterations of Ca^{2+} homeostasis and accumulation of peroxidized CL in mitochondria, such as heart ischemia/reperfusion, aging and other degenerative diseases.

Melatonin's action in preventing MPTP opening induced by oxidative stress caused by t-BuOOH was shown in another study carried out in primary skeletal muscle cultures (Hibaoui et al. 2009). Melatonin (1–100 μM) fully prevented myotube death induced by t-BuOOH as assessed by acid phosphatase, caspase 3 activities and cellular morphological changes. Using fluorescence imaging, it

was shown that the mitochondrial protection provided by melatonin was associated with an inhibition of t-BuOOH-induced ROS generation. In isolated mitochondria, melatonin desensitized the MPTP to Ca^{2+} and prevented t-BuOOH-induced mitochondrial swelling, pyridine nucleotide and GSH oxidation. The inhibition of MPTP opening by melatonin was suggested as an explanation for the protective action of this indoleamine against oxidative stress in myotubes (Hibaoui et al. 2009).

MPTP may occur in the cell through two modes, the transient MPTP and the prolonged MPTP, having different outcomes of survival or death. The transient MPTP protects mitochondria, whereas the prolonged MPTP triggers the "point of no return" for apoptosis or necrosis. It has been shown that melatonin targets mitochondrial Ca^{2+}-mediated MPTP for protection during mitochondrial Ca^{2+} mediated apoptosis in astrocytes (Jou 2011). With the application of fluorescence laser scanning imaging microscopy, it was demonstrated that melatonin does not inhibit the MPT pore, rather it preserves the pore in its protective mode of transient MPT during mitochondrial Ca^{2+} stress. In addition, the melatonin-preserved transient MPT allowed mitochondria to release the toxic overloaded Ca^{2+} to sublethal levels, thus preventing Ca^{2+}-mediated fission of mitochondria, Ca^{2+}-dependent prolonged MPT and possibly improving Ca^{2+}-dependent ATP synthesis through activation of mitochondrial dehydrogenases. This unique melatonin-dependent modulation of MPTP has been suggested to have important therapeutic potential in the treatment of mitochondrial Ca^{2+}-mediated astrocyte-associated neurodegenerative disorders (Jou 2011).

Melatonin and mitochondrial dysfunction in heart ischemia/reperfusion

Ischemia/reperfusion (I/R) leads to myocardial dysfunction and irreversible damage characterized by cardiomyocyte hypercontracture, reduction of left ventricular pressure and elevated incidence of ventricular fibrillation. Mitochondria are known to be involved in the processes that lead to cell death following I/R and are therefore potential target for protective intervention (Camara et al. 2011). ROS are recognized as an important factor in producing lethal cell injury associated with cardiac I/R (Chen and Zweier 2014). Mitochondria isolated from I/R rat heart exhibit altered bioenergetic function, associated with CL abnormalities. In fact, results obtained in our laboratory have shown that mitochondria isolated from I/R rat heart exhibit decreased rate of mitochondrial oxygen consumption, reduced activity of C I and CIII and increased basal rate of H_2O_2 production (Petrosillo et al. 2003a; Paradies et al. 2004). These changes in the mitochondrial bioenergetic parameters were associated with an oxidation/depletion of CL. The defect in the respiratory chain complexes activity observed in mitochondria isolated from I/R rat heart has been ascribed, at least in part, to ROS-induced oxidation of CL, a phospholipid required for the optimal activity of these enzymatic complexes. Melatonin treatment had strong protective effect against I/R-associated mitochondrial bioenergetic alterations (Petrosillo et al. 2006b). In fact, melatonin administration to I/R rat heart counteracted the reduction in C I and C III activity and the associated decrease in state 3 respiration in isolated rat heart mitochondria as well as CL alterations. Similarly, melatonin prevented alterations to C I and C III as well as to CL in in vitro experiments on isolated rat heart mitochondria subjected to oxidative stress conditions. The melatonin's protective effect against I/R-induced mitochondrial dysfunction could be ascribed to its ability to preserve CL integrity and/or to directly improve the activity of the respiratory chain complexes. This effect was associated with an improvement of post-ischemic hemodynamic function of the heart. These results emphasize that melatonin-induced mitochondrial adaptive changes are likely of great value for the cardioprotective actions of the indoleamine (Dominguez-Rodriguez et al. 2012; Yang et al. 2014).

A large body of experimental evidence supports a crucial role of MPTP in cardiomyocyte cell death occurring with I/R (Halestrap 2009; Ong et al. 2015). It has been suggested that MPTP remains closed during the ischemic period, because of the low pH due to lactate accumulation. At reperfusion, there is an uptake of Ca^{2+} by mitochondria, a burst of ROS production and rapid correction of acidosis, all events that contribute to increase the likelihood of MPTP opening. Pharmacological intervention aimed to protect the heart from the damaging effect of MPTP opening are of considerably importance in attenuating mitochondrial dysfunction associated with I/R injury. Recent studies have shown that melatonin and several of its metabolites have significant protective actions against cardiac damage and altered physiology during I/R contributing to the rehabilitation of the heart contractile function during reperfusion (Sahna et al. 2005; Petrosillo et al. 2006b; Paradies et al. 2010a, b; Dominguez-Rodriguez et al. 2012; Lochner et al. 2013). Other studies demonstrated that melatonin plays a role in the mitochondrial adaptive changes and that cytochrome c is a significant mediator of this process (Giacomo and Antonio 2007). Very recently, we have demonstrated that melatonin protects against mitochondrial dysfunction associated with heart I/R injury by inhibiting MPTP opening (Petrosillo et al. 2009a). Melatonin treatment significantly improves the functional recovery of Langendorff hearts on reperfusion, reduces

the infarct size and decreases necrotic damage, as shown by the reduced release of lactate dehydrogenase. All these effects were accompanied by the inhibition of the MPTP opening as detected in situ by the mitochondrial release of NAD^+. Furthermore, melatonin desensitizes mitochondria isolated from melatonin-reperfused heart to Ca^{2+}-induced MPTP opening, as assessed by the CRC (calcium retention capacity), a sensitive and quantitative measure of the ability of mitochondria to open the MPTP in response to of Ca^{2+} uptake. Together, these results demonstrate that melatonin protects against heart I/R injury by inhibiting MPTP opening, thus improving the post-ischemic hemodynamic function of the heart. The possible mechanism underlying the inhibition of MPTP opening during I/R by melatonin treatment was also investigated (Petrosillo et al. 2009a). It is now accepted that, in addition to Ca^{2+} overload, other factors may contribute to the MPTP opening during heart I/R. As described above, oxidized CL, together with Ca^{2+}, promotes the induction of MPTP in isolated rat heart mitochondria, suggesting that an increased level of oxidized CL may increase the probability of MPTP opening during I/R. We found an increased level of oxidized CL in mitochondria isolated from rat heart subjected to I/R, which was prevented by melatonin treatment. Thus, it is plausible that CL oxidation, together with Ca^{2+} overload, synergistically contribute to MPTP opening during I/R and that melatonin treatment inhibits MPTP opening by preserving CL integrity by ROS attack. Melatonin was reported to inhibit linoleate peroxidation in a model system in vitro (Mekhloufi et al. 2005). The inhibitory effect of melatonin on mitochondrial CL peroxidation during reperfusion can be reasonably explained on the ability of this indoleamine to inhibit the oxidation of linoleic fatty acid which is the main constituent of heart CL.

It has been shown that CL binds cytochrome c to the outer surface of the IMM (Rytomaa et al. 1992). Oxidation of CL results in the detachment of this hemoprotein from mitochondrial membrane and this event is considered an important initial step in the cytochrome c release from mitochondria (Petrosillo et al. 2001; Ott et al. 2007a, b). Our results have shown that, in addition to inhibit MPTP opening, melatonin prevents also the release of cytochrome c from mitochondria upon I/R. This effect of melatonin can be explained on the ability of this indoleamine to inhibit CL oxidation, thus preventing cytochrome c detachment from IMM. The inhibitory effect of melatonin on cytochrome c release and MPTP opening may contribute to the protective effect exerted by this indoleamine against mitochondrial dysfunction associated with I/R. These beneficial effects of melatonin reinforce the therapeutic potential of this compound to combat a variety of oxidative stress-induced oxidative dysfunctions as well as mitochondrial-mediated apoptosis in various cardiovascular disorders.

Melatonin and mitochondrial dysfunction in aging

Aging is a multifactorial process, which is genetically determined and influenced epigenetically by environment (Kirkwood 2005). This biological process is characterized by impairment of bioenergetic functions, increased oxidative stress and increased risk of contracting age-associated diseases. Mitochondria are intimately involved in the aging process because these organelles are recognized as the main intracellular source of ROS as well as the major target of their oxidative attack. According to the mitochondrial theory of aging, ROS produced by the mitochondrial respiratory chain, attack mitochondrial constituents including proteins, lipids and mitochondrial DNA (mtDNA; Harman 1972; Miquel et al. 1980; Pak et al. 2003). As mtDNA encodes essential components of OXPHOS and protein synthesis machinery, accumulation of mtDNA mutations may lead to impairment of either the assembly or the function of the respiratory chain, leading to further ROS generation and subsequent accumulation of more mtDNA mutations. This triggers a vicious cycle which leads to the progressive decline of mitochondrial and cellular bioenergetics functions as results of insufficient supply of energy and/or increased susceptibility to cell death (Judge and Leeuwenburgh 2007; Paradies et al. 2010b). Although there is a large consensus on the mitochondrial free radical theory of aging (Harman 1972), recent findings, obtained in particular in Caenorhabditis elegans and in rodents, suggest that ROS generation may not be the main factor involved in the aging process (Hekimi et al. 2011).

A number of studies have shown a decreased electron transport activity in mitochondria isolated from rat and mouse tissues upon aging (Navarro and Boveris 2007; Judge and Leeuwenburgh 2007; Paradies et al. 2010b, 2011). Of the five respiratory chain complexes, C I and C IV show a selectively reduced enzymatic activity in mitochondria isolated from various tissues of rats and mice upon aging (Lenaz et al. 1997; Navarro and Boveris 2007; Petrosillo et al. 2008b, 2009b). One possible factor responsible for the age-related impairment of C I and C IV activity might be the oxidation/depletion of mitochondrial CL as supported by the following experimental observations. The content of normal CL declines, while the level of oxidized CL increases with aging (Petrosillo et al. 2008b, 2009b). Cardiolipin molecules are specifically bound to C I and C IV of the respiratory chain and required for their functional activity (Lange et al. 2001; Musatov and Robinson 2012). In addition, mitochondrial-mediated ROS generation affects C I and C IV activity through CL peroxidation in beef heart submitochondrial particles (Paradies et al. 2000, 2002). These results suggest that the age-associated defects in mitochondrial C I and C IV activities could be ascribed, at least in part, to ROS-induced oxidative damage

to mitochondrial CL. Complex I is considered a rate-limiting factor in the mitochondrial respiratory chain and also the main source of ROS during the aging process. Thus, the impairment of mitochondrial C I activity, in addition to that of C IV, may increase the electron leak from the ETC, generating more superoxide radical, triggering a cycle of oxidative damage that ultimately leads to mitochondrial bioenergetic decay in aging (Judge and Leeuwenburgh 2007; Paradies et al. 2010b, 2011). The age-associated impairment of mitochondrial C I e C IV activity observed in heart and brain tissues may have important implications in the etiopathology of age-associated cardiovascular and neurodegenerative disorders and may represent an important target for the development of potential therapeutic strategies (Beal 2005; Boveris and Navarro 2008).

Growing evidence indicate that the individual components of the mitochondrial ETC may exist as large macromolecular assemblies, or so-called supercomplexes (Zhang et al. 2002; Schagger 2002; Genova and Lenaz 2014). A general role for CL in respiratory supercomplexes formation and stability in mammalian mitochondria has been suggested (Zhang et al. 2002; Bazán et al. 2013). Recently, age-associated destabilization of rat heart mitochondrial supercomplexes has been reported (Gómez and Hagen 2012). Due to the oxidation/depletion of CL with aging, it is possible that abnormalities in CL might be involved in the destabilization and dysfunction of mitochondrial respiratory supercomplexes, thus contributing to mitochondrial bioenergetics decay with aging.

Several properties of melatonin indicate that this compound may have beneficial effects in aging. Serum levels of melatonin significantly decrease in aged animals compared with young animals (Reiter et al. 1980, 1981). In humans, the total antioxidative capacity of serum correlates well with its melatonin levels. Thus, the decreased level of melatonin in aging has been associated with the increased oxidative damage observed in the elderly. The mechanism of aging process can be studied in experimental animal models like the senescent accelerated mouse (SAM; Takeda 1999). Recently, using SAM mice, the effects of chronic administration of physiological doses of melatonin on mitochondrial oxidative stress and mitochondrial function in heart tissue were investigated (Rodríguez et al. 2007). Mitochondrial oxidative stress was determined by measuring the levels of lipid peroxidation, GSH and GSSG and the activities of GPx and GRd. The activities of several mitochondrial bioenergetic parameters including ETC complexes and ATP levels were also evaluated. The results of these studies showed an age-dependent mitochondrial oxidative damage in the heart tissue of SAM mice, which was associated with a reduction in the ETC complexes activity and in ATP levels. Chronic melatonin administration normalized these age-associated alterations in mitochondrial bioenergetic parameters and increased ATP level. Moreover, melatonin treatment had beneficial effect on longevity of SAM mice (Rodríguez et al. 2008). Together, these studies indicate that melatonin treatment counteracts age-related oxidative damage and mitochondrial dysfunction in heart tissue of SAM mice by improving mitochondrial bioenergetic function as reflected by increase in ATP production and prolonged longevity.

Experimental evidence indicates that mitochondrial decay is a major contributor to brain tissue alterations associated with aging (Navarro and Boveris 2007). Aged mammalian brain has a decreased capacity to produce ATP by OXPHOS and it is considered that this decreased capacity for energy production becomes limiting under physiological conditions in aged individuals. The current knowledge indicates that the impairment of brain mitochondrial function in aging is mainly due to decreased electron transfer rates by C I and C IV, among other decreased mitochondrial activities (Navarro and Boveris 2007; Paradies et al. 2011). Impaired mitochondrial respiration with NAD-dependent substrates has been consistently observed in brain mitochondria isolated from aged rats and mice (Navarro and Boveris 2007; Petrosillo et al. 2008a). Given the brain's high energy requirements, the impairment in brain ETC complexes activity could have a significant impact on brain function in aging and on the etiology of age-associated neurodegenerative disorders (Beal 2005).

A potential role of melatonin in mitigation of mitochondrial decay in brain aging has been described (Bondy et al. 2004; Bondy and Sharman 2007). Moreover, melatonin has been identified as a potential mitochondria-targeted protector against several oxidative stress-associated brain disorders (Caballero et al. 2008). Results obtained in our laboratory have shown that brain aging is associated with an impairment in C I and C IV activity, decrease in oxygen consumption and membrane potential and with an increase in mitochondrial ROS production (Petrosillo et al. 2008a, b; Paradies et al. 2011). These age-related mitochondrial bioenergetic alterations were largely attenuated by melatonin administration. Melatonin administration did not affect these bioenergetic parameters when administered to young rats, suggesting that the observed protective effects of this indoleamine are related to changes produced by aging. The melatonin's ability to prevent the age-related alterations of mitochondrial bioenergetic parameters in rat brain could be ascribed, at least in part, to its protective effect against CL peroxidation (Paradies et al. 2011) as also supported by in vitro experiments on isolated rat brain mitochondria (Petrosillo et al. 2008a).

Other studies carried out in brain mitochondria isolated from male and female SAM mice indicated that there was significant age-dependent mitochondrial dysfunction with a reduced efficiency of the ETC and diminished ATP

production associated with elevated oxidative-nitrosative stress (Carretero et al. 2009; Öztürk et al. 2012). Melatonin administration between 1 and 10 months of age prevented these mitochondrial alterations, maintaining ATP production. It was also shown that aging causes membrane rigidity in synaptosomal and mitochondrial membranes isolated from brain of SAM mice, which was prevented by chronic treatment with melatonin (García et al. 2014). Thus, long-term administration of melatonin could be accompanied by an improvement in mitochondrial bioenergetics and brain function.

Melatonin and MPTP in aging

The MPTP may be involved in mitochondrial dysfunction associated with aging (Paradies et al. 2013). Mitochondria isolated from liver, brain and heart are more prone to Ca^{2+}-induced MPTP opening with aging (Mather and Rottenberg 2000; Crompton 2004). As mentioned above, oxidized CL sensitizes rat heart mitochondria to Ca^{2+}-induced MPTP opening and promotes the release of cytochrome c from mitochondria (Petrosillo et al. 2006a). It is thus conceivable that an increased level of oxidized CL with age could enhance the susceptibility to Ca^{2+}-induced MPTP opening and promote the release of cytochrome c from mitochondria. We investigated the effect of aging and long-term treatment with melatonin on the susceptibility of rat heart mitochondria to Ca^{2+}-induced MPTP opening and on the cytochrome c release from mitochondria (Petrosillo et al. 2010). Mitochondria from aged rats displayed an increased susceptibility to Ca^{2+}-induced MPTP opening associated with an increased release of cytochrome c. These events may be related to the observed increased extent of apoptosis in aged heart (Kajstura et al. 1996). Long-term treatment with melatonin counteracts both these events. This protective effect of melatonin may be explained on the ability of this indoleamine to inhibit CL oxidation, thereby preventing MPTP opening, the detachment of cytochrome c from the IMM and its release from mitochondria. The fact that heart mitochondria from aged rats are more susceptible to Ca^{2+}-induced MPTP opening and to cytochrome c release might have important implications in necrotic and apoptotic myocyte cell death associated with aging and age-related cardiovascular disorders. Pharmacological intervention with melatonin might be beneficial against these age-related cardiovascular disorders.

Melatonin, mitochondria and neurodegenerative disease

Mitochondria play a pivotal role in several biochemical processes in the neuron including energy metabolism and ATP production, intracellular Ca^{2+} homeostasis and cell signaling. Therefore, mitochondrial dysfunction has emerged as a hallmark of aging and various age-related neurodegenerative diseases in which a progressive functional decline of mitochondria has been observed (Beal 1998, 2005).

Parkinson's disease (PD) is a complex, chronic, progressive and debilitating neurodegenerative disorder characterized by progressive degeneration of the dopamine neurons The molecular mechanisms underlying this disease are still not well understood. Considerable evidence supports mitochondrial dysfunction and α-synuclein as two of the major contributors to PD (Gautier et al. 2014; Mullin and Schapira 2015). Functional impairment of mitochondrial C I, reduced levels and altered assembly of C I subunits, associated with oxidative damage and alterations in OXPHOS activity, occur in PD brain (Mizuno et al. 1989, Schapira et al. 1990). α-Synuclein is the major structural constituent of cytoplasmic inclusion bodies (Lewy bodies). Recently, it has been reported that α-synuclein interacts with mitochondrial membranes, suggesting that both these two factors might have a synergistic interrelationship that contributes toward the complexities of PD (Protter et al. 2012). In vitro studies have shown that liposomes derived from mitochondrial membranes as well as liposomes rich in CL bind α-synuclein, while liposomes containing neutral lipids do not bind this protein (Pranke et al. 2011). α-Synuclein binding to CL resulted in several recent studies focused on involvement of α-synuclein in inhibiting the mitochondrial fission and fusion cycle (Büeler 2009). In fact, CL is enriched in fusion events zones and appears to play an important role in mitochondrial dynamics, including fusion and fission processes.

The observed specific binding of α-synuclein to CL in mitochondria suggests an important molecular role of this phospholipid in PD. α-Synuclein knockout mice show a decrease in mitochondrial CL content and CL precursors, associated with impairment of ETC complexes activity, without reductions in mitochondrial number (Ellis et al. 2005). Reduced complex I activity is thought to be a critical factor in the etiopathology of PD. Thus, altered membrane composition and impaired ETC activity in mice lacking α-synuclein brain suggest a relationship between α-synuclein's role in brain phospholipid metabolism, mitochondrial function and development of PD. Recently, it was reported that α-synuclein forms complex with cytochrome c and CL to protect neurons against apoptosis in PD (Bayir et al. 2009). There is no evidence for an impairment of CL remodeling and/or CL oxidation/depletion in PD, but it is possible that CL abnormalities occurred in the affected neurons. Due to the well-documented interaction between CL and α-synuclein, and similarity between disorders caused by loss of CL and overexpression of α-synuclein, further investigation into their interrelationship seems well warranted.

Very recently, the effects of overexpressing the N terminus of α-synuclein (α-Syn/N) on mitochondrial structure and function and cell viability in dopaminergic MN9D cells were investigated. A decrease in cell viability was observed in cells transfected with α-Syn/N. In addition, α-Syn/N induced alteration in mitochondrial morphology, increase in the level of intracellular ROS and decrease in mitochondrial membrane potential associated with the activation of MPTP and a decline in mitochondrial CL content (Shen et al. 2014).

Melatonin has been tested as potential neuroprotective agent in various experimental PD models.

Several studies report that melatonin is neuroprotective in animal experimental models of PD (Sharma et al. 2006; Saravanan et al. 2007). Recently, long-term melatonin treatment on striatal mitochondrial and dopaminergic functions and on locomotor performance in a chronic mouse model of PD was examined (Patki and Lau 2011). It was shown that pretreatment of chronic PD mice with melatonin maintained mitochondrial function, ATP and antioxidant enzymes at normal level.

Functional impairment of mitochondrial complex I has been associated with PD (Schapira et al. 1990). The pathogenic role of this mitochondrial dysfunction is supported by the demonstration that natural and synthetic complex I antagonists provoke neuronal death in animals. Moreover, it has been reported that complex I deficiency, which occurs in numerous neurodegenerative situations, sensitizes neurons to the action of death agonists such as Bax, through mitochondrial CL peroxidation (Perier et al. 2005). A protective effect of melatonin on Complex I activity and oxidative stress in mitochondria from substantia nigra and striatum in a mouse model of PD was reported (Acuña-Castroviejo et al. 1997; Ortiz et al. 2001). This protective effect of melatonin seems to be related to the ability of this indoleamine to reduce the activity of mitochondrial mtNOS, thus decreasing mitochondrial NO levels and preventing the inhibition of respiration produced by this compound. Melatonin may interact with the ETC complexes by electron exchange, thereby modulating electron flux, enabling a recycling of electrons (Hardeland 2005). Moreover, melatonin was found to bind with high affinity to C I, and this binding could be involved in the protection exerted by melatonin on this respiratory chain complex in the PD (Srinivasan et al. 2011a, b). Preservation of mitochondrial CL integrity could be considered as an additional mechanism by which melatonin protects against mitochondrial dysfunction associated with PD.

Several studies have demonstrated the involvement of mitochondrial dysfunction in the etiopathology of Alzheimer's disease (AD; Melov et al. 2007; Massaad et al. 2009; Santos et al. 2010). AD is characterized by accumulation of extracellular senile plaques of aggregated β-amiloide (Aβ) and intracellular neurofibrillary tangles that contain hyperphosphorylated tau. The resulting clinical effect of this disease is a progressive loss of memory and deterioration of cognition. There is substantial evidence proving that mitochondrial toxicity is linked to the progressive accumulation of mitochondrial Aβ (Chen and Yan 2010). Studies in cultured neuronal cells or in astrocytes exposed to Aβ have shown that this peptide induces mitochondrial swelling, decreases ATP synthesis and impairs the activity of several mitochondrial enzymes (Casley et al. 2002; Aliev et al. 2003; Clementi et al. 2005). Several actions of melatonin have been described, which antagonize the deleterious effect of AD (Cardinali et al. 2013; Pandi-Perumal et al. 2013). Protection from Aβ toxicity was observed especially at mitochondrial level. Melatonin prevention of damage induced by Aβ was evaluated in young and senescent hippocampal neurons (Dong et al. 2010). Exposure of neurons to $A\beta_{25–35}$ resulted in alterations of mitochondrial bioenergetic parameters such as impairment of respiratory chain complexes activity, decreased mitochondrial membrane potential and depletion of ATP. Melatonin administration attenuated all these mitochondrial bioenergetic alterations. Other studies in transgenic AD mice and cultured cells have shown that melatonin inhibits the Aβ-induced increases in the levels of mitochondria-related Bax and suppresses Aβ-induced caspase-3 activity (Wang 2009 Feng et al. 2006). Therefore, melatonin may provide an effective means of treatment for AD through its antiapoptotic activities.

Conclusions

Mitochondrial dysfunction is considered an important causative factor in a variety of disorders such as heart ischemia/reperfusion, diabetes, various forms of hepatic disorders, neurodegenerative and cardiovascular diseases as well as in aging. Alterations in mitochondrial function such as defects in the ETC, OXPHOS process and MPTP opening have all been suggested to play a role in the pathogenesis of these disorders. A large body of experimental evidence indicates a beneficial effect of melatonin in a number of physiopathological situations that involve abnormal mitochondrial function as a primary cause of disease. Most of the beneficial effects of melatonin may depend on its effect on mitochondrial bioenergetics mediated via different mechanisms including general antioxidant actions at level of ETC dysfunction, electron leakage and mitochondrial oxidative damage and a more direct action of this indoleamine on MPTP opening. There is now clear evidence that CL plays a key role in several mitochondrial bioenergetic processes, in mitochondrial step of cell death as well as in mitochondrial morphology and dynamics. Mitochondrial dysfunction

due to CL oxidation/depletion has been associated with in a variety of pathological conditions and aging. The ability of melatonin to prevent CL oxidation/depletion in mitochondria thereby preserving mitochondrial membranes integrity and function may have important implications in mitochondrial physiopathology. Melatonin may emerge as a valid therapeutic candidate to preserve the bioenergetic function of mitochondria. In addition, due to the lack of side effects of melatonin, administration of this indoleamine may represent an effective therapeutic strategy to combat a variety of oxidative stress-induced mitochondrial-related diseases.

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