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Minireview

Melatonin and mitochondrial function

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Abstract

Melatonin is a natural occurring compound with well-known antioxidant properties. In the last decade a new effect of melatonin on mitochondrial homeostasis has been discovered and, although the exact molecular mechanism for this effect remains unknown, it may explain, at least in part, the protective properties found for the indoleamine in degenerative conditions such as aging as well as Parkinson's disease, Alzheimer's disease, epilepsy, sepsis and other injuries such as ischemia-reperfusion. A common feature in these diseases is the existence of mitochondrial damage due to oxidative stress, which may lead to a decrease in the activities of mitochondrial complexes and ATP production, and, as a consequence, a further increase in free radical generation. A vicious cycle thus results under these conditions of oxidative stress with the final consequence being cell death by necrosis or apoptosis. Melatonin is able of directly scavenging a variety of toxic oxygen and nitrogen-based reactants, stimulates antioxidative enzymes, increases the efficiency of the electron transport chain thereby limiting electron leakage and free radical generation, and promotes ATP synthesis. Via these actions, melatonin preserves the integrity of the mitochondria and helps to maintain cell functions and survival.

Keywords: Melatonin; Mitochondria; Reactive oxygen species; Reactive nitrogen species; Parkinson's disease; Alzheimer's disease; Aging; Sepsis; Ischemia-reperfusion

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Introduction

Mitochondria play a central role in energy-generating processes within the cell through the electron transport chain (ETC), the primary function of which is ATP synthesis via oxidative phosphorylation (OXPHOS). The ETC, located in the inner mitochondrial membrane, comprises a series of electron carriers grouped into four enzyme complexes: complex I (NADH ubiquinone reductase); complex II (succinate ubiquinone reductase); complex III (ubiquinol cytochrome c reductase); and complex IV (cytochrome c oxidase). According to the chemiosmotic hypothesis, the ETC converts redox energy into an electrochemical gradient of protons (termed proton-motive force, Δp, when expressed in voltage units) which subsequently drives ATP formation from ADP and phosphate by ATP synthase (Mitchell and Moyle, 1967). The proton-motive force comprises an electrical component, the membrane potential ($\Delta\Psi_m$), and a transmembrane pH gradient (ΔpH). $\Delta\Psi_m$ (which normally accounts for about 80% of Δp)

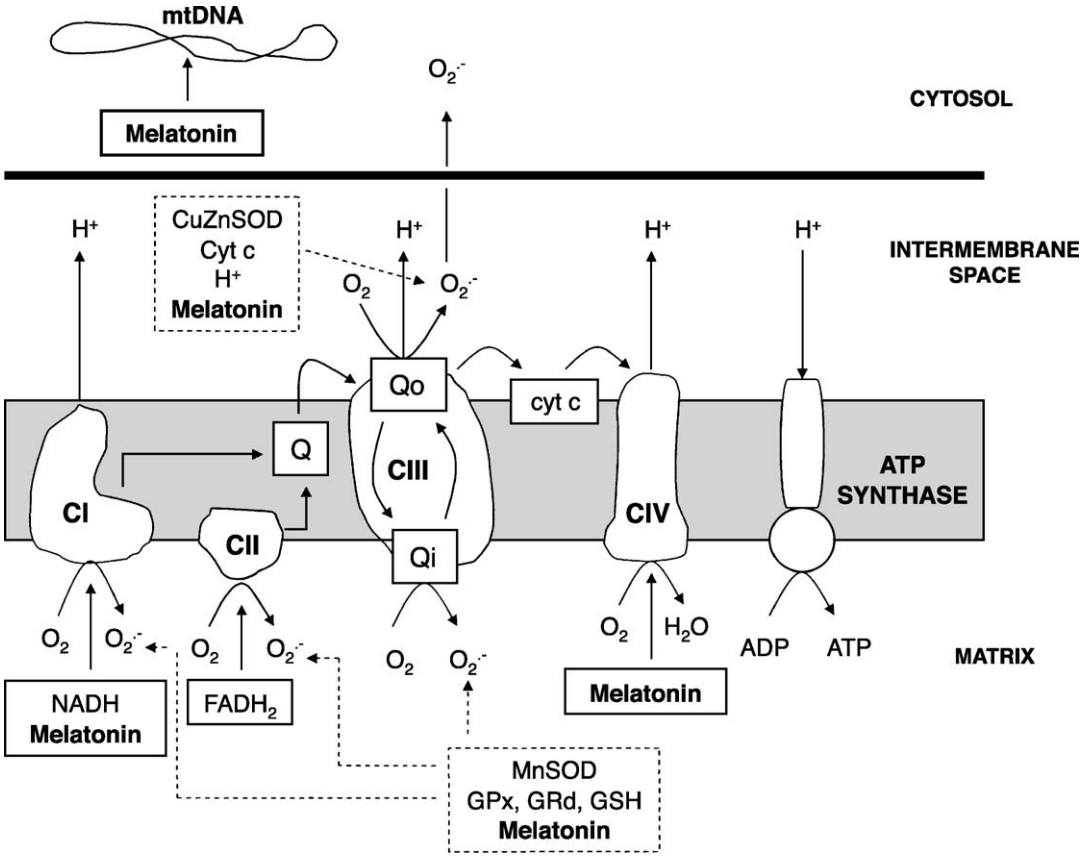

Fig. 1. Components of the ETC. In cells under aerobic conditions, OXPHOS is responsible for production of 90–95% pf the total amount of ATP, the remainder being synthesized by glycolytic phosphorylation. The synthesis of ATP via the respiratory chain is the result of two coupled process: electron transport and OXPHOS. CI, CIII and CIV function as proton pumps acting in series with respect to the electron flux and in parallel with respect to the proton circuit. The antioxidant defense system controls ROS production during normal metabolism. Melatonin acts as antioxidant but also can interact with the complexes.

provides the driving force for the accumulation of calcium in the mitochondrial matrix (Nicholls and Budd, 2000) (Fig. 1).

The end product of the respiratory chain is water that is generated in a four-electron reduction of molecular oxygen (O_2) by complex IV. However, a small proportion of O_2 is involved in generation of reactive oxygen species (ROS), in particular, superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the extremely reactive hydroxyl radical (•OH) (Lee et al., 2001; Lenaz, 2001). Mitochondria can also produce nitric oxide (NO•) from mitochondrial nitric oxide synthase (mtNOS) (Ghafourifar and Richter, 1997; Giulivi et al., 1998). Depending on the environment, NO• can be converted to various reactive nitrogen species (RNS) such as nitrosonium cation (NO^+), nitroxyl anion (NO^-) or peroxynitrite ($ONOO^-$) (Stamler et al., 1992).

Normally, free radicals are decomposed or their peroxidation products are neutralized by the natural antioxidative defense system (Chance et al., 1979; Halliwell and Gutteridge, 1989; Fridovich, 1995; Ursini et al., 1999; Genova et al., 2003). While small fluctuations in the steady state concentration of ROS/RNS may play a role in intracellular signaling (Dröge, 2002), uncontrolled increases in these metabolites lead to free radical-mediated chain reactions which indiscriminately target proteins (Stadtman and Levine, 2000), lipids (Rubbo et al., 1994) and DNA (Richte, 1988; LeDoux et al., 1999). Mitochondria, being a primary site of ROS/RNS generation in the cell, are also a main target (Raha and Robinson, 2000). This in turn results in damage to the mitochondrial respiratory chain and, as a consequence, a further increase in free radical generation. A vicious cycle thus results (Lenaz, 2001) and, under these conditions of oxidative stress, the final consequence is cell death via necrosis or apoptosis (Kim et al., 2003).

In the recent years, several findings support the antioxidant effect as well as a direct role of melatonin in mitochondrial homeostasis (Martin et al., 2000a,b, 2002); this latter action of melatonin may contribute to melatonin's protective effects in degenerative disorders such as Parkinson's disease, Alzheimer disease, epilepsy, aging, ischemia-reperfusion and sepsis, all of which involve mitochondrial dysfunction as a primary or secondary cause of the disease (Acuña-Castroviejo et al., 2001, 2002; Reiter et al., 2002).

Mitochondrial production of free radicals

During normal metabolism, the ETC is the main source of ROS within the cell (Lenaz, 2001). Two principal sites of $O_2^{\bullet -}$ generation have been identified in mitochondria: complex I (Barja and Herrero, 1998) and complex III (Turrens et al., 1982), although complex II may also contribute to ROS production (Lenaz, 2001). The contribution of each of these sites to the $O_2^{\bullet -}$ production depends both on the organ and on whether mitochondria are actively respiring (State 3) or whether the respiratory chain is highly reduced (State 4) (Barja, 1999). Complex III appears to be responsible for the majority of $O_2^{\bullet -}$ produced in heart and lung mitochondria (Turrens and Boveris, 1980; Turrens et al., 1982) while $O_2^{\bullet -}$ formation by complex I appears to be the primary source of free radicals in the brain under normal conditions (Barja and Herrero, 1998). $O_2^{\bullet -}$ is not produced during reduction of dioxygen by cytochrome c oxidase, because of the almost simultaneous transfer of four electrons to O_2 (Ludwig et al., 2001).

In complex I, the primary source of $O_2^{\bullet -}$ appears to be one of the iron-sulfur clusters, either N2 or N1a (Genova et al., 2001; Kushnareva et al., 2002). Oxidant production from complex I is directed into the mitochondrial matrix, but not into the intermembrane space (Han et al., 2003b), and they are inactivated by matrix antioxidant enzyme systems (Chen et al., 2003).

In complex III, most of the $O_2^{\bullet -}$ appears to be formed as a result of the autoxidation of ubisemiquinone, an intermediate produced in complex III during the Q-cycle (Trumpower, 1990). This complex has two centers: the Q_o center, oriented toward the intermembrane space, and the Q_i center, located in the inner membrane and facing the mitochondrial matrix. $O_2^{\bullet -}$ generated at the Q_o center is released into the intermembrane space (Han et al., 2001), a portion of which diffuses into the cytoplasm through voltage dependent anion channels (Han et al., 2003a), whereas $O_2^{\bullet -}$ generated at the Q_i center is likely to enter the matrix (Chen et al., 2003). ROS from the complex III Q_o center are basically unaffected by the antioxidant defense system whereas ROS from complex III Q_i center are rapidly inactivated by the antioxidant system of the mitochondrial matrix (Chen et al., 2003; Han et al., 2003b).

The free radical NO• may be also produced by mitochondria via the activity of mitochondrial nitric oxide synthase (mtNOS) (Ghafourifar and Richter, 1997; Giulivi, 1998; Giulivi et al., 1998). MtNOS, localized in the inner mitochondrial membrane, requires Ca^{2+} and, in a stereo-selective manner, uses L-arginine to produce NO• and L-citrulline (Ghafourifar and Richter, 1997), although other studies describe an enzyme that is similar to iNOS (Giulivi et al., 1998). Purified mtNOS in the absence of L-arginine and supplemented with NADPH leads to the production of $O_2^{\bullet -}$ (Giulivi et al., 1999). Although, under basal conditions the contribution of mtNOS to the total rate of toxic reactant generation from mitochondria could be considered negligible (Sarkela et al., 2001), under certain pathological situations it may be important due to the generation of the toxic $ONOO^-$ (Ghafourifar et al., 1999). The oxidizing reactivity of $ONOO^-$ is generally considered equivalent to that of •OH (Pryor and Squadrito, 1995). On the other hand, mitochondrial production of ROS is also modulated by endogenous NO•. At low levels NO· can increase $O_2^{\bullet -}$ and H_2O_2 production by modulating the rate of O_2 consumption at the cytochrome c oxidase level (Sarkela et al., 2001), whereas at higher levels NO• inhibits H_2O_2 production by coupling with $O_2^{\bullet -}$ resulting in $ONOO^-$ formation (Cleeter et al., 1994).

Free radicals and reactive non-radical species derived from radicals are continually generated in cells and tissues at low but measurable concentrations (Halliwell and Gutteridge, 1989). Higher organisms have evolved the use of NO• and ROS also as signaling molecules for physiological functions (Dröge, 2002). In these cases, temporary exposure to increased ROS or RNS concentrations is necessary for normal cellular physiology (Dröge, 2002). For example, ROS mainly produced by mitochondria have been implicated in the cell death transduction pathways (Banki et al., 1999). Under basal conditions, NO• produced by mtNOS modulates O_2 consumption, ATP production and free radical generation by mitochondria by the reversible inhibition of cytochrome c oxidase (Giulivi, 2003).

Several mechanisms control ROS/RNS production by mitochondria. One of them is the antioxidant defense system that neutralizes ROS/RNS or their oxidation products (Halliwell and Gutteridge, 1989). Enzymatically, $O_2^{\bullet -}$ is converted to H_2O_2 by superoxide dismutase (SOD), although this process can also occur spontaneously (Fridovich, 1995). The mitochondrial matrix contains a specific form of SOD, with manganese in the active site (MnSOD), which removes the $O_2^{\bullet -}$ formed in the matrix or on the inner side of the inner membrane (Fridovich, 1995). The concentration of $O_2^{\bullet -}$ in the intermembraneous space is controlled by three different mechanisms. First, this compartment contains a different SOD isozyme which contains copper and zinc instead of manganese (Cu ZnSOD); this enzyme is also found in the cytoplasm of eukaryotic cells (Okado-Matsumoto and Fridovich, 2001). Second, the intermembrane space contains cytochrome c which can be reduced by $O_2^{\bullet -}$ regenerating O_2 in the process (Butler et al., 1975). Finally, the spontaneous dismutation of $O_2^{\bullet -}$ in the intermembrane space is facilitated by the lower pH in this compartment, resulting from the extrusion of H^+ coupled to respiration (Guidot et al., 1995).

H_2O_2, the main precursor of •OH, in the presence of reduced transition metals (Halliwell and Gutteridge, 1989) is mostly decomposed by the enzyme glutathione peroxidase (GPx) (Chance et al., 1979). This process metabolizes reduced glutathione (GSH) to its disulfide (GSSG), which in turn is reduced to GSH by matrix glutathione reductase (GRd). A second GPx associated with the mitochondrial membrane, known as phospholipid-hydroperoxide glutathione peroxidase, is specifically involved in reducing lipid peroxides associated with the membrane (Ursini et al., 1999). Mitochondria do not synthesize GSH but rather take it up from cytosol through a multicomponent transport system (McKernan et al., 1991). In addition, mitochondria do not contain catalase, a major H_2O_2 detoxifying enzyme found in peroxisomes. Because of this, mitochondria are largely dependent on reduced glutathione (GSH) and its recycling enzymes for its antioxidant protection (Phung et al., 1994).

Coenzyme Q is a source of $O_2^{\bullet -}$ when partially reduced (semiquinone form) and an antioxidant when fully reduced (Genova et al., 2003). The inner mitochondrial membrane also contains vitamin E, a powerful antioxidant that interferes with the propagation of free radical-mediated chain reactions (Ham and Liebler, 1995).

An effective way of diminishing mitochondrial production of ROS is the control of the $\Delta\Psi_m$ (Kadenbach, 2003). ROS formation in mitochondria occurs at high $\Delta\Psi_m$ and increases exponentially above 140 mV, but is absent below this value (Korshunov et al., 1997). The parameters controlling the $\Delta\Psi_m$ in vivo, however, are complex and not fully understood. The inhibition of respiration at high $\Delta\Psi_m$ values can occur via the stimulation of respiration by the uptake of ADP, followed by its decrease after conversion of ADP into ATP (Nicholls and Ferguson, 1992). Also, classical uncouplers of OXPHOS, fatty acids, an unspecific "proton leak" of the inner mitochondrial membrane, the uncoupling proteins (UCPs) and any active transport of cations or anions across the membrane via carrier are able to decrease $\Delta\Psi_m$ (Kadenbach, 2003). In contrast to the above mechanisms (extrinsic uncoupling), the inhibition of respiration can also occur by high ATP/ADP ratios via allosteric ATP inhibition of cytochrome c oxidase (Kadenbach and Arnold, 1999). In this case (intrinsic uncoupling), a decrease of the efficiency of the proton pumps (i.e., decrease of the H^+/e^- stoichiometry or slip) would also result in a reduced of $\Delta\Psi_m$ (Papa et al., 1997).

Several control mechanisms are present in mitochondria for modulation of mtNOS activity. These include the dependence of the availability of L-arginine, NADPH, end-product inhibition and calcium levels (Giulivi, 2003). High concentrations of L-arginine inhibit the production of NO• by intact mitochondria. The reversal of this inhibition by the addition of oxymyoglobin shows NO• to be responsible for enzyme activity inhibition (Sarkela et al., 2001). Mitochondria maintained in either State 3 or 4 exhibit high levels of NADPH and have significant rates of NO• production. Conversely, mitochondria in States 3 or 2, with low levels or no NADPH, have a negligible production of NO• (Giulivi, 2003).

Mitochondria respiring complex II substrate generate NO• without an additional supplement of calcium, indicating that the concentration of this cation in mitochondrial preparations seem to be sufficient to sustain mtNOS activity (Ghafourifar and Richter, 1997). Uptake of calcium by respiring mitochondria may lead to increased $ONOO^-$ formation, which in turn causes calcium release via the pyridine nucleotide-dependent pathway followed by mtNOS deactivation. Therefore, stimulation of mtNOS by additional calcium is considered as part of the mechanism that prevents calcium overloading and allows its release, thereby preserving $\Delta\Psi_m$ (Ghafourifar and Richter, 1997).

Mitochondria and cell death

In the mitochondrial-mediated cell death pathway, a non-specific increase in the permeability of the inner mitochondrial membrane can occur, when mitochondrial matrix calcium is greatly increased (Szalai et al., 1999). This phenomenon, known as the mitochondrial permeability transition (MPT), is associated with opening of a non-specific "megachannel" in the mitochondrial inner membrane, which transports any molecule of <1500 Daltons. Under these conditions, mitochondria become uncoupled and hydrolyze ATP rather than synthesize it. Not only does its opening prevent ATP synthesis, it also causes the loss of ions and metabolites from the mitochondrial matrix and induces extensive swelling of the mitochondria as a result of the colloidal osmotic pressure exerted by the matrix proteins. If the MPT remains open, ATP levels can be totally depleted leading to cell necrosis. On the contrary, transient opening of MPT may be involved in the intrinsic pathway or mitochondrial mediated-apoptosis (Halestrap et al., 2000; Kim et al., 2003). In this process, cytochrome c moves from the intermembrane space into the cytoplasm (Bossy-Wetzel et al., 1998) where it joins another factor (Apaf-1). In the presence of dATP this complex polymerizes into an oligomer known as the apoptosome. The apoptosome activates a protease (caspase-9), which in turn activates caspase-3. The cascade of proteolytic reactions also activates DNAses with the process resulting in cell death (Zamzami and Kroemer, 2001).

Several factors are known to greatly enhance the sensitivity of the pore to Ca^{2+}, of which the most potent and relevant to the cellular setting are oxidative stress, adenine nucleotide depletion, increased inorganic phosphate concentrations and loss of $\Delta\Psi_m$ (Chernyak, 1997; Kim et al., 2003).

The ability of oxidative stress to produce necrotic cell death as a result of massive cellular damage associated to lipid peroxidation and alterations of proteins and nucleic acids has been well documented (Halliwell and Gutteridge, 1989). However, the possible implication of ROS as signaling molecules in apoptosis is a more recent concept (Schulze-Osthoff et al., 1993). Indeed, several observations suggest that ROS might mediate apoptosis. First, the addition of ROS or the depletion of endogenous antioxidants can promote apoptosis (Sato et al., 1995). Second, apoptosis can be delayed or inhibited by antioxidants (Mayo et al., 1998). Third, some data raise the possibility that ROS are also required for the execution of the death program (Kroemer et al., 1995). An increased mitochondrial formation of ROS triggers the mitochondrial pathway of apoptosis by opening of transition pores due to the oxidation of intracellular glutathione and other critical sulfhydryl groups present in the channel (Fig. 2).

NO• and RNS kill cells by two main ways: (i) energy depletion-induced necrosis, and (ii) oxidant-induced apoptosis. In addition, in neurons, NO• induces excitotoxic cell death (Brown and Borutaite, 2002).

Cells exposed to NO• (or NO•-producing cells) for several hours show an irreversible inhibition of mitochondrial respiration, probably due to conversion of NO• to RNS (Cassina and Radi, 1996). $ONOO^-$ can inhibit complex I, complex II, complex IV, ATP synthase, aconitase, MnSOD, creatine kinase, and probably many other proteins (Murphy, 1999). This nitrogen-based reactant is a strong oxidant and can also cause DNA damage, induce lipid peroxidation, and increase mitochondrial proton permeability (Klotz and Sies, 2003). If DNA damage is extensive, both cytosolic NAD^+ (causing inhibition of glycolysis) and adenine nucleotides are depleted due the activation of the PARP. But activation of this nuclear protein may contribute to energy depletion of the cell, possibly in synergy with inhibition of respiration, inhibition of glycolysis, and/or activation of MPT (Brown and Borutaite, 2002).

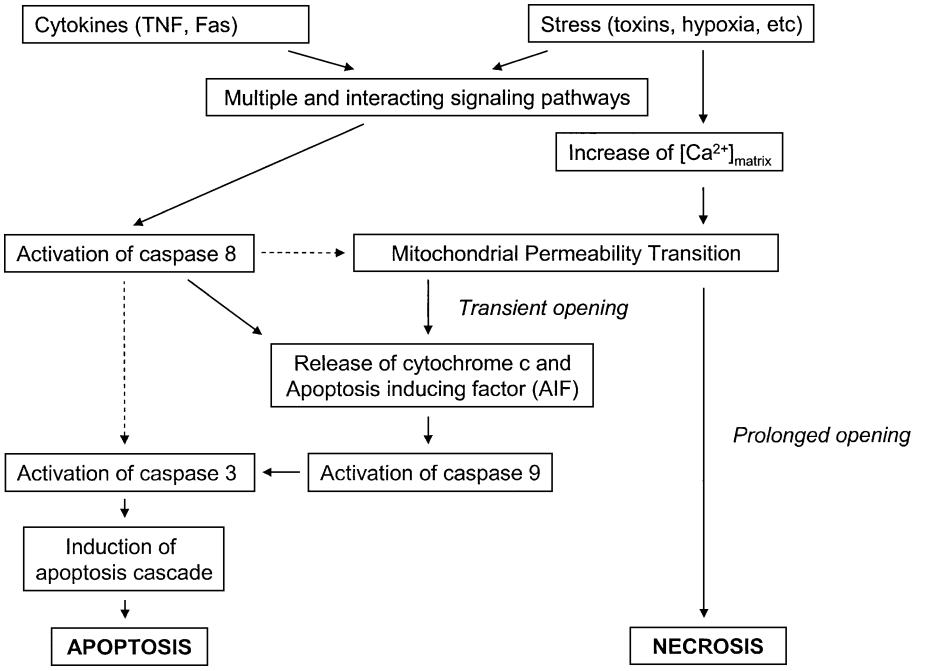

Fig. 2. Mitochondrial pathways of apoptosis. Apoptosis requires ATP levels to be elevated, whereas in necrosis ATP levels fall.

NO• can induce apoptosis in cells under conditions where respiratory or glycolytic ATP production is sufficient. NO•-induced apoptosis is mediated by caspase activation (such as caspase-3) and is blocked by caspase inhibitors (Borutaite et al., 2000). Release of cytochrome c has been observed, suggesting that NO•-induced apoptosis is normally mediated by mitochondria (Yabuki et al., 2000), although in some cell types, early activation of caspase-8 or caspase-2 is found, indicating that NO•-induced apoptosis may be triggered by non-mitochondrial pathways (Moriya et al., 2000).

It has also been reported that the addition of calcium to isolated mitochondria stimulates mtNOS, causing $ONOO^-$ production and (cyclosporine-insensitive) cytochrome c release associated with peroxidation of mitochondrial lipids (Ghafourifar et al., 1999).

Melatonin and free radicals

Melatonin is a highly conservative compound found in non-vertebrates, including bacteria (Tilden et al., 1997), eukaryotic unicells (Macias et al., 1999), macroalgae (Hardeland and Poeggeler, 2003), plants (Manchester et al., 2000; Reiter et al., 2001; Reiter and Tan, 2002), invertebrates (Meyer-Rochow and Vakkuri, 2002; Vivien-Roels and Pevet, 1993) and vertebrates. In mammals, the synthesis of melatonin in the pineal gland functions as a message encoding for the duration of darkness and it is responsible for the picomolar/nanomolar concentration of the indoleamine in serum (Reiter, 1991a). However, there are fluids and tissues, some of which may produce their own melatonin, where it can be found at much higher concentrations (Reiter and Tan, 2003; Tan et al., 2003b). Within subcellular organelles as well, the concentrations of melatonin may vary, and some authors report that the levels of this indole in nuclei

and in mitochondria may significantly exceed those in the serum (Menendez-Pelaez and Reiter, 1993; Martin et al., 2000a). Certainly, melatonin in multicellular organisms is not in equilibrium (Reiter and Tan, 2003).

There is evidence demonstrating the complexity of melatonin's role in modulating a diverse number of physiological processes including circadian entrainment (Reiter, 1991b), the control of seasonal reproduction (Reiter, 1980), retinal physiology (Dubocovich et al., 1997), blood pressure regulation (Doolen et al., 1998), regulation of the immune system (Guerrero and Reiter, 2002), oncogenesis (Reiter, 2003) and tumor growth (Blask et al., 2002) among others. This complex of actions likely requires different mechanisms of action. Melatonin membrane receptors have been identified and belong to two distinct classes of proteins, that is, the G-protein coupled receptor super family (MT_1, MT_2) and the quinone reductase enzyme family (MT_3) which makes them unique at the molecular level (Reppert et al., 1994, 1995; Nosjean et al., 2000). Also, within the G-protein coupled receptor family of proteins, the MT_1 and MT_2 receptors can couple to multiple and distinct signal transduction cascades whose activation can lead to unique cellular responses (Witt-Enderby et al., 2003). Furthermore, nuclear receptors for melatonin, referred as RZR/RORα (Wiesenberg et al., 1995) and RZRβ (Carlberg et al., 1994) have been also proposed (Acuña-Castroviejo et al., 1993, 1994) and physiologically documented (Guerrero and Reiter, 2002). However, the two kinds of receptors described may not act separately and the existence of a membrane-nuclear signaling pathway has been suggested (Carlberg, 2000).

Some of the actions of melatonin depend on non-receptor mediated processes. Included in this group is its interaction with cytosolic proteins, such as calmodulin (Benitez-King et al., 1993) and protein kinase c (Anton-Tay et al., 1998), and its actions as a direct free radical scavenger. Melatonin reacts with •OH, singlet oxygen ($^1O_2$), H_2O_2, hypochlorous acid (HClO), NO•, $ONOO^-$ and oxoferryl-derived species (Tan et al., 1993a,b; Reiter et al., 2002; Allegra et al., 2003). Some of the products that are produced when melatonin detoxifies reactive species are also efficient antioxidants. N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), a product of melatonin oxidation (Rozov et al., 2004), and N-acetyl-5-methoxykynuramine (AMK), the resulting metabolite from reaction between melatonin and H_2O_2, also scavenge H_2O_2 (Tan et al., 2000, 2001, 2003a; Carampin et al., 2003). These metabolites are also formed by the enzymatic metabolism of melatonin in the brain (Hirata et al., 1974). Another metabolite of melatonin, cyclic 3-hydroxymelatonin, generated in the reaction between •OH and melatonin (Tan et al., 1998), also scavenges 2 mol of •OH yielding AFMK as a final product (Lopez-Burillo et al., 2003). As AFMK is also a free radical scavenger, the action of melatonin as a scavenger is a sequence of scavenging reactions in which the products are themselves scavengers, resulting in a cascade of protective reactions (Lopez-Burillo et al., 2003). Melatonin is primarily metabolized in the liver to 6-hydroxymelatonin and this hydroxylated indole has also been found to function as a •OH scavenger and, additionally, to possess some prooxidative potential (Matuszak et al., 1997).

Besides the direct antioxidant effects described above, other data support an indirect antioxidant action of melatonin. The indoleamine can regulate the production of NO• through its interaction with the enzymes that synthesize it. In vitro studies have shown that melatonin inhibits nNOS activity in cerebellum (Pozo et al., 1994), hypothalamus (Bettahi et al., 1998) and striatum (Leon et al., 1998) due its binding to the calcium-calmodulin complex (Leon et al., 2000). Some compounds structurally related with the brain metabolite, AMK, can also inhibit nNOS activity in rat striatum in a dose-dependent manner, suggesting that the effect of melatonin on cerebral nNOS may take place, at least in part, through its metabolites (Leon et al., 1998, 2000). Other in vivo studies document that melatonin inhibits

iNOS and mtNOS expression and activity in an experimental model of sepsis in young and old rats (Crespo et al., 1999; Escames et al., 2003).

Both physiological and pharmacological doses of melatonin have been shown to increase gene expression and enzyme activities of GPx, GRd, SOD and CAT (Antolin et al., 1996; Pablos et al., 1998; Reiter et al., 2000; Kilanczyk and Bryszewska, 2003; Rodriguez et al., 2004). Furthermore, increased oxidative stress diminishes the activities of the toxic reactant-metabolizing enzymes, responses that are reversed by melatonin (Martin et al., 2000a; Reiter et al., 2000). Since melatonin stimulates GRd, it benefits the recycling of GSH and helps to maintain a high GSH:GSSG ratio (Hara et al., 2001). Melatonin also promotes the de novo synthesis of glutathione by stimulating the activity of its rate-limiting enzyme, γ-glutamyl-cysteine synthetase (Urata et al., 1999).

Melatonin and mitochondria: the relationship

Several molecular characteristics of melatonin are decisive for it effects on mitochondria. Melatonin is a highly lipophilic molecule that crosses cell membranes to easily reach subcellular compartments (Menendez-Pelaez and Reiter, 1993) including mitochondria, where it seems to accumulate in high concentrations (Martin et al., 2000a). In addition, melatonin interacts with lipid bilayers (Costa et al., 1997) and stabilizes mitochondrial inner membranes (Garcia et al., 1999), an effect that may improve ETC activity (Acuña-Castroviejo et al., 2001).

Studies performed in rats reveal that OXPHOS displays a circadian rhythm (Simon et al., 2003), possibly regulated by the daily fluctuations in melatonin, although further experiments are necessary to confirm this point. Melatonin is known to inhibit mitochondrial gene expression in isolated brown adipocyes obtained from Syrian hamsters (Prunet-Marcassus et al., 2001).

A direct potential relation between melatonin and mitochondria was found by Yuan and Pang (1991) and by Poon and Pang (1992). They described the pharmacological characteristics of melatonin-binding sites labeled with 2-[125]-Iodomelatonin in mitochondrial membrane preparations from pigeon brain and the spleen of guinea pigs, respectively.

The ability of melatonin to influence mitochondrial homeostasis has been tested using both in vivo and in vitro experiments. Melatonin reportedly increases the activities of the brain and liver mitochondrial respiratory complexes I and IV in a time-dependent manner after its administration to rats, whereas the activities of complexes II and III were not affected (Martin et al., 2000b). Melatonin administration also prevented the reduction in the activity of complexes I and IV due to mitochondrial damage and oxidative stress induced by ruthenium red administration in rats. At the dose used, ruthenium red did not cause lipid peroxidation but it significantly reduced the activity of the antioxidant enzyme GPx, an effect also counteracted partially by melatonin (Martin et al., 2000b).

Other studies performed using mitochondrial subparticles obtained from rat brain and liver also show that melatonin influences complexes I and IV in a concentration dependent-manner (Martin et al., 2000a, 2002). Melatonin at a concentration 1 nM significantly increased the activity of the complexes I and IV in rat liver mitochondria, whereas 10–100 nM melatonin stimulated the activities of these same complexes in brain mitochondria. The effects on complex I were also studied using a BN-PAGE histochemical procedure to measure changes in its activity induced by melatonin; this study documented the increase of complex I activity after melatonin treatment (Martin et al., 2002). Melatonin counteracts the inhibition of the complex IV caused by a dose of cyanide sufficient to decrease the enzyme activity

by 50%. However, melatonin was unable to counteract the inhibition of the complex IV when it was totally inactivated by cyanide despite the high concentration of the indoleamine used (up to 5 mM). These findings suggest that melatonin does not compete with cyanide binding to complex IV, and that it only improves the activity of the complex IV when the enzyme is in the active form (Martin et al., 2002). Based on these results, it is possible to conclude that the effect of melatonin in regulating the activity of complexes I and IV likely do not only rely on the antioxidant role of the indoleamine. In fact, the high redox potential of melatonin (0.94 V) (Tan et al., 2000), suggests that melatonin may interact with the complexes of the ETC and may donate and accept electrons thereby increasing the electron flow, an effect not possessed by other antioxidants (Martin et al., 2002).

Some authors claim that compounds which behaved either as electron donors to the mitochondrial ETC or as mitochondrial respiring substrates support the reduction of GSSG formed during oxidative stress (Liu and Kehrer, 1996); melatonin is an example of this. The indoleamine, but not other endogenous antioxidants such as vitamins C and E, regulates the glutathione redox status in isolated brain and liver mitochondria, correcting it when it is disrupted by oxidative stress (Martin et al., 2000a). Under normal conditions, melatonin (100 nM) increases the mitochondrial GSH pool, decreases GSSG levels, reduces mitochondrial hydroperoxide levels and stimulates the activity of the two enzymes involved in the GSH-GSSG balance, i.e., GPx and GRd (Martin et al., 2000a). Melatonin also is able to counteract the oxidative damage induced by high doses of tert-buthyl hydroperoxide (*t*-BHP), restoring GSH levels and GPx and GRd activities and scavenging hydroperoxides. However, vitamin C and vitamin E have no such effect under these conditions (Martin et al., 2000a). These results are in agreement with other data showing the effects of melatonin on GSH homeostasis in brain tissue (Floreani et al., 1997) and in gastric mucosa and testis in which the intragastric administration of melatonin also restores the activity of both Mn- and CuZnSOD in rats treated with indomethacin (Othman et al., 2001).

As a result of the interaction of melatonin with complexes I and IV and the subsequent promotion of electron flux through the ETC, a major physiological consequence of the melatonin action on mitochondria is revealed, i.e., increase in ATP production (Martin et al., 2002). Melatonin also has been shown to counteract cyanide-induced depletion of ATP associated with complex IV inhibition. The indolamine also stimulates metabolism in isolated mitochondria from frog oocytes (de Atenor et al., 1994). Other studies reveal another effect of melatonin on mitochondrial metabolism. Thus, experiments carried out in isolated rat liver mitochondria demonstrate that the basal respiratory index was inhibited by melatonin with a threshold at 10^{-7} M concentration. Melatonin inhibited state 3 respiration at concentrations of 100 nM–1 mM whereas state 4 was not affected. The ability of melatonin to reduce acutely the stimulation of oxygen consumption in liver mitochondria may protect this organelle from excessive oxidative damage (Reyes-Toso et al., 2003). In fact, melatonin reduces oxidative stress induced by the administration of thyroid hormones that can affect the metabolism of oxygen under aerobic conditions (Sewerynek et al., 1999) (Fig. 3).

Melatonin maintains GSH homeostasis in isolated mitochondria by a mechanism independent of its free radical scavenging properties (Martin et al., 2000a). However, other effects of melatonin on mitochondria are related with its direct antioxidant activities. Following the discovery that melatonin, in a cell free system, scavenges •OH (Tan et al., 1993a), Tan and colleagues (Tan et al., 1993b) conducted a series of in vivo experiments in which melatonin was tested for its ability to protect nuclear DNA from oxidative damage. However, studies of the protective effects of melatonin on mtDNA damage induced by free radicals are more recent. In vivo and in vitro exposures to cyanide and kainic acid induce damage

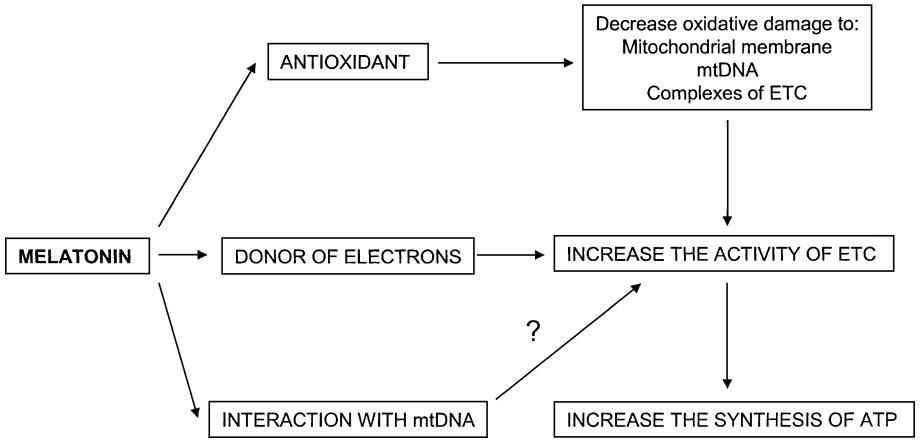

Fig. 3. Differential effects of melatonin on mitochondria.

to mtDNA and increased lipid peroxidation in isolated brain mitochondria of mice caused because these agents induce the generation of ROS, including •OH. These effects were abolished when the mitochondria were treated with melatonin (Yamamoto and Mohanan, 2002, 2003). In a model of stress induced by the acute administration of ethanol in mice, mtDNA depletion and degradation were attenuated in liver, brain, heart and skeletal muscle by the administration of the antioxidants melatonin, vitamin E or coenzyme Q. This study shows for the first time that ethanol metabolism causes oxidative degradation of the mitochondrial genome in brain, heart, and skeletal muscles and that the effect is prevented by melatonin (Mansouri et al., 2001).

Many studies demonstrate the protective effect of melatonin on lipid peroxidation induced by oxidative stress in mitochondrial membranes. In human placenta, melatonin inhibits NADPH-dependent mitochondrial lipid peroxidation (Milczarek et al., 2000). In vivo experiments performed in rats treated with Δ-aminolevulinic acid show that melatonin counteracted oxidative damage to nuclear DNA and microsomal and mitochondrial membranes in liver (Karbownik et al., 2000a) and kidney (Karbownik et al., 2000b). Melatonin also protects neuronal parikarya, axons, myelin and intracellular organelles, including mitochondria, and the nucleus by inhibiting lipid peroxidation during spinal cord injury in adult rats (Kaptanoglu et al., 2000). A melatonin metabolite, 6-hydroxymelatonin, also protects against cyanide-induced oxidative stress in rat brain homogenates. This compound significantly reduced KCN-induced $O_2^{\bullet -}$ generation which was accompanied by a reduction in lipid peroxidation (Maharaj et al., 2003).

The content of fatty acids in testes is very high with a prevalence of polyunsaturated fatty acids (PUFA); this renders these organs highly susceptible to lipid peroxidation. Consistent with melatonin's ability to reduce lipid peroxidation, supplementation of rats with melatonin reduced the quantity of damaged PUFA present in rat testicular microsomes and mitochondria (Gavazza and Catala, 2003).

ROS/RNS have been reported to initiate a wide range or irreversible oxidative damage in mitochondria which results in mitochondrial protein and DNA synthesis inhibition, membrane lipid peroxidation and DNA deletion and mutation (Halliwell and Gutteridge, 1989). Mitochondrial dysfunction can lead to $\Delta\Psi_m$ depolarization, ATP depletion and initiation of the apoptotic processes including opening of the MPT pore, release of proteins from the intermitochondrial space to the cytosol

to either activate caspases or directly cause nuclear DNA fragmentation (Halestrap et al., 2000; Zamzami and Kroemer, 2001; Kim et al., 2003). Melatonin is capable of reducing membrane lipid peroxidation, mtDNA and nuclear DNA oxidation under a variety of conditions of elevated oxidative stress. Because of this, it is possible that the indoleamine acts as an antiapoptotic agent in ROS/RNS mitochondrial-mediated cell death. In fact, the antiapoptotic effect of melatonin has been described in several systems and in a variety of different situations (Sainz et al., 2003). Melatonin protects against cyclosporine A-induced hemolysis in human erytrocytes due to the depuration resulting from $O_2^{\bullet -}$ produced by mitochondria (Kwak et al., 2002). Melatonin is also highly protective against cardiotoxicity induced by doxorubicin (DOX). DOX causes cardiac damage via production of ROS and mitochondria in DOX-treated myocytes exhibit a collapse of the $\Delta\Psi_m$. Pretreatment with melatonin prevented the release of lactate dehydrogenase (LDH) and restored $\Delta\Psi_m$. The data support the hypothesis that DOX induces damage to mitochondria via mechanisms that involve free radicals, and this is reflected in depolarization of $\Delta\Psi_m$, a change that is prevented by melatonin (Xu and Ashraf, 2002).

Laser irradiation-induced phototoxicity has been intensively applied in clinical photodynamic therapy for the treatment of a variety of tumors. However, the precise laser damage sites as well as the underlying mechanisms at the subcellular level are unknown. In rat brain astrocytes, radiation induces mitochondrial swelling and nuclear condensation and fragmentation indicating that the laser-irradiated cells die from apoptosis. In laser-irradiated cells, the level of ROS in the mitochondrial compartment was found to be higher than in other parts of the cell. Treatment with the antioxidants melatonin and vitamin E largely attenuated mitochondrial ROS formation and prevented apoptosis, indicating that mitochondrial ROS contribute significantly to laser irradiation-induced apoptosis via an MPT-independent pathway (Jou et al., 2002).

Interestingly, proapoptotic effects of melatonin have been proposed in a number of tumor cell lines (Sainz et al., 2003). Also, in MCF-7 breast tumor cell studies conducted in the absence of exogenous steroid hormones, treatment with melatonin produced a 64% reduction in the cellular ATP levels through a membrane receptor-modulated pathway (Scott et al., 2001). These findings in tumor cells are in contrast to the described actions of melatonin in normal cells and suggest melatonin's potential use in killing cancer cells while preserving the function of normal cells.

Melatonin, mitochondria and aging

Forty-five years ago it was proposed that free radicals are the major factor in the aging process (Harman, 1956). Since mitochondria are a major source of free radicals in the cell, Harman (1981) later refined the hypothesis and suggested that mitochondria are the major target of free radical attack that leads to aging. This ''mitochondrial theory of aging'' emphasized that enhanced production of ROS and accumulation of mtDNA mutations in mitochondria of postmitotic cells are a contributory factor to age-related deterioration (Miquel et al., 1980). MtDNA with oxidative modification and/or mutation are transcribed and translated to produce defective protein subunits that are assembled to form defective ETC proteins, especially in complexes I and IV (Cooper et al., 1992). The impaired ETC not only works less efficiently in ATP synthesis (Harper et al., 1998), but it also generates more ROS, which further enhances oxidative damage to various biomolecules in mitochondria (Sohal and Dubey, 1994). The high levels of oxidants can induce apoptosis by changing cellular redox potentials, depleting reduced glutathione, reducing ATP levels, and decreasing reducing equivalents such as NADH and NADPH

(Esteve et al., 1999; Wei and Lee, 2003). These changes facilitate lipid peroxidation and the opening of permeability transition pores, leading to apoptosis (Lenaz, 2001).

Although a direct relationship between melatonin and aging has been difficult to document, several properties of the indolamine indicate that it may be beneficial in aging. Serum levels of melatonin significantly decrease in aged animals compared with the young to the population, including humans (Reiter, 1992). In humans, the total antioxidative capacity of serum correlates well with its melatonin levels (Benot et al., 1999). Thus, the reduction in melatonin with age may be a factor in the elevated oxidative damage observed in the elderly (Reiter et al., 2002).

Chronic treatment with melatonin counteracts the increase in complex IV activity of aged C57Bl/6 mice, restoring its activity at the level of young animals. The activity of the other respiratory complexes did not change as a consequence of melatonin treatment. Other antioxidants that were tested were unable to modify the activities of complexes I and IV, although they influenced complex II (Sharman and Bondy, 2001). The inhibition of the age-dependent increase in complex IV activity by melatonin is in contract to other studies which reported a stimulatory effect of the indoleamine (Martin et al., 2000a,b). However, it was suggested that the decay in mitochondrial respiration with age is preceded by a hyperactive but inefficient mitochondrial state, which may depend on the elevation in free radical generation. Thus, scavenging free radicals by melatonin may account for the normalization of the activity of the complex IV in old mice reported in the study of Sharman and Bondy (2001).

Other recent studies using the senescence-accelerated mouse (SAM) investigated the effects of chronic administration of physiological doses of melatonin on mitochondrial function in SAMP8 mice, a strain prone to accelerated senescence (SAM) and from SAMR1, a senescence-resistant strain, at 3, 6 and 12 months (Okatani et al., 2002a,b, 2003a). These studies demonstrated that the activities of complex I and IV in liver mitochondria from SAMP8 show an age-related reduction. In contrast, preparations from SAMR1 displayed no reduction in respiratory function (Okatani et al., 2002a,b). Likewise, the concentrations of products of lipid peroxidation in liver and brain homogenates from SAMP8 were significantly higher than those in the SAMR1 at the age tested. In contrast, the activity of the antioxidative enzyme GPx at 12 months of age from SAMP8 was significantly lower than that measured in SAMR1 mice. These results suggest that oxidative stress as a result of excessive free-radical generation combined with a less effective defense against oxidative stress may lead to the alteration in mitochondrial function seen in SAMP8 mice (Okatani et al., 2002a,b). Malondialdehyde levels also were significantly higher in the liver and brain of 12-month-old SAMP8 mice than in the SAMR1 animals. Although liver homogenates of SAMP8 mice have been reported to have lower SOD activity than homogenates from SAMR1, the values did not differ significantly (Okatani et al., 2002a). Chronic treatment with melatonin (2 mg/mL of drinking fluid) significantly reduced (30%) neural and hepatic lipid peroxidation products (TBARS) content in both strains and also lowered the protein carbonyl content in brain of SAMP8 mice. Furthermore, melatonin significantly augmented (>20%) GPx activity in both strains, but it had no effect on SOD activity (Okatani et al., 2002a,b).

Melatonin also prevents age-related oxidative DNA damage in the brain of female senescence-accelerated SAM-P/6 mice (Morioka et al., 1999), restores mitochondrial respiratory control index, ADP/O ratio, state 3 and DNP-dependent uncoupled respiration (Okatani et al., 2002b). Furthermore, melatonin induces increases, with respect to the levels in young animals, the senescence-associated reductions of complex I and IV activities in liver mitochondria from SAMP8 (Okatani et al., 2002a,b). Additionally, injections of pharmacological doses of melatonin modifed mitochondrial respiratory

activity and respiratory chain complex I and IV activities in liver mitochondria from SAMP8 mice (Okatani et al., 2003a).

Increasing evidences suggests that NO• contributes to the aging process (McCann, 1997) and changes in the activities of different isoforms, i.e., endothelial (eNOS), neuronal (nNOS) and inducible (iNOS), of the enzyme occur with aging (Siles et al., 2002); however, a recent study in rats shows no changes in liver and lung mtNOS activity and expression in aged rats (Escames et al., 2003). Lipopolysaccharide (LPS) administration (10 mg/kg, i.v.) significantly increased mtNOS expression and activity and NO• production in lung mitochondria, effects that was larger in old that in young rats. LPS administration also reduced the age-dependent decreases of respiratory complexes I and IV. Melatonin administration (60 mg/kg, i.p.) prevented the toxic effects of LPS and reduced both mtNOS activity and NO• production. Melatonin also counteracted LPS-induced inhibition of complexes I and IV. In general, the actions of melatonin were greater in old than in young animals. The results suggest also that an inducible component of mtNOS, together with mitochondrial damage, occurs during sepsis, and melatonin prevents the mitochondrial failure that occurs as a result of endotoxemia (Escames et al., 2003).

Melatonin, mitochondria and neurodegenerative disorders

Parkinson's disease (PD) is a progressive neurodegenerative condition characterized clinically by bradykinesia, rigidity, resting tremor, and ataxia. PD is characterized primarily by the death of dopaminergic neurons in the pars compacta of the substantia nigra, causing reduced dopamine release in the striatum along with the formation of ubiquitin- and α-synuclein-positive cytoplasmic inclusions (Lewy bodies). The molecular mechanisms responsible for these changes are not clearly understood. Numerous studies have reported reduced complex I activity in the substantia nigra (Hattori et al., 1991) and in platelets (Shults et al., 1997) of patients with PD accompanied by a loss of GSH (Schapira et al., 1989). Other evidence shows that oxidative damage is a feature of the disease. Analysis of postmortem brain specimens has revealed oxidative damage to proteins, lipids, and mtDNA in the substantia nigra (Zhang et al., 2000). The source of this oxidative damage is unknown, but dopamine neurons are believed to exist in a permanent state of oxidative stress, due the activities of tyrosine hydroxylase and monoamine oxidase, which cause the formation of H_2O_2 as a normal byproduct (Strolin Benedetti and Dostert, 1989; Adams et al., 1997). Auto-oxidation of dopamine also yields H_2O_2, which can produce •OH, a reaction that is accelerated in the presence of iron, which is abundant physiologically in the substantia nigra (Khaldy et al., 2000).

Despite an abundance of information about biochemical changes in this disease, the initiating factor of this one addition remains unknown. Mitochondrial changes have been reported. Thus, complex I activity and GSH content are decreased in in vitro models of oxidative stress (Antunes et al., 2002). GSH depletion in PC12 cells results in selective inhibition of complex I (Jha et al., 2000). Inhibition of complex I activity induces GSH depletion (Seyfried et al., 2000), increases ROS and cell death by apoptosis (Li et al., 2003). 1-methyl-4-phenyl-tetra hydropyridine (MPTP) toxicity represents a useful tool to study dopaminergic degeneration in models of Parkinson's disease both in vivo and in vitro. MPTP crosses the blood–brain barrier, is converted to MPP^+ in glial cells by monoamine oxidase B and is then taken up by catecholaminergic neurons via the dopamine reuptake system. MPP^+ concentrates in mitochondria where it inhibits complex I leading to ATP depletion and to cell death of dopaminergic neurons (Zigmond and Stricker, 1989). Evidence for TUNEL-positive dopaminergic

cells after application of MPP^+ in cell culture has been demonstrated (Dodel et al., 1998). Likewise, caspase-3 activation has been documented after the application of MPP^+ to cerebellar neurons (Leist et al., 1998) or after MPTP administration in vivo (Hartmann et al., 2000). Activated caspase-3 was also demonstrated in postmortem tissues of human Parkinson's disease substantia nigra (Hartmann et al., 2000). Finally, in cultured cells, MPP^+ induces MTP, cytochrome c liberation and apoptosis (Cassarino et al., 1998).

Melatonin ameliorates the reduction of tyrosine hydroxylase-positive fibers and the lipid peroxidation in the striatum which follows the injection of MPTP into mice (Acuña-Castroviejo et al., 1997) but the indoleamine does not restore enzyme activity after it is lost (Khaldy et al., 2003). Melatonin, unlike deprenyl, prevents the inhibition of mitochondrial complex I activity induced by MPTP (Khaldy et al., 2003) and inhibits dopamine autooxidation (Khaldy et al., 2000). Melatonin also potentiates the effect of deprenyl on catecholamine turnover and ambulatory activity of mice treated with MPTP (Khaldy et al., 2003). These results suggest a dissociation of complex I inhibition from DA depletion in this model of Parkinson's disease. The data also support the idea that a combination of melatonin, which improves mitochondrial ETC and reduces oxidative damage, and deprenyl, which promotes the specific function of the rescued neurons, i.e. DA turnover, may be a promising strategy for the treatment of PD (Khaldy et al., 2003).

Experiments on isolated rat liver mitochondria and striatal synaptosomes treated with MPP^+ reveal that melatonin prevents the inhibition of mitochondrial respiration associated to complex I inhibition due to the fact that melatonin limits MPP^+ interaction with complex I (Absi et al., 2000). Melatonin also inhibits mouse brain DNA fragmentation and apoptosis associated with both acute (Ortiz et al., 2001) or chronic (Ortiz et al., 2001; Antolin et al., 2002) MPTP treatment.

The catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA), also produces loss in nigro-striatal dopaminergic neurons after its intracerebral administration in rats. In an in vitro model with PC12 cells, melatonin inhibited apoptosis and the structural changes produced by treatment with 6-OHDA (Mayo et al., 1998). The administration of melatonin corrects a hemi-Parkinson condition in rats caused by intranigral application of the 6-OHDA (Dabbeni-Sala et al., 2001a). The indolamine also limits the alterations in the motor activity due to the injection of the neurotoxin possibly as a consequence of its ability to restore complex I activity.

NO has been implicated in the toxicity of amphetamine and methamphetamine, two drugs that induce a PD like condition via free radical formation, mitochondrial damage and a decrease in ATP synthesis (Virmani et al., 2002, 2003). Melatonin restores mitochondrial parameters and reduces the production of $ONOO^-$ stress marker, 3-nitrotyrosine, in PC12 cells, and also in vivo in the striatum of adult mice.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by amyloid deposits as components of senile plaques in the walls of cerebral and meningeal blood vessels (Robakis, 1994). The amyloid is composed of a 40–42 amino acid peptide and is referred to as amyloid β protein (Aβ), a small region of a larger amyloid precursor protein (APP) (Glenner and Wong, 1984); Aβ is toxic to neurons (Behl et al., 1992; Zatta et al., 2003). Several studies demonstrate that •OH is involved in the cytotoxic properties of Aβ. Decreased levels of cytochrome c oxidase activity are present in AD brain tissue, which suggests possible free radical injury to mitochondria (Maurer et al., 2000).

A decrease in the cerebrospinal fluid (CSF) melatonin was found in patients with AD and subsequently it was suggested that the loss of melatonin allowed neuronal mitochondria to be

damaged by endogenously-generated •OH, with injury noted first in the most active tissues (Maurizi, 1997, 2001; Cardinali, 2003). In fact, results from initial therapeutic trials of melatonin in AD patients have demonstrated improved function, decreased "sundowning", improved sleep, and a significant slowing of the progression of the disease (Maurizi, 2001; Cardinali, 2003). In cultured cells, melatonin prevents nuclear and mtDNA cleavage and apoptosis induced by amyloid protein (Pappolla et al., 1997, 1999, 2000). This effect may dependent not only of the antioxidant properties of the melatonin but also of its capacity of increase the activity of the complex IV of the ETC (Martin et al., 2000a,b).

Many reports have shown a protective effect of melatonin on epilepsy in humans (Molina-Carballo et al., 1997) and in experimental models in rats (Bikjdaouene et al., 2003). These antiepileptic properties of melatonin may be due to the increase the in GABAergic neurotransmission due to the regulation of the central GABA-benzodiazepine receptor complex and inhibition of the glutamate-mediated response (Acuña-Castroviejo et al., 1995) through the inhibition of the nNOS activity (Leon et al., 2000) and NO• production (Bikjdaouene et al., 2003). Besides the regulation of the activity of the receptors of inhibitory and excitatory amino acids, melatonin also influences the liberation of the amino acids responsible for their activation in the model of pentylenetetrazole (PTZ)-induced seizures in rats (Bikjdaouene et al., 2003). Other studies reveal mitochondrial dysfunction in experimental models of epilepsy in vivo (Mohanan and Yamamoto, 2002; Yamamoto and Mohanan, 2003) and in cultured cells (Dabbeni-Sala et al., 2001b). In vivo, melatonin reduces the damaging effects of kainic acid on mtDNA, mitochondrial lipid peroxidation and seizures (Mohanan and Yamamoto, 2002; Yamamoto and Mohanan, 2003). Melatonin also protects against the increase in ROS production and the decreases in GSH levels, complex II activity and the consequent cell viability in cerebellar granule neurons after the treatment with kainic acid (Dabbeni-Sala et al., 2001b).

Melatonin, mitochondria and ischemia-reperfusion

Ischemia-reperfusion (I/R) is a common problem encountered in a variety of clinical situations. For example, it is one of the most critical problems in liver transplantation, hepatic failure after shock and liver surgery and I/R is also important in the brain damage during the perinatal period. The pathogenesis of I/R is multifactorial and includes overproduction of ROS (Fosslien, 2001). In liver, I/R induces deterioration of hepatic mitochondrial function and energy metabolism (Okatani et al., 2003b), with a reduction in the levels of ATP, caused, at least in part, by the activation of iNOS and an increase in the production of TNF-α (Rodriguez-Reynoso et al., 2001). In vitro treatment of hepatocytes with TNF-α suppresses mitochondrial respiration by inhibiting complex I of the respiratory chain, and this may be associated with the generation of ROS (Stadler et al., 1992), whereas NO• induces inhibition of mitochondrial aconitase and complexes I and II (Stadler et al., 1991). Treatment with melatonin before the injury protects against mitochondrial dysfunction induced by I/R of rat liver (Okatani et al., 2003b) and restores hepatic energetic status by inhibiting both activation of iNOS and the production of TNF-α (Rodriguez-Reynoso et al., 2001).

ROS produced during I/R in fetal rat brain induce mitochondrial damage, decreasing mitochondrial respiratory activity and the activity of GPx and mitochondrial SOD, parameters that can be restored with melatonin treatment before the injury (Wakatsuki et al., 2001a,b).

Conclusions

Melatonin is a compound with two effects on mitochondria. First, the indoleamine is an antioxidant that can directly scavenge ROS produced during the normal metabolism of this organelle and it indirectly promotes the activity of the antioxidant enzymes including SOD, GPx, GRd, and catalase. Second, melatonin increases the activities and the expression of complexes I and IV of the ETC under normal conditions and restores their activities in some pathological situations. A major consequence of these effects is that melatonin maintains GSH homeostasis in mitochondria and increases ATP production.

Further studies are necessary to identify the mechanisms by which melatonin increases the activity of ETC complexes. Besides its antioxidant properties, which can prevent oxidative damage to the proteins of the complexes, an effect on the expression of these proteins has been found. Additionally, melatonin is able to donate electrons to certain proteins. This property and the possibility of its interaction with the mitochondrial genome should be considered in subsequent studies related to the interaction of melatonin with mitochondria.

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