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Melatonin as a stabilizer of mitochondrial function: role in diseases and aging

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Abstract: It is now almost 60 years since the discovery of melatonin and new physiological functions of the indole continuously appear in the most recent studies worldwide. Experimental evidence emphasizes its importance as a stabilizer of the mitochondrial bioenergetics, which could be related to the prevention of development of aging and several diseases. In the next years, conscientious investigation about this topic should be undertaken by scientists of different research areas to achieve a better understanding of the molecular mechanisms implied. This will ultimately allow the development and clinical application of efficacious treatments.

Key words: Melatonin, mitochondria, diseases, aging, Alzheimer and Parkinson diseases

1. Introduction

It is now almost 60 years since the discovery of melatonin and new physiological functions of the indole continuously appear in the most recent studies worldwide. Besides the pineal gland, the existence and value of other sources of synthesis force us to rethink the established premises about the biological role of this molecule, such as the well-known regulation of circadian and reproductive cycles (Hardeland et al., 2008). In the last few years, other properties of melatonin such as antioxidant power, immunoregulatory capacity, and oncostatic action have enriched our knowledge about the pleiotropic nature of the hormone. The role of melatonin in mitochondrial homeostasis has gained strength in the scientific community. Experimental evidence emphasizes its importance as a stabilizer of organular bioenergetics, which could be related to the prevention of development of aging and several diseases.

2. Melatonin and mitochondria: origin and status of a relationship

Recently, Tan et al. (2014) proposed that ability to synthesize melatonin in eukaryotes could be inherited from prokaryotic cells, particularly from photosynthetic alpha-proteobacteria. As the authors noted, the alpha-proteobacteria are a group of bacteria currently accepted as being the precursors of mitochondria and probably the oldest organisms with the capacity to synthesize melatonin. Hence, following the endosymbiotic theory, mitochondria may be the original site of melatonin production in eukaryotic cells and may still possess this capacity at present. Interestingly, it has been recorded that the indolamine is concentrated particularly in subcellular compartments (to levels of 100 nM) such as the nucleus and mitochondria, the latter showing higher concentrations than cytosol and serum (Escames et al., 2010; Phillipson, 2014).

Besides being sites of important metabolic reactions (steroid hormone/porphyrin synthesis, urea cycle, lipid metabolism, amino acid interconversion, xenobiotic metabolism, glucose sensing/insulin regulation, and cellular Ca^{2+} homeostasis), mitochondria are the major generators of cellular energy (Brookes et al., 2004). This last feature converts them also into the major sources of potentially harmful reactive species. However, some of these radicals are key elements for basic cell signaling processes. For this reason, steady-state maintenance of free radical/antioxidant ratio appears to be the key for optimal mitochondrial functioning (Sheu et al., 2006; Morley et al., 2012).

This is where the close relationship between mitochondria and melatonin lies. Once indole reaches the organelle, its lipophilicity allows it to accumulate in the inner mitochondrial membrane, close to the electron transport chain (ETC) (Petrosillo et al., 2006). The mitochondrial ETC is a system of oxidoreductant protein complexes (C-I, -II, -III, and -IV), responsible for approximately 90%–95% of cell ATP production through the metabolic process known as oxidative

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phosphorylation (OXPHOS) in aerobic cells (Reiter et al., 2003). It is thought that melatonin counteracts the leakage of electrons derived from deficiencies in the ETC function in several ways; for example, it has been seen that melatonin administration increases C-I and C-IV activities in a dose-dependent manner (Martin et al., 2000a, 2000b) and even enhances gene expression of C-IV components (Hardeland et al., 2005). Single-electron exchange may be the basis for these beneficial interactions, an effect not shared by other antioxidants (Paradies et al., 2010; Srinivasan et al., 2010). On balance, melatonin would make mitochondrial respiration and ATP synthesis more efficient. Thus, quasicatalytic amounts of melatonin would be required for radical formation avoidance; additionally, one of its metabolites, N1-acetyl-5-methoxykynuramine (AMK), could also be implicated in this kind of single-electron transfer reaction (Hardeland et al., 2005).

This feature leads us to the next mechanism of action of melatonin on mitochondrial homeostasis. As it has been mentioned, mitochondria are major sites of free radical production and it is largely recognized that melatonin acts as a scavenger of free radicals already formed. Thereby, high concentrations of melatonin within mitochondria would be advantageous to avoid oxidative damage, due to the antioxidant properties of the molecule (Reiter et al., 2008). In this way, related to inner mitochondrial membrane essential components, not only are the above mentioned protein complexes of ETC protected from the harmful effects caused by reactive oxygen/nitrogen species (ROS/RNS), but also phospholipidic elements are safeguarded from lipid peroxidation (Stetinová et al., 2002). Among them, cardiolipin (CL) is biosynthesized and almost exclusively located in the inner mitochondrial membrane, where it seems to be involved in the regulation of several bioenergetic and dynamic processes of the organelle. Nevertheless, its high content of unsaturated fatty acids and its location near the site of production of radicals (C-I and C-III) make it particularly susceptible to peroxidative attack by ROS. This phenomenon negatively impacts the structural properties (membrane fluidity and permeability) and function (respiration and oxidative phosphorylation) of the mitochondrial membrane, leading to cellular dysfunction and even cell death (Paradies et al., 2010). Some studies show that melatonin, at micromolar concentrations, would prevent the oxidation/depletion of CL (Petrosillo et al., 2006, 2008).

On the other hand, there are some other aspects that cannot be forgotten in relation to melatonin's effects on mitochondria. Scientific evidence reflects that melatonin and its metabolite AMK could be able to inhibit production of nitric oxide (NO) and consequently the formation of peroxynitrite radical (ONOO-), regulating the activity of mitochondrial nitric oxide synthase. Besides being a signalization molecule, NO has been demonstrated to be a potent reversible inhibitor of cell respiration (at the C-IV level), inducing impairment of ATP synthesis and, eventually, apoptosis and cell death (Freitas et al., 2006; Luchetti et al., 2010). Nevertheless, nowadays is not clear if this effect could differ according to melatonin concentration, affected tissues, or metabolic requirements. In fact, recently Sarti et al. (2013) observed that mRNA expression of neuronal nitric oxide synthase (nNOS) in HaCat cells (a spontaneously transformed human keratinocyte cell line, widely used for its high capacity to differentiate and proliferate in vitro) (Boukamp et al., 1988; Schoop et al., 1999) is increased at nanomolar concentrations of the indole. Conversely, at higher intracellular concentrations of melatonin (and/or its metabolites), a progressive enzymatic inhibition is recorded; simultaneously, the consequent lowered ATP_{OXPHOS} production is balanced by glycolysis. As the authors pointed out, mechanisms involved in this biphasic behavior remain unclear, but they could be related to competition between melatonin and nNOS for binding with calmodulin.

Melatonin also exerts indirect antioxidant actions through promotion of antioxidant enzymatic activity in mitochondria, which ultimately has positive repercussions on ETC function. Glutathione (GSH) and its related enzymes glutathione peroxidase (GPx) and reductase (GRd) are the main mitochondrial antioxidant system; a lack in catalase activity makes mitochondria almost entirely dependent on cytosolic GSH and its recycling enzymes (Martin et al., 2000a; Reiter et al., 2003). Some studies have shown that melatonin regulates GSH redox status in brain and liver mitochondria (Martin et al., 2000a). Furthermore, it seems that melatonin may promote de novo synthesis of GSH by stimulating the activity of the enzyme γ-glutamyl-cysteine synthetase (Srinivasan et al., 2011). In relation to this, melatonin has the ability to preserve the nicotinamide nucleotides NADPH and NADH, essential for the activity of GRd. Thus, this could be another mechanism implied in the maintenance of GSH levels, which would prevent in turn both inhibition of C-I by NO and extensive mitochondrial damage produced by hydrogen peroxide (H_2O_2) (Martin et al., 2000a; Phillipson, 2014). Moreover, NAD recycling to NADH by melatonin facilitates ATP_{OXPHOS} production, since NADH acts as a substrate for C-I (Tan et al., 2005; Phillipson, 2014). In summary, as can be noted, the indolamine at mitochondrial level acts as a multifactorial and powerful antioxidant, influencing several components of a complex network that need to be totally clarified.

It is well established that mitochondria are arbiters of life and death, playing a decisive role in signaling of apoptosis and necrosis in the function of oxidative damage grade

suffered. Consequently, antioxidants able to reduce the production of ROS/RNS at the mitochondrial level would be expected to limit cellular death (Reiter et al., 2008). In normal cells exposed to different experimental conditions, melatonin has the ability to avoid apoptotic cascade. Some authors have demonstrated that the indolamine prevents opening of the mitochondrial permeability transition pore (mPTP) caused by alterations of Ca^{2+} homeostasis (del Castillo-Vaquero et al., 2010; Santofimia-Castaño et al., 2014) and also restores mitochondrial membrane potential ($\Delta\psi m$) (Xu et al., 2002; Espino et al., 2011). According to Espino et al. (2010), melatonin could also exert its antiapoptotic effects through prevention of Bax activation, release of cytochrome C, and activation of caspases 3 and 9. For others, protection of CL from lipid peroxidation could be another suitable molecular mechanism implied, because this lipid is responsible for the anchorage of cytochrome C in the inner membrane of mitochondria (Luchetti et al., 2010). Interestingly, melatonin has shown a prooxidant effect on tumor cell lines, thus promoting intracellular ROS generation and apoptosis (Bejarano et al., 2011a). In this case, the events described above are induced in the presence of millimolar concentrations of melatonin, which strongly suggests involvement of the mitochondrial-mediated (intrinsic) pathway of apoptosis (Bejarano et al., 2009). Thereby, the indolamine seems to enhance the viability of normal cells at low pharmacological concentrations (1 μM to 10 nM), being prooxidant at high concentrations (1–0.1 mM) in several human tumoral cells (Bejarano et al., 2011a). It should be noted that antioxidant and scavenger capacities of melatonin may also intervene (Espino et al., 2010; Luchetti et al., 2010). This dual action could be exploited not only to protect healthy cells but also to increase selective effects of anticancer agents during chemotherapy (Bejarano et al., 2011b).

Oxidative stress may damage other important components of mitochondria, leading to lethal cell injury. Mitochondrial DNA (mtDNA) is especially susceptible to attack by ROS because of the close proximity to the ETC and lack of protective histones (Paradies et al., 2010). Once more, it has been registered that even in cells with a common deletion of mtDNA, melatonin would be still capable of reducing oxidative damage and thus preventing apoptotic cascade (Jou et al., 2007).

Finally, as Luchetti et al. (2010) mentioned, melatonin metabolism by itself could contribute to the defense against oxidative stress. Specifically, phase I detoxification components such as mitochondrial cytochrome P450 metabolize the O-demethylation and hydroxylation at position 2 and 6 of the indolamine, generating several antioxidant metabolites (N1-acetyl-N2-formyl-5-methoxykynuramine, N-acetylserotonin, and 2-hydroxymelatonin). This evidence might suggest a functional linkage between the control of melatonin metabolism and its mitochondrial-protective function.

In the Figure a summary of different levels of action of melatonin on mitochondrial function of normal cells is shown.

3. Role of melatonin on mitochondrial dysfunction and diseases

The idea that mitochondrial dysfunction is implicated in the etiology of various diseases has been strengthened after several years of research. Initially, studies of mitochondrial diseases have focused on mitochondrial respiratory-chain diseases associated with mutations of mtDNA. However, more recent evidence shows that oxidative damage is responsible for the impairment of mitochondrial function, leading to a self-induced vicious cycle that finally culminates in necrosis and apoptosis of cells and organ failure. We are now starting to understand the mechanisms of a large list of mitochondrial-related diseases (cancer, diabetes, obesity, cardiovascular and neurodegenerative diseases, and aging); all of them seem to share the common features of disturbances of mitochondrial Ca^{2+}, ATP, or ROS metabolism (Sheu et al., 2006). Therefore, selective prevention of such phenomena should be an effective therapy in a wide range of human diseases (Smith et al., 1999; Sheu et al., 2006). Melatonin, as was described in the previous section, has many of the characteristics of a perfect candidate for the treatment of these kinds of illnesses. Recent insights about this interesting topic are summarized below and in the Table.

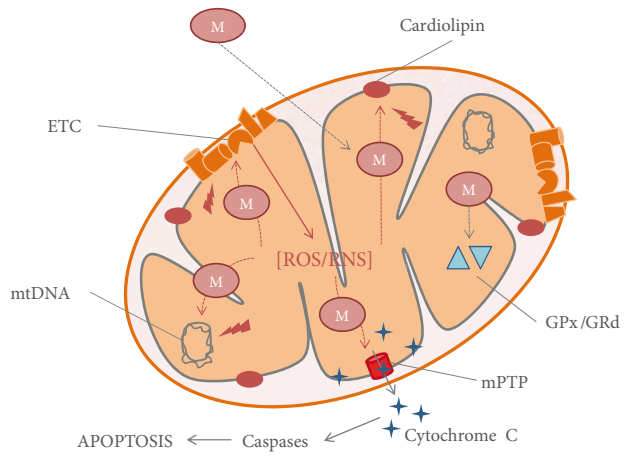

Figure. Summary of melatonin and mitochondrial function in a normal cell: action levels (ETC = electron transport chain; GPx/GRd = glutathione peroxidase/glutathione reductase; M = melatonin; mPTP = mitochondrial permeability transition pore; mtDNA = mitochondrial DNA; ROS/RNS = reactive oxygen species/reactive nitrogen species).

Table. Summary of experimental articles on melatonin and mitochondrial function on diseases and aging.

Disorders	References
Aging	Rodríguez et al., 2007
	Espino et al., 2011
	Acuña-Castroviejo et al., 201
Neurodegenerative diseases	
Alzheimer disease	Morley et al., 2012
Parkinson disease	Dabbeni-Sala et al., 2001
	Patki et al., 2011
Huntington disease	Wang et al., 2012
Amyotrophic lateral sclerosis	Zhang et al., 2013
Cancer	Bejarano et al., 2009
	Bejarano et al., 2011a
	Uguz et al., 2012
	Wang et al., 2013
Gastrointestinal diseases	
Chronic diabetes mellitus	Zavodnik et al., 2011
Obesity	Stacchiotti et al., 2014
Ischemia/reperfusion	
Liver	Freitas et al., 2006
Heart	Petrosillo et al., 2006
	Petrosillo et al., 2009
Brain	Andrabi et al., 2005
	Han et al., 2006
	Wang et al., 2009
Sepsis	Lowes et al., 2013
Others	
Photodamage	Kleszczyński et al., 2011
	Argun et al., 2014
Alcoholism	Mansouri et al., 2001

3.1. Aging

Mitochondrial dysfunction plays a major role in the physiological aging process (Morley et al., 2012). Although not fully understood, a reduction in mitochondrial respiration (in terms of efficiency of the ETC (C-I and C-IV), ATP production, underexpression of genes associated with energy metabolism, etc.) and GPx activity has been recorded, accompanied by an increase in oxidation of CL and activation of mPTP, with the consequent release of proapoptotic factors (Mather and Rottenberg, 2000; Paradies et al, 2010; Phillipson, 2014). According to the free radical theory of aging proposed by Harman (1956), leakage of electrons, overproduction of ROS/RNS, and possibly accumulation of mtDNA mutations in postmitotic cells (beginning in adults after their mid-thirties) are considered to be contributory factors to age-related degeneration (Rodríguez et al., 2007; Srinivasan et al., 2011). Additionally, the gradual reduction in the nocturnal secretion of melatonin throughout life may contribute to the persistent oxidative damage and apoptosis signaling, due to failure of antioxidant protection exerted by the indolamine on the mitochondria (Reiter et al., 2008; Escames et al., 2010; Phillipson, 2014). This phenomenon is considered to be the signal for switching on the mammalian senescence program, which would decrease the number of cells in organs and tissues and functional resources until organic senescence of the organism (Skulachev, 2009; Espino et al., 2012). It has been demonstrated that huge interindividual variation exists in circulating levels of melatonin; this phenomenon likely has

a genetic origin localized in the gene for hydroxyindole-O-methyltransferase, the rate-limiting enzyme in melatonin synthesis. Thus, different genotypes may cause different grades of predisposition to age-related diseases in populations (Srinivasan et al., 2011). Furthermore, as Acuña-Castroviejo et al. (2012) pointed out, females seem to be more protected against oxidative stress than males. The authors hypothesize that this feature could be related, at least in part, to the regulatory action of sex hormones in the activities of several complexes of the ETC. Altogether, this evidence must be taken into account in the future for melatonin supplementation in the elderly.

In general, the studies conducted in this sense have shown that chronic treatment with melatonin counteracts some of the aspects related to the decaying mitochondrial function in senescence-accelerated mice, such as decreased ETC and GPx activities (Phillipson, 2014), increased lipid peroxidation, and reduction of the GSH:GSSG ratio through promotion of GPx and GRd activity (Rodríguez et al., 2007; Acuña-Castroviejo et al., 2012), favoring prosurvival pathways (sirtuins and PI3K/Akt signaling) versus the intrinsic apoptotic pathway (Espino et al., 2011).

3.2. Neurodegenerative diseases

The mammalian brain is a tissue with high energy demands, therefore with an active mitochondrial metabolism that consumes 20% of the total oxygen inspired. Consequently, ROS generation is intense, as well as the oxidative damage suffered by mitochondria, a situation worsened by the fact that the brain contains low concentrations of cytosolic antioxidants. Oxidative damage to different structural and functional components of mitochondria such as mtDNA, proteins (ETC complexes), and membrane lipids has been reported in a variety of organisms during the aging process. For instance, cognitive functions, motor ability, exploratory capacity, and neuromuscular coordination in mice are decreased upon aging in parallel with these oxidative phenomena (Escames et al., 2010). In the last few decades, mitochondrial dysfunction and oxidative stress have been linked to many neurodegenerative diseases, as explained below.

Alzheimer disease (AD) is characterized by extracellular senile plaques of aggregated β-amyloid (Aβ) and intracellular neurofibrillary tangles that contain hyperphosphorylated tau protein. Clinical manifestations include loss of memory and deterioration of cognition. This feature has led to the classical belief that the pathology of AD is due to overproduction of Aβ (Srinivasan et al., 2010). In 2004, Swerdlow and Kahn proposed an alternative hypothesis for AD called the mitochondrial cascade hypothesis. This new insight is based on the premise that damage to mtDNA results in mitochondrial dysfunction, leading to an increase in oxidative stress and subsequent activation of Aβ. In addition, it has been observed that brains of AD patients have a deficiency in C-IV activity and increased levels of oxidation and/or nitration of certain proteins. Thus, a diminished synthesis of ATP results in synaptic dysfunction, apoptosis, and tau hyperphosphorylation. At present, there is not enough information to confirm whether Aβ causes mitochondrial dysfunction and oxidative damage or whether mitochondrial dysfunction leads to inflammation resulting in excess Aβ production (Escames et al., 2010; Morley et al., 2012). Other evidence suggests that amyloid precursor protein (APP) and Aβ are taken up by mitochondria using the transporter outer membrane. Once there, only a small fraction is maintained in the mitochondrial matrix and the rest particularly accumulates in the inner membrane. The consequent alteration of fatty acid composition and the derived oxidative stress might influence the membrane permeabilization, leading to mitochondrial dysfunction (Rosales-Corral et al., 2012).

Parkinson disease (PD) is an age-related disorder characterized by a progressive degeneration of dopaminergic neurons of the substantia nigra pars compacta and by formation of Lewy bodies (Dabbeni-Sala et al., 2001; Srinivasan et al., 2010). Oxidative stress, loss of antioxidant defenses and GSH, and underexpression of genes associated with energy metabolism are considered as plausible and interrelated causes of this neurodegenerative disease. Thus, a deficit in mitochondrial C-I could contribute to cell degeneration via a direct generation of ROS, leading to disruption of Ca^{2+} homeostasis, decrease in ATP synthesis, and apoptosis (Dabbeni-Sala et al., 2001; Srinivasan et al., 2010; Patki et al., 2011; Phillipson, 2014). Deficiencies in C-II, C-III, and C-IV have also been observed in platelets of PD patients (Escames et al., 2010). Although there is no convincing proof demonstrating a primary role of inherited mtDNA mutations (Escames et al., 2010), they must not be discarded as well as implications of environmental factors in the neurodegenerative disorder (Patki et al., 2011).

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by ataxia, chorea, and dementia (Escames et al., 2010; Wang et al., 2012). It is well known to be caused by an alteration in a nuclear gene encoding huntingtin, a protein of unknown function but associated with inappropriate apoptosis. Mutant huntingtin protein first kills selectively vulnerable medium spiny neurons in the striatum and thereafter the cortical neurons (Wang et al., 2012). Additionally, multiple mtDNA depletions have also been found in HD patients, although their significance is unclear (Escames et al., 2010). Current evidence from genetic models supports mitochondrial dysfunction as a major cause of the disease, including deficiencies in the activities of C-II, C-III, and C-IV and calcium dyshomeostasis, impaired

ATP production, and anomalous mitochondrial dynamics (Escames et al., 2010; Srinivasan et al., 2010).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive and selective loss of motor neurons, resulting in progressive paralysis and death. Although the exact pathophysiological mechanisms are not fully understood, the occurrence of apoptosis-related mitochondrial dysfunction in the spinal cord-associated disease progression has been observed (Zhang et al., 2013). On the other hand, in patients with the familial form of ALS (5%–10%) mutations have been found in the *SOD1* gene that encodes the Cu, Zn-superoxide dismutase 1; thus, mutant forms of this mitochondrial antioxidant enzyme may damage mitochondria by some misunderstood mechanism (Escames et al., 2010).

Since the role of mitochondria in neural cell proliferation and/or differentiation seems to be essential, melatonin's effects in such cases might be related to its close relation with mitochondrial function (Escames et al., 2010). Therefore, the strategy in neurodegenerative diseases may be the use of pharmacological agents with antioxidant and free radical scavenging properties, leading to this goal attained by endogenous melatonin (Patki et al., 2011). Data obtained in animal models of different neural disorders point out the ability of melatonin supplementation to limit disfiguration of mtDNA (Reiter et al., 2008); restore GSH levels; promote GPx and GRd activities (Phillipson, 2014); prevent loss in mitochondrial C-I activity, mitochondrial respiration, and ATP production (Dabbeni-Sala et al., 2001; Morley et al., 2012); inhibit $\Delta\Psi m$ loss and release of proapoptotic factors (Wang et al., 2012; Zhang et al., 2013); and increase the numerical density and surface volume of mitochondria (Morley et al., 2012). Furthermore, melatonin supplementation could counteract cellular vulnerability to proapoptotic challenge due to altered melatonin receptors MT1 and MT2 observed in AD, PD, HD, and ALS, increasing resistance to cellular stress (Wang et al., 2012; Zhang et al., 2013). Other aspects such as melatonin treatment that could also directly alter the expression of the huntingtin gene, affecting disease onset, progression, and survival of HD, must not be excluded (Wang et al., 2012).

3.3. Cancer

Today in vitro evidence clearly shows that cancer cells exhibit altered metabolic regulation and mitochondrial morphology and physiology compared with normal cells (Sheu et al., 2006). For instance, a study conducted in rat prolactinoma cells showed elevated activities of C-I, C-III, and C-IV along with higher production of ATP, an important requirement for dividing cells. However, as the authors noted, it cannot be forgotten that it is possible that the ETC complexes display different degrees of increasing activities in different tumor cells lines (Wang et al., 2013). In this sense, the question of how cancer cells modulate oxygen consumption by OXPHOS during the different stages of tumorigenesis is still being discussed (Chen et al., 2009).

As was previously emphasized, melatonin shows proapoptotic effects on tumor cell lines compared to normal cells (Bejarano et al., 2011a; Uguz et al., 2012). In this sense, melatonin seems to be able to block ETC complexes and decrease ATP production (Wang et al., 2013), as well as activate the intrinsic apoptotic pathway (Bejarano et al., 2009). Furthermore, antioxidant and scavenger capacities of melatonin may also intervene (Espino et al., 2010; Luchetti et al., 2010). As many studies suggest, low melatonin levels observed during aging could be linked to cancer progression (Wang et al., 2013).

3.4. Gastrointestinal and metabolic diseases

Compared to the pineal gland, the gastrointestinal tract contains several hundred times more melatonin, where it is believed to act as a synchronizer of the digestive processes, a regulator of liver metabolism, and a protector from the oxidant effects of biliary salts (Freitas et al., 2006).

Regarding chronic diabetes mellitus, enhanced oxidative stress and declines in antioxidant capacity are considered to play important roles in the pathogenesis of this disease. At the mitochondrial level, ROS formation, excessive oxidative damage, and reduced organular biogenesis contribute to mitochondrial disruption and, subsequently, to insulin resistance and associated diabetic complications (Zavodnik et al., 2011). According to Zavodnik et al. (2011), long-term melatonin administration to diabetic rats reverts the oxygen consumption rate V3 and prevents the mitochondrial glutathione-S-transferase inhibition in liver cells.

On the other hand, in metabolic diseases, such as obesity, it has been observed that inflammatory cytokines stimulate the generation of mitochondrial ROS and induce oxidative/nitrosative stress that causes defective aggregation of respiratory chain complexes; alteration in organular bioenergetic has been also related to misshaped mitochondria in renal proximal tubules of obese mice. In this case, mitochondria were roundish when compared to the elongated shape of mitochondria in control conditions, as a consequence of renal oxidative stress. Melatonin oral treatment modified mitochondrial morphology and distribution in proximal tubules and greatly reduced specific markers of the intrinsic apoptotic pathway (Stacchiotti et al., 2014).

Regarding acute pancreatitis, the protective effect of antioxidant supplementation in both oxidative and inflammatory processes implied in this gastrointestinal disease has been demonstrated. Thus, melatonin and resveratrol are shown as chemopreventive agents against oxidative phenomena such as lipid peroxidation and protein

oxidation, increasing antioxidant defenses (Carrasco et al., 2013, 2014b) and regulating Ca^{2+} homeostasis at the pancreatic level (Carrasco et al., 2014a).

3.5. Ischemia/reperfusion challenge in organ transplantation and cerebrovascular accidents

Induced injury by cold ischemia/reperfusion (I/R) plays a critical role in the occurrence of primary nonfunction and delayed graft function, being limiting factors in different organ transplantations such as heart and liver (Freitas et al., 2006). Similarly, most cerebral ischemia induced by various factors can be transiently reversed, although reperfusion produces further neuron damage (Han et al., 2006). After I/R, the oxidative stress, derived from impairment of ETC function (C-I and C-III) among other sources, is worsened by the concomitant depletion of endogenous antioxidants. Mitochondrial ROS generation occurs in part during ischemia and more abundantly during early reperfusion. The decrease in ETC activity has been ascribed to ROS-induced CL oxidation (Freitas et al., 2006; Petrosillo et al., 2006). Moreover, in cardiomyocytes and neurons it has also been observed that at reperfusion the influx of Ca^{2+} into the mitochondria combined with an oxidative environment and higher pH causes the opening of mPTP channels and therefore triggers the pathway of cell death described above (Andrabi et al., 2004; Petrosillo et al., 2009; Srinivasan et al., 2010).

For this reason, the use of melatonin could be a good therapeutic option in I/R. Some experiments showed that reperfusion with a melatonin-containing medium significantly improved the restoration of liver function after cold storage (Freitas et al., 2006). Furthermore, melatonin reperfusion of ischemic hearts significantly lowered the degree of mitochondrial lipid peroxidation, prevented the loss in ETC function and the increase of H_2O_2 production (Petrosillo et al., 2006), inhibited the formation of mPTP and related events, and preserved the content and integrity of CL molecules, suggesting a possible link between these processes (Petrosillo et al., 2009; Paradies et al., 2010). Focusing on the apoptotic pathway, it has been observed that the indole inhibits cytochrome C release and caspase 1 and 3 activation, thus preventing DNA fragmentation (Andrabi et al., 2004; Han et al., 2006; Wang et al., 2009). In other words, scientific evidence indicates that melatonin's effect during I/R is not only due to its direct/indirect antioxidant properties, but also due to its influence over mitochondrial homeostasis and dynamics (Srinivasan et al., 2010; Huang et al., 2013). Thus, in the case of a stroke, it must be considered that elderly individuals may be increasingly vulnerable to the damaging effects of this accident because they lack appropriate protective levels of endogenous melatonin (Andrabi et al., 2004).

3.6. Sepsis and septic shock

Sepsis is the major cause of death in critical care units worldwide and is characterized by systemic dysregulated inflammatory response and oxidative stress, which can culminate in organ failure and death (Lowes et al., 2011). At the cellular level, data show mitochondrial dysfunction with subsequent reduced C-I, C- II, and C-IV activities and decreased ATP production, CL peroxidation, dissociation of cytochrome C, and more ROS generation. These events lead to an increased inflammatory response, resulting in a self-sustaining and amplifying cycle. In fact, it is well known that mitochondrial ROS drive inflammatory cytokine responses such as IL-1b and IL-6 via both inflammasome-dependent and -independent mechanisms acting together with NFκβ. Septic experimental models show that melatonin treatment restores mitochondrial respiration and reduces both oxidative stress and IL-6 levels (Lowes et al., 2013).

On the other hand, septic shock is a lethal condition caused by a complex chain of pathogen-induced events. It has been associated with the production and release of numerous proinflammatory mediators as well as massive cellular apoptosis; as a result, a decrease in mitochondrial respiration and in GSH levels as well as an overproduction of ROS and NO is observed. Thus, it is suspected that melatonin might exert a protective effect in mitochondrial failure via regulation of mtiNOS activity, among other mechanisms (Srinivasan et al., 2010).

3.7. Other diseases

UV-induced ROS generation is tightly connected with skin photodamage. Melatonin shows protective effects in photobiological disturbances not only for its strong antioxidative properties, but also for its antiapoptotic properties observed in keratinocytes and retinal pigment epithelium cell cultures. The indole has been shown to inhibit caspase 3 and 9 activation and to reduce dissipation of mitochondrial transmembrane potential (Kleszczyński et al., 2011; Argun et al., 2014).

Regarding alcoholism, ROS and other free radicals generated during ethanol metabolism cause oxidative stress and lipid peroxidation in the liver, brain, heart, skeletal muscles, and exocrine pancreas. Mitochondria are major targets of this oxidative damage, and in particular some of their components such as proteins, CL, and mtDNA. Scientific evidence shows that alcoholic binging causes extensive mtDNA degradation and mtDNA depletion in mouse heart, skeletal muscles, and brain, followed by increased mtDNA replication and increased mtDNA levels. Antioxidants such as melatonin exert protective effects against ethanol-mediated hepatic mtDNA depletion, likely due to its ability to incorporate into mitochondrial membranes and bind to C-I (Mansouri et al., 2001).

In Barth syndrome, a genetic cardiomyopathy in children, peroxidation of CL appears to be responsible, at least in part, for the loss of the activity of ETC complexes (C-I, C-III, and C-IV), mitochondrial dysfunction, and subsequent heart injury (Paradies et al., 2010).

Finally, sudden infant death syndrome (SIDS), one of the most mysterious disorders in medicine, could have a mitochondrial origin. It has been hypothesized that some infections, both viral and bacterial, can produce profound alterations in the mitochondrial function of the diaphragm, precipitating a significant reduction in the ventilatory capacity of this muscle. Newborns and infants born prematurely are especially prone to oxidative stress and have reduced antioxidant defense mechanisms. Since young infants exhibit transient melatonin deficiency for the first 2–4 months of life and pineal dysfunction and impaired melatonin metabolism have been associated with SIDS, the role of the tryptophan–serotonin–melatonin pathway in the etiology of SIDS should be investigated in the future (Siren et al., 2011).

4. Conclusion

Mitochondrial dyshomeostasis and related events have begun to reveal themselves as possible etiologies of several diseases of unknown origin. In the next years, conscientious investigation about this topic should be undertaken by scientists of different research areas to achieve a better understanding of the molecular mechanisms implied, which will ultimately allow the development and clinical application of efficacious treatments.

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