Melatonin, Mitochondrial Homeostasis and Mitochondrial-Related Diseases

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Abstract: The recently described 'hydrogen hypothesis' invokes metabolic symbiosis as the driving force for a symbiotic association between an anaerobic, strictly hydrogen-dependent organism (the host) and an eubacterium (the symbiont) that is able to respire, but which generates molecular hydrogen as an end product of anaerobic metabolism. The resulting proto-eukaryotic cell would have acquired the essentials of eukaryotic energy metabolism, evolving not only aerobic respiration, but also the cost of oxygen consumption, i.e., generation of reactive oxygen species (ROS) and oxidative damage. Mitochondria contain their own genome with a modified genetic code that is highly conserved among mammals. Control of gene expression suggests that transcription of certain mitochondrial genes may be regulated in response to the redox potential of the mitochondrial membrane. Mitochondria are involved in energy production and conservation, and they have an uncoupling mechanism to produce heat instead of ATP. Also, mitochondria are involved in programmed cell death. Increasing evidence suggests the participation of mitochondria in neurodegenerative and neuromuscular diseases involving alterations in both nuclear (nDNA) and mitochondrial (mtDNA) DNA. Melatonin is now known as a powerful antioxidant and increasing experimental evidence shows its beneficial effects against oxidative stress-induced macromolecular damage and diseases, including those in which mitochondrial function is affected. This review summarizes the data and mechanisms of action of melatonin in relation to mitochondrial pathologies.

INTRODUCTION

Mitochondrial Function

Mitochondria are specialized for the rapid oxidation of NADH and FADH produced during glycolysis, Krebs cycle and β-oxidation of fatty acids by the transfer electrons from these precursors to oxygen. The electron transport chain (ETC) is a system of oxido-reductant protein complexes (complexes I, II, III and IV) in the inner mitochondrial membrane. According to the chemiosmotic hypothesis, C-I, C-III and C-IV pump protons yielding a proton gradient along the mitochondrial inner membrane, which is a source of free energy that is dissipated when protons enter the inner mitochondrial membrane through ATP synthase [1]. During this process, ADP is phosphorylated to ATP. Mitochondrial DNA encodes several components of the respiratory complexes: 7 of C-I; cyt b_{560} corresponding to a cofactor of C-II; 3 of C-IV and 2 of ATP synthase [2]. In aerobic cells, oxidative phosphorylation (OXPHOS) is responsible for production of 90-95% of the total amount of ATP, and more than 90% of respiratory phosphorylation is catalyzed by ATP synthase, an enzyme converting the respiratory chain-produced electrochemical proton potential difference ($\Delta\mu_H^+$) into ATP [3]. The respiration-produced $\Delta\mu_H^+$ can be utilized by mitochondria not only to form ATP but also to support other energy-consuming processes such as transport of certain solutes from the cytosol to the matrix. Mitochondria are also of central importance for physiological Ca^{2+} handling, acting as a reservoir for Ca^{2+}. Mitochondrial Ca^{2+} regulates the activity of mitochondrial dehydrogenases as well as nucleic acid and protein synthesis [4]. Several factors have been proposed to regulate respiration including ATP (respiratory control), Ca^{2+} and proton leak [5].

Dissipation of energy as heat to maintain body temperature at a level higher than in the environment is another important function of mitochondria. The mechanism is referred to as thermoregulatory uncoupling of respiration and phosphorylation. Uncoupling results in dissipation of the respiratory chain-produced $\Delta\mu_H^+$ due to increased proton conductance of the inner membrane. Thus, energy released by respiration is dissipated immediately as heat without formation and hydrolysis of ATP. Non-esterified fatty acids have been proven as the compounds mediating the thermoregulatory uncoupling. They operate as protonophorous uncouplers with the help of special uncoupling proteins (UCPs) [6]. It is known that T_3 influences in rat the expression of nine nuclear-encoded respiratory genes, regulates mitochondrial RNA synthesis through both the activation of a mitochondrial transcription factor (mtTFA) and the specific mitochondrial T_3 receptors,

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and stimulates the expression of the mRNA for UCP2 and UCP3 [7-9].

UCPs are elementary proton transporters across the inner mitochondrial membrane. Proton transport is driven only exclusively by the membrane potential [10]. Fatty acids provide one or more carboxyl groups along the translocation channel, and deliver their protons to an acceptor group (carboxyl groups of the other fatty acid), which in turn delivers protons into the matrix. While UCP1 is known to play an important role in regulating heat production during cold exposure, possible roles for UCP2 and UCP3 include: regulation of ATP synthesis; control of reactive oxygen species (ROS) production by mitochondria; control of adaptative thermogenesis in response to cold and diet; and regulation of fatty acid oxidation [11, 12]. A failure to control ROS damage can cause the collapse of multiple vital functions, including mitochondrial energy conservation which culminates in the loss of membrane integrity and cell death by necrosis and/or apoptosis [6]. UCP2-dependent uncoupling mitochondrial depolarization reduces ROS production and thus inhibits the permeability transition pore (PTP) avoiding the proapoptotic cascade. It was suggested that superoxide anion ($O_2^{-\bullet}$) production decreases because uncoupling increases the rate of electron transport, diminishing the probability that electrons will escape from the respiratory chain and interact with molecular oxygen. Also, a direct interaction between UCP2 with the antiapoptotic bcl-2 family was also proposed [6].

Mitochondrial DNA

Mitochondrial DNA consists of a closed-circular, doubled-stranded DNA molecule of about 16.6 kbp. Most information is encoded on the heavy (H) strand, with genes for two rRNAs, 14 tRNAs, and 12 polypeptides. The light (L) strand codes for 8 tRNA, and a single polypeptide. All 13 polypeptides are constituents of the enzyme complexes of the ETC. The genes lack introns and, except for one regulatory region, intergenetic sequences are absent or limited to a few bases. Replication and transcription in mitochondria depend upon trans-acting nuclear-encoded factors [2, 13].

Transcription of mtDNA is controlled by a human dissociable transcription factor (mtTFA) acting in concert with the mitochondrial RNA polymerase and a factor mediating attenuation of transcription (mtTERM). As no intron sequences are present in vertebrate mtDNA, and intergenetic sequences are minimal, processing of the long polycistronic H- and L-strand messengers is thought to be a relatively simple process requiring only a few enzymes. Genes for tRNAs flank the two rRNAs genes and nearly every protein gene, suggesting that the secondary structure of the tRNA sequences provide the punctuation marks in the reading of the mtDNA information. One initiation factor (mtIF-2) and three mitochondrial elongation factors (mtEFs) have been identified and participate in the polypeptidic chain elongation. Mammalian mtDNA replication is a slow process and replication is unidirectionally. DNA polymerase γ is the only DNA polymerase present in mitochondria, and it is required for mtDNA synthesis. In addition to its role in transcription, mtTFA appears to have a function in maintenance of mtDNA [2]. Because the mitochondrial genes encode only a few proteins, almost all of the mitochondrial proteins must be imported into the mitochondria after their synthesis by cytoplasmic free ribosomes as preproteins [14]. These usually have 20 amino acid N-terminal extensions (presequences), which can direct the preproteins to the mitochondria [15]. Cytoplasmic import factors deliver the preproteins to the outer surface of the mitochondria; then import systems of the outer membrane (Tom, translocase of the outer membrane) and the inner membrane (Tim, translocase of the inner membrane) transport the preproteins to their final destinations [16, 17]. Fundamental mechanisms of mitochondrial protein import seem to be conserved from eukaryotes to mammals.

Mitochondria and Apoptosis

Although some aspects remain to be elucidated [18, 19], the mitochondria exert a central role in eukaryote life and death [20]. These organelles are involved in apoptosis (and necrosis) by promoting the release of proapoptotic factors including cytochrome c and other "death factors" in the intermembrane space [21]. The activation of the apoptotic cascade leads to cell death [22]. In some situations, Ca^{2+} overload leads to mitochondrial swelling, loss of respiratory control, collapse of $\Delta\psi_m$, and release of matrix Ca^{2+} caused by a permeabilization of the mitochondrial inner membrane (PTP) to molecules up to 1.5 kDa. Structurally, the PTP is formed by the adenine nucleotide translocase (ANT), and electrophysiological studies also have shown an interaction of PTP with the membrane porins and with the mitochondrial benzodiazepine receptor [4, 23].

The PTP can switch from low- to high-conductance states. The conformational switch appears to be dependent on the saturation of the internal Ca^{2+} binding of the channel. The low-conductance state of PTP may be responsible for mitochondrial volume homeostasis and contributes to a significant part of the final cytosolic Ca^{2+} signalling [23]. Thus, under its low-conductance conformation, the PTP does not impair mitochondrial function and is operated by changes in matrix pH accompanying mitochondrial Ca^{2+} uptake. The high-conductance state of the PTP is activated by the cooperative binding of two Ca^{2+} ions to its matrix domain, with a molecular cutoff of 1.5 kDa. This induces a complete collapse of the proton gradient, allowing for the efflux of a variety of other ions and of small molecules such as pyrimidine and adenine nucleotides, and promotes the diffusion of components from the incubation medium into the matrix, such as sucrose. The high-conductance state of PTP is highly regulated and exhibits the features of a Ca^{2+}-, voltage- and pH-gated channel [23], modulated by the redox and phosphate potentials. Opening of the PTP appears to be regulated by direct binding of a mitochondrial cycloplilin (cyclophilin D) to its matrix domain, accounting for the inhibitory effect of cyclosphorin A (CsA).

The PTP may be also regulated by ROS leaking from the ETC. The shift from a low to a high-conductance state can be promoted by the oxidation of NADPH by oxidative stress. This impairs the antioxidant function of glutathione

(GSH) [24]. The participation of ROS in opening PTP is clear, since no PTP opening occurs in the absence of molecular oxygen [24]. The PTP possesses at least two redox-sensitive sites and both increase the probability of opening after oxidation: the S-site, a dithiol in apparent redox equilibrium with matrix GSH, and the P-site, in apparent redox equilibrium with the pyridine nucleotides [25]. Glutathione disulfide (GSSG) is probably the immediate oxidant of the S-site and many pore inducers such as hydrogen peroxide (H_2O_2) appear to affect the pore through changes in the level of the GSH rather than direct oxidation of the S-site. In turn, oxidation of the P-site by oxidized pyridine nucleotides can induce PTP under conditions where the GSH pool is maintained in a fully reduced state. Under conditions of oxidative stress, the mitochondrial levels of GSH and reduced pyridine nucleotides are connected through energy-linked transhydrogenase and glutathione reductase (GRd) and thus it is difficult for these compounds to independently modulate the S- and P-site in vivo [25].

Apoptosis may be visualized as a process of the glycolyzing host cell to punish respiring guests if they formed excessive ROS [3]. In fact, the protomitochondria brought respiration to the partnership and with it the power to kill the new cell through the production of ROS [26]. It is obvious that in modern organisms the functions of apoptosis (at mitoptosis) are not restricted by the elimination of ROS-overproducing mitochondria and cells. However, apoptotic stimuli are processed inside the cell in such a way that an increase in intramitochondrial (intracellular) levels of ROS is initiated. The production of $O_2^{-\bullet}$ and H_2O_2 by the ETC is the inevitable side effect of ETC induced by the one or two electron reduction of O_2 [3]. In some instances, the production of ROS increases and may induce PTP opening. The increased PTP mitochondria cannot survive due to the collapse of $\Delta\psi_m$, since PTP permits the efflux of molecules up to 1.5 kDa; this causes the high molecular mass compounds in the matrix to exert an osmotic effect and water reaches the matrix causing its swelling. As a result, mitochondrial cristae straighten and the outer membrane is broken since it is much smaller that the inner membrane. The loss of outer membrane integrity causes all the intermembrane proteins to be released into the cytosol including some involved in apoptosis including cyt c, apoptosis-inducing factor (AIF) and some procaspases [3, 27, 28]. Cyt c and AIF form a complex with the cytosolic Apaf-1 and ATP. The complex hydrolyzes inactive procaspase 9 to active caspase 9, which in turn hydrolyzes procaspase 3 to caspase 3. Caspase 3 attacks some other key proteins resulting in controlled cell death [29]. To avoid apoptosis, the bcl-2 family of proteins block death by preventing the mitochondrial release of the intermembrane proteins [29, 30]. Once the process gets past the mitochondria, the anti-apoptotic proteins are not effective. [22].

Mitochondrial Pathologies

The functional activity of mitochondria depends on a precise cross-talk between two different genetic systems, i.e., nuclear and mitochondrial genomes [31]. A series of mechanisms controlling the mitochondrial genetic system and intergenomic communication have been recently summarized [32]. Thus, defects in mitochondrial metabolism may be associated with mutations of mtDNA or nDNA. Abnormalities of mitochondrial metabolism that cause human disease have been recognized for more than 30 years. They encompass defects in fatty acid oxidation, Krebs cycle enzymes and the OXPHOS system. Some of these pathologies do not induce, as a primary cause, an alteration in mitochondrial metabolism, but result from it. That is the case during energy alterations due to changes in hormones (hyper and hypothyroidism-induced changes in UCPs expression altering $\Delta\psi_m$) [3, 9, 33], or as a consequence of ischemia/reperfusion, excitotoxicity, or sepsis. In each of these, there is an increase in ROS production and an alteration in mitochondrial function that may lead to cell death [34, 35]. Moreover, as mtDNA encodes proteins of the OXPHOS, such mutations frequently result in a deficiency in one or more constituents of these enzymes.

A recently-described group of alterations involves mitochondrial transmembrane carrier deficiencies that constitute the mitochondriopathies [36]. Increasing amounts of ROS damage DNA and produces an increase in poly-(ADP-ribose) synthetase (PARS) to repair the damaged DNA. This enzyme ADP-ribosyates proteins depleting the intracellular concentration of its substrate, NAD^+, slowing the ETC and ATP production [37, 38]. Primary OXPHOS defects [39] are caused by mutations of mtDNA or nDNA genes encoding subunits of the ETC complexes, including mutations affecting mitochondrial targeting of protein, i.e., the N-terminus sequence. A defect in the import of the Rieske iron-sulphur center has been postulated. Other alterations of mtDNA including tRNA and protein encoding genes of the ETC complexes, and cyt b. In turn, OXPHOS deficiencies may result in increased ROS [39]. Secondary, OXPHOS deficiencies are both due to genetic and environmental factors. Alterations in mtDNA transcription, translation and replication are also included [39]. Endogenous and exogenous toxins may impair OXPHOS. Aging itself may be due to ROS over production and mitochondrial damage. Toxins such as MPTP simulate neurodegenerative diseases further demonstrating the involvement of ROS in this pathology [39, 40].

Neurodegenerative Pathologies

Neurodegenerative diseases of different etiologies may share mitochondrial dysfunction as a final common pathway. Recent studies using cybrid cell lines certainly support this possibility [41]. Parkinson's disease (PD) is characterized by bradykinesia, rigidity and tremor. Mitochondrial involvement in PD was suggested by deficiencies in mitochondrial C-I in the substantia nigra [42], with a parallel reduction in GSH levels, suggesting the existence of oxidative stress. In platelets of PD patients, C-I activity is also reduced, and in some cases are accompanied by C-II, C-III and C-IV deficiencies. Studies with cybrids have shown that alterations in C-I is due to a defect in the mtDNA [42]. This defect is accompanied by an alteration in the expression of C-IV activity and a decreased $\Delta\psi_m$, which lowers the apoptotic threshold.

Environmental factors influence PD, as shown by the C-I inhibitory effects of MPTP and paraquat. The C-I inhibition is prevented by free radical scavengers indicating oxidative damage to C-I. MPTP also stimulates NMDA-dependent nNOS activity thereby increasing nitric oxide (NO) production [43], and decreasing the content of mtDNA [44].

Huntington's disease (HD) is a neurodegenerative disorder characterized by ataxia, chorea and dementia. It is known to be caused by alterations in a gene for nDNA encoding huntinin, a widely expressed protein of unknown function. The pathology of HD involves mainly the GABA-containing neurons of the caudate nucleus [42]. Excitotoxicity has been suggested to play an important role in this disease. This includes activation of NMDA-dependent neuronal nitric oxide synthase (nNOS) and NO production. NO and particularly peroxynitrite ($ONOO^-$) mediate the oxidative damage. There are also deficiencies in the activities of mitochondrial C-II, C-III and C-IV in caudate and in a lesser extend in putamen in HD. Aconitase, an iron-sulphur-containing enzyme is particularly susceptible to inhibition by superoxide and $NO/ONOO^-$, as are C-II and C-III, which are FeS-containing enzymes [42, 45]. The subsequent oxidative damage to proteins, lipids and mtDNA reduces $\Delta\psi_m$ and induces apoptosis.

Hereditary spastic paraparesis (HSP) is another hereditary disease involving a nDNA mutation. It may be present in children or adults. A new gene defect has been recently described encoding paraplegin which contains an N-terminus sequence and which is imported into mitochondria. Muscle biopsies show mitochondrial alterations including cytochrome oxidase negative fibers.

Wilson's disease, usually present in children and adolescences, shows liver failure with movement disorders (dystonia, parkinsonism), and is caused by a mutation in the gene encoding a mitochondrial P-type ATPase, leading to copper accumulation and ROS generation [42, 45].

Friedreich's ataxia (FA) is an adolescent autosomal disease with progressive ataxia, dysarthria, skeletal deformations, hyporeflexia, pyramidal features and cardiomyopathy. The pathology also includes distal axonopathy affecting the large sensory axons of the dorsal root ganglia and the spinocerebellar and pyramidal tracts in the cord with loss of neuronal parikarya. The genetic defect results in a deficiency of frataxin protein whose function is not known. Since it has a N-terminus sequence and is associated with mitochondrial membranes, a role of mitochondria was proposed. There are several deficiencies of complexes I-III and in the Krebs cycle enzyme aconitase. There is a parallel increase in the mitochondrial iron levels and, through the Fenton reaction, oxidative damage to mtDNA may also result [42, 45].

Alzheimer's disease (AD), is associated with a decrease in mRNA expression of mtDNA encoding cytochrome oxidase (COX) subunit II, although it has been proposed that other nDNA-encoded COX subunits may be also altered [46]. Also, β-amyloid peptide generates ROS in a metal-catalyzed reaction inducing neuronal cell death in a ROS-mediated process resulting in damage to neuronal membrane lipids, proteins and nucleic acids. This suggests that the use of antioxidants such as vitamin E, melatonin and estrogens may be beneficial in AD [45, 47].

Epilepsy may involve mitochondrial dysfunction which may contribute to neuronal damage during seizures, as in the case of myoclonic epilepsy and generalized tonic-clonic seizures. The OXPHOS defects, reduced ATP production, free radical generation and altered Ca^{2+} handling may all contribute to neuronal damage and epileptogenesis [45].

Neuromuscular Disorders

Whereas nuclear mutations can affect genes encoding enzymatic or structural mitochondrial proteins, translocases, mitochondrial protein importation, and intergenomic signalling, mtDNA mutations fall in three main categories: sporadic rearrangements (deletions/duplications), maternally inherited rearrangements (duplications), and maternally inherited point mutations.

Disorders Due to Defects of mtDNA

Several mtDNA-related diseases are frequent. The mitochondrial genetic code differs from Mendelian genetic code in several ways: polyplasmy, which means there are several genomes in each mitochondrin (5-10); heteroplasmy, means that a mutation may affect all or only one mtDNA genome; threshold effect, which requires a minimal critical number of mutant mtDNAs to express an alteration; mitotic segregation, since at cell division, the proportion of mutant mtDNA in daughter cells may vary, as does maternal inheritance. These alterations include: a) sporadic rearrangements of mtDNA, which are single deletions of duplications. There are three main clinical syndromes: Kearns-Sayre syndrome, a subtype of progressive external ophthalmoplegia (PEO) with early onset (before 20 years), limb weakness and fatigue; Pearson syndrome, which is manifested in infancy as a severe hematopoietic disorder with sideroblastic anemia and exocrine pancreas dysfunction, and sporadic PEO with ragged-red fibers (RR) [48, 49]; b) maternally inherited rearrangements of mtDNA. Although there is no evidence that single mtDNA deletions are inherited, there are a few disorders in which duplications/deletions are maternally transmitted. These conditions are usually associated with diabetes and myopathy [48]; c) point mutations of mtDNA.

Over 50 pathological point mutations have been documented, including myoclonus epilepsy with RR fibers, characterized by myoclonus, generalized seizures, cerebellar ataxia, and myopathy. In this condition, muscle biopsy shows RRF, which are typically COX negative; also included in this group is mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), characterized by stroke-like episodes with hemiparesia or hemianopia, before 40 years and often in childhood. Common features include generalized seizures, migraine-like headache and vomiting, and dementia. There is also a mutation in the $tRNA^{Leu(UUR)}$ associated with myoclonic epilepsy with ragged red fibers (MERRF) showing myopathy and myoclonus and generalized seizures. An

alteration in $tRNA^{Lys}$ produces neuropathy, ataxia, retinitis pigmentosa (NARP) consisting in a multisystem disorder of young adult life comprising neuropathy, ataxia, seizures, dementia and retinitis pigmentosa. An alteration in ATPase 6 leads to the maternally inherited Leigh syndrome (MILS) or a more severe condition than NARP syndrome manifested in infancy by developmental delay, hypotonia, seizures, pyramidal signs, ataxia, retinitis pigmentosa, and with the neurological factors of LS. There are several biochemical conditioning including a pyruvate dehydrogenase deficiency and OXPHOS defects, and Leber hereditary optic neuropathy (LHON), which causes loss of vision in young adults, and is associated with a C-I deficiency [48, 49].

Disorders due to defects of nDNA

This group of pathologies includes: a) defects of genes encoding enzymatic or structural proteins. Many neuromuscular or generalized syndromes are due to mutations of mtDNA-encoded subunits of the ETC. Examples of this situation are three pediatric syndromes associated with C-IV deficiency. One of them is Leigh syndrome, a devastating encephalopathy with characteristically symmetric lesions of the basal ganglia and the brain stem. COX deficiency appears to be the most common [48]. Two other syndromes associated with COX deficiency are tissue-specific and cause severe generalized myopathy in infancy: one of them is invariably fatal within a year (fatal infant myopathy), whereas the other is spontaneously reversible (benign infantile myopathy), implying a COX subunit. Another Mendelian disease which is due to an ETC chain defect, a primary ubiquinone (CoQ10) deficiency, responds well to CoQ10 administration [48]; b) disorders due to defects in translocases, proteins that are essential for the trafficking of metabolites across the inner membrane. Most frequent is the defect of carnitine-acylcarnitine translocase and deficiencies in ANT, mainly of the subunit ANT1. Children with dysmorphic features, hypotonia, developmental delay, seizures and hydrocephalus show deficits in the outer membrane protein porin, also called the voltage-dependent anion channel (VDAC). In this case, pyruvate oxidation and ATP synthesis are decreased in muscle mitochondria. [48]; c) disorders due to defects in mitochondrial protein importation. Transporting proteins to mitochondria is accomplished through targeting signals localized at the N-terminus of polypeptides. Some alterations involving this disorder have been described, although considering the complexity of the multistep process involving protein import, it seems likely more defects will be uncovered [48]; d) disorders due to defects of intergenic signalling. In this group, the primary genetic lesion is in the nDNA but the consequence of the nuclear mutation is an abnormality of mtDNA. Included are defects in mtDNA replication, including deletions that cause congenital myopathy or hepatopathy. The severity depends on the quantity of mtDNA remaining. Depletion of 60-80% mtDNA is usually associated with myopathy starting at 1 year of age and causing respiratory failure and death in childhood. Serum creatine kinase is usually elevated, which is an unusual finding in other mitochondrial myopathies. Another group includes multiple mtDNA deletions, with paralysis of eye muscles and associated in some cases to exercise intolerance, hearing loss and psychosis. A special from of this disease is the MNGIE (mitochondrial neurogastrointestinal encephalomyopathy) syndrome showing both strong gastrointestoinal dysfunction and peripheral neuropathy [48].

In all mitochondrial encephalomyopathies, cells die because the lack of an adequate energy supply and the decrease in $\Delta\psi_m$ which triggers PTP and apoptosis, although small amounts of the non-mutant genome seem to be sufficient to protect tissue from defects of ETC. In neurons, inability to maintain adequate ATP levels leads to a partial neuronal depolarization and excitotoxicity, and muscle cells seem to die mainly by apoptosis [48, 50].

Mitochondria and Aging

Two views have been inked to genetic models of aging. One is that aging is a genetically programmed event. Specific aging genes, functioning as hierarchical clocks, might exist to cause aging and death of the individual. The alternative view is that environmental insults and/or endogenous ROS and reactive nitrogen species (RNS) may cause genetic damage and mutations [51]. The proposal that free radicals, produced by normal aerobic metabolism, cause, at subcellular locations, random tissue damage that impairs cellular function and proliferative capacity was proposed as a cause of aging by Harmon in 1956 [52]. Aging is then the result of the failure of various protective mechanisms to counteract the radical-induced damage [51].

The mitochondrial theory of aging states that the accumulation of impaired mitochondria is the driving force of the aging process [53-56]. This theory has gained new support in recent years with the discovery of age-related mtDNA delections. The mtDNA inherited variability could play a role in successful aging and longevity in humans [57], whereas continuous damage to mtDNA leads to a bioenergetic crisis. It has been demonstrated that the levels of mitochondrial transcripts in *Drosophila* during aging are significantly reduced, which means that the ability of mtDNA to perform transcriptional activity decreases [58]. However, an increase in mtDNA damage in response to oxidative stress in human cells has been recently reported [59]. Experimental accumulation of mtDNA deletion mutations have been observed in several species including mice and humans [60]. Additionally, some genes encoded by nDNA involved in aging have been identified [51].

There is increasing consensus that ROS and RNS are a major cause of aging [55, 61]. Aging is accompanied by structural changes in mitochondria including their reduction in number and increase in size (97), and a decrease in C-IV and C-V activities. These changes may impair energy-dependent neurotransmission, contributing to senescent decline in memory and other brain functions [62, 63]. The mutation rate of mtDNA is much higher than that of nDNA because expression of the whole genome is essential for the maintenance of mitochondrial bioenergetic function, while only about 7% of the nuclear genome is expressed at any cell differentiation [63]. Oxidative injury is not limited to mtDNA but also occurs in mitochondrial membranes. This may lead to a progressive lipid peroxidation and

crosslinking damage, with concomitant changes in respiration rate, ATP synthesis, membrane fluidity and permeability, Ca^{2+} homeostasis and apoptosis. The free radical theory of aging provides a rationale for intervention by means of antioxidant administration [64, 65]. In fact, mitochondrial aging may be due to chronic oxidative stress, with and the $O_2^{-\bullet}$ generated by mitochondria leading to the formation of other ROS/RNS [66, 67]. All these oxidants reduce GSH availability thereby producing oxidative damage to mtDNA, lipids and proteins, which is manifested as mitochondrial aging and in turn, cell aging.

Mitochondria repair mechanisms

The increase in mitochondrial mass and mtDNA content are the early molecular events of human cells in response to oxidative stress [59]. Most of the O_2 taken up by cells is reduced to water via the action of mitochondrial C-IV by the addition of 4 electrons per O_2 molecule. The intermediate steps of O_2 reduction are formation of $O_2^{-\bullet}$, H_2O_2 and hydroxyl radical ($HO^{\bullet}$), corresponding to reduction by one, two and three electrons, respectively. NO and its metabolite $ONOO^-$ are RNS produced in the mitochondria. Mitochondrial DNA is not protected by histones and lies in close proximity to the free radical-producing ETC. Primary (mtDNA) or secondary (nDNA) mitochondrial mutations and/or changes in ROS production may induce mitochondrial damage which is the basis of aging and several conditions including neurodegenerative diseases and mitochondriopathies.

A large number of DNA base modifications caused by oxidative stress have been detected. On of the most widely studied is 8-hydroxydeoxyguanosine (8-oxo-dG). This mutagenic lesion also accumulates with age. Mitochondria defend against oxidative stress by two main mechanisms: eliminating ROS/RNS (antioxidants and scavengers) and repairing the damaged molecules. The former include SOD which actively dismutates $O_2^{-\bullet}$ to H_2O_2, which in turn is transformed to water by glutathione peroxidase (GPx). In this process, GSH is oxidized to GSSG and the enzyme GRd restores GSH levels. The glutathione recycling system is very active in mitochondria because these organellos do not synthetize GSH and they do not possess catalase. Thus, they mainly depend on their own GSH pool, although they can also import GSH from cytosol. ROS/RNS are involved in tissue injury associated with aging and a number of inflammatory and neurodegenerative diseases. In addition, mitochondrial respiratory chain deficiencies lead to overexpression of antioxidant enzymes [68]. Thus, under physiological conditions, an equilibrium should exist between the mechanisms generating and scavenging $O_2^{-\bullet}$ and other ROS.

Endogenous metabolic processes generate ROS yielding oxidized bases that are removed from the DNA mainly by the base excision repair (BER) pathway. Adducts due to UV exposure are removed by the nucleotide excision repair (NER) pathway [69]. Mitochondria are able to carry out BER. The first repair enzyme detected was uracil DNA glycosylase. Homologes of the yeast repair enzymes, OGG1, which excise 8-oxo-dG from DNA, have been found in mouse and human mitochondria. The formamidopyridine DNA glycosylase, an enzyme that deletes 8-oxo-dG, has been reported in rat hepatic mitochondria. Removal of 4-nitroquinoline lesions from mtDNA, have also been reported; these are generally removed via NER pathways. However, NER as it exists in the nucleus, does not exist in mitochondria, and thus, the role of NER protein in mitochondrial repair remains unclear [69]. It was found that the endonucleolytic activity of the enzyme that specifically cleaves 8-oxo-dG oligonucleotides is higher in 12 and 23-month old rats than in 6-month old rats. Thus, the mitochondrial capacity to repair 8-oxo-dG seems to increase with age [69].

Melatonin and mitochondrial pathology

In the last decade, an increasing amount of evidence supports new roles and mechanisms for the actions of melatonin. The actions of melatonin depend on receptor- and non-receptor-mediated processes, the latter accounting for the antioxidant properties of melatonin. Receptor-mediated events for melatonin involve both membrane and nuclear receptors [70-73], and the existence of a membrane-nuclear signaling pathway has been proposed [74]. Some of the protective effects of melatonin on the cell seem to be mediated by genomic regulation, and some genes, including the 5-lipoxygenase gene in the human B lymphocyte, seem to be regulated by melatonin [75]. In addition, the expression of some genes, mainly related to the cell redox and inflammatory responses including GPx, GRd, SOD, inducible nitric oxide synthase (iNOS) and cytokines are also under genomic regulation by melatonin [76-78]. In addition, the specific binding of melatonin to Ca^{2+}-calmodulin (CaCaM) appears to regulate some CaCaM-dependent enzymes such as nNOS [79, 80]. The recent discovery of the mitochondrion being a target for melatonin action opens new perspectives to understand the mechanism of action of melatonin, and may help to explain the antiapoptotic and thermogenic effects of the indoleamine [81, 82].

In a number of experimental and clinical situations, a beneficial effect of melatonin has been reported in those pathologies which involve mitochondria dysfunction as a primary or secondary cause of the disease, including ROS-induced DNA damage, excitotoxicity and neurodegenerative diseases such as PD, AD and epilepsy, and aging [83-88]. Melatonin's ability to counteract excitotoxicity and ROS-induced DNA damage has been described under a variety of different experimental paradigms. Melatonin prevents DNA damage in human blood cells exposed to ionizing radiation, and reduces genetic damage in lymphocytes which were exposed to ionizing radiation after their removal from individuals who had consumed melatonin [89]. Oxidation of guanine bases in DNA from rat liver induced by whole body ionizing radiation also was prevented by melatonin administration [90]. Furthermore, the DNA damage caused by the chemical carcinogen safrole or by chromium is reduced by melatonin [91, 92]. Using the comet assay, it was shown that treatment with melatonin reduced neural DNA fragmentation induced by exposure in rats to extremely low frequency magnetic fields [93]. Melatonin counteracts

paraquat-induced genotoxicity in mice [94], as well as ferric nitrilotriacetate-induced DNA damage and H_2O_2-induced DNA damage in U-937 cells [95, 96]. The protective effects of melatonin against DNA damage was shown by measuring the 8-oxo-dG levels in the brain of kainic acid-treated rats [97].

When DNA repair mechanisms are induced, the activation of the nuclear enzyme PARS triggers an energy-consuming repair cycle reducing cellular NAD^+ levels. This also occurs in rats treated with zymosan, a non-bacterial agent causing cellular injury by inducing the production of $ONOO^-$ and consequent PARS activation. In this case there is also an inhibition of mitochondrial respiration due to $ONOO^-$. The administration of melatonin protects against cellular energy depletion and prevents the appearance of DNA damage [98]. Renal and hepatic DNA damage induced by the carcinogen δ-aminolevulinic acid was assessed by measuring the levels of 8-oxo-dG; these levels were reduced by melatonin [99, 100]. Rat lung and spleen concentrations of 8-oxo-dG induced by δ-aminolevulinic acid are also lowered by melatonin [101].

Interesting findings were described when the effect of melatonin on mitochondrial membrane fluidity was tested. Mitochondrial membrane fluidity decreased after the animals were treated with δ-aminolevulinic acid with these changes being prevented by melatonin co-treatment [99]. However, no changes in mitochondrial membrane lipid peroxidation (LPO) levels were reported and thus, the effects of melatonin on mitochondrial membrane fluidity may be independent of its ability to counteract lipid damage [99, 100]. The effects of melatonin in maintaining optimal membrane fluidity of mitochondrial membranes may depend on its ability to localize into the membrane itself, in a superficial position in lipid bilayers near the polar heads of membrane phospholipids. In this position melatonin would be near the mitochondrial proteins which then would be protected from ROS. It should be note that δ-aminolevulinic acid damage to mitochondria results in the disruption of the $\Delta\psi_m$ and enhanced permeability [101, 102] leading to decrease of ATP, PTP opening and apoptosis. Thus, melatonin may protect protein complexes in the inner mitochondrial membrane and thereby improve ETC.

A recent series of experiments have provided strong evidence for the antiexcitotoxic properties of melatonin both in vivo and in vitro. Anticonvulsant activity of melatonin was initially shown to be related to its effects on both brain GABA-benzodiazepine receptor complex and Na^+, K^+-ATPase activity [103-107]. However, due to the inhibitory effect of melatonin on the NOS/NO system, an effect of the indoleamine on glutamate-induced excitotoxicity was soon proposed. A melatonin deficiency was related to increased brain damage after stroke or excitotoxic seizures in rats [108], and an anticonvulsant activity of melatonin against seizures induced by a variety of drugs in mice was reported [109]. Melatonin protects cultured cerebellar neurons from kainate excitotoxicity [110]. Quinolinic acid, a neuroactive metabolite of tryptophan implicated in some neurodegenerative diseases [111], induces neuronal degeneration when injected into animals, an effect counteracted by melatonin administration [112].

Electrophysiological experiments document the antagonism of melatonin on the N-methyl-D-aspartate (NMDA) receptor involved in excitotoxicity [113-116]. The effect of melatonin was specific, dose-dependent and was independent of melatonin receptors. Thus, an intracellular action of melatonin in inhibiting NMDA-dependent excitotoxic events was further demonstrated with synthetic kynurenamines suggesting an inhibition of the NOS/NO system, the main mediator of glutamate-dependent excitotoxicity [117-119]. The effects of melatonin against brain excitotoxicity were the basis for the clinical use of melatonin in infantile seizures [120, 121]. Melatonin also protects against excitotoxicity by reducing the autoxidation of dopamine (DA) which occurs in some degenerative diseases such as PD [122]. These effects were documented in 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced PD in mice [123, 124]. The effect of melatonin in reducing DA autoxidation was tested elsewhere and it showed a greater potency than other antioxidants including vitamin E and C, and that of L-deprenyl, a monoamine oxidase (MAO) B inhibitor which also has antioxidant properties [122].

The neuroprotective effects of melatonin were also tested against neurodegenerative manifestations in AD [125]. When neuroblastoma cells were incubated with β-amyloid, more than 80% of the neurons died due to apoptosis, but the presence of melatonin reduced cellular death and DNA damage in a dose-related manner [126]. In human platelets melatonin also protected against AB-induced damage [127, 128]. The protective properties of melatonin were extensively tested in models of aging, which involves extensive cell damage. In different models of aging and age-related diseases including cancer and cataracts, melatonin administration has been shown to be protective. The fact that melatonin decreases with age was then suggested as one of the potential causes of aging in mammals [87, 129-137].

The importance of melatonin as an antioxidant depends from several characteristics; it is both lipophilic and hydrophilic and it passes all bio-barriers with ease. It is available to all tissues and cells, where it scavenges free radicals [138-141]. Melatonin distributes in all subcellar compartments, being especially high in the nucleus and mitochondria. This means that melatonin is available at the sites in which free radicals are being generated, thus decreasing their toxicity [142, 143]. Melatonin, identified by Lerner as a product of the mammalian pineal gland [144], is also found in several tissues including the retina, cells of the immune system, gut, bone marrow, ovary and testes [145-152]. It seems that these tissues may produce melatonin required for antioxidant regulation [153], since this melatonin does not enter the circulation. Also, most of these tissues have much higher levels of melatonin than those in the blood. Levels of melatonin 2-3 orders of magnitude higher than maximal blood melatonin concentrations are present in bile [154]. Another fluid that contains very high levels of melatonin is the cerebrospinal fluid (CSF) [155].

It was recently reported that expression of the genes of the key enzymes for melatonin synthesis, i.e., N-acetyltransferase (NAT) and hydroxyindole-O-methyl-transferase (HIOMT), are found in many organs [156]. Thus,

these organs may synthesized their own melatonin and do not depend on the circulation to provide this indoleamine. This suggests that each organ may in part produce the melatonin that it needs independently of its the circulating levels. Thus, the concept of what constitutes a physiological level of melatonin is changing, and physiological levels must be defined based on specific fluids and subcellular organelles.

Melatonin and Apoptosis

The observation that melatonin influences apoptotic cell death is a documented regulatory effect of melatonin on cell survival. The possibility that the antioxidant properties of melatonin account for inhibition of apoptosis was investigated in vivo and in vitro by measuring DNA fragmentation. These experiments showed that melatonin administration counteracts apoptosis in rat thymus. In cultured thymocytes, 1 nM of melatonin decreased cell death by 20% [157]. It was suggest that melatonin down-regulates the glucocorticoid receptor in thymocytes, which may explain its antiapoptotic effect in the thymus [158]. In primary cultures of cerebellar granule neurons, melatonin protects them from singlet oxygen-induced apoptosis [159]. Melatonin also inhibited pre-B-cell apoptosis during lymphopoiesis in mouse bone marrow; this has implications for neoplasia since boosting the formed B cells would have effects on humoral immunity [160]. Melatonin was also shown to protect bovine cerebral endothelial cells from hyperoxia-induced DNA damage and apoptotic death [161].

Since apoptosis is a possible mechanism involved in the neuronal death described in several neurodegenerative diseases such as PD, AD and epilepsy, it would be expected that melatonin may exert antiapoptotic effects in these diseases. In fact, in neuroblastoma cells exposed to the Alzheimer β-amyloid peptide, melatonin prevented cell death [126]. Melatonin also prevents apoptosis induced by MPTP in the mouse [124] and by 6-hydroxydopamine in PC12 cells [162]; these finding could be of potential clinical importance in the treatment of PD. Melatonin also abrogated cell death induced by cystamine pretreatment of PC12 cells; cystamine treatment involves mitochondrial iron sequestration [163]. The age-associated accumulation of redox-active iron in subcortical astrocytes may facilitate the bioactivation of DA to neurotoxic free radical intermediates and thereby predispose the nervous system to PD and other neurodegenerative diseases. Melatonin counteracts very efficiently DA autoxidation, by reducing iron-dependent ROS production by mitochondria [122]. In rats injected with kainic acid to produced excitotoxicity-induced apoptotic cell death, melatonin significantly attenuated apoptosis, an effect linked to the reduction in oxidative damage and an increased GSH content [164]. In a spontaneous, age-induced model of apoptosis using cerebellar granule cells, it was shown that melatonin and Ca^{2+}-channel blockers such as amlodipine, inhibited spontaneous apoptosis [165]. The antagonism between melatonin and Ca^{2+}-channels was also demonstrated in electrophysiological and binding experiments [116]. Striatal neurons growing in low density culture on serum-free medium and in the absence of glia die within 3 days by apoptosis. The presence of melatonin rescues striatal neurons from impending cell death, which may have important consequences in neurodegenerative diseases involving the nigrostriatal pathway such as in PD [166].

The relation of melatonin to cell death was tested in several cell cancer models as well. In an ovarian carcinoma cell line it was found that melatonin exerts an oncostatic action linked to a nuclear effect of the indoleamine, since the melatonin nuclear receptor agonist CGP 52608 caused a similar effect [167]. Interestingly, melatonin seems to enhance apoptosis in carcinoma cells, as has been demonstrated with Ehrlich ascites carcinoma cells. In this case, changes in GSH were not detected during the proapoptotic effects of melatonin [168]. Similarly, in colon mucosa and colon tumors induced by 1,2-dimethylhydrazine in rats, melatonin behaves as a potent stimulator of apoptosis [169]. It was recently shown that melatonin also inhibited LOOH-triggered cell death, in a similar manner to that of CsA, an inhibitor of the permeability transition pore [170]. While melatonin counteracted H_2O_2-induced DNA damage in U-937 cells [96], other authors were unable to confirm the antiapoptotic role of melatonin against 7-ketocholesterol-induced apoptosis in the same cell type, although melatonin prevented $O_2^{-\bullet}$ generation by mitochondria [171]. In general, it seems that the antioxidant and to some extend the GSH-enhancing effects of melatonin may account for melatonin's antiapoptotic activity in non-cancerous cells.

Melatonin Actions on Mitochondria

Three main considerations suggest a role for melatonin in mitochondrial homeostasis. First, the mitochondrion is the organelle with the highest ROS/RNS production in the cell, and melatonin is a powerful scavenger of ROS and RNS. Secondly, mitochondria depend on GSH uptake from the cytosol, although they have GPx and GRd to maintain the GSH redox cycling; melatonin improves GSH redox cycling and increases GSH content by stimulating its synthesis in the cytosol. Third, melatonin exerts important antiapoptotic effects (Fig. **1**) and most of the apoptotic signals originate from the mitochondria [81].

The relationships between melatonin and mitochondria have been known for several years, but to date the existence of a specific role of the indoleamine in mitochondrial homeostasis remains enigmatic. Following the lines of evidence that an aerobic organism entered an anaerobic one, the subsequent symbiosis had beneficial consequences for the two organisms [172]. However, the anaerobic one had an unexpected problem, i.e., oxygen is highly toxic and oxidizes many molecules of the host. The hybrid organisms had to acquire new antioxidant mechanisms not only to protect itself from O_2 toxicity but also to preserve the enzymatic machinery required to produce ATP highly efficiently. Also, when ROS produced by the guest were excessive, the host organism evolved a trigger to initiate mitoptotic signals to eliminate the damaging symbiotic organelle [3]. Since melatonin was present in the invading unicellular organisms, what was the function of melatonin in the mitochondria of multicellular organisms?

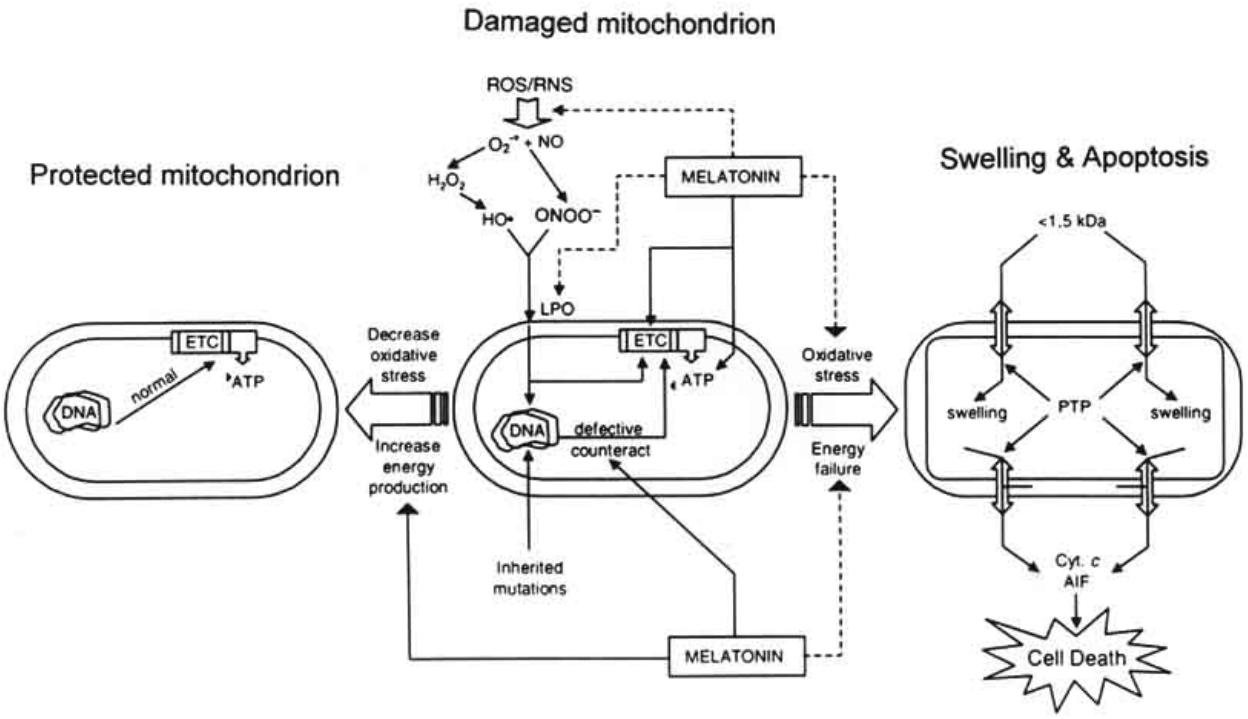

Fig. (1). Schematic representation of the effects of melatonin on the mitochondrion. Nuclear and/or mitochondrial DNA mutations lead to a defective electron transport chain (ETC) and subsequent energy depletion. Energy depletion can also result from free radical-induced mtDNA and/or ETC damage. Oxidative stress and ATP depletion favor opening of the mitochondrial permeability transition pore (PTP), which in turn induces mitochondrial swelling and release of proapoptotic factors such as cytochrome *c* and AIF (apoptosis-inducing factor). These proapoptotic factors activate a caspase cascade in the cytosol leading to cell death. Melatonin scavenges free radicals and other reactive species including H_2O_2 and the peroxynitrite anion ($ONOO^-$) and reduces lipid peroxidation (LPO), thus lowering free radical damage to mtDNA and the ETC. Moreover, melatonin improves the activity of the ETC complexes I and IV and ATP synthesis. Finally, melatonin increases transcriptional activity of mtDNA, improving mitochondrial physiology. As a consequence of the effects of melatonin, the mitochondrion is protected and recovers almost normal function thereby avoiding PTP opening and apoptosis.

Chronic melatonin administration increases the number and size of mitochondria in the pineal and ependymal epithelium of choroids plexuses [173, 174]. Binding experiments with ^{125}Iodomelatonin also revealed a high percentage of specific binding sites in the mitochondrial fraction of the pigeon brain and in spleens of guinea pigs [175, 176]. In the hamster hypothalamus, higher binding of ^{125}Iodomelatonin was recorded in the mitochondrial pellet than in the nuclear pellet [177]. Soon thereafter, it was shown that melatonin influenced mitochondrial activity throughout the circannual cycle [178]. Milczarek [179] showed that melatonin inhibits NADPH-dependent lipid peroxidation in human placental mitochondria. Melatonin protects fetal rat brain against oxidative mitochondrial damage [180]. Finally, a protective effect for melatonin against the MPP^+-induced inhibition of C-I of the ETC was also shown [181].

The ability of melatonin to influence mitochondrial homeostasis was initially tested in vivo. In this study it was shown that the injection of melatonin into normal rats significantly increased the activity of the complex C-I and C-IV of the mitochondrial ETC measured in mitochondria obtained from brain and liver, whereas the C-II and C-II were unaffected [182]. Melatonin also counteracted ruthenium red-induced inhibition of the C-I and C-IV in brain and liver mitochondria when melatonin was given simultaneously with ruthenium red [182].

To further test the antioxidant ability of melatonin against mitochondrial oxidative stress, in vitro experiments with isolated mitochondria prepared from rat brain and liver were performed. Oxidative stress was induced by incubation of these mitochondria with t-butyl hydroperoxide (t-BHP), which oxidizes pyridine nucleotides and depletes the mitochondrial GSH pool and inhibits both GPx and GRd activities [183]. In this situation, 100 nM melatonin counteracted these effects, by restoring basal levels of GSH and the normal activities of both GPx and GRd. N-acetylcysteine (NAC) and vitamins E and C were unable to exert any significant effect on t-BHP-induced oxidative stress in mitochondria despite the high doses of these compounds used [184]. Interestingly, melatonin increased the activity of C-I and C-IV in a dose-dependent manner, the effect being significant at 1 nM melatonin [184]. Melatonin was also able to counteract cyanide-induced inhibition of the C-IV, and restored the levels of cyt $a+a_3$. Melatonin also increased the activity of isolated C-I by blue native polyacrylamide gel electrophoresis (PAGE). These effects of melatonin are of physiological significance since the indoleamine increased the ETC activity coupled to OXPHOS, which was reflected in an increase of ATP

synthesis, either in normal mitochondria or in mitochondria depleted of ATP by cyanide incubation (D. Acuña-Castroviejo *et al.*, unpub-lished observations).

These results suggest a direct effect of melatonin on mitochondrial energy metabolism (Fig. **1**), providing a new homeostatic mechanism regulating mitochondrial function [81, 137, 182, 184]. First, melatonin scavenges H_2O_2 [140] the most important ROS produced into the mitochondria from $O_2^{-\bullet}$, This reduces the loss of the intramitochondrial GSH pool and lowers mitochondrial damage [99, 100]. This effect is also supported by the observation that melatonin increases mitochondrial membrane fluidity thereby at least partially protecting against protein oxidative damage. Due to the high content of proteins in the inner mitochondrial membrane, this effect of melatonin may also account for the improvement in ETC activity. Second, improving mitochondrial respiration and ATP synthesis increases the rate of electron transport across the ETC and reduces ROS production. Due to the high redox potential of melatonin [141], this molecule may donate an electron to C-I of the ETC. Thus, melatonin improves ETC and reduces mitochondrial oxidative damage. These effects reflect an ability of melatonin to reduce the harmful decrease in $\Delta\psi_m$ that may trigger PTP opening and the apoptotic cascade. Another important consequence of the effects of melatonin on mitochondria is its role in thermogenesis [82]. Since the data indicated that melatonin exerts an opposite effect to UCPs, melatonin reduces heat production by mitochondria and induces a more efficient use of substrates in terms of ATP production.

An important question becomes apparent from these data. If melatonin improves OXPHOS and ATP synthesis, does melatonin exert some effect on mtDNA transcriptional and/or translational activity? It was shown in tumor cell studies that melatonin exerts an oncostatic effect unrelated to nascent DNA synthesis [185]. However, when melatonin was added to cultured J774 macrophages, the indoleamine reduced the suppression of mitochondrial respiration and inhibited the development of DNA single strand breaks in response to $ONOO^-$ [186]. In another set of experiments, it was shown that melatonin administration prevented oxidative degradation of mtDNA and reduction of mtDNA transcripts in several tissues including liver, heart, skeletal muscle and brain [187, 188]. In addition, a direct effect of melatonin on mitochondrial genome expression in brown adipocytes of Siberian hamster was documented [189].

Because of these findings, we performed a series of experiments to analyze the possible effects of melatonin of the expression on the mtDNA encoded polypeptide subunits of the C-IV in both in vivo and in vitro. Starting from the mtDNA-encoded subunits I, II and III of the C-IV, a quantitative analysis of the mRNAs of these subunits by means of quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed (D. Acuña-Castroviejo *et al.*, unpublished). Rats were intraperitoneally injected with melatonin (10 mg/kg body weight) or vehicle and sacrificed at different times after treatment to obtain the livers used for the determinations. The results show a significant increase in the expression of the mRNAs for the three subunits tested. The increases in the mRNA content were time-dependent, peaking at 90 min after melatonin injection. This time-dependence of melatonin action agrees well with the time-dependent changes in complex IV activity after melatonin injection into rats as reported elsewhere [182]. Thus, it seems that mtDNA transcriptional activity and complex IV enzyme activity are two melatonin-related events. To further analyze the effects of melatonin, another set of rats was intraperitoneally injected with melatonin (10 mg/kg) or vehicle for 10 days; at this point their livers were used for mitochondria preparations. Fresh mitochondria were incubated as described [190], and incorporation of labelled UTP into mRNA was analyzed. The results showed that the animals treated with melatonin have lower mRNA levels than the controls, an effect partially counteracted in pinealectomized animals. Given the effect of melatonin on ATP production [81] and the effect of ATP on mitochondrial mRNA synthesis [190], a 10-day regimen of melatonin treatment in rats may increase significantly ATP production which in turn inhibits mRNA synthesis.

CONCLUDING REMARKS

The observations described herein suggest that melatonin acts as a coupling agent in mitochondria to reduce heat production, to increase ATP synthesis, and to increase mtDNA expression. These effects may be the basis for the alleged anti-aging properties of melatonin. The reduction of melatonin levels during aging [191] presumably promotes an increase in oxidative stress [135] that impairs mitochondria metabolism, favoring apoptosis. Additionally, the age-dependent reduction in mtDNA transcriptional activity may also partially depend on the age-dependent loss of melatonin. Additionally, the neuroprotective properties of melatonin in many degenerative disorders which exhibit mitochondrial alterations may also relate to melatonin's mitochondrial homeostatic role. Considering this, melatonin may be useful for the treatment of some mitochondrial dysfunctions which involve mtDNA damage and/or other mitochondriopathies. The mitochondria are now considered a potentially important target for drug delivery, and strategies to prevent mitochondrial damage or to manipulate mitochondrial function may provide new therapies for these disorders [192]. Moreover, the application of antioxidant therapy in oxidative stress-related diseases is now of increasing clinical interest [193, 194]. On the basis of data summarized in this report, melatonin becomes an interesting pharmacological tool in mitochondrial-related diseases since it easily reaches the mitochondria, it regulates the mitochondrial redox status and mtDNA transcriptional ability, and it is metabolized to other compounds with strong antioxidant ability [195]. Finally, the lack of significant toxic effects of melatonin treatment at pharmacological doses [120, 196], allows for a wide margin of safety in clinical trials.

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ABBREVIATIONS

8-oxo-dG	=	8-Hydroxydeoxyguanosine
AD	=	Alzheimer's disease
AIF	=	Mitochondrial apoptosis-inducing factor
ANT	=	Adenine nucleotide translocase
BER	=	Base excision repair mechanism
CaCaM	=	Calcium-camodulin complex
C-I, C-II, C-III, C-IV	=	Complex I, II, III and IV of the etc.
CoQ10	=	Ubiquinone
COX	=	Cytochrome oxidase (complex IV of the ETC)
CsA	=	Cyclosphorin A
Cyt	=	Cytochrome
DA	=	Dopamine
$\Delta\mu_{H^+}$	=	Mitochondrial electrochemical proton potential
$\Delta\psi_m$	=	Mitochondrial membrane potential
ETC	=	Mitochondrial electron transport chain
FA	=	Friedreich's ataxia
GPx	=	Glutathione peroxidase
GRd	=	Glutathione reductase
GSH	=	Glutathione
GSSG	=	Glutathione disulfide
H_2O_2	=	Hydrogen peroxide
HD	=	Huntington's disease
HIOMT	=	Hydroxyindol-*O*-methyl-transferase
$HO^{\bullet}$	=	Hydroxyl radical
HSP	=	Hereditary spastic paraparesis
iNOS	=	Inducible nitric oxide synthase
LHON	=	Leber hereditary optic neuropathy
LPO	=	Lipid peroxidation
MAO	=	Monoamine oxidase
MELAS	=	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF	=	Myoclonic epilepsy with ragged red fibers
MILS	=	Maternally inherited Leigh syndrome
MNGIE	=	Mitochondrial neurogastrointestinal encephalomyopathy
MPTP	=	1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine
mtDNA	=	Mitochondrial DNA
mtEF	=	Mitochondrial elongation factor
mtIF-2	=	Mitochondrial translation initiation factor
mtTERM	=	Factor mediating attenuation of mitochondrial transcription
mtTFA	=	Mitochondrial transcription factor
NAC	=	*N*-acetyl cystein
NAT	=	*N*-acetyl-transferase
nDNA	=	Nuclear DNA
NER	=	Nucleotide excision repair mechanism
NMDA	=	*N*-methyl-*D*-aspartate
nNOS	=	Neuronal nitric oxide synthase
NO	=	Nitric oxide
$O_2^{-\bullet}$	=	Superoxide anion
$ONOO^-$	=	Peroxynitrite
OXPHOS	=	Oxidative phosphorilation
PAGE	=	Polyacrylamide gel electrophoresis
PARS	=	Poly-(ADP-ribose) synthetase
PD	=	Parkinson's disease
PEO	=	Progressive external ophthalmoplegia
PTP	=	Mitochondrial permeability transition pore
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
RR	=	Ragged-red fibers
RT-PCR	=	Reverse transcription polymerase chain reaction
SOD	=	Superoxide dismutase
T_3	=	Triiodo tyronine
t-BPH	=	t-Butyl hydroperoxide

Tim = Translocase of the inner mitochondrial membrane

Tom = Translocase of the outer mitochondrial membrane

UCP = Uncoupling proteins

VDAC = Voltage-dependent anion channel

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