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Melatonin role in the mitochondrial function

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1. ABSTRACT

Melatonin is an ancient molecule present in unicellular organisms at the very early moment of life. Initially identified as a secretory product of the pineal gland in mammals and in other species, it was considered a hormone related to reproduction. The evidence that melatonin is produced in many organs and tissues of the body, reaching concentrations higher than in the blood, support the multiplicity of the melatonin actions. The best-known actions of melatonin, currently supported by experimental and clinical data, include antioxidant and anti-inflammatory abilities, some of them involving genomic regulation of a series of enzymes. Besides, melatonin displays anticonvulsant and antiexcitotoxic properties. Most of the beneficial consequences resulting from melatonin administration may depend on its effects on mitochondrial physiology. The physiological effects of melatonin on normal mitochondria, its role to prevent mitochondrial impairment, energy failure, and apoptosis in oxidatively-damaged mitochondria, and the beneficial effects of the administration of melatonin in experimental and clinical diseases involving mitochondrial dysfunction and cell death, are revised.

2. INTRODUCTION

Increasing evidence supports the antioxidant and free radical scavenging properties of melatonin and its metabolites (1, 2). The relationship between oxidative stress and mitochondria, the existence of circadian and seasonal variations in mitochondria, and the presence of high amounts of melatonin into these organelles, suggest a physiological role of melatonin on mitochondrial function (3-7). Subsequent studies concerning the role of melatonin in health and disease have provided a lot of information regarding its physiological role and therapeutic applications due to its antioxidant and anti-inflammatory properties (4-9). It is now presumed that mitochondrial dysfunction is the main cause of cell death in most of neurodegenerative diseases (10, 11). Initially, reactive oxygen species (ROS) were considered the most aggressive molecules against mitochondrial function. The recent discovery of the presence of a nitric oxide synthase (mtNOS) in the mitochondria points that the NO produced in these organelles may be related to the impairment of mitochondrial function in situations such as inflammation (12, 13). Although it is difficult to know what is the initial event in the diseases coursing with mitochondrial

pathologies, there is agreement that those therapies directed to maintain a good mitochondrial function are of great importance to prevent cell death. In this chapter we present evidences regarding the role of melatonin in mitochondrial homeostasis that supports the therapeutic utility of the indoleamine in multiple diseases.

3. MELATONIN

3.1. Melatonin production

Melatonin, a product of tryptophan metabolism, was primarily isolated in 1956 from the pineal gland and its structure identified soon after (14). Two main enzymes control the last steps in the melatonin synthesis, N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT). Although NAT was considered by years the rate limiting enzyme in melatonin production (15), there are now evidences suggesting that this role correspond to HIOMT (16). Melatonin production by the pineal gland exhibits a circadian rhythm increasing at night, when the activity of these enzymes is higher (15). The pattern of the circadian production of melatonin by the pineal is under the control of the photoperiod. Whereas in total darkness the rhythm of melatonin production runs with a period greater than 24 hour, continuous light blunts this rhythm. This photoneuroendocrine pathway induces the production of melatonin at night, whereas prevents its production during the day. Depending on the species, the nighttime rise in pineal (and blood) melatonin levels are usually on the order of 2- to 12 folds (from 10 pg/ml during the day to 120 pg/ml at night) (15). Melatonin produced by the pineal gland is released to blood and the cerebrospinal (CSF) fluid and thus, melatonin reaches to every cellular compartment in the body. Because the pineal gland is outside the blood-brain barrier, it is accessible to any molecule in the blood that may modify pineal activity. Besides, melatonin displays a half-life in the blood of 20-40 min (15), with 90% cleared during a single passage through the liver. Liver transforms most of the uptake melatonin into 6-hydroxymelatonin that is mainly conjugated to 6-sulfatoxymelatonin that is the main metabolite of melatonin in urine (15).

Melatonin, identified initially as a secretory product of the pineal gland (17), was considered for several decades as a regulator of reproduction, mainly in seasonal-breeding animals (15). But it is now evident that melatonin is also synthesized in many organs and tissues of the body (18). Several extrapineal tissues, including retina, cerebellum, harderian gland, gut, skin, ovary, testes, lymphocytes, and platelets, airway epithelium, liver, pancreas, adrenals, kidney, thymus, thyroid, carotid body, placenta, endometrium, bone marrow, mast cells, natural killer cells, eosinophilic leukocytes, platelets and endothelial cells contain melatonin, and some of them are able to produce the hormone (18-23). These tissues express the enzymes necessary for melatonin synthesis (24). The concentration of melatonin in these tissues is higher than in blood and in some of them, such as bone marrow, bile and CSF, exceeds 2-3 orders of magnitude its blood levels (18, 25). A common characteristic of extrapineal melatonin is that it does not leave the organ. The broad extracellular and intracellular distribution of melatonin, in addition to its extrapineal production, may explain the role of this hormone to modulate a number physiological process through a variety of mechanisms.

3.2. Melatonin functions

The pineal-dependent rhythm in melatonin is clearly driven by the photoperiodic environment under which animals are living. Due to the precisely regulated melatonin circadian rhythm, which it is synchronized to 24 h, it provides information concerning the day- and night-times, used for synchronization of many other rhythms in the body (3). It was proposed also that seasonal variations in the melatonin rhythm may serve as a calendar for regulatory purposes (26). Brain electrical activity, neurotransmitter production, secretion, and their receptors, display circadian rhythms controlled by melatonin. (27-29). Alterations in melatonin production, either in blind people, by its reduction with age, or by some pathology, disrupt the melatonin-dependent rhythms. Thus, alterations in the behavior, intellectual and cognitive abilities may also reflect an abnormal pineal melatonin production. This is not surprising taking in mind that melatonin controls the rhythm of the neurotransmitters involved in the superior functions of the brain (30).

Because extrapineal melatonin does not leave the organ where it is produced, extrapineal melatonin is considered as a paracrine hormone or signaling molecule. Three are the main aspects of the extrapineal melatonin functions studied to date. First, the antioxidant activity of the hormone, initially reported in 1993 (31), is now supported by a number of experiments *in vitro* and *in vivo* (1-3, 8, 9). The existence of an inverse relationship between melatonin content in different tissues and their degree of oxidative damage has been recently reported (32). Second, the immunoenhancing properties of melatonin and the production of the hormone by immune cells are now accepted (23). Third, an increasing body of evidence suggests that melatonin exerts homeostatic roles on the mitochondrion. Melatonin effects on mitochondria, that have been reported *in vitro* and *in vivo*, include increased membrane fluidity, increased activity of the electron transfer chain (ETC) complexes and ATP production, increased mitochondrial membrane potential, reduced oxidative stress, and closing of the mitochondrial permeability pore (PTP) (3, 4, 6, 7, 33). Since mitochondria produce both ROS and reactive nitrogen species (RNS) including the highly toxic peroxynitrite ($ONOO^-$), the presence of melatonin, a scavenger of both ROS and RNS in mitochondria, guarantees their protection against oxidative damage by these reactive molecules, maintaining at the same time a good mitochondrial function.

After *in vivo* administration to rats, melatonin reaches every organ and tissue of the body, but its concentration varies among subcellular compartments, such as nucleus and mitochondria (5, 34). Experiments with rat liver and brain mitochondria were conducted *in vitro* to assess their ability to concentrate melatonin. Mitochondria take up melatonin in a time- and concentration-dependent manner, with a first order kinetics and a K_M= 32 nM,

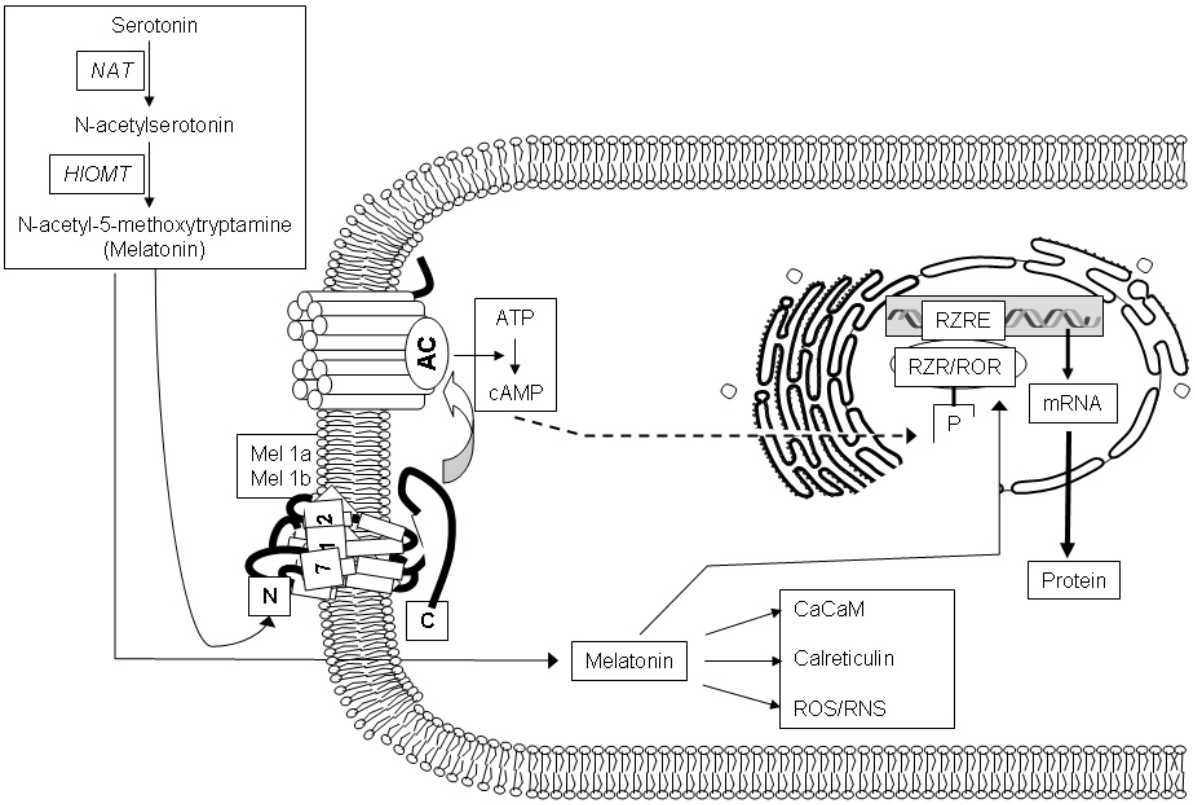

Figure 1. Mechanisms of action of melatonin. Melatonin, a hormone derived from serotonin, exerts its effects through both membrane and nuclear receptors. Cooperation between these receptors has been proposed. Besides, melatonin exerts non-receptor-mediated effects through its interaction with cytosolic proteins such as Ca^{2+}-calmodulin complex and calreticulin. Finally, melatonin can directly scavenge free radicals.

reaching its maximal concentration 60-90 min after addition. At this time, the concentration of melatonin in mitochondria was in the range of 100-200 nM. These data indicate the existence of an active transport system and suggest a role for melatonin in mitochondrial homeostasis.

3.3. Melatonin mechanisms of action

Due to the multiplicity of effects of melatonin, it is not surprising that the hormone may exert its actions by multiple mechanisms. Several examples of energy economy during phylogeny leading to the use of one molecule for multiple purposes include thyroid hormones, steroid hormones, and peptidic hormones such as prolactins. The mechanisms of action of melatonin may be classified into three main groups (Figure 1): a) those actions related to either membrane or nuclear receptors; b) the actions linked to cytosolic proteins, and c) the antioxidant and free radical scavenger activities. Nevertheless, some of the antioxidant properties of melatonin are related to genomic, nuclear receptor-related events. Although there is not full confirmation to date, recent experiments suggest that melatonin also exerts some of its effects in the mitochondria through the activation of mtDNA transcriptional activity, although whether this effect is related to a mitochondrial receptor of melatonin remain unclear. Since receptor-mediated actions of melatonin are out the scope of this chapter, we will not discuss this topic here.

3.3.1. Antioxidant and free radical scavenger properties of melatonin

The importance of melatonin as antioxidant depends on several characteristics: its lipophilic and hydrophilic nature, its ability to pass all bio-barriers with ease, and its availability to all tissues and cells. Melatonin distributes in all cell compartments, being especially high in the nucleus and mitochondria (5, 34). Melatonin maintains membrane function and permeability by preventing lipid peroxidation (LPO) (35) and increasing its fluidity (33) and maintains mitochondrial function by reducing hydroperoxide levels and maintaining GSH homeostasis in both normal conditions and under oxidative stress (3, 6, 7, 36). This means that melatonin is available in the sites in which free radicals are forming, thus decreasing their toxicity (1, 2). Moreover, extrapineal melatonin is produced for *in situ* protection against oxidative damage. This suggests that each organ may produce the amount of melatonin that it needs independently of the circulating fluctuations.

Melatonin is a powerful antioxidant and free radical scavenger and directly scavenges both ROS and

RNS (1, 2, 9). Several experiments have been done to compare the antioxidant activity of melatonin with other known antioxidants. In vitro, the efficacy of melatonin to prevent DA autoxidation was significantly higher than other antioxidants including vitamin E and C (37). In isolated mitochondria, 100 nM melatonin counteracted t-butyl hydroperoxide (BPH)-induced GSH depletion, whereas vitamins C and E and N-acetylcysteine (NAC) were unable to counteract BPH-induced oxidative stress at doses of 1 mM (38). In other studies it was reported that melatonin prevented the oxidation of the radical-trapping agent 2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid more effectively that did ascorbate, GSH, or trolox (water-soluble vitamin E) (1). Melatonin reacts with $HO^{\cdot}$ at near-diffusion-controlled rate (1). The calculated second order rate constant *k* for the scavenging of the $HO^{\cdot}$ by melatonin was 2.7 x 10^{10} M/s (1). This value is similar or even higher than other well-known $HO^{\cdot}$ scavengers (1). Melatonin also scavenges H_2O_2, precursor of $HO^{\cdot}$, with a *k* of 2.3 x 10^6 M/s. Melatonin also reacts with both NO and $ONOO^-$ *in vitro* (1), whereas *in vivo* markedly reduced nitrite levels and nitrotyrosine, reflecting NO levels and tyrosine nitration by $ONOO^-$, respectively (1). Besides, several metabolites formed when melatonin functions as a free radical scavenger, *i.e.* N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) also possess significant antioxidative and anti-inflammatory activity (1, 39, 40). Thus not only the parent molecule, melatonin, but its metabolites as well are protective against oxidative stress (41). The levels of melatonin and its metabolites determine the redox status in human serum. Changes in the cellular redox state mediate the binding activities of some critical transcription factors such as AP-1 and NF-kappaB and regulate the gene expression of antioxidant enzymes (42). Thus, melatonin and its oxidative metabolites may play a role in modifying the signal transduction pathways and gene regulation to protect organisms from oxidative stress

3.3.1.1. Melatonin and reactive oxygen species

Melatonin is an efficient scavenger of hydroxyl radical ($HO^{\cdot}$), and as a consequence of this interaction 3-hydroxymelatonin is produced (40). Interestingly, this metabolite is excreted in the urine and it was proposed as a biological marker of oxidative stress. As it is well known, $HO^{\cdot}$ is a highly reactive compound that rapidly reacts with proteins, lipids, and DNA inducing oxidative damage. By scavenging $HO^{\cdot}$, melatonin provides a good mechanism for protecting against $HO^{\cdot}$ -induced oxidative damage in cells (43). It should be noted here that the effects of melatonin result more effective than those due to the antioxidant activity of vitamins E and C (37, 38). In contrast to vitamins E and C, melatonin not only does not deplete the cell from GSH, but prevents or even increases its content (36, 38). The solubility of melatonin, which is not restricted to a specific cellular compartment as vitamin E, and the antioxidant cascade of the indoleamine, account for the antioxidant efficiency of melatonin in comparison with other antioxidants (40). Melatonin regulates the expression and activity of several antioxidant enzymes, including glutathione peroxidase (GPx) and reductase (GRd), superoxide dismutase (SOD), catalase (CAT), and glucose-6-phosphate dehydrogenase (G-6-PDH) (40, 44, 45). Melatonin also increases the cellular content of GSH through the activation of the gammaglutamylcysteine synthase, the rate limiting enzyme in GSH synthesis (46). Melatonin and some of its metabolites comprise the so-called melatonin family of antioxidants (41). The melatonin family includes melatonin and the metabolites which are generated by the interaction of melatonin with ROS and RNS (1, 2). Similar to other antioxidants, melatonin possesses a specific electron reduction potential of 0.73 V (38). Thus, melatonin donates one electron transforming into a melatonyl cation radical. Another possibility also proposed is that melatonin might donate a hydrogen atom from the NH group of the pyrrole ring to generate a neutral melatonin radical, which in turn could scavenge one O_2^- to form the final product N^1-acetyl-N^2-formyl-5-methoxykynurenamine (AFMK), just as does the melatonyl cation radical (2). The other metabolite, N^1-acetyl-5-methoxykynurenamine (AMK), is a deformyl product of AFMK. In the presence of high levels of ROS, cyclic 3-hydroxymelatonin interacts with them to form AFMK. Alternatively, both, AFMK and AMK can be formed through the action of the indoleamine-2,3-dioxygenase (47). AFMK and melatonin protect against $HO^{\cdot}$ -induced DNA damage in a similar extend, whereas the ability of the former to protect against lipid peroxidation is lesser than that of melatonin (48). AFMK also significantly reduces neuronal cell death induced by H_2O_2, glutamate or beta-amyloid peptide (48). AMK also prevents $HO^{\cdot}$-induced DNA damage even more effectively than did melatonin. Thus, melatonin, AFMK and AMK constitute an antioxidant cascade produced during the interaction of melatonin with ROS (41). Together with cyclic 3-hydroxymelatonin, it was calculated that one melatonin molecule, via this cascade scavenges possibly up to four reactive species (48).

3.3.1.2. Melatonin and reactive nitrogen species

One of the most quantitative important ROS in the body is O_2^- that it is mainly produced by one-electron reduction of O_2 in the mitochondria; O_2^- is not a highly toxic radical, but it easily reacts with NO forming $ONOO^-$ (49). The toxicity of $ONOO^-$ is similar to the one of $HO^{\cdot}$, and both compounds damage lipids, proteins and DNA at similar extend. *In vitro* and *in vivo* studies reported that melatonin reduces the production of $ONOO^-$ (1, 50). It was shown that $ONOO^-$ reacts with the nitrogen of the pyrrole ring of melatonin yielding 1-nitrosomelatonin and 1-hydroxymelatonin (50). Neutralization of $ONOO^-$ by melatonin protects against protein oxidation more efficiently than GSH, vitamin E or mannitol (41). Other authors also showed that melatonin reacts with peroxynitrous acid, yielding 6-hydroxymelatonin that is excreted normally in the urine, and could serve as marker of nitrosative stress (41). *In vitro* and *in vivo* studies have shown that 6-hydroxymelatonin protects against oxidative tissue damage (51). The scavenging mechanism of 6-hydroxymelatonin may be similar to melatonin although its antioxidant capacity is even more potent than that of the later (50). However, under *in vivo* conditions, the tissue protective effect of melatonin is always better than that of

6-hydroxymelatonin, probably due to the formation of a series of metabolites with antioxidant properties.

Besides scavenging RNS, melatonin also inhibits the expression and activity of the iNOS (52). At physiological concentrations, melatonin administration also reduces nNOS activity in the brain of different animal species (53). *In vitro*, melatonin inhibits nNOS in a dose-dependent manner, with 1 nM inhibiting 20% of the activity, and reaching a 40% inhibition at 3 mM (53). *In vivo*, melatonin administration at doses of 20-30 mg/kg inhibits 100% of the activity of iNOS but also a 25% activity of nNOS. The inhibition of iNOS activity depends on the reduction of the gene expression, because *in vitro*, melatonin has not effect on iNOS activity (54). These results agree with the different mechanisms of inhibition of melatonin on nNOS/iNOS activities. Pharmacological experiments with purified nNOS revealed that melatonin behaves as a noncompetitive antagonist of nNOS, binding to Ca^{2+}-calmodulin (CaCaM) and impeding the CaCaM-dependent activation of nNOS (53). Because iNOS is synthesized with the CaCaM subunit bound to its molecule, melatonin does not further interact with CaCaM, and melatonin does not inhibit iNOS activity directly. The inhibition of iNOS activity by melatonin depends on the inhibition of the iNOS gene expression (52).

The ability of AFMK and AMK to interact with nNOS and iNOS was recently tested. Interestingly, AMK, but not AFMK, inhibits nNOS activity *in vitro* by the same mechanism that melatonin, *i.e.* through its binding to CaCaM. The difference is that AMK has an IC_{50} of 70 microM, whereas the IC_{50} for melatonin was > 3 mM (53). In vivo administration of AMK to normal rats also inhibits nNOS activity more efficiently than melatonin and so, 10 mg/kg b.w. AMK inhibits 25% the activity of rat brain nNOS, a similar percentage than 20 mg/kg melatonin. Interestingly, when the enzyme indoleamine 2,3-dioxygenase was inhibited with norharmane or 1-methyltryptophan , the inhibitory activity of melatonin on nNOS activity both *in vitro* and *in vivo* disappeared. It was then proposed that the inhibitory activity of melatonin on nNOS activity does not depend on the melatonin directly but through its transformation to AMK (54). AMK was also more potent than melatonin to inhibit the iNOS activity in vivo. In the model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) administration to mice, the induction of iNOS in the *substantia nigra* caused by the neurotoxin was reduced at the same extend after the administration of 30 mg/kg melatonin or 15 mg/kg AMK (unpublished data from this lab). The data suggest that besides their antioxidant activity, melatonin metabolites also exert protective activities through the regulation of the NO/NOS system. Under oxidative stress, the inflammatory reaction that follows to oxidative damage induces iNOS expression and NO production. The parallel transformation of melatonin to AFMK and AMK yield an additional mechanism of defense against oxidative stress and inflammation, producing a cascade of antioxidant and anti-inflammatory molecules.

4. MITOCHONDRIA

4.1. Mitochondria and energy conservation

Mitochondria possesses an electron transfer chain coupled to a phosphorylation system that enables the cell to obtain most of its energy requirements (55). In aerobic cells, oxidative phosphorylation produces 90-95% of the total amount of ATP, and more than 90% of the respiratory phosphorylation is catalyzed by ATP synthase. The chemiosmotic hypothesis (56) indicates that electron transfer runs parallel to the proton pumping by the complexes I, III and IV, yielding a proton gradient (proton-motive force) across the mitochondrial inner membrane. Whereas the full four proton reduction transforms O_2 to water at complex IV, the dissipation of the protonic gradient through complex V (ATP synthase) yields the energy enough to phosphorylate ADP and to form ATP. Regulation of respiration includes ADP (respiratory control), Ca^{2+} and proton leak (57). Recently, the regulation of mitochondrial respiration by NO has been reported (58). Mitochondria also posses a constitutive proton leak across the inner membrane that limits the basal metabolic rate and the production of potentially dangerous ROS. Besides, dissipation of energy as heat instead of ATP formation, permits mitochondria to maintain body temperature at a level higher than in the environment. This mechanism, named thermoregulatory uncoupling, causes dissipation of the mitochondrial membrane potential by an increased proton conductance of the inner membrane. Non-esterified fatty acids operate as protonophorous uncouplers with the help of the special uncoupling proteins (UCPs) (59).

4.2. Mitochondrial production of ROS and RNS

Most of the O_2 taken up by mammalian cells is processed in the mitochondria and reduced to water via mitochondrial complex IV. The complete elimination of every O_2 molecule requires its reduction by 4 electrons. However, O_2 can be partially reduced by one, two, or three electrons, yielding $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$, respectively. Superoxide ($O_2^{\cdot-}$) is primarily produced at the level of ubisemiquinone both at complex I and III (99). To prevent $O_2^{\cdot-}$-dependent damage, this radical is dismutated by mitochondrial Mn-SOD to H_2O_2. In turn, H_2O_2 has to be removed to prevent its transformation to the highly toxic $HO^{\cdot}$. Reduced glutathione (GSH) is of main importance to maintain the redox status of the mitochondria. Due to the significance of GSH in mitochondrial physiology, the redox cycling of GSH in this organelle is normally very active to avoid any significant lost of GSH (60).

Mitochondria also contain RNS, including NO and $ONOO^{-}$. It is now recognized that mitochondrial NO acts as a reversible antagonist of complex IV competing with O_2 for its binding site. Normal tissue levels of NO and O_2 are in the ranges of 100-500 nM and 10-30 microM, respectively. At these concentrations, the NO/O_2 ratio causes approximately half-maximal inhibition of mitochondrial respiration rate, suggesting that NO serves to the physiological regulation of respiration and of the rate of energy supply to the cell (57). High concentrations of NO may inhibit not only complex IV but also complexes III and I, reducing the electron transfer rates through the

mitochondrial electron transfer chain 58) and favoring electron leakage and production of O_2^-, with a simultaneous activation of the mitochondrial antioxidant defense. However, NO reacts very quickly with O_2^- yielding $ONOO^-$ that is able to irreversibly damage the respiratory complexes (61). The effects of $ONOO^-$ are to be considered as potentially highly toxic for mitochondria, leading to mitochondrial dysfunction and cell death (57). The existence of a specific mitochondrial isoform of NOS (mtNOS) has been reported, and its role as the enzyme responsible for the intramitochondrial production of NO is under active consideration and debate (12, 62, 63). It was suggested that this constitutively expressed mtNOS isoform presumably derives from a nNOS isoform (12). Most recently, we were able to show that mitochondria also express constitutively another NOS isoform (i-mtNOS) that is induced in inflammation (13, 64, 65). It is likely that the NO produced by these isoforms in normal conditions may serve for regulatory purposes, and that the excess of NO produced by i-mtNOS in inflammation may deeply inhibit respiration, in a way that O_2 is redistributed to other mitochondria or cells where it can be used for energy production. Mitochondria seem to contain some mechanisms to reduce RNS. Among them, the reaction between NO and cytochrome c may serve to remove an excess of NO (66). Also, the O_2 redistribution due to NO-dependent electron transfer chain impairment may constitute an adaptive mechanism to prevent O_2 consumption by these mitochondria and to reduce ROS production.

5. MELATONIN AND MITOCHONDRIA IN HEALTH AND DISEASE

Mitochondrial diseases started to be described 30 years ago, and they are nowadays mainly related to myopathies (67). Soon after, however, a relationship between mitochondrial alterations and neurological symptoms led to group these pathologies in mitochondrial neuromyopathies. Biochemical analysis of the presence of mitochondrial alterations have revealed the existence of electron transfer chain defects, impairment of ATP production, oxidative stress, and/or high sensitivity to PTP in different degree. Since the recent studies with melatonin have been mainly related to its antioxidant and anti-inflammatory properties, we will describe here some of those mitochondrial alterations in which ROS/RNS are present.

5.1. Melatonin actions on mitochondria

The relationships between melatonin and mitochondria are known from several years, and increasing evidence supports now a physiological role for the hormone on the organelles. During the last years, experimental and clinical studies have proved the protective role of melatonin against mitochondrial failure not only in pathologies coursing with oxidative stress but also in those neurodegenerative diseases in which electron transfer dysfunction is present (68). Experiments *in vivo* have shown that intraperitoneal administration of melatonin significantly enhanced the activity of complexes I and IV in brain and liver mitochondria of normal rats (69). Interestingly, the activity of these electron transfer complexes was maximal after 30 min in liver mitochondria and after 60 min in brain mitochondria. This difference depends on the faster availability of melatonin to liver than to brain after its intraperitoneal administration. After systemic injection, melatonin appears in cells in the central nervous system within 30 min and at higher concentrations than plasma levels (34). The time-dependence of melatonin also correlates with its half-life in blood. The administration of melatonin protects mitochondria against ruthenium red-induced inhibition of the complexes I and IV (69). To further assess the mechanism of action of melatonin on mitochondrial bioenergetics, *in vitro* experiments were conducted. Melatonin counteracted butyl hydroperoxide -induced mitochondrial damage, recovering the GSH pool and the activities of the complexes I and IV (38). In dose-dependent experiments, it was found that melatonin significantly increased the activity of these complexes I and IV at concentrations starting from 1 nM in rat brain and liver mitochondria (70). These effects of melatonin may be related, at least in part, to the increase of the mitochondrial membrane fluidity (33). Thus, *in vivo* and *in vitro*, in normal mitochondria and in oxidatively-damaged mitochondria, melatonin positively affects the mitochondrial bioenergetic functions, a finding of great importance for the extrapolation to pathological situations (3, 4, 6, 7). The data provide a new homeostatic mechanism regulating mitochondrial function. First, melatonin scavenges H_2O_2 (1, 41), the most important ROS produced into the mitochondria from O_2^-, avoiding the waste of the intramitochondrial GSH pool and subsequent mitochondrial damage (3, 6, 7). Second, improving mitochondrial function and ATP synthesis increases the rate of electron transport and reduces ROS production. These actions reflect an effect of melatonin to avoid a harmful decrease in mitochondrial membrane potential that may trigger the PTP. In some circumstances, melatonin reduces O_2 consumption by mitochondria, an effect that may protect this organelle from excessive oxidative damage (71). Other important consequence of the effects of melatonin on mitochondria is its role in thermogenesis. Since from these data it becomes apparent that melatonin exerts an opposite effect to UCPs, melatonin supplementation may reduce heat production and produce a more efficient use of substrates in terms of ATP production.

In view of the multiple actions of melatonin on mitochondria, it cannot be discarded an effect of the hormone on mtDNA. In preliminary experiments, it was found that melatonin increases the activity of the complex I isolated by blue native polyacrylamide gel electrophoresis (70). In other set of experiments, it was shown that melatonin administration prevents oxidative degradation of mtDNA and reduction of mtDNA transcripts in several tissues including liver, heart, skeletal muscle and brain (72). Besides, a direct effect of melatonin on mitochondrial genome expression in brown adipocytes of the Syberian hamster was reported (73). With these antecedents, we analyzed the possible effect of melatonin of the expression of the mtDNA-coded polypeptide subunits I, II, and III of the complex IV in both *in vivo* and *in vitro*, by reverse transcription polymerase chain reaction. Rats were

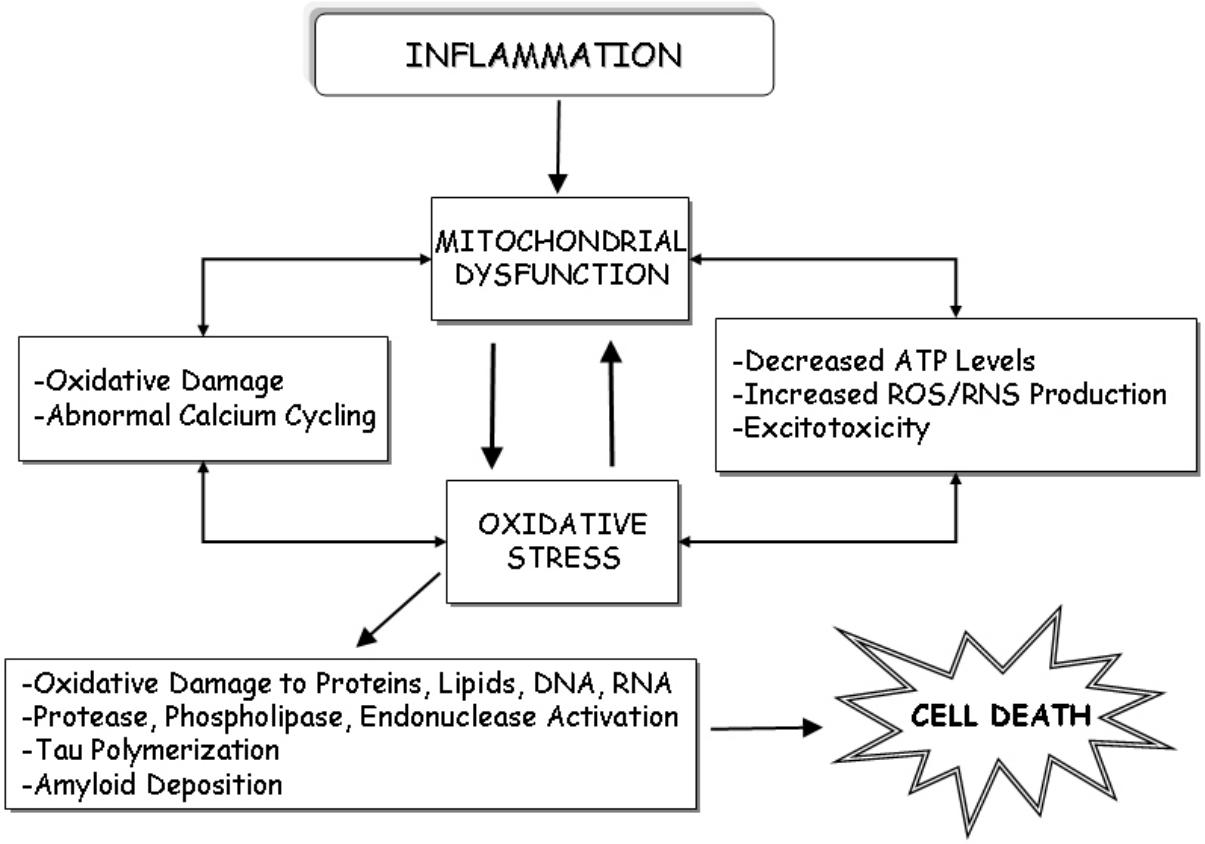

Figure 2. A diagram showing the relationship between inflammatory reaction, mitochondrial impairment, and cell death.

intraperitoneally injected with melatonin (10 mg/kg body weight) or vehicle and sacrificed at different times after treatment to obtain the liver mitochondria used for the determinations. The results showed a significant increase in the expression of the mRNAs for the three subunits tested (5). These results were confirmed by real-time quantitative PCR, demonstrating that the maximal effect of melatonin to increase the mRNA content of the three subunits of the complex IV coded by mtRNA peaked at 8 h after melatonin injection (unpublished data from this lab).

5.2. Melatonin, inflammation and mitochondria

Sepsis and the consequences of the response to sepsis are the leading cause of death in medical intensive care units (74). Common causes of sepsis include bacterial pathogens, fungi, viruses and parasites. Lipopolysaccharide (LPS), a component of the cell walls of Gram-negative bacteria, is the main responsible for the initiation of sepsis (75). Among other actions, LPS activates a number of intracellular signaling pathways, including nuclear factor kappaB (NFkappaB), thereby allowing rapid gene induction and the expression of inflammatory mediators which include, besides cytokines, chemokines, lipid mediators, inducible nitric oxide synthase (iNOS), enzyme activities, adhesion molecules, myocardial depressant substances and heat shock proteins (76). The progression of the inflammatory response leads to multiple organ dysfunction syndrome (MODS). Mitochondria are primary targets of injury in systemic organs during the first stage of sepsis, leading to MODS (13, 52, 77). Septic patients exhibit an impaired capacity to increase tissue O_2 consumption in response to O_2 delivery. There are a number of possible mechanisms through which cellular O_2 utilization may be impaired during sepsis, although it is now considered that NO and RNS play a central role in this process (Figure 2). In sepsis, the induction of i-mtNOS and subsequent RNS production were also accompanied by ROS increase and oxidative damage, with a significant depletion of GSH (64, 65). Experimental data have probed the beneficial effects of melatonin in restoring mitochondrial homeostasis in sepsis; melatonin administration produced a dose-dependent inhibition of the activity of iNOS, and a parallel reduction of NO and lipid peroxidation (52) and a reduction in the expression of iNOS protein and mRNA, suggesting a genomic effect of the hormone (Figure 3). Melatonin also recovers the mitochondrial GSH pool in different organs in experimental sepsis (64, 65) and prevented mitochondrial oxidative-nitrosative damage with inhibition of the i-mtNOS expression and activity induced by LPS in rat lung and liver mitochondria (13) (Figure 4). The reduction of NO and $ONOO^-$ also depends on the ability of melatonin to scavenge both NO and $ONOO^-$ (41). Together, these effects of melatonin explain many of its protective actions against endotoxemia, including the normalization of the electron transfer activity and ATP production. (64, 65, 72).

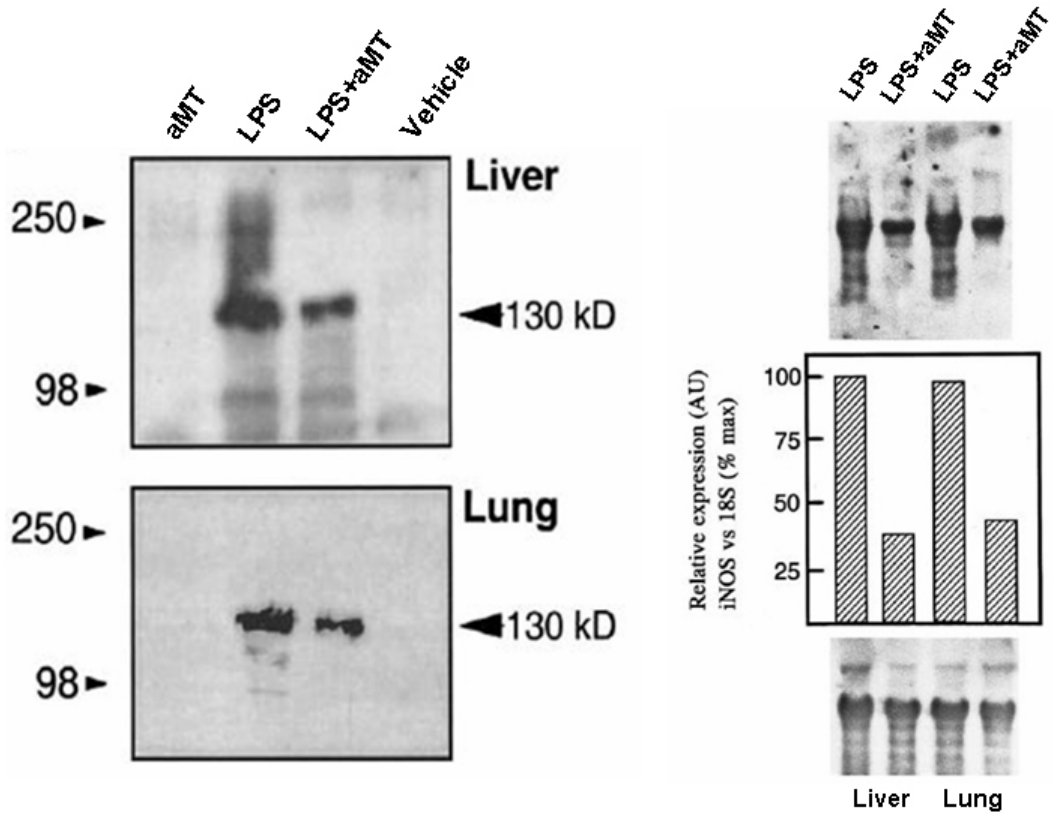

Figure 3. Effects of melatonin on the expression of the iNOS protein (left) and mRNA (right) in liver and lungs of rats. Melatonin (aMT) administration reduced significantly the expression of both protein and mRNA of iNOS induced by administration of LPS.

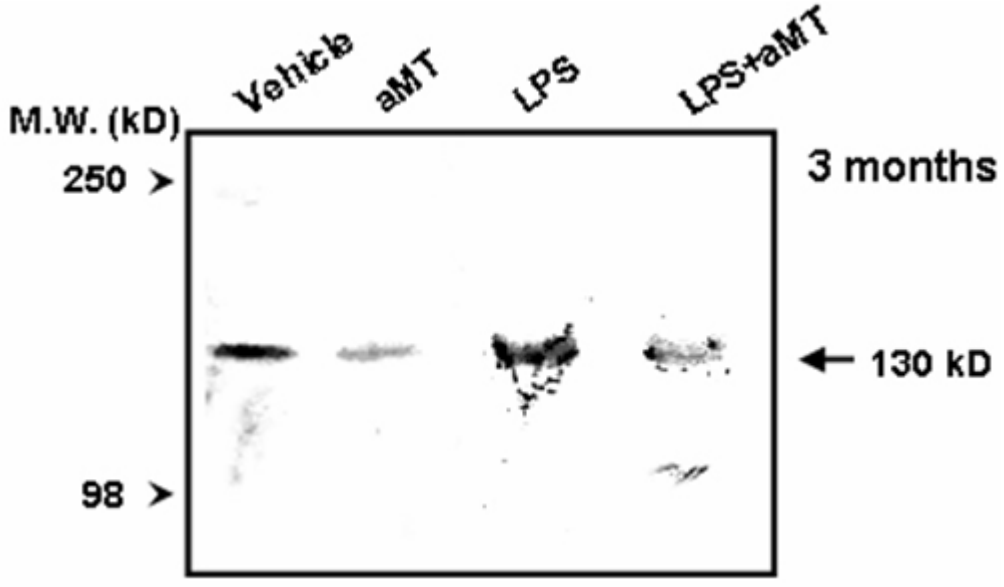

Figure 4. Western blot analysis of the effect of melatonin (aMT) on inducible mitochondrial NOS (i-mtNOS) protein expression in the mitochondria from rat lungs treated with LPS. Melatonin (aMT) administration prevents most of the i-mtNOS induction produced by LPS.

Severe septic patients show a significant alteration of the circadian melatonin secretion (76). Cytokines such as IL-1beta and TNF-alpha drastically reduce pineal serotonin content in pineal explants or cell cultures from neonate animals (78). Because serotonin is a precursor for melatonin synthesis, a reduction of the former in the pineal would likely limit melatonin production and alters its circadian rhythm, two findings related to the pathophysiology of septic shock (79). Based on these data and the multiple protective roles of melatonin in sepsis, melatonin was used to treat septic human newborns (80). This study, which constitutes the first report on septic patients, yielded important data that confirm the beneficial effects of melatonin against sepsis in humans. This study demonstrated that melatonin reduced several parameters of sepsis such as lipid peroxidation. The mortality of newborns with sepsis is high, usually between 30 and 50%. In this report (80), three of 10 septic children who were not treated with melatonin died within 72 h after diagnosis of sepsis, and more important, none of the 10 septic newborns treated with melatonin died. The dose of melatonin used in these treatments was a total of 20 mg orally distributed in two doses of 10 mg each, with a 1 hour interval, within the first 12 hours after diagnosis. The comparison of serum parameters between melatonin-treated and untreated septic newborns indeed confirmed the antioxidant (reduction in serum lipid peroxidation products) and the anti-inflammatory (reduction in C reactive protein) effects of melatonin. Other parameters including the white blood cell count and the absolute neutrophil count also significantly decreased with melatonin treatment, and they remained elevated in the untreated newborns. In view of the excellent results with melatonin, the hormone was also used to treat newborn humans suffering from hypoxia or respiratory distress. In these studies as well, melatonin improved the clinical outcome (81).

5.3. Melatonin, neurodegenerative diseases and mitochondria

The brain is especially sensitive to free radical damage due to the high utilization of O_2, its relatively poorly developed antioxidant defense, and the fact that it contains large amounts of easily oxidizable fatty acids. Brain oxidative damage has been considered a common link in the pathogenesis of a variety of neurodegenerative disorders (68, 82). Although it is yet unclear whether oxidative damage is the primary event in the pathophysiology of neurodegenerative disease, an increasing body of evidence implicates both ROS and RNS in the neuronal injury and cell death that occurs in these pathologies. Moreover, it is now clear that most of the neurodegenerative diseases course with an increased activity of iNOS, reflecting the participation of an inflammatory reaction in these diseases (10). On the other hand, excitotoxicity is also involved in neuronal death of a number of neurodegenerative diseases. Excitotoxicity is associated with mitochondrial dysfunction, loss of Ca^{2+} homeostasis, and enhanced oxidative stress (83). The mechanisms of overstimulation of the NMDA receptors, which increases Ca^{2+} influx into the cell triggering the excitotoxicity events and apoptosis have been previously commented. Thus, the sequence of events leading to cell death in neurodegerative diseases may involve NMDA-dependent excitotoxicity, and cytosolic and mitochondrial Ca^{2+} overload (most of the mitochondria are located closed to the NMDA receptors). Moreover, mitochondrial Ca^{2+} initiates oxidative stress and can inhibit respiration (11). Microglia, activated in response to neuronal death, initiates an inflammatory reaction, with iNOS and i-mtNOS induction and NO production, which contributes to the excitotoxic events and to mitochondrial dysfunction, thus establishing a vicious cycle concluding in cell death. It is then likely that the elevated mitochondrial NO produced by i-mtNOS, together with the product of reaction with O_2^-, $ONOO^-$, could be the major responsible for the mitochondrial impairment, ATP depletion, and subsequent cell death in neurodegenerative diseases. In fact, neurodegenerative diseases of different etiology share the common feature of oxidative stress, chronic inflammation, and mitochondrial dysfunction. Thus, mitochondrial oxidative/nitrosative damage probably constitutes the core of neurodegeneration. Anti-inflammatory drugs, including corticosteroids and immunodepressive agents, are used for the treatment of neurodegenerative diseases, but the efficacy of these treatments is unclear. The depression that follows to the anti-inflammatory therapy may increase the vulnerability of brain tissues to attack by virus such as adenovirus or herpes virus. In turn, tissue may generate autoantigens and create autoantibodies.

Biochemical findings revealed the existence of mitochondrial dysfunction in neurodegenerative diseases and a protective effect of melatonin administration. In the case of Parkinson's disease (PD), there is a deficiency of the complex I in *substantia nigra*, and sometimes a reduction in the activity of the four respiratory complexes in platelets (84). Inhibition of complex I activity is accompanied by a reduction of GSH levels, suggesting the existence of oxidative stress in these mitochondria. The defect in complex I is accompanied by a defect, with a lower degree, in complex IV activity and by a decreased mitochondrial membrane potential. Besides genetic predisposition to PD, epidemiological studies have indicated that environmental factors, including exposure to pesticides, are associated with an increased risk of PD. In this sense, MPTP, rotenone and paraquat, inhibit mitochondrial complex I and reproduce most of the features of PD including mitochondrial oxidative stress and energy loss, apoptosis initiation and selective dopaminergic neurodegeneration in the nigrostriatal pathway. The increase in O_2^- production, probably dependent of the complex I inhibition, and in H_2O_2 concentration in mitochondria from *substantia nigra*, are consistently reported.

Several observations account for the neuroprotective properties of melatonin in PD. Melatonin regulates the circadian rhythm of DA and DOPAC, its main metabolite (85). Melatonin also protects against excitotoxicity avoiding the autoxidation of dopamine (DA) that occurs in PD (86). The efficacy of melatonin to prevent DA autoxidation in vitro was significantly higher than other antioxidants including vitamin E and C, and that of L-deprenyl, a MAO B inhibitor that also has antioxidant

properties (37). Melatonin administration ameliorated the reduction of tyrosine hydroxylase-positive fibers, reduced lipid peroxidation in the striatum (86, 87), and prevented the MPTP-induced inhibition of the complex I in striatum and *substantia nigra* of mice. There was a synergism between melatonin plus L-deprenyl to increase the locomotor activity of the mice treated with MPTP, restoring their normal activity (87). After MPTP administration, there is an induction of iNOS in *substantia nigra* but not in the striatum, whereas the activity of nNOS increased in the later (88). We found that MPTP administration induced i-mtNOS without changes in mtNOS activity in the *substantia nigra* of MPTP-treated mice. These changes were accompanied by increased mitochondrial lipid peroxidation and NO levels (unpublished data from this lab). Melatonin administration, besides normalizing complex I activity, lipid peroxidation and NO levels in mitochondria from *substantia nigra* of MPTP-treated mice, counteracted both i-mtNOS and cytosolic iNOS activity and expression. These effects of melatonin were accompanied by a total normalization of the locomotor activity of the mice (87). In parallel to these studies, we tested a series of synthetic compounds structurally related to AMK, in search of new neuroprotective agents. Some of these compounds showed more potency than AMK to neutralize MPTP toxicity, although their utility for the treatment of PD in humans require further studies (89).

To characterize the sequence of pathophysiological events in PD, a series of experiments were done in cultured PC12 cells, differentiated with NGF and treated with rotenone, a selective inhibitor of complex I. Under these circumstances, PC12 cells show depletion of DA, increased oxidative stress and reduction of TH activity. The mitochondria of these cells show an inhibition of complex I activity, a depletion of the GSH pool and a reduction in the GRd activity. These mitochondria also show a loss of mitochondrial membrane potential, ATP depletion and PTP opening, and most of them die by apoptosis. At concentrations of 10-100 nM, melatonin counteracted in a dose-dependent manner the alterations induced by rotenone: melatonin normalized complex I activity, counteracted the oxidative stress recovering the GSH pool and the activity of GRd, and normalized the mitochondrial membrane potential. As a consequence of melatonin actions, the ATP production increased and PTP remained closed, preventing apoptosis almost completely. Melatonin was much more effective than cyclosporin A to close the PTP, since 100 nM melatonin displayed the same effect than 3 microM cyclosporin A (unpublished data from this lab). The results suggest that the chronology of events are the following: a) the primary event seems to be the inhibition of complex I, independently of the cause of PD; b) as a consequence, an increases in O_2^- and ROS production; c) then, the mitochondrial membrane potential decreases and ATP depletion occurrs; d) PTP opening induces the first events of apoptosis; e) ROS and cell death induce microglial reaction and an inflammatory response increasing the activity of iNOS and i-mtNOS; f) i-mtNOS (and perhaps iNOS) increases mitochondrial levels of NO and $ONOO^-$, and ROS/RNS induce mitochondrial injury and electron transfer dysfunction; g) consequently, a further reduction in mitochondrial membrane potential and ATP levels with increased levels of ROS/RNS multiply the apoptotic events, and h) the process enters in a vicious cycle, increasing neuronal death. Because the significant consequences of melatonin administration acts at several steps of these events including electron transfer activity activity, ROS/RNS production, mitochondrial membrane potential, and ATP production, clinical studies, aimed to improve mitochondrial function in PD, should be conducted to test the beneficial role of melatonin in PD patients.

Alzheimer's disease (AD) is another common neurodegenerative disease coursing with decreased mRNA expression of mtDNA encoding cytochrome oxidase subunit II, although it has been proposed that other nDNA-encoded cytochrome oxidase subunits may be also altered (90). In AD the beta-amyloid peptide accumulates extracellular, forming typical insoluble structures or senile plaques. Beta-amyloid neurotoxicity is associated with increased oxidative stress, mitochondrial complex IV inhibition, energy impairment, disruption of Ca^{2+} homeostasis, excitotoxicity, apoptosis and necrosis. Beta-amyloid induces ROS in a metal-catalyzed reaction which damage neuronal membrane lipids, proteins and nucleic acids. These process leads to microglia proliferation and activation, inflammatory reaction and NO/iNOS system. The subsequent events triggered by NO are similar to those in other neurodegenerative diseases. Again, the participation of i-mtNOS induction in the mitochondrial dysfunction in AD is yet unknown.

In experimental models of Alzheimer's disease, melatonin prevented neurodegenerative changes (91-93), whereas in humans melatonin administration significantly slowed the progression of the disease (94, 95). *In vitro* experiments were performed in murine neuroblastoma cells incubated with the beta-amyloid peptide. In these conditions, oxidative damage to lipids and DNA, and mitochondrial damage were found. Besides, more than 80% of the neurons died by apoptosis. The presence of melatonin in the incubation medium counteracted the beta-amyloid-induced oxidative stress. The reduction of ROS levels and the melatonin effect seem associated with an inhibition of the amyloid molecule capacity to organize itself into sheets, a process that is required to favor an enhanced free radical generation. Melatonin was also able to reduce in parallel cellular death and DNA damage in a dose-related manner (96). In human platelets melatonin protected against beta-amyloid-induced damage. Clinical studies were conducted with melatonin in AD patients. Melatonin administration at dose of 6 mg daily, delayed significantly the development of the signs of AD after 3 years of treatment. In other study, at dose of 9 mg daily, melatonin delayed the signs of AD and prevented cognitive and behavioral deterioration (95). Although no studies on mitochondrial function were performed in these studies, the improvement of mitochondrial function was one of the main mechanisms supposedly involved in the beneficial effects of melatonin.

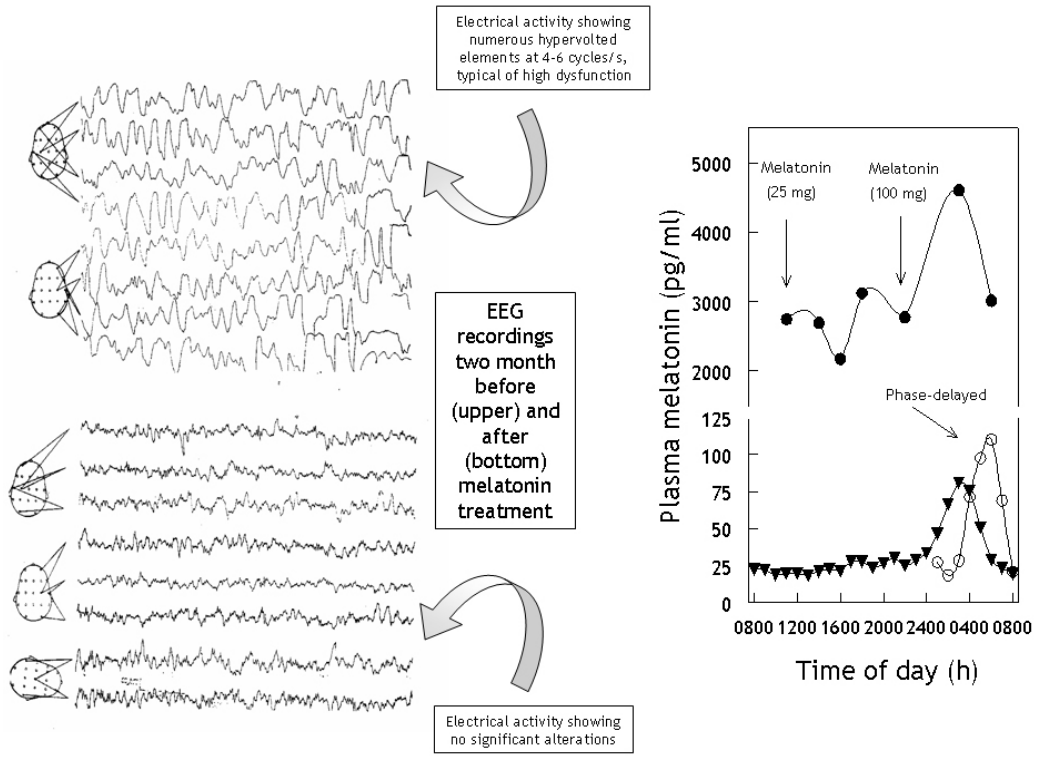

Figure 5. EEG recordings (left) and plasma levels of melatonin (right) before and after melatonin treastment in a 29-months-old patient with myoclonic epilepsy. Melatonin treatment induced a phase-advance of its rhythm, normlaizing the circadian rhythm of the hormone in blood. The pharmacological treatment with melatonin yielded a good control of the brain excitability, normalizing the EEG recordings.

Epilepsy may involve mitochondrial dysfunction through the excitotoxic pathway that may contribute to neuronal damage during seizures, as in the case of myoclonic epilepsy and generalized tonic-clonic seizures. Oxidative phosphorylation defects, reduced ATP production, ROS/RNS production, and altered Ca^{2+} handling may all contribute to neuronal damage and epileptogenesis (97). The acute neurodegenerative process that occurs during seizures dramatically induces neuronal death and microglia activation, reflecting the presence of an inflammatory reaction and NO production. Both, experimental and clinical anticonvulsant activities of melatonin were reported (97, 98). The anticonvulsant activity of melatonin was initially related to its effects on both brain GABA-benzodiazepine receptor complex and Na^+, K^+-ATPase (27-29). Reduced melatonin levels were related to increased brain damage after stroke or excitotoxic seizures in rats (177), whereas anticonvulsant activity of melatonin against seizures induced by a series of drugs in mice was reported (99, 100). However, due to the inhibitory effect of melatonin on the NO/NOS system, an effect of the indoleamine on glutamate-induced excitotoxicity was soon proposed. Electrophysiological experiments further showed the antagonism of melatonin against NMDA-induced excitotoxicity, an effect involving the inhibition of nNOS (101, 102). The effect of melatonin was specific, dose-dependent and was independent of melatonin receptors (101, 102). An intracellular action of melatonin in inhibiting the NMDA-dependent excitotoxic events was further reported with synthetic kynurenamines, supporting an inhibition of the NO/NOS system (103). In pentylenetetrazole-induced generalized convulsions, melatonin administration significantly increased the latency of the first seizure, and reduced the number and intensity of the seizures *in vivo*. The effects of melatonin were related to increased GABA and reduced glutamate activity, and to a reduction of brain NO levels in a dose-dependent manner (103). Some of synthetic kynurenines, structurally related to melatonin, were also effective to counteract pentylenetetrazole-induced seizures in vivo (100). The effects of melatonin against brain excitotoxicity were the basis for the clinical use of melatonin in infantile seizures. A 29-months old child having severe myoclonic epilepsy without response to conventional anticonvulsant was treated with melatonin (98). Severe neurological and psychomotor deterioration combined with increased seizure activity showed a lack of response to the treatment. Imaging studies including computerized tomography (CT), single-photon emission computed tomography (SPECT), and magnetic nuclear resonance (MNR), electroencephalography (EEG) recordings, blood biochemistry, and hematological analyses, including measures of the circadian rhythm of melatonin, were done. At the moment of the treatment, the patient was in a pre-comatose stage. After 1 month of melatonin (125 mg daily) plus phenobarbital therapy for a year, the child seizures were under control (Figure 5). All analyses, including EEG recordings and SPECT, were normal. As far as the results of neurological examination are concerned, only mild hypotony without focalization remained. Seizures returned

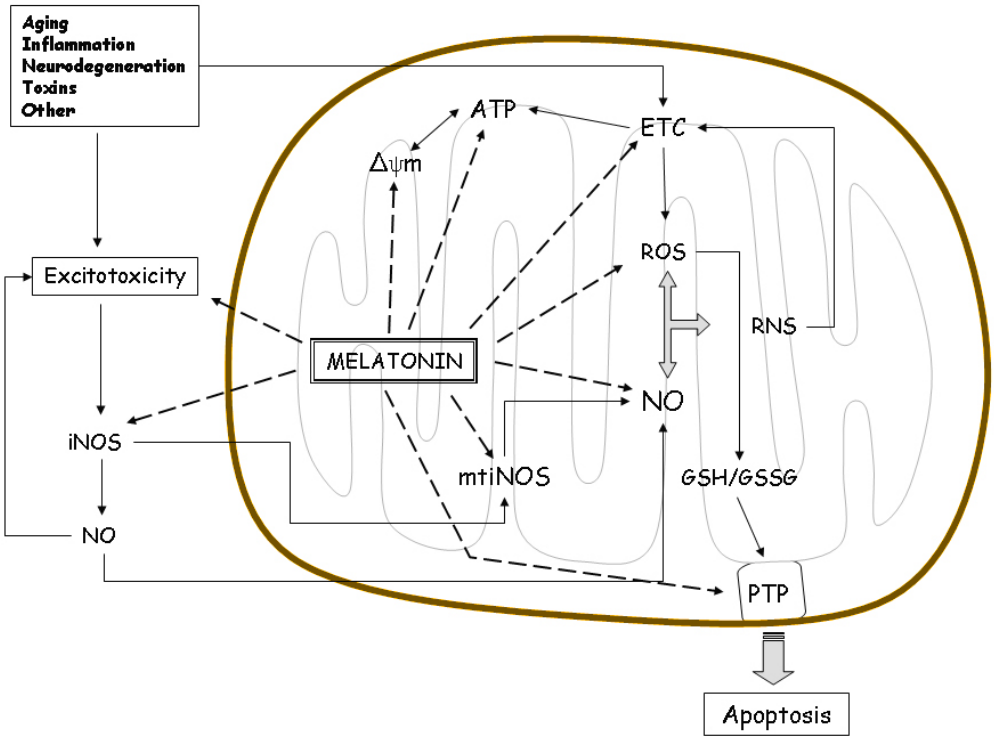

Figure 6. Effects of melatonin in the mitochondria. Melatonin displays a series of actions into the mitochondria that reflect its protective role. Melatonin counteracts excitotoxic events reducing nNOS and iNOS activities; increases electron transfer activity and AT production, leading to mitochondrial membrane potential increase and thus, ROS/RNS reduction . Besides, melatonin inhibits the activity of i-mtNOS decreasing RNS production. These actions of melatonin recover GSH pool and close PTP, preventing apoptosis.

when melatonin was removed from the treatment, but seizures resumed and the patient's condition was re-stabilized after restoring melatonin. During the second year of melatonin treatment the child progressively became satisfactorily controlled. The results suggest that neuroprotection by melatonin also includes excitotoxic events from epileptic seizures. Further studies with more patients and placebo-treatment would be beneficial in understanding the potential use of melatonin as a co-therapy in some cases of seizures (98).

6. PERSPECTIVE

From the data reviewed here it can be summarized that mitochondrial dysfunction is a common feature of most of the diseases related to ROS/RNS excess, independently of the cause of the disease. Pharmacological intervention with antioxidants and anti-inflammatory agents may ameliorate the severity of these diseases. But classical antioxidants, although may improve some signs of neurodegeneration, have limited efficacy by several causes. Antioxidants such as vitamin E are limited by the blood-brain barrier in terms of their ability to enter the central nervous system. Vitamin C may be toxic in some circumstances, specially when Fe^{2+} levels are increased, a frequent finding in neurodegenerative diseases. In any case, high doses of these vitamins would be necessary for therapeutic effects, and these doses have been reported as pro-oxidant and/or genotoxic (104). Mitochondrial studies have shown that these vitamins and other antioxidants such as N-acetylcysteine frequently lack significant effects in the recovery of mitochondria from ROS/RNS-induced damage (38). Experimental and clinical data have shown that melatonin has low toxicity (98, 105, 106) and the studies conducted under the guidelines of U.S. National Toxicity Program found little evidence of melatonin toxicity in rats treated throughout pregnancy with massive doses (10 to 200 mg/kg daily) (107). In addition to maternal health, prenatal survival, fetal body weight, and incidence of fetal malformations were recorded. None of these indices indicated that melatonin had any significant toxicity. Thus, melatonin is a secure pharmacological agent that may be safety used in human therapy. The activity of melatonin metabolites and synthetic related compounds against mitochondrial impairment is now being tested. These studies yield a considerable information regarding the pharmacophore of melatonin related to it interaction with nNOS (53, 54, 89). Thus, a promising future research in this field is the design of new drugs that, acting through the melatonin pharmacophore, may selectively antagonize the familyof nNOS, iNOS and i-mtNOS with even higher potency than melatonin. Such new compounds could have high therapeutic efficacy in mitochondrial diseases with reduced side effects resulting from their lack of receptor-mediated actions. Taking in mind that mitochondria are now considered a target for drug development, and that the drugs used for this purpose in neurological diseases should cross the blood-brain barrier and reach brain mitochondria (108), melatonin, its metabolites and synthetic analogs, may be the drugs of election (Figure 6).

7. ACKNOWLEDGMENTS

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8. REFERENCES

1. Reiter R J, D.-X. Tan, L. Manchester, & W. Qi: Biochemical reactivity of melatonin with reactive oxygen and nitrogen species. *Cell Biochem Biophys* 34, 237-256 (2001)

2. Tan D-X, R. J. Reiter, L. Manchester, M.-t. Yan, M. El Sawi, R. M. Sainz, J. C. Mayo, R. Kohen, M. Allegra & R. Hardeland: Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr Top Med Chem* 2, 133-151 (2002)

3. Acuña-Castroviejo D, M. Martín, M. Macías, G. Escames, J. León, H. Khaldy & R. J. Reiter: Melatonin, mitochondria and cellular bioenergetics. *J Pineal Res* 30, 65-74 (200)

4. Acuña-Castroviejo D, G. Escames, A. Carazo, J. León, H. Khaldy & R. J. Reiter: Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. *Curr Top Med Chem* 2, 133-151 (2002)

5. Acuña-Castroviejo D, G. Escames Rosa, J. León López, A. Carazo Gallego & H. Khaldy: Mitochondrial regulation by melatonin and its metabolites. *Adv Exp Med Biol* 527, 549-557 (2003)

6. León J, D. Acuña-Castroviejo, R. M. Sainz, J. C. Mayo, D. X. Tan & R. J. Reiter: Melatonin and mitochondrial function. *Life Sci* 75, 765-790 (2004)

7. León J, D. Acuña-Castroviejo, G. Escames, D. X. Tan & R. J. Reiter: Melatonin mitigates mitochondrial malfunction. *J Pineal Res* 38, 1-9 (2005)

8. Reiter R J, D.-X. Tan & M. A. Pappolla: Melatonin relieves the neural oxidative burden that contributes to dementia. *Ann NY Acad Sci USA* 1035, 179-196 (2004)

9. Reiter R J, D. Acuña-Castroviejo, D. X. Tan & S. Burkhardt: Free radical-mediated molecular damage: Mechanisms of melatonin's protective actions in the central nervous system. *Ann NY Acad Sci* 939, 200-215 (2001)

10. Andersen J K: Oxidative stress in neurodegeneration: cause or consequence? *Nat Med* Suppl S18-25 (2004)

11. Rego C A & C. R: Oliveira: Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: implications for the pathogenesis of neurodegenerative diseases. *Neurochem Int* 28, 1593-1574 (2003) Azul 1

12. Tatoyan A & C. Giulivi: Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. *J Biol Chem* 273, 11044-11048 (1998)

13. Escames G, J. León, M. Macías, H. Khaldy & D. Acuña-Castroviejo: Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. *FASEB J* 17, 8, 932-934 (2003)

14. Lerner A B, J. D. Case & R. V. Heinzelmann: Structure of melatonin. *J Am Chem Soc* 81, 6084 (1959)

15. Reiter R J: Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. *Endocr Rev* 12, 151-180 (1991)

16. Liu T & J. Borjigin: N-acetyltransferase is not the rate-limiting enzyme of melatonin synthesis at night. *J Pineal Res* 39, 91-96 (2005)

17. Lerner A B, J. D. Case, Y. Takahasi, T. H. Lee & W. Mori: Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 80, 587 (1958)

18. Kvetnoy L: Extrapineal melatonin in pathology: new perspectives for diagnosis, prognosis and treatment of illness. *Neuro Endocrinol Lett* 23, 92-96 (2002)

19. Ivanova T N & P. M. Iubvone: Melatonin synthesis in retina: circadian regulation of arylalkylamine N-acetyltransferase activity in cultured photoreceptor cells of embryonic chicken retina. *Brain Res* 973, 56-63 (2003)

20. Bubenik G A: Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci* 47, 2336-2348 (2002)

21. Slominski A, J Worstman & D. J. Tobin: The cutaneous serotoninergic/melatoninergic system: securing a place under the sun. *FASEB J* 19, 176-94 (2005)

22. Conti A, S. Conconi, E. Hertens, K. Skwarlo-Sonta, M. Markowska & J. M. Maestroni: Evidence for melatonin synthesis in mouse and human bone marrow cells. *J Pineal Res* 28, 193-202 (2000)

23. Carrillo-Vico A, P. J. Lardone, J. M. Fernández-Santos, I. Martín-Lacave, J. R. Calvo, M. Karasek & J. M. Guerrero: Human lymphocyte-synthesized melatonin is involved in the regulation of the interleukin-2/interleukin-2 receptor system. *J Clin Endocrinol Metab* 90, 992-1000 (2005)

24. Stefulj J, M. Hortner, M. Ghosh, K. Schauenstein, I. Rinner, A. Wolfler, J. Semmler & P. M. Liebmann: Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. *J Pineal Res* 30, 243-7 (2001)

25. Tan, D.-X, L. C. Manchester, R. J. Reiter, W. Qi, M. A. Hanes & N. J. Farley: High physiological levels of melatonin in the bile of mammals. *Life Sci* 65, 2523-2529 (1999)

26. Reiter R. J: The melatonin rhythm: Both a clock and a calendar. *Experientia* 49, 654-664 (1993)

27. Acuña-Castroviejo D, H. E. Romero & D. P. Cardinali: Changes in gamma-aminobutiryc acid hight affinity binding to cerebral cortex membranes after pinealectomy or melatonin administrations to rats. *Neuroendocrinology* 43, 24-31 (1986)

28. Acuña-Castroviejo D, P. R. Lowenstein, R. E. Rosenstein & D. P. Cardinali: Diurnal variations of benzodiapine binding in rat cerebral cortex: disruption by pinealectomy. *J Pineal Res* 3, 101-109 (1986)

29. Acuña-Castroviejo D, J. L. Castillo, B. Fernández, M. D. Gomar & C. M. Del Aguila: Modulation by pineal gland of ouabain high affinity binding sites in rat cerebral cortex. *Am J Physiol* 262, R698-R706 (1992)

30. Roush W: Can "resetting" hormonal rhythms treat illness? *Science* 269, 1220-1221 (1995)

31. Reiter R J, D.-X. Tan, B. Poeggeler, A. Menendez-Pelaez, L.-D. Chen & D. Saarela: Melatonin as a free radical scavenger: Implications for aging and age-related diseases. *Ann NY Acad Sci* USA 32, 1-12 (1993)

32. Lardone P J, O. Alvarez-García, A. Carrillo-Vico, I. Vega-Naredo, B. Caballero, J. M. Guerrero & A. Coto-Montes: Inverse correlation between endogenous melatonin levels and oxidative damage in some tissues of SAM P8 mice. *J Pineal Res* 40, 153-157 (2006)

33. García J J, R. J. Reiter, J. M. Guerrero, G. Escames, B. P. Yu, C. S. Oh & A. Muñoz-Hoyos: Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett* 408, 297-300 (1997)

34. Menéndez-Peláez A & R. J. Reiter: Distribution of melatonin in mammalian tissues: The relative importance of nuclear versus cytosolic localization. *J Pineal Res* 15, 59-69 (1993)

35. Escames G, J. M. Guerrero, R. J. Reiter, J. J. García, A. Muñoz, G. G. Ortiz & C. S. Oh: Melatonin and vitamin E prevent nitric oxide-induced lipid peroxidation in rat brain homogenates. *Neurosci Lett* 230, 147-150 (1997)

36. Barlow-Walden L R, R. J. Reiter, M. Abe, M. I. Pablos, A. Menéndez-Peláez, L. D. Chen, & B. Poeggeler: Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int 26, 497-502 (1995)

37. Khaldy H, G. Escames, J. León, F. Vives, J. Luna & D. Acuña-Castroviejo: Comparative effects of melatonin, L-deprenyl, Trolox and ascorbate in the suppression of hydroxyl radical formation during dopamine autoxidation in vitro. *J Pineal Res* 2, 100-107 (2000)

38. Martín M, M. Macías, G. Escames, J. León & D. Acuña-Castroviejo: Melatonin but not vitamins C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. *FASEB J* 14, 1677-1679 (2000)

39. Ressmeyer A R, J. C. Mayo, V. Zelosko, R. M. Sainz, D.-X. Tan, B. Poeggeler, I. Antolin, B. K. Zsizsik, R. J. Reiter & R. Hardeland: Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep* 8, 205-213 (2003)

40. Tan D X, L. C. Manchester, R. J. Reiter, B. F. Plummer, L. J. Hardies, S. T. Weintraub, Vijayalaxmi & A. M. M. Shepherd: A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. *Biochem Biophys Res Commun* 253, 614-620 (1998)

41. Tan D-X, L. C. Manchester, R. J. Reiter, W.-B. Qi, M. Karbownik & J. R. Calvo: Significance of melatonin in antioxidative defense system: reactions and products. *Biol Signals Recept* 9, 137-159 (2000)

42. Schenk H, M. Klein, W. Erdbrugger, W. Droge & K. Schulze-Oathoff: Distinct Effects of Thioredoxin and Antioxidants on the Activation of Transcription Factors NF-kB and AP-1. *Proc Natl Acad Sci* 91, 1672-1676 (1994)

43. Reiter R J, S. Burkhardt, J. Cabrera & J. J. García: Beneficial neurobiological effects of melatonin under conditions of increased oxidative stress. *Curr Med Chem* 2, 45-58 (2002)

44. Rodriguez C, J. C. Mayo, R. M. Sainz, I. Antolin, F. Herrera, V. Martin & R. J. Reiter: Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 36, 1-9 (2004)

45. Urata Y, S. Honma, S. Goto, S. Todoroki, T. Iida, S. Cho, K. Honma & T. Kondo: Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic Biol Med* 27, 838-847 (1999)

46. Albarrán M T, S. López-Burillo, M. I. Pablos, R. J. Reiter & M. T. Agapito: Endogenous rhythms of melatonin, total antioxidant status and superoxide dismutase activity in several tissues of chick and their inhibition by light. *J Pineal Res* 30, 227-33 (2001)

47. Kelly R W, F. Amato & R. F. Seamark: N-acetyl-5-methoxy kynurenamine, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin biosynthesis. *Biochem Biophys Res Commun* 121, 372-379 (1984)

48. Tan D-X, L. C. Manchester, S. Burkhardt, R. M. Sainz, J. C. Mayo, R. Kohen, E. Shohami, Y. S. Huo, R. Hardeland & R. J. Reiter: N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. *FASEB J* 15, 2294-2296 (2001)

49. Packer M A, C. M. Porteous & M. P. Murphy: Superoxide production by mitochondria in the presence of nitric oxide forms peroxynitrite. *Biochem Mol Biol Int* 40, 527−534 (1996)

50. Zhang H, G. L. Squadrito & W. A. Pryor: The reaction of melatonin with peroxynitrite: formation of melatonin radical cation and absence of stable nitrated products. *Biochem Biophys Res Commun* 251, 83-87 (1998)

51. Maharaj D S, H. Maharaj, E. M. Antunes, D. M. Maree, T. Nyokong, B. D. Glass & S. Daya: 6-Hydroxymelatonin protects against quinolinic-acid-induced oxidative neurotoxicity in the rat hippocampus. *J Pharm Pharmacol* 57, 877-81 (2005)

52. Crespo E, M. Macías, D. Pozo, G. Escames, M. Martín, F. Vives, J. M. Guerrero & D. Acuña-Castroviejo: Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. *FASEB J* 13, 1537-1546 (1999)

53. León J, M. Macías, G. Escames, E. Camacho, H. Khaldy, M. Martín, A. Espinosa, M. A. Gallo & D. Acuña-Castroviejo: Structure-related inhibition of calmodulin-dependent nNOS activity by melatonin and synthetic kynurenines. *Mol Pharmacol* 58, 967-975 (2000)

54. León J, G. Germaines, M. I. Rodríguez, L. C. López, V. Tapias, A. Entrena, E. Camacho, M. D. Carrión, M. A. Gallo, A. Espinosa, D.-X. Tan, R. J. Reiter & D. Acuña-Castroviejo: Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynurenamine, a brain metabolite of melatonin. Submitted (2006)

55. Skulachev V. P: Mitochondrial physiology and pathology: Concepts or programmed death of organelles, cells and organisms. *Mol Aspects Med* 20, 139-184 (1999)

56. Mitchell P: Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol Rev* 71, 445-502 (1966)

57. Brown G. C: Control of respiration and ATP synthesis in mammalian mitochondria and cells. *Biochem J* 284, 1-13 (1992)

58. Brown G. C & V. Borutaite: Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitosothiols. *Biochim Biophys Acta* 1658, 44-49 (2004)
59. Andrews Z. B, S. Diano & T. L. Horvath: Mitochondrial uncoupling proteins in the CNS: in support of function and survival. *Nat Rev Neurosci* 6, 829-840 (2005)
60. Fernández-Checa J. C & N. Kaplowitz: Hepatic mitochondrial glutathione: transport and role in disease and toxicity. *Toxicol Appl Pharmacol* 204, 263-273 (2005)
61. Cadenas E, J. J. Poderoso, F. Antunes, A. Boveris, I. Lizasoain, M. A. Moro, R. G. Knowles, V. Darley-Usmar & S. Moncada: Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem J* 314, 877-880 (1996)
62. Brookes P. S: Mitochondrial nitric oxide synthase. *Mitochondrion* 3:187-204, 2004.
63. Ghafourifar P & E. Cadenas: Mitochondrial nitric oxide synthase. *Trends Pharmacol Sci* 26, 190-195 (2005)
64. López L. C, G. Escames, V. Tapias, M. P. Utrilla, J. León & D. Acuña-Castroviejo: Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice. Its relation with mitochondrial dysfunction and revention by melatonin. *Int J Biochem Cell Biol* 38, 267-278 (2005)
65. Escames G, L. C. López, V. Tapias, M. P. Utrilla, R. J. Reiter, A. B. Hitos, J. León, M. I. Rodríguez & D. Acuña-Castroviejo: Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J Pineal Res* 40, 71-78 (2006)
66. Pearce L. L, A. J. Kanai, L. A. Birder, B. R. Pitt & J. Peterson: The catabolic fate of nitric oxide. *J Biol Chem* 277, 13556-13562 (2002)
67. DiMauro S: Mitochondrial diseases. *Biochim Biophys Acta* 1658, 80-88 (2004)
68. Reiter R J: Oxidative damage in the central nervous system: Protection by melatonin. *Prog Neurobiol* 56, 359-3841 (1998) 69. Martín M, M. Macías, G. Escames, R. J. Reiter, M. T. Agapito, G. G. Ortiz & D. Acuña-Castroviejo: Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J Pineal Res 28, 242-248 (2000)
70. Martín M, M. Macías, J. León, G. Escames, H. Khaldy & D. Acuña-Castroviejo: Melatonin increases the activity of the complexes I and IV of the electron transport chain and the ATP production in rat brain and liver mitochondria. *Int J Biochem Cell Biol* 34, 348-357 (2002)
71. Reyes-Toso C F, C. R. Ricci, I. R. de Mignone, P. Reyes, L. M. Linares, L. E. Albornoz, D. P. Cardinali & A. Zaninovich: In vitro effect of melatonin on oxygen consumption in liver mitochondria of rats. *Neuro Endocrinol Lett* 24, 341-344 (2003)
72. Acuña-Castroviejo D, G. Escames, L. C. López, A. B. Hitos & J. León: Melatonin and nitric oxide: Two required antagonists for mitochondrial homeostasis. *Endocrine* 27, 159-168 (2005)
73. Prunet-Marcassus B, L. Ambid, N. Viguerie-Bascands, L. Pénicaud & L. Casteilla: Evidence for a direct effect of melatonin on mitochondrial genome expression of Siberian hamster brown adipocytes. *J Pineal Res* 30, 108-115 (2001)
74. Calandra T & J. Cohen: The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Crit Care Med* 33, 1538-1548 (2005)
75. Peters K, R. E. Unger, J. Brunner & J. Kirkpatrick: Molecular basis of endothelial dysfunction in sepsis. *Cardiovasc Res* 60, 49-57 (2003)
76. Escames G, D. Acuña-Castroviejo, L. C. López, D.-X. Tan, M. D. Maldonado, M. Sánchez-Hidalgo, J. León & R. J. Reiter: The pharmacological utility of melatonin in the treatment of septic shock: experimental and clinical evidence. *J Pharm Pharmacol* 2006, in press
77. Boveris A, S. Alvarez & A. Navarro: The role of mitochondrial nitric oxide synthase in inflammation and septic shock. *Free Radic Biol Med* 33, 1186-93 (2002)
78. Tay S. Y, T. E. OìBrien & J. A. McNulty: Microglia play a role in mediating the effects of cytokines on the structure and function of the rat pineal gland. *Cell Tissue Res* 303, 423-431 (2001)
79. Jiang-Shieh Y. F, C. H. Wu, H. F. Chien, I. H. Wei, M. L. Chang, J. Y. Shieh & C. Y. Wen: Reactive changes of interstitial glia and pinealocytes in the rat pineal gland challenged with cell wall components from gram-positive and−negative bacteria. *J Pineal Res* 38, 17-26 (2005)
80. Gitto E, M. Karbownik, R. J. Reiter, D.-X. Tan, S. Cuzzocrea, P. Chiurazzi, S. P. Cordaro, G. Corona, G. Trimarchi & I. Barberi: Effects of melatonin treatment in septic newborns. *Pediatr Res* 50, 756-760 (2001)
81. Gitto E, R. J. Reiter, S. P. Cordaro, M. La Rosa, P. Chiurazzi, G. Trimarchi, P. Gitto, M. P. Calabro & I. Barberi: Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin. *Am J Perinatol* 21, 209-216 (2004)
82. Reiter R. J, D.-X. Tan & M. A. Pappolla: Melatonin relieves the neural oxidative burden that contributes to dementia. *Ann NY Acad Sci USA* 1035, 179-196 (2004)
83. Nicholls D. G: Mitochondrial dysfunction and glutamate excitotoxicity studied in primary neuronal cultures. *Curr Mol Med* 4, 149-177 (2004)
85. Khaldy H, J. León, G. Escames, M. Martín, M. Macías & D. Acuña-Castroviejo: Circadian rhythm of dopamine and their metabolites in mouse striatum: Effects of pinealectomy and melatonin replacement. *Neuroendocrinology* 75, 201-208 (2002)
86. Acuña-Castroviejo D, A. Coto-Montes, M. Gaia Monti, G. G. Ortiz & R. J. Reiter: Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life Sci* 60, 23-29 (1997)
87. Khaldy H, J. León, G. Escames, L. Bikjdaouene & D. Acuña-Castroviejo: Synergistic effects of melatonin and deprenyl protect against MPTP-induced mitochondrial damage and DA depletion. *Neurobiol Aging* 24, 491-500 (2003)
96. Reiter R J, J. Cabrera, R. M. Sainz, J. C. Mayo, L. Manchester & D.-X. Tan: Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alhzeimer's disease annd Parkinsonism. *Ann NY Acad Sci USA* 890, 471-485 (1999)
84. Schapira A H V: Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic

paraplegia and Friedreich's ataxia. *Biochim Biophys Acta* 1410, 159-170 (1999)

88. Liberatore G. T, V. Jackson-Lewis, S. Vukosavic, A. S. Mandir, M. Vila, W. G. McAuliffe, V. L. Dawson, T. M. Dawson & S. Przedborski: Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. *Nat Med* 5, 1403-1409 (1999)

89. Entrena A, M. E. Camacho, M. D. Carrión, L. C. López-Cara, G. Velasco, J. León, G. Escames, D. Acuña-Castroviejo, V. Tapias, M. A. Gallo, A. Vivó & A. Espinosa: Kynurenamines as Neural Nitric Oxide Synthase Inhibitors. *J Med Chem* 48, 8174-8181 (2005)

90. Bonilla E, K. Tanji, M. Hirano, T. H. Vu, S. DiMauro & E. A. Schon: Mitochondrial involvement in Alzheimer's disease. *Biochim Biophys Acta* 1410, 171-182 (1999)

91. Lahiri D K, D. Chen, Y. W. Ge, S. C. Bondy & E. H. Sharman: Dietary supplementation with melatonin reduces levels of amyloid beta-peptides in the murine cerebral cortex. *J Pineal Res* 36, 224-31 (2004)

92. Pappolla M, P. Bozner, C. Soto, H. Shao, N. K. Robakis, M. Zagorski, B. Frangione & J. Ghiso: Inhibition of Alzheimer beta-fibrillogenesis by melatonin. *J Biol Chem* 273, 7185-7188 (1998)

93. Reiter R J, J. Cabrera, R. M. Sainz, J. C. Mayo, L. C. Manchester & D.-X. Tan: Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism. *Ann NY Acad Sci* 890, 471-485 (1999)

94. Wu Y H & D. F. Swaab: The human pineal gland and melatonin in aging and Alzheimer's disease. *J Pineal Res* 38, 145-152 (2005)

95. Brusco L I, M. Marquez & D: P. Cardinali: Monozygotic twins with Alzheimer's disease treated with melatonin: Case report. *J Pineal Res* 25, 260-263 (1998)

97. Lapin I P, S. M. Mirzaev, I. V. Ryzov & G. F. Oxenkrug: Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and pentylenetetrazole in mice. *J Pineal Res* 24, 215-218 (1998)

98. Molina-Carballo A, A. Muñoz-Hoyos, R. J. Reiter, M. Sanchez-Forte, F. Moreno-Madrid, M. Rufo-Campos, J. A. Molina-Font & D. Acuña-Castroviejo: Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic experience: Two years' experience. *J Pineal Res* 23, 97-105 (1997)

99. Bikjdaouene L, G. Escames, J. León, J. M. R. Ferrer, H. Khaldy, F. Vives & D. Acuña-Castroviejo: Changes in brain amino acids and nitric oxide after melatonin administration to pentylenetetrazole-induced seizures in rats. *J Pineal Res* 35, 54-60 (2003)

100. Bikjdaouene L, G. Escames, E. Camacho, J. León, J. M. R. Ferrer, A. Espinosa, M. A. Gallo, J. Luna & D. Acuña-Castroviejo: Effects of some synthetic kynurenines on brain amino acids and nitric oxide after pentylenetetrazole administration to rats. *J Pineal Res* 36, 267-277 (2004)

101. Escames G, M. Macías, J. León, J. J. García, H. Khaldy, M. Martín, F. Vives & D. Acuña-Castroviejo: Calcium-dependent effects of melatonin inhibition of glutamatergic response in rat striatum. *J Neuroendocrinol* 13,:459-466 (2001)

102. Escames G, J. León, L. C. López & D. Acuña-Castroviejo: Mechanisms of the NMDA receptor inhibition by melatonin in the rat brain striatum. *J Neuroendocrinol* 16, 929-935 (2004)

103. León J, F. Vives, E. Crespo, E. Camacho, A. Espinosa, A. Gallardo, G. Escames & D. Acuña-Castroviejo: Modification of nitric oxide synthase activity and neuronal response in rat estriatum by melatonin and kynurenine derivatives. *J Neuroendocrinol* 10, 297-302 (1998)

104. Lee S. H, O. T & I. A. Blair: Vitamin C-induced decomposition of lipid hydroperoxyides to endogenous genotoxins. Science 292, 2083-2086 (2001)

105. Jan J. E, D. Hamilton, N. Seward, D. K. Fast, R. D. Freeman & M. Laudon: Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. *J Pineal Res* 29, 34-39 (2000)

106. Seabra M. L. V, M. Bignotto, L. R. Pinto & S. Tufik: Randomized, double-blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. *J Pineal Res* 29, 193-200 (2000)

107. Jahnke G, M. Marr, C. Myers, R. Wilson, G. Travlos & C. Price: Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. *Toxicol Sci* 50, 271-9 (1999)

108. Gilgun-Sherki Y, E. Melamed & and D. Offen: Oxidative stress induced-neurodegenerative diseases: The need for antioxidants that penetrate the blodd brain barrier. *Neuropharmacology* 40, 959-975 (2001)

Abbreviations: AD, Alzhemier's disease; AFMK, N1-acetyl-N2-formyl-5-methoxykynuramine; AMK, N1-acetyl-5-methoxykynuramine; CaCaM, calcium-calmodulin complex; CAT, catalase, CLP, cecal ligation and puncture; CSF, cephalospinal fluid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid ; GABA, gamma-aminobutyric acid; GPx, glutathione peroxidase; GRd, glutathione reductase; GSH, glutathione; GSSG, disulfide glutathione; H_2O_2, hydrogen peroxide; HD, Huntington's disease; HIOMT, hidroxyindole-O-methyltransferase; $HO^{\cdot}$, hydroxyl radical; iNOS, nitric oxide synthase II, inducible; LPS, lipopolysaccharide; MODS, multiple organ dysfunction syndrome; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mtNOS, mitochondrial nitric oxide synthase; NAC, N-acetylcysteine; NAT, N-acetyltransferase; NFkB, nuclear factor kappa beta; NK, natural killer; NMDA, N-methyl-D-aspartate; nNOS, nitric oxide synthase I, constitutive; NO, nitric oxide; O_2^{-}, superoxide anion; $ONOO^{-}$, peroxynitrite; PD, Parkinson's disease; PTP, permeability transition pore; QA, quinolinic acid; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TNFalpha, tumor necrosis factor alpha; UCPs, uncoupling proteins

Key Words: Melatonin, Melatonin Metabolites Mitochondria, Antioxidant, Oxidative Stress, Reactive Oxygen Species, Reactive Nitrogen Species, Excitotoxicity, Mitochondrial Dysfunction, Mitochondrial Diseases, Neurodegeneration, Review

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