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Melatonin and Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It is characterized by a progressive loss of dopamine in the substantia nigra and striatum. However, over 70% of dopaminergic neuronal death occurs before the first symptoms appear, which makes either early diagnosis or effective treatments extremely difficult. Only symptomatic therapies have been used, including levodopa (L-dopa), to restore dopamine content; however, the use of L-dopa leads to some long-term pro-oxidant damage. In addition to a few specific mutations, oxidative stress and generation of free radicals from both mitochondrial impairment and dopamine metabolism are considered to play critical roles in PD etiology. Thus, the use of antioxidants as an important co-treatment with traditional therapies for PD has been suggested. Melatonin, or *N*-acetyl-5-methoxy-tryptamine, an indole mainly produced in the pineal gland, has been shown to have potent endogenous antioxidant actions. Because neurodegenerative disorders are mainly caused by oxidative damage, melatonin has been tested successfully in both in vivo and in vitro models of PD. The present review provides an up-to-date account of the findings and mechanisms involved in neuroprotection of melatonin in PD.

Key Words: Melatonin; Parkinson's disease; antioxidant; oxidative damage; dopaminergic neurons; neurodegeneration.

Introduction

First described by James Parkinson in 1817, Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting roughly 1.8% of people over 65 *(1)*. PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) in the midbrain and a subsequent loss of dopamine. It is clinically manifested by defective motor function, a decline in cognitive function, and depression. Histologically, its features include the presence of Lewy bodies and cytoplasmic inclusions that are composed predominantly of fibrillar α-synuclein *(2)*. Most biochemical studies suggest that, directly or indirectly, reactive oxygen/nitrogen species (ROS/RNS) are important mediators in the pathogenesis of PD.

Given the difficulties in studying the molecular events that precede the onset of PD in patients, several in vivo and in vitro models have been developed. These models are primarily based on free-radical-generating toxins with a specific target in the nigrostriatal system. Some of the dopaminergic neuronal loss in PD has been postulated to be via apoptosis, given the morphological characteristics exhibited by what appear to be dying cells in postmortem brains of parkinsonism patients. Furthermore, in vivo experimental models of PD strongly suggest a role for apoptosis in the pathology of this human disease.

The initial original observations of PD patients reported that about 15% of PD patients had a family background of the disease. Since then, the role of genetic factors in PD has been the subject of intense research *(3)*. To date, a few specific mutations have been identified to be responsible for rare familial forms of the disease: α-synuclein, parkin, UCH-L1, DJ-1, and PINK1 are genes found to be related to PD. As is the case in Alzheimer's disease, these genetic defects seem to affect a common molecular pathway related to the ubiquitin–proteasome system *(4)* with the exception of PINK1, which is related to mitochondrial metabolism. Analysis of the products of these genes are beyond the scope of the present review and have been reviewed extensively elsewhere *(3,4)*. Some (if not all) of these mutations are partially related to free-radical generation. Thus, in addition to its genetic basis, it is widely believed that oxidative stress and free radicals play a critical role in idiopathic PD etiology.

A "Radical" View to PD

In eukaryotic cells, reactive ROS/RNS are generated as part of the normal metabolism by the chemical reduction of oxygen by cellular oxidases, peroxidases, and mono- and dioxygenases, by exposure to ultraviolet light or other environmental agents and by incomplete reduction of oxygen to water in the mitochondrial respiratory chain *(5)*.

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Cells must thus keep free-radical generation under control using cellular machinery that includes antioxidant enzymes and small scavenging molecules. However, under some conditions, there is an overproduction of ROS/RNS or a diminished level of antioxidants and the balance of free-radical production overruns the cellular antioxidant defense system and excessive damage occurs referred to as oxidative stress (OS). ROS/RNS are harmful to most macromolecules and can damage lipids, proteins, and DNA *(6)*. Halliwell and Whiteman *(7)* have defined the criteria for associating oxidative damage to a particular tissue injury: (i) OS should occur at the primary site of injury; (ii) the time course of OS should be consistent with the time course observed for the injury; (iii) OS induced by pro-oxidants should reproduce all or most of the features observed; and (iv) elimination of free radicals by inhibiting OS should diminish the injury to an extent proportional to the degree of oxidative damage observed. Considering these premises, several authors have proposed free radicals as one of the mechanisms involved in the pathogenesis of a variety of diseases, e.g., cancer and a variety of neurodegenerative conditions.

Although, as noted above, some genes are involved in PD, many research findings and clinical evidence support the involvement of free radicals and oxidative stress in dopaminergic neurodegeneration and, consequently, in PD etiology. The importance of OS as a pathogenic factor in PD is based on (i) biochemical features of dopaminergic neurons, which make them vulnerable to OS; (ii) evidence of OS in post-mortem studies including increased iron content *(8,9)* and depletion of reduced glutathione (GSH) in the brain of PD patients *(10)*; (iii) increased lipid peroxidation index *(11)*; and (iv) damage to protein and DNA *(12)*. Additionally, some clinical and biochemical studies also support the idea that other factors not directly related to OS may also be involved in the pathogenesis of PD, such as inflammation, excitotoxicity, excessive nitric oxide generation, and mitochondrial dysfunction *(13–15)*. Interestingly, these additional factors are also somehow related to OS. The presence of neuromelanin in the dopaminergic cells of SNpc is due to the autoxidation of dopamine, semiquinone formation, and further polymerization and, as a consequence, the production of free radicals *(16)*. Also, hydrogen peroxide (H_2O_2) is produced during the oxidation of dopamine by monoamine oxidase. This increase in H_2O_2 causes the oxidation of GSH, which ultimately leads to OS and the impairment of a major antioxidant system *(17)*, a feature repeatedly observed in PD. Increased concentrations of iron observed in SN of PD patients also supports the OS hypothesis, because Fe^{3+} catalyzes, via the Fenton reaction, the formation of free radicals from H_2O_2 and iron accumulation may also increase the toxicity of environmental toxins *(18)*.

Toxicity from PD-inducing toxins comes also, at least partially, from overproduction of nitric oxide (NO), which also plays an important role in neuroinflammation. NO is present both within cells and in the extracellular space surrounding dopaminergic neurons and it is produced by either neural nitric oxide synthase (nNOS) or by inducible NOS (iNOS). Under physiological conditions, NO levels are low, as are levels of the superoxide anion radical ($O_2^{\bullet -}$) or the peroxynitrite anion ($ONOO^-$) levels. However, in PD there is an increased concentration of $O_2^{\bullet -}$ caused by mitochondrial dysfunction, and NO levels are also higher resulting in $ONOO^-$ overproduction *(19)*, which impairs mitochondrial function and promotes protein nitration. Furthermore, oxidized and nitrated proteins are preferentially targeted for fast proteolytic degradation *(20)*. Proof that this might be crucial in PD comes from evidence that selective inhibitors of nNOS protects dopaminergic neurons in in vivo PD models *(21)* and nNOS knockout mice are less vulnerable to some PD-inducing toxins *(22)*. Finally, the inducible isoform, iNOS, mainly expressed after immune activation during inflammation, also seems to play a role during microglial activation *(23)*.

Models of Study for PD

Parkinson's signs begin to appear when neuronal damage exceeds a threshold of 70–80% of dopamine nerve terminals in the striatum and 50–60% of dopaminergic neurons in SNpc. Thus, only symptomatic treatments of PD such as levodopa (L-dopa) are conventionally used *(24)*, because it is usually too late to prevent neuronal death when the first symptoms of the disease are observed. Numerous research groups haves focused their attention on animal models of PD, which would eventually allow the testing of neuroprotective strategies that would identify the key molecular steps in the neurodegenerative process *(25,26)*. These animal models may provide additional clues for therapeutic strategies that slow or halt the neurodegenerative process; this could have a major impact in the treatment of PD. Nonetheless, to date, no drug has yet been established to have a neuroprotective effect nor has any drug been approved as a neuroprotective against PD *(27)*.

In 1982, Langston and co-workers *(28)* first described that young heroin users developed a rapidly progressive parkinsonian-like syndrome. It was further confirmed that the drug contained a contaminant, 1-methyl-4-phenyl-1,2,3,6-tetrahydopyridine (MPTP), which was ultimately the chemical responsible for the PD syndrome *(28)*. In humans and monkeys, MPTP elicits irreversible and severe parkinsonian-like signs characterized by all the features observed in PD, i.e., tremor, rigidity, slowness of movement, and postural instability. MPTP readily crosses the blood–brain barrier and is converted into the active toxin 1-methyl-4-phenylpyridinium (MPP^+) by monoamine oxidase B (MAO-B), present in glial and serotonergic cells. MPP^+ is a high-affinity substrate for the dopamine transporter and can be concentrated in the synaptosomal vesicles *(29)*. Additionally, MPP^+ also accumulates in mitochondria where it causes oxidative phosphorylation impairment by inhibiting complex

I of the mitochondrial electron transport chain *(30)*. These findings explain the induction of specific neurodegenerative signs in the dopaminergic neurons.

Other toxin-based models, frequently used to induce dopaminergic neurodegeneration, include the neurotoxin 6-hydroxydopamine (6-OHDA) and, more recently, the pesticide paraquat and the herbicide rotenone. Each of these toxins triggers the formation of ROS/RNS. 6-OHDA was the first agent to be used in experimental animals to induce PD signs more than 30 yr ago *(31)*. Although very selective for monoaminergic neurons, resulting in a extensive loss of neurons in the SNpc, the pathology elicited by 6-OHDA differs slightly from that of PD. In the cytosol 6-OHDA generates ROS and inactivates macromolecules by producing quinones *(32)*. The herbicide paraquat (*N*,*N*'-dimethyl-4-4'-bipiridinium), which shares a structural similarity to MPP^+ and is present in the environment, is also a toxin model of PD which causes the formation of $O_2^{\bullet -}$ *(33)*. To date, it remains uncertain whether this is a specific dopaminergic toxin or whether other cell types are also affected. Finally, the herbicide rotenone was recently found to be a parkinsonism-inducing toxin. This compound, extracted from tropical plants, inhibits mitochondrial complex I at the same site as MPP^+ and selectively induces nigrostriatal dopaminergic degeneration and causes the formation of fibrillar elements similar to those seen in Lewy bodies *(34)*. In addition, rotenone-treated animals develop motor and behavioral abnormalities similar to those observed in PD. Taken together, the results obtained to date with rotenone indicate that it causes complex I inhibition and leads to marked nigrostriatal dopaminergic cell death with its toxicity being not solely derived from ATP depletion but rather involving ROS-mediated mechanisms *(35)*.

Mitochondrial Dysfunction, Programmed Cell Death, and Neuroinflammation in PD

In addition to the formation of ROS from dopamine metabolism, another candidate for the source of free radicals is dysfunctional mitochondria. Mitochondria are thought to be the major source of ROS, mostly at the first enzyme complex of the electron-transfer chain, complex I (NADPH:ubiquinone oxidoreductase) *(5)*. $O_2^{\bullet -}$ formation in mitochondria derive from either H_2O_2 or $ONOO^-$, the latter depending on intracellular NO concentrations. $ONOO^-$ participates in many harmful reactions with proteins, lipids, and DNA, e.g., forming 3-nitrotyrosine, an excellent biochemical biomarker of oxidative damage in proteins, which has been recently found in Lewy bodies of PD patients *(36)*. Increased nitrotyrosine formation has also been observed in mitochondria of MPTP-treated rats, with this being prevented by administration of a specific nNOS inhibitor *(37)*. Some studies have highlighted the possible existence of abnormal function in mitochondrial complex I, given that 30–40% of idiopathic PD patients show a reduction in the activity of this complex *(38)*. The possibility exists that an oxidative phosphorylation defect plays a role in the pathogenesis of PD. Gu and co-workers *(39)* showed that a mitochondrial DNA–encoded defect from PD platelets is transferable into mitochondrial-deficient cell lines. Cybrids formed with this technique exhibited decreased complex I activity and increased ROS production *(40)*. These defects are related not only to ROS overproduction but also to increased vulnerability to MPP^+, and impaired mitochondrial calcium buffering *(41)*. However, to date all the efforts to identify mitochondrial DNA mutations associated with PD have failed. Accumulation of PD-inducing toxins such as pesticides (e.g., rotenone or paraquat) or MPP^+ in the mitochondrial matrix has suggested an interesting link between environmental toxins and complex I defects as a pathogenic cause of PD.

Programmed cell death (PCD) is a physiological process in which an intrinsic molecular program leading to cell death is triggered. PCD is sometimes accompanied by a particular morphology that includes membrane blebbing, cell shrinkage, and nuclear condensation, known as apoptosis. PCD does not usually trigger an immune reaction in contrast to necrosis, another "passive" form of cell death. Although etiologically it was assumed that dopamine-containing neurons in PD would degenerate most likely via PCD, only a few post-mortem studies have indicated this, owing to many conceptual and technical difficulties *(42)*. Alterations in energy metabolism and formation of ROS usually occurs hours before the cell death induced by MPTP is observed, raising the possibility of a cascade of signals that precedes cellular demise, as it takes place in PCD. Bax and Bcl-2 are up- and downregulated, respectively, after MPTP administration in the SNpc *(43)* while overexpression of Bcl-2 protects dopaminergic cells from MPTP-induced neurodegeneration *(44)*. Furthermore, several studies have confirmed the presence of apoptotic markers in dying cells after MPTP injection in vivo, as well as MPP^+ incubation in vitro *(45)*. Therefore, approaches to inhibit PCD at several levels have been suggested as potential therapeutic strategies in PD *(42)*.

Data collected in the last few years suggest a role for the inflammatory process in PD. Inflammation involves glial activation, especially microglia and astroglia. Microglia, the resident mononuclear phagocytes of the brain, become readily activated in response to a number of injuries or to immunological stimuli *(46)*. Upon activation, microglial cells undergo striking morphological changes, exhibiting increased expression of both surface molecules and classical pro-oxidant enzymes such as cyclooxygenase-2 (inducible form, COX-2) or iNOS as well as other cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Most of the soluble and/or trophic factors released from activated microglia posseses proinflammatory and potentially cytotoxic properties *(47)*. Likewise, astrocytes, under physiologic conditions, provide the necessary glia–neuron contacts, maintain ionic homeostasis, provide a buffer against

excess neurotransmitters, and secrete neurotrophic factors. However, in response to immunologic challenges and/or brain injury, astrocytes, like microglia, become activated. During activation, astrocytes up-regulate the expression of cell-type-specific proteins such as the glial fibrillary acidic protein, neurotrophic factors, and several proinflammatory cytokines *(48)*. While astrocytes seem to depend on a secondary response to produce certain proinflammatory substances, activated microglia secrete a much more complex repertoire of factors *(47)* and respond to a variety of stimuli including lipopolysaccharide (LPS) and β-amyloid peptide, thereby generating TNF-α and $O_2^{\bullet -}$ superoxide radicals among other factors *(49)*. Stimulation of these proinflammatory factors by glial cells, e.g., NO, makes a crucial contribution to the neuronal death, in particular to dopaminergic neurons, because this cell population is specially vulnerable to oxidative damage and the SN is especially rich in microglia *(50)*.

The presence of iNOS-positive activated microglia has been reported to occur in parkinsonian brains *(51)* and in animal models of PD *(23)*. Other indicators of microglial activation in post-mortem analysis from PD brains have been reviewed elsewhere *(47,52)*. Some authors raised the question as to whether glial activation is a cause or a mere consequence of neurodegeneration. More recently, other groups have shown that COX-2 is instrumental in a mouse model of PD *(53,54)*, therefore demonstrating that this pro-inflammatory enzyme plays a critical role in the neurodegenerative process of SN in PD. Also, using chronic treatment with LPS to evoke inflammation of the rat brain, microglial activation was triggered within 2 wk, followed by significant degeneration of SN dopaminergic neurons within 4–6 wk *(55)*.

Melatonin as a Free Radical Scavenger

The indoleamine melatonin (*N*-acetyl-5-methoxytryptamine) is synthesized from tryptophan in the pineal gland following a circadian rhythm, with a peak of melatonin production always occurring during the dark period in all vertebrates species studied to date *(56)*. However, production in sites other than pineal gland have also been reported; these extra pineal sites include the retina, Harderian glands, gut, ovary, testes, bone marrow, and lens *(57–59)*. In addition to its production at multiple sites in vertebrate tissues, melatonin is also synthesized in non-mammalian vertebrates, in invertebrates, and also in unicellular organisms, including dinoflagellates, algae, and bacteria, although in some of these organisms not always following a circadian rhythm *(60)*. The evidence of such a broad range of organisms and different sites of biosynthesis of melatonin points to a basic function of this ubiquitous indoleamine. Classically, melatonin has been associated with the organismal adjustments to circadian and seasonal rhythms *(56,61)*. Nevertheless, the functional repertoire of melatonin is not confined to the circadian/circannual–related functions; other properties have also been demonstrated, i.e., as an oncostatic agent *(62)*, in immunoregulation *(63,64)*, and as an antiaging substance.

Since the early 1990s when melatonin was discovered to function as an antioxidant, i.e., to directly scavenge free radicals *(65)*, the number of described functions and potential applications have expanded rapidly. Melatonin directly reacts with reactive species during which it is metabolized to several metabolites including N_1-acetyl-N_2-formyl-5-methoxykynuramine (AFMK) and N_1-acetyl- 5-methoxykynuramine (AMK) and cyclic-3-hydroxymelatonin; these metabolites also are effective scavengers, and this greatly enhances melatonin's ability to reduce OS *(66,67)*. In addition to its direct scavenging activity, melatonin also confers indirect protection against OS through its ability to increase antioxidant enzyme gene expression and/or activities *(68, 69)*. The regulatory role of melatonin on gene expression and/or activity of antioxidant enzymes might also mediate protection afforded by the metabolites of melatonin *(70)*. It is presumed that melatonin's ability to promote the activities of antioxidant enzyme is mediated by specific receptors (membrane or nuclear) for the indoleamine.

In vitro free-radical-scavenging abilities of melatonin have been tested in an uncommonly large number of studies by different groups. Recently, Sofic and co-workers *(71)* analyzed the antioxidant capacity of melatonin using the oxygen radical absorbance capacity (ORAC) assay with different free-radical generators; this assay is based on the fluorescence emitted by the protein β-phycoerytrin. The ORAC assay indicated that melatonin was twice as potent as vitamin E and four times more effective than vitamin C or GSH. Other authors have shown similar results using different assays either in vitro or in vivo *(72)*. Melatonin's reaction with the highly reactive $^{\bullet}OH$ shows a rate constant similar to that of other antioxidants *(73)*. While tocopherols (vitamin E) are excellent in protecting membrane lipids from oxidation, they are not very effective against free-radical damage to proteins and DNA. On the other hand, melatonin readily protects lipids *(74)*, mitochondrial and nuclear DNA *(75,76)*, and proteins *(77)* from oxidative damage. How melatonin is able to protect this range of molecules is presumably due to its wide intracellular distribution; however, specifically how the indole (or its metabolites) reach all these intracellular targets is yet unknown and might be due to its dual lipophilic and hydrophilic nature.

Recent observations by Stefulj and colleagues *(78)* have brought up some interesting potential insights into the widespread antioxidant activity of melatonin. Using the RT-PCR technique, this group showed that the mRNAs that encode for the two enzymes in the synthesis of melatonin, i.e., *N*-acetyl-transferase (NAT) and hydroxyindole-*O*-methyltransferase (HIOMT), are present in the intestine, fundic stomach, testis, spinal cord, raphe nuclei of brain stem, and striatum. This suggests that melatonin synthesis may occur in many tissues and could help to explain its numerous anti-

oxidant, intracrine, autocrine, and/or paracrine action, as has been suggested *(79)*. Clearly, the widespread intracellular distribution of melatonin and its autocrine/paracrine production could help to explain the large number of tissues in which melatonin has been shown to afford free-radical-scavenging protection.

Melatonin as a Neuroprotector in PD Models

Within the last decade, hundreds of reports provide scientific evidence for the protective role of melatonin in a number of OS-related diseases with the protective actions being attributable to the direct and indirect antioxidative properties of the indole. Melatonin has been used in a remarkably large number of experimental models in which the pathogeny is thought to be mediated by free radicals in one way or another. Recovery after melatonin treatment for ischemia–reperfusion in a variety of organs and in experimental models of stroke has repeatedly been demonstrated *(80–82)*, as well as protective effects in the central nervous system in situations where either neurons or glia are challenged with ionizing radiation, diabetes, toxins, viral infections, excitotocicity, metals, seizures, homocysteine, or during aging, among others *(73)*. By far some of the most compelling evidence related to the protective actions of melatonin are obvious when it is tested as a neuroprotector, especially in Huntington's, Alzheimer's, and Parkinson's disease models *(83–85)*.

The first evidence of a special relationship between melatonin and parkinsonism came from findings of a diminished pineal activity and a subsequent reduction in circulating melatonin concentrations in PD patients *(86)*. After melatonin scavenging properties were uncovered, numerous studies were conducted in order to test the effectiveness of its antioxidant capabilities in several PD models, both in vivo and in vitro. Initially, melatonin was found to inhibit the prooxidant effects of dopamine and L-dopa in vitro *(87)*. These data were further confirmed by Khaldy et al. *(88)*, who additionally showed that melatonin was more effective than the vitamin E analog, trolox, in preventing dopamine autooxidation. Soon after this in vitro study, the same group *(89)* reported that melatonin administration prevented lipid peroxidation induced by MPTP treatment in corpus striatum and hippocampus of mice. A year later using MPTP in rats rather than mice, Jin and colleagues *(90)* showed protection of nigral dopaminergic neurons by melatonin (10 mg/kg bw). Consistent with the earlier mouse study, they showed that melatonin prevents the rise in lipid peroxidation products in the SN of MPP^+-treated rats and, additionally, preserves tyrosine hydroxylase (TH) activity, which is normally decreased after toxin treatment. Both acute and chronic treatments with the indoleamine were equally effective in reducing damage mediated by MPP^+ *(90)*. This group also extended their research by using the 6-OHDA model instead of MPTP to induce dopaminergic degeneration. In this report, melatonin administration restored the motor deficits elicited by apomorphine co-treatment with 6-OHDA and, furthermore, first showed that the indoleamine also prevented the behavioral changes associated with this PD model *(91)*. They also reported that melatonin completely prevented a rise in neural lipid peroxidation products and partially rescued striatal dopaminergic levels after lesioning with 6-OHDA *(92)*.

Melatonin's protection is not limited to a reduction of lipid damage in these models of PD, but also to DNA fragmentation induced by MPTP *(93)* and to the mitochondrial complex I deficiency observed after 6-OHDA administration *(94)*. Mitochondria are thought to play a critical role in the neuroprotective function of melatonin in neurodegenerative diseases *(95)*. One of the most compelling findings of the ability of melatonin to reduce the MPTP-induced loss of TH-reactive dopaminergic neurons comes from the report by Antolin and co-workers *(83)*. They used chronic injection of MPTP into mice for 35 d to cause a gradual reduction in the number of dopaminergic neurons. When melatonin (500 μg/kg bw) was co-administered daily with MPTP, it prevented dopaminergic cell death in the SNpc, and greatly reduced the loss of TH immunoreactivity in SN and striatum, thus demonstrating the beneficial role of the indoleamine in protecting against dopaminergic neuronal degeneration. These findings were supported by others *(96,97)* who reported that melatonin partially preserves the glutathione (GSH) concentrations in SN of MPTP-treated rats. The antioxidant activities of melatonin were surmised to be the major mechanism underlying melatonin's protection in these models of PD. A recent study showed that melatonin reduces in vivo $^{\bullet}OH$ formation after MPTP treatment, without affecting monoamino oxidase-B (MAO-B) activity *(98)*. Despite the uniformity of the results summarized above, one report claimed an incomplete recovery of dopamine levels after melatonin co-treatment with MPTP *(96)*.

A new in vivo approach has been exploited to show the beneficial effects in a model of PD where another toxin was chronically administered. Given its numerous advantages (e.g., genetic familiarity, rapid screening, and so on) the fruit fly *Drosophila melanogaster* was used as a model for neurodegenerative diseases, including a genetic model of PD. Because the pesticide rotenone has been used successfully to generate PD-like behavior in rats *(34)*, Coulom and Birman *(99)* used this environmental toxin to cause a selective loss of dopaminergic neurons in the brain and the induction of severe locomotor impairments in the fruit fly. They further found that behavioral defects and neuronal loss were rescued by melatonin more efficiently than by L-dopa *(99)*.

Several of cell culture models have been used to identify clues as to the mechanisms of dopaminergic cell death. Many of these models have also provided insights as to how melatonin confers protection against toxins that produce Parkinson-like signs. Although large doses of drugs that generate oxidative stress are generally considered to promote acci-

Table 1
Summary of Many of the Reports Documenting Protective Actions of Melatonin in PD[a]

Species / cell line	Model used	Melatonin effects	Ref.
Human	—	Reduction of pineal activity and melatonin concentrations	*86,124*
Human	—	Modification of secretion pattern of melatonin in PD patients receiving L-dopa	*125*
Human	—	Prevention of RBD, hypersomnolence and visual hallucinations	*121,122*
In vivo			
C57/Bl mouse	MPTP	Inhibition of lipid peroxidation in corpus striatum and hippocampus	*89*
C57/Bl mouse	MPTP	Prevention of DNA fragmentation in SN; reduction of oxidative stress in SN	*93*
C57/Bl mouse	MPTP (chronic)	Prevention of dopaminergic cell death and preservation of TH immunoreactivity	*83,96,97*
Balb/c mouse	MPTP	Blockade of $^{\bullet}OH$ formation in striatum	*98*
Rat	6-OHDA	Inhibition of complex I deficiency	*94*
Rat	MPTP	Protection of nigral dopaminergic neurons; prevention lipid peroxidation in SN; preservation of TH activity	*90*
Rat	6-OHDA/ apomorphin	Prevention of lipid peroxidation, recovery of motor impairment and dopaminergic function	*91,92*
Drosophila melanogaster	Rotenone	Rescuing of dopaminergic cell death and prevention of behavioral defects (locomotor impairments)	*99*
In vitro			
Cell-free	Dopamine	Inhibition of pro-oxidant effects of dopamine and L-dopa	*87,88*
Mesencephalic cultures	—	Rescuing of dopamine neurons from spontaneous cell death in low-density seeding cultures	*100,101,126*
PC12 cells	6-OHDA	Protection from both apoptosis and necrosis, increasing antioxidant enzymes	*104,105*
Astrocytes	H_2O_2	Avoidance of ROS formation and prevention of cytochrome *c* release	*110*
C6 glioma cells	—	Induction of GDNF expression	*116*
Mitochondrial fraction	MPP^+	Reduction of $^{\bullet}OH$ formation	*98*

[a]Included are clinical data and in vitro and in vivo data from experimental studies.

dental cell death or necrosis, lower doses of such drugs given over long periods of time enhance the frequency of apoptosis. The initial evidence of melatonin protection against apoptotic events was provided by Iacovitti and colleagues *(100)*, who reported that the indoleamine rescued dopaminergic cells in culture. In their work, embryonic mesencephalic cultures (mostly dopaminergic) were seeded at low density and they underwent apoptosis due to the absence of growth factors. Melatonin prevented cell death, mostly in the neuronal population, as assayed by the specific marker *Tau (100)*. In a similar model using striatal neurons in culture, melatonin, as well as other antioxidants, protected these cells from cell death induced by low-density plating. The effect was not mimicked by growth factors such as BDNF, bFGF, GDNF, NGF, NT3, or EGF *(101)*, which indicates that it was likely the antioxiant properties of the indole that account for the major portion of the protection. Neurotoxins MPP^+, 6-OHDA, and dopamine itself also cause apoptosis of dopaminergic PC12 cells as well as in cultured primary midbrain mesencephalic neurons *(102,103)*. It was shown that melatonin protects both naive PC12 cells and NGF-differentiated neuronal PC12 cells from the toxicity of 6-OHDA *(104)*. Furthermore, not only did melatonin inhibit apoptosis caused by low doses of 6-OHDA, but also cellular necrosis induced by high doses of 6-OHDA in PC12 was likewise inhibited by the indole *(105)*. Similarly, melatonin also protects PC12 and neuroblastoma cells from amyloid peptide in in vitro Alzheimer's models *(106,107)* as well as against NMDA excitotoxicity *(108,109)*; these findings confirm the general neuroprotective effect of the indole under a variety of challenges that induce neuronal death. Table 1 summarizes the reports regarding the protective role of melatonin in PD models.

Cellular toxicity induced by 6-OHDA is mediated by increased free-radical generation. For this reason, the antioxidant properties of melatonin presumably account for its ability to suppress both necrosis and apoptosis. Compelling evidence of the direct antioxidant actions of melatonin have recently been reported. Jou and colleagues *(110)* showed, using confocal microscopy, that melatonin abolishes the generation of mitochondrial ROS during H_2O_2-induced cell damage of rat astrocytes; this inhibition precedes cell death, which occurs hours later in cells treated with H_2O_2 only. Other proof of the direct action of melatonin have been iden-

tified by Imam and co-workers *(111)*, who reported a reduction in nitrotyrosine residues due to melatonin treatment during dopaminergic neurotoxicity *(111)*. Some of the molecular pathways involved in melatonin's neuroprotective actions have been elucidated. Hence, the antioxidant actions of the indole abolish NFκB activation and c-Jun phosphorylation *(112,113)*, events that usually precede 6-OHDA–induced apoptotic death.

In addition to its direct scavenging activity, melatonin prevents the reduction in mRNA levels for antioxidant enzymes, i.e., CuZn-superoxide dismutase (SOD), Mn-SOD, and glutathione peroxidase (GPx), which normally occur several hours after cells are incubated with 6-OHDA. Furthermore, melatonin stimulates gene expression of a variety of antioxidant enzymes *(69)*; promotion of antioxidant enzyme activities increases the repertoire of mechanisms whereby melatonin limits free-radical damage. It is likely that several of melatonin's antioxidant actions play a role in neuroprotection, including direct scavenging, antioxidant enzyme stimulation, and GSH synthesis promotion *(114)*. Melatonin also preserves protein integrity by scavenging free radicals and preventing direct oxidative damage to the antioxidant enzymes *(115)*. The possibility also exists that melatonin might exert some physiological protection by activating GDNF gene expression *(116)*. Clearly both direct or indirect antioxidant actions are thought to be important mediators of melatonin's antiparkinsonian actions; furthermore, it is likely that yet to be identified genomic actions of melatonin are also involved. A function of the indole in inflammation may not be ruled out; we have recently found that melatonin prevents COX-2 and iNOS activation in LPS-stimulated macrophage cells (J.C. Mayo et al., unpublished data) and some authors have demonstrated that melatonin prevents microglial activation in vivo *(117)*. Hence, immunomodulation of microglial activation might also play a central role in the neuroprotective actions of the indoleamine. Whether this is an antioxidative or a genomic-based action remains unknown and should be addressed in order to assess the potential therapeutic use of melatonin in PD.

There are numerous data suggesting a role for melatonin in mitochondrial homeostasis *(95)*. Melatonin increases the activities of respiratory complexes I and IV in a time-dependent manner after in vivo administration to rats *(118)* and maintains GSH homeostasis in the mitochondrial matrix under increased oxidative stress; these actions are not shared by either vitamin C or vitamin E *(119)*. Mitochondria are the major source of ROS production in the cell, owing to the leakage of electrons through the electron-transport chain. Given the critical role of mitochondria in PCD and in PD, it is conceivable that actions at the mitochondria level mediate at least some of melatonin's antiapoptotic effects *(94, 95,120)*. It has been reported that melatonin increases ATP production via the induction of mitochondrial complexes *(118)*; this might confer some protection in case of oxidative challenges in dopaminergic cells. Additionally, melatonin prevents ROS-induced calcium overload and mitochondrial membrane depolarization *(110)*. The indoleamine also protects mitochondrial DNA, which is particularly vulnerable to oxidative damage, thus indirectly helping to preserve mitochondrial metabolism. Collectively, the results identify the mitochondria as one of the main targets for melatonin, in terms of its neuroprotective actions. Figure 1 summarizes the potential pathways involved in the neuroprotective effects of melatonin in PD, using MPTP as a general parkinsonian model.

Melatonin as a Sleep Adjuster in PD

The antioxidant actions only may not account for the beneficial actions of melatonin in PD. There is growing evidence of sleep–wake boundary dysfunction in PD. Rapid-eye-movement (REM) sleep behavior disorder (RBD) is characterized by loss of normal skeletal muscle tone with prominent motor activity and dreaming. Interestingly, RBD has been associated with PD and/or other forms of dementia, with a tendency for RBD to precede the onset of parkinsonism. Boeve and colleagues *(121,122)* have found that treatment of humans with clonazepam or melatonin, drugs used to treat RBD, prevents hypersomnolence and visual hallucinations associated with PD. Additionally, spontaneous circadian patterns of motor symptoms in PD patients include dysfunction typically less severe in the early morning than in the afternoon, suggesting circadian fluctuations of the action of dopamine in the central nervous system. Many biological indices including cortisol, catecholamines, and melatonin are also altered in PD patients, which suggests the possibility of using melatonin as a circadian adjuster in these subjects *(123)*.

Concluding Remarks

PD is a highly debilitating condition that concerns thousands of families and cost millions of dollars for treatment annually. While the disease occasionally has a genetic basis, the signs of PD likely develop, at least in part, after free-radical damage to the SNpc. Additionally, neuroinflammation and mitochondrial malfunction participate in the etiology of this neurodegenerative disorder and contribute to the increase of oxidative damage to the dopaminergic neuronal population. Once a large percentage of these cells are lost, PD signs appear. Currently, the most common therapeutic approach is symptomatic treatment with L-dopa to restore dopamine content. Unfortunately, this treatment usually begins late in the progression of the disorder and it may even cause further damage when the drug is used in the long term *(24)*. Thus, alternative therapies or supporting treatments for traditional drugs have been suggested, including the use of antioxidants like vitamin E or C, among others. Melatonin has been tested in many in vitro and in vivo models of PD and these studies uniformly show it is

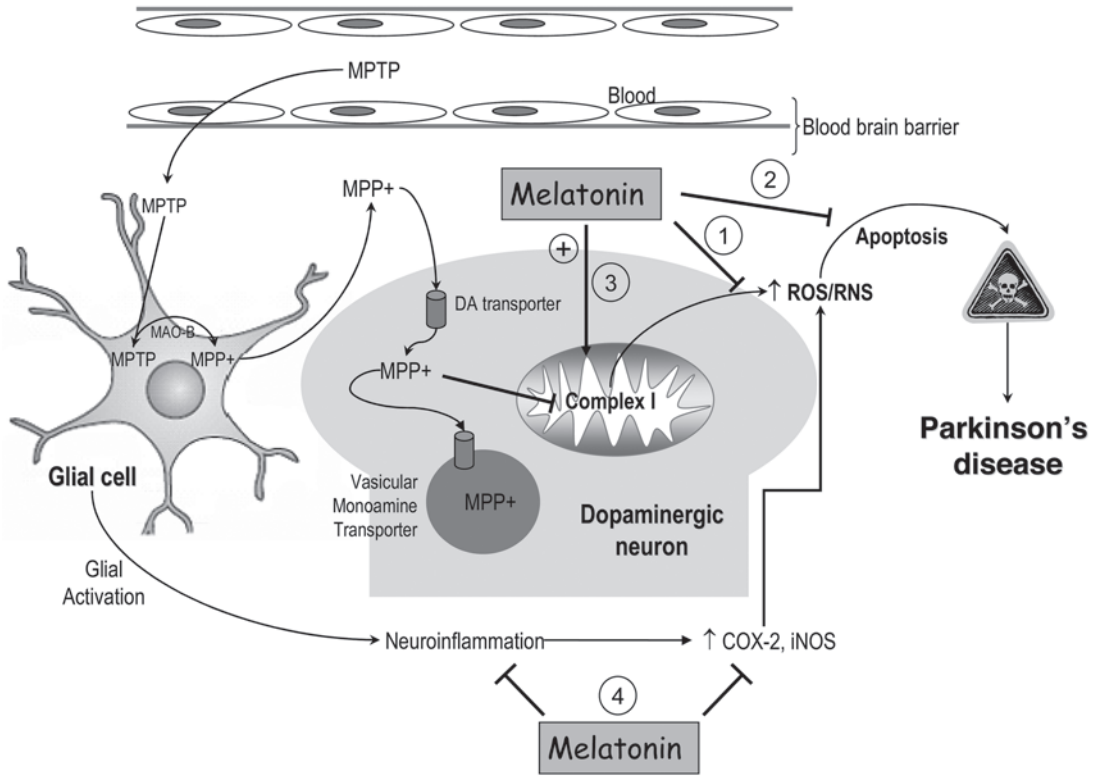

Fig. 1. Potential pathways involved in the neuroprotective actions of melatonin in the MPTP model of PD, which recapitulates most of the features of PD in humans. MPTP crosses the blood–brain barrier and is converted by glial cells into MPP^+ by MAO-B; MPP^+ then enters into dopamine terminals through the dopamine transporter and accumulates in vesicles or in the mitochondria of dopaminergic neurons. In these cells, MPP^+ blocks complex I of the electron-transport chain thereby generating ROS/RNS, which eventually triggers apoptosis of dopaminergic neurons. Possible protective functions of melatonin include (1) antioxidant actions, scavenging ROS/RNS which are harmful to macromolecules and which trigger apoptosis; (2) antiapoptotic effects, blockade of the apoptotic cascade; (3) activation of mitochondrial metabolism; (4) anti-inflammatory actions, by either blocking microglia activation or inhibiting both COX-2 and iNOS enzymes, which produce pro-inlammatory mediators and NO, respectively. Thus, melatonin may block the pro-apoptotic cascade at different levels and prevent dopaminergic neuronal death which eventually causes parkinsonian signs.

highly efficacious. Considering that melatonin is an endogenous, non-toxic, antioxidant substance without known side effects, the indoleamine should be seriously considered as a useful agent in PD patients as a co-treatment with conventional therapies. The experimental data collectively suggest melatonin use by PD subjects would reduce their disease burden. Additionally, epidemiological studies of individuals who use melatonin daily regularly should be carried out. The devastating nature of PD and the lack of currently available means of preventing the disease, call for the use of more imaginative treatments. Melatonin, as noted, has very low toxicity and is much less expensive than prescription drugs and, thus, it should be tested against the development or progression of PD.

References

1. de Rijk, M. C., Launer, L. J., Berger, K., et al. (2000). *Neurology* **54,** S21–S23.
2. Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., and Goedert, M. (1998). *Proc. Natl. Acad. Sci. USA* **95,** 6469–6473.
3. Huang, Y., Cheung, L., Rowe, D., and Halliday, G. (2004). *Brain Res. Brain Res. Rev.* **46,** 44–70.
4. Vila, M. and Przedborski, S. (2004). *Nat. Med.* **10(Suppl.),** S58–S62.
5. Halliwell, B. and Gutteridge, J. M. C. (eds.) (1999). *Free radicals in biology and medicine*. Oxford University Press: Oxford, UK.
6. Halliwell, B. and Gutteridge, J. M. (1984). *Lancet* **1,** 1396–1397.
7. Halliwell, B. and Whiteman, M. (2004). *Br. J. Pharmacol.* **142,** 231–255.
8. Dexter, D. T., Sian, J., Jenner, P., and Marsden, C. D. (1993). *Adv. Neurol.* **60,** 273–281.
9. Youdim, M. B., Stephenson, G., and Ben Shachar, D. (2004). *Ann. NY Acad. Sci.* **1012,** 306–325.
10. Sian, J., Dexter, D. T., Lees, A. J., et al. (1994). *Ann. Neurol.* **36,** 348–355.
11. Dexter, D. T., Carter, C. J., Wells, F. R., et al. (1989). *J. Neurochem.* **52,** 381–389.
12. Sanchez-Ramos, J., Overvik, E., and Ames, A. B. (1994). *Neurodegeneration* **3,** 197–204.
13. Schapira, A. H. (1998). *Biochim. Biophys. Acta* **1366,** 225–233.
14. Beal, M. F. (2003). *Ann. NY Acad. Sci.* **991,** 120–131.
15. Beal, M. F. (1998). *Ann. Neurol.* **44,** S110–S114.

16. Graham, D. G. (1978). *Mol. Pharmacol.* **14,** 633–643.
17. Spina, M. B. and Cohen, G. (1988). *J. Pharmacol. Exp. Ther.* **247,** 502–507.
18. Zecca, L., Youdim, M. B., Riederer, P., Connor, J. R., and Crichton, R. R. (2004). *Nat. Rev. Neurosci.* **5,** 863–873.
19. Tieu, K., Ischiropoulos, H., and Przedborski, S. (2003). *IUBMB Life* **55,** 329–335.
20. Carreras, M. C., Franco, M. C., Peralta, J. G., and Poderoso, J. J. (2004). *Mol. Aspects Med.* **25,** 125–139.
21. Schulz, J. B., Matthews, R. T., Muqit, M. M., Browne, S. E., and Beal, M. F. (1995). *J. Neurochem.* **64,** 936–939.
22. Przedborski, S., Jackson-Lewis, V., Yokoyama, R., Shibata, T., Dawson, V. L., and Dawson, T. M. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 4565–4571.
23. Wu, D. C., Jackson-Lewis, V., Vila, M., et al. (2002). *J. Neurosci.* **22,** 1763–1771.
24. Olanow, C. W., Agid, Y., Mizuno, Y., et al. (2004). *Mov. Disord.* **19,** 997–1005.
25. Dauer, W. and Przedborski, S. (2003). *Neuron* **39,** 889–909.
26. Meissner, W., Hill, M. P., Tison, F., Gross, C. E., and Bezard, E. (2004). *Trends Pharmacol. Sci.* **25,** 249–253.
27. Stocchi, F. and Olanow, C. W. (2003). *Ann. Neurol.* **53(Suppl. 3),** S87–S97.
28. Langston, J. W., Ballard, P., Tetrud, J. W., and Irwin, I. (1983). *Science* **219,** 979–980.
29. Liu, Y., Roghani, A., and Edwards, R. H. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 9074–9078.
30. Nicklas, W. J., Vyas, I., and Heikkila, R. E. (1985). *Life Sci.* **36,** 2503–2508.
31. Ungerstedt, U., Ljungberg, T., and Steg, G. (1974). *Adv. Neurol.* **5,** 421–426.
32. Cohen, G. and Kesler, N. (1999). *J. Neurochem.* **73,** 2310–2315.
33. Day, B. J., Patel, M., Calavetta, L., Chang, L. Y., and Stamler, J. S. (1999). *Proc. Natl. Acad. Sci. USA* **96,** 12760–12765.
34. Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., and Greenamyre, J. T. (2000). *Nat. Neurosci.* **3,** 1301–1306.
35. Sherer, T. B., Betarbet, R., Testa, C. M., et al. (2003). *J. Neurosci.* **23,** 10756–10764.
36. Good, P. F., Hsu, A., Werner, P., Perl, D. P., and Olanow, C. W. (1998). *J. Neuropathol. Exp. Neurol.* **57,** 338–342.
37. Dennis, J. and Bennett, J. P. Jr. (2003). *J. Neurosci. Res.* **72,** 89–97.
38. Schapira, A. H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., and Marsden, C. D. (1990). *J. Neurochem.* **54,** 823–827.
39. Gu, M., Cooper, J. M., Taanman, J. W., and Schapira, A. H. (1998). *Ann. Neurol.* **44,** 177–186.
40. Swerdlow, R. H., Parks, J. K., Miller, S. W., et al. (1996). *Ann. Neurol.* **40,** 663–671.
41. Sheehan, J. P., Swerdlow, R. H., Parker, W. D., Miller, S. W., Davis, R. E., and Tuttle, J. B. (1997). *J. Neurochem.* **68,** 1221–1233.
42. Vila, M. and Przedborski, S. (2003). *Nat. Rev. Neurosci.* **4,** 365–375.
43. Hartmann, A., Hunot, S., Michel, P. P., et al. (2000). *Proc. Natl. Acad. Sci. USA* **97,** 2875–2880.
44. Yang, L., Matthews, R. T., Schulz, J. B., et al. (1998). *J. Neurosci.* **18,** 8145–8152.
45. Tatton, N. A. and Kish, S. J. (1997). *Neuroscience* **77,** 1037–1048.
46. Kreutzberg, G. W. (1996). *Trends Neurosci.* **19,** 312–318.
47. Liu, B., Gao, H. M., and Hong, J. S. (2003). *Environ. Health Perspect.* **111,** 1065–1073.
48. Lindsay, R. M., Wiegand, S. J., Altar, C. A., and DiStefano, P. S. (1994). *Trends Neurosci.* **17,** 182–190.
49. Qin, L., Liu, Y., Cooper, C., Liu, B., Wilson, B., and Hong, J. S. (2002). *J. Neurochem.* **83,** 973–983.
50. Kim, W. G., Mohney, R. P., Wilson, B., Jeohn, G. H., Liu, B., and Hong, J. S. (2000). *J. Neurosci.* **20,** 6309–6316.
51. Hunot, S., Boissiere, F., Faucheux, B., et al. (1996). *Neuroscience* **72,** 355–363.
52. Hunot, S., Dugas, N., Faucheux, B., et al. (1999). *J. Neurosci.* **19,** 3440–3447.
53. Teismann, P., Vila, M., Choi, D. K., et al. (2003). *Ann. NY Acad. Sci.* **991,** 272–277.
54. Teismann, P., Tieu, K., Choi, D. K., et al. (2003). *Proc. Natl. Acad. Sci. USA* **100,** 5473–5478.
55. Gao, H. M., Jiang, J., Wilson, B., Zhang, W., Hong, J. S., and Liu, B. (2002). *J. Neurochem.* **81,** 1285–1297.
56. Reiter, R. J. (1991). *Mol. Cell Endocrinol.* **79,** C153–C158.
57. Gern, W. A. and Ralph, C. L. (1979). *Science* **204,** 183–184.
58. Menendez-Pelaez, A., Reiter, R. J., Howes, K. A., et al. (1988). *Endocr. Res.* **14,** 121–130.
59. Tan, D. X., Manchester, L. C., Reiter, R. J., et al. (1999). *Biochim. Biophys. Acta* **1472,** 206–214.
60. Hardeland, R. and Poeggeler, B. (2003). *J. Pineal Res.* **34,** 233–241.
61. Reiter, R. J. (1991). *Endocr. Rev.* **12,** 151–180.
62. Blask, D. E., Sauer, L. A., Dauchy, R. T., Holowachuk, E. W., and Ruhoff, M. S. (1999). *Adv. Exp. Med. Biol.* **460,** 337–343.
63. Fraschini, F. and Reiter, R. J. (eds.) (1991). *Role of melatonin and pineal peptides in neuroimmunomodulation*. Plenum Press: New York.
64. Guerrero, J. M. and Reiter, R. J. (2002). *Curr. Top. Med. Chem.* **2,** 167–179.
65. Tan, D. X., Chen, L. D., Poeggeler, B., Manchester, L. C., and Reiter, R. J. (1993). *Endocr. J.* **1,** 57–60.
66. Tan, D. X., Manchester, L. C., Reiter, R. J., et al. (1998). *Biochem. Biophys. Res. Commun.* **253,** 614–620.
67. Tan, D. X., Manchester, L. C., Burkhardt, S., et al. (2001). *FASEB J.* **15,** 2294–2296.
68. Barlow-Walden, L. R., Reiter, R. J., Abe, M., et al. (1995). *Neurochem. Int.* **26,** 497–502.
69. Mayo, J. C., Sainz, R. M., Antoli, I., Herrera, F., Martin, V., and Rodriguez, C. (2002). *Cell Mol. Life Sci.* **59,** 1706–1713.
70. Antolin, I., Rodriguez, C., Sainz, R. M., et al. (1996). *FASEB J.* **10,** 882–890.
71. Sofic, E., Rimpapa, Z., Kundurovic, Z., et al. (2005). *J. Neural Transm.* **112,** 349–358.
72. Tan, D. X., Reiter, R. J., Manchester, L. C., et al. (2002). *Curr. Top. Med. Chem.* **2,** 181–197.
73. Reiter, R. J., Tan, D. X., and Pappolla, M. A. (2004). *Ann. NY Acad. Sci.* **1035,** 179–196.
74. Sewerynek, E., Melchiorri, D., Ortiz, G. G., Poeggeler, B., and Reiter, R. J. (1995). *J. Pineal Res.* **19,** 51–56.
75. Mohanan, P. V. and Yamamoto, H. A. (2002). *Toxicol. Lett.* **129,** 99–105.
76. Lopez-Burillo, S., Tan, D. X., Mayo, J. C., Sainz, R. M., Manchester, L. C., and Reiter, R. J. (2003). *J. Pineal Res.* **34,** 269–277.
77. Mayo, J. C., Tan, D. X., Sainz, R. M., Natarajan, M., Lopez-Burillo, S., and Reiter, R. J. (2003). *Biochim. Biophys. Acta* **1620,** 139–150.
78. Stefulj, J., Hortner, M., Ghosh, M., et al. (2001). *J. Pineal Res.* **30,** 243–247.
79. Tan, D. X., Manchester, L. C., Hardeland, R., et al. (2003). *J. Pineal Res.* **34,** 75–78.
80. Borlongan, C. V., Yamamoto, M., Takei, N., et al. (2000). *FASEB J.* **14,** 1307–1317.
81. Cheung, R. T. (2003). *J. Pineal Res.* **34,** 153–160.
82. Reiter, R. J., Tan, D. X., Leon, J., Kilic, U., and Kilic, E. (2005). *Exp. Biol. Med. (Maywood.)* **230,** 104–117.
83. Antolin, I., Mayo, J. C., Sainz, R. M., et al. (2002). *Brain Res.* **943,** 163–173.

84. Pappolla, M. A., Simovich, M. J., Bryant-Thomas, T., et al. (2002). *J. Pineal Res.* **32,** 135–142.
85. Reiter, R. J., Cabrera, J., Sainz, R. M., Mayo, J. C., Manchester, L. C., and Tan, D. X. (1999). *Ann. NY Acad. Sci.* **890,** 471–485.
86. Sandyk, R. (1990). *Int. J. Neurosci.* **50,** 37–53.
87. Miller, J. W., Selhub, J., and Joseph, J. A. (1996). *Free Radic. Biol. Med.* **21,** 241–249.
88. Khaldy, H., Escames, G., Leon, J., Vives, F., Luna, J. D., and Acuna-Castroviejo, D. (2000). *J. Pineal Res.* **29,** 100–107.
89. Acuna-Castroviejo, D., Coto-Montes, A., Gaia, M. M., Ortiz, G. G., and Reiter, R. J. (1997). *Life Sci.* **60,** L23–L29.
90. Jin, B. K., Shin, D. Y., Jeong, M. Y., et al. (1998). *Neurosci. Lett.* **245,** 61–64.
91. Kim, Y. S., Joo, W. S., Jin, B. K., Cho, Y. H., Baik, H. H., and Park, C. W. (1998). *Neuroreport* **9,** 2387–2390.
92. Joo, W. S., Jin, B. K., Park, C. W., Maeng, S. H., and Kim, Y. S. (1998). *Neuroreport* **9,** 4123–4126.
93. Ortiz, G. G., Crespo-Lopez, M. E., Moran-Moguel, C., Garcia, J. J., Reiter, R. J., and Acuna-Castroviejo, D. (2001). *Neuroendocrinol. Lett.* **22,** 101–108.
94. Dabbeni-Sala, F., Di Santo, S., Franceschini, D., Skaper, S. D., and Giusti, P. (2001). *FASEB J.* **15,** 164–170.
95. Leon, J., Acuna-Castroviejo, D., Escames, G., Tan, D. X., and Reiter, R. J. (2005). *J. Pineal Res.* **38,** 1–9.
96. Khaldy, H., Escames, G., Leon, J., Bikjdaouene, L., and Acuna-Castroviejo, D. (2003). *Neurobiol. Aging* **24,** 491–500.
97. Chen, S. T., Chuang, J. I., Hong, M. H., and Li, E. I. (2002). *J. Pineal Res.* **32,** 262–269.
98. Thomas, B. and Mohanakumar, K. P. (2004). *J. Pineal Res.* **36,** 25–32.
99. Coulom, H. and Birman, S. (2004). *J. Neurosci.* **24,** 10993–10998.
100. Iacovitti, L., Stull, N. D., and Johnston, K. (1997). *Brain Res.* **768,** 317–326.
101. Iacovitti, L., Stull, N. D., and Mishizen, A. (1999). *Brain Res.* **816,** 276–285.
102. Walkinshaw, G. and Waters, C. M. (1994). *Neuroscience* **63,** 975–987.
103. Andersen, J. K. (2001). *Bioessays* **23,** 640–646.
104. Mayo, J. C., Sainz, R. M., Uria, H., Antolin, I., Esteban, M. M., and Rodriguez, C. (1998). *J. Pineal Res.* **24,** 179–192.
105. Mayo, J. C., Sainz, R. M., Antolin, I., and Rodriguez, C. (1999). *Brain Res.* **818,** 221–227.
106. Pappolla, M. A., Sos, M., Omar, R. A., et al. (1997). *J. Neurosci.* **17,** 1683–1690.
107. Feng, Z. and Zhang, J. T. (2004). *J. Pineal Res.* **37,** 257–266.
108. Cazevieille, C., Safa, R., and Osborne, N. N. (1997). *Brain Res.* **768,** 120–124.
109. Herrera, F., Sainz, R. M., Mayo, J. C., Martin, V., Antolin, I., and Rodriguez, C. (2001). *J. Pineal Res.* **31,** 356–362.
110. Jou, M. J., Peng, T. I., Reiter, R. J., Jou, S. B., Wu, H. Y., and Wen, S. T. (2004). *J. Pineal Res.* **37,** 55–70.
111. Imam, S. Z., el Yazal, J., Newport, G. D., et al. (2001). *Ann. NY Acad. Sci.* **939,** 366–380.
112. Lezoualc'h, F., Sparapani, M., and Behl, C. (1998). *J. Pineal Res.* **24,** 168–178.
113. Chetsawang, B., Govitrapong, P., and Ebadi, M. (2004). *Neurosci. Lett.* **371,** 205–208.
114. Martin, V., Sainz, R. M., Antolin, I., Mayo, J. C., Herrera, F., and Rodriguez, C. (2002). *J. Pineal Res.* **33,** 204–212.
115. Mayo, J. C., Tan, D. X., Sainz, R. M., Lopez-Burillo, S., and Reiter, R. J. (2003). *Free Radic. Res.* **37,** 543–553.
116. Armstrong, K. J. and Niles, L. P. (2002). *Neuroreport* **13,** 473–475.
117. Chung, S. Y. and Han, S. H. (2003). *J. Pineal Res.* **34,** 95–102.
118. Martin, M., Macias, M., Leon, J., Escames, G., Khaldy, H., and Acuna-Castroviejo, D. (2002). *Int. J. Biochem. Cell Biol.* **34,** 348–357.
119. Martin, M., Macias, M., Escames, G., Leon, J., and Acuna-Castroviejo, D. (2000). *FASEB J.* **14,** 1677–1679.
120. Acuna, C. D., Escames, G., Carazo, A., Leon, J., Khaldy, H., and Reiter, R. J. (2002). *Curr. Top. Med. Chem.* **2,** 133–151.
121. Boeve, B. F., Silber, M. H., and Ferman, T. J. (2003). *Sleep Med.* **4,** 281–284.
122. Boeve, B. F., Silber, M. H., and Ferman, T. J. (2004). *J. Geriatr. Psychiatry Neurol.* **17,** 146–157.
123. Bruguerolle, B. and Simon, N. (2002). *Clin. Neuropharmacol.* **25,** 194–201.
124. Sandyk, R. (1990). *Int. J. Neurosci.* **51,** 73–77.
125. Bordet, R., Devos, D., Brique, S., et al. (2003). *Clin. Neuropharmacol.* **26,** 65–72.
126. Stull, N. D., Polan, D. P., and Iacovitti, L. (2002). *Brain Res.* **931,** 181–185.