Melatonin Reduces Oxidative Catastrophe in Neurons and Glia

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Abstract

Melatonin, the chief secretory product of the pineal gland, is an uncommonly effective direct free radical scavenger and indirect antioxidant. It detoxifies both reactive oxygen (ROS) and reactive nitrogen species (RNS), both groups of which are abundantly produced in the brain. Endogenously-generated melatonin is discharged from pinealocytes into the rich capillary plexus in the gland and may also be released directly into the cerebrospinal fluid (CSF). In the few species where it has been investigated, CSF levels of melatonin greatly exceed its concentrations in the blood. From both the CSF and the blood melatonin readily enters the brain to protect neurons and glia from molecular damage induced by ROS/RNS. Melatonin's efficacy in reducing molecular damage resulting from toxic oxygen and nitrogen derivatives in both the brain and spinal cord supports the notion that this non-toxic molecule may have utility in forestalling and/or delaying the development and progression of neurodegenerative diseases that have a massive free radical component. The disease models of interest and in which melatonin has been most thoroughly tested include Alzheimer disease, Parkinson disease and to a lesser extent Huntington disease and amyotrophic lateral sclerosis. The experimental findings summarized herein clearly document that melatonin readily prevents oxidative damage to both neurons and glia. In doing so, it greatly reduces apoptosis of critical cells within the central nervous system.

INTRODUCTION

The chemical reduction of molecular oxygen (O_2) within cells generates derivatives that can be highly damaging to subcellular structures and organelles (Lambeth 2007). These O_2 by-products are generally referred to as reactive oxygen (ROS) and reactive nitrogen species (RNS). Some ROS and RNS are free radicals, i.e., molecules or portions thereof that have an unpaired electron in their valence orbital (Yap *et al.* 2009). This feature makes them highly reactive and damaging.

Fortunately, only a small percentage (estimated to be up to 4%) of the O_2 taken up by cells is normally converted to these toxic derivatives. Under some circumstances, e.g., when tissues experience an episode of ischemia (often followed by reperfusion), the number of free radicals and related products produced becomes overwhelming and, therefore, catastrophic (Reiter *et al.* 2009). Such calamities often result in massive molecular destruction and either severely compromise cellular functions and, in the worst case

scenario, cause cellular death either due to apoptosis or necrosis (Jou *et al.* 2010; Lee *et al.* 2010b).

Cells do have protective mechanisms to ward off the potential damage normally inflicted by ROS/RNS. Thus, they are equipped with a large number of molecules that directly neutralize free radicals. These are referred to as free radical scavengers, the best known of which are probably vitamins E and C and reduced glutathione (GSH) (Wojcik 2010). There are, however, many other molecules that incapacitate free radicals. Their individual concentrations within cells vary widely and, likewise, different cell types harbor different concentrations of these agents.

While direct free radical scavengers obviously help cells to resist molecular damage, it is not the only means they employ to combat the molecular destruction normally meted out by ROS/RNS. Likewise, enzymes are used to metabolically convert reactive species to innocuous products (Reiter *et al.* 2000). Some of the best known of these catalytic agents are the superoxide dismutases, glutathione peroxidases and catalase. As with the direct free radical scavenges, the activities of these antioxidative enzymes are not uniformly distributed among all cells.

Transition metals such as free iron (Fe) also assist in the generation of the most devastatingly toxic hydroxyl radical ($\cdot$OH) in what is referred to as the Haber-Weiss and Fenton reactions (Vidrio *et al.* 2008). Transition metals are usually bound to other molecules within cells and are therefore unable to initiate the reactions that lead to the formation of the $\cdot$OH. Some molecules are especially effective in binding transition metals; perhaps the best known of these metal chelators are transferrin and ferritin (Schmid 2009). Because of their capacity to bind transition metals, chelators reduce molecular damage normally inflicted by free radicals and, thereby, they function as antioxidants.

In the current brief review the role of an endogenously-produced indoleamine, melatonin (N-acetyl-5-methoxytryptamine), in detoxifying and metabolizing ROS/RNS will be summarized. The antioxidative actions of melatonin were uncovered less than two decades ago (Tan *et al.* 1993). In the interviewing years, numerous publications have repeatedly documented the ability of melatonin to scavenge free radicals (Stascia *et al.* 1998, 2000; Turjanski *et al.* 1998, 2001; Bandyopadhyay *et al.* 2000; Brömme *et al.* 2000; Zavodnik *et al.* 2006; Velkov *et al.* 2009). Moreover, metabolites of melatonin also function as radical scavengers (Zhang *et al.* 1999; Ceraulo *et al.* 1999; Blanchard *et al.* 2000; Tan *et al.* 2000, 2007; Rosen *et al.* 2006; Ressmeyer *et al.* 2003). These indoleamines are uncommonly effective in limiting molecular destruction by ROS/RNS. They have proven especially effective in reducing free radical damage and cellular death normally associated with catastrophic circumstances where ROS/RNS are massively generated (Maldonado *et al.* 2007; Mayo *et al.* 2005; Brusco *et al.* 1998; Samantaray *et al.* 2009; Hardeland *et al.*

2009). Besides the fact that melatonin detoxifies ROS/RNS directly, it is also capable of stimulating antioxidative enzymes which contributes to its ability to forestall molecular damage that is the result of O_2 derivatives (Pablos *et al.* 1995; Rodriguez *et al.* 2004; Reiter *et al.* 2000; Barlow-Walden *et al.* 1995; Pablos *et al.* 1998); these enzymes metabolize reactive species to harmless products before they have an opportunity to interact with and harm essential molecules.

Melatonin in the CNS

A major source of melatonin is the pineal gland which, in the human, is located near the anatomical center of the brain on the posterior dorsal aspect of the third ventricle. While the nocturnal release of melatonin into the rich capillary plexus in the gland has been known for several decades (Pelham *et al.* 1973; Pelham 1975; Vaughan *et al.* 1976; Arendt *et al.* 1978), it has also been surmised that the indoleamine may be discharged directly or indirectly into the cerebrospinal fluid (CSF) of the ventricular system in mammals (Mess & Trentini 1974; Reiter *et al.* 1975; Smith *et al.* 1976). This suspicion stemmed from the fact that in some non-mammalian vertebrates the morphophysiological evidence is suggestive of a CSF route of secretion of melatonin (Vigh & Vigh-Teichman 1988; Quay 1970a). This hypothesis is consistent with the fact that in addition to the pineal recess, a ventricular outpouching that penetrates the pineal (Kappers 1960; Hülsemann 1967; Quay 1970b), there are other morphophysiological features, e.g., the suprahabenular recess, that puts the CSF of the third ventricle virtually in direct contact with the pinealocytes (Sheridan *et al.* 1969). In each of these cases, a single layer of absorptive/secretory cells, the ependyma of the third ventricle, separates the pinealocyte processes from the CSF. Assuming that morphology portends physiology, a number of authors predicted a CSF route of secretion of melatonin in mammals.

A common misconception is that the pineal gland in vertebrates is a single well-circumscribed organ that is situated either directly on the habenula of the epithalamus (as in the human) (Hülsemann 1967) or in a superficial position beneath the junction of the superior sagittal and transverse sinus as in most rodents (Quay 1965). There are, however, many variations on these themes with some mammals, e.g., the Syrian hamster (Sheridan & Reiter 1970), having both a deep and superficial pineal. The presence of the deep pineal gland in the hamster along with an evagination of the third ventricle, i.e., the pineal recess, penetrating the base of the deep portion of the pineal gland leaves open the possibility for the direct release of melatonin into the ventricular CSF. Additionally, the existence of a prominent suprahabenular recess which makes contact with the superficial pineal may even allow this portion of the pineal complex to release melatonin into the third ventricle (Sheridan *et al.* 1969).

A technically-difficult and elegant series of studies carried out in the sheep (which has exclusively a deep pineal gland) by Skinner and Malpaux (1999) and Tricoire and colleagues (2002) have convincingly shown that the CSF route of secretion of pineal melatonin is highly likely. In the initial study, cannulae were placed into the third ventricle of sheep allowing for ventricular CSF to be collected periodically throughout a light:dark cycle. Not only did the findings reveal that the CSF exhibits a nighttime rise in melatonin as in the plasma, but the amplitude of the nocturnal levels of melatonin in the former fluid are orders of magnitude higher than those measured in simultaneously-collected blood samples. This provides a compelling argument that the pineal discharges melatonin not only into the blood vascular system but also, presumably via the pineal recess, into the third ventricle.

In a subsequent series of studies, the same group provided additional evidence to strengthen their argument for a CSF route of secretion of melatonin (Tricoire *et al.* 2002). They, thus reported that blockade of the pineal recess (by the installation of a biological glue) not only eliminated the marked day:night rhythm in CSF melatonin in the sheep but also levels of the indoleamine were reduced in the ventricular fluid. This provided proof that the melatonin rhythm in the CSF was not a consequence of the secretion of the indoleamine first into the blood and thereafter, via the choroid plexus, into the CSF. Despite the high importance of the discoveries by Skinner and Malpaux (1999) and Tricoire *et al.* (2002), the findings seem to have generated rather little interest among scientists working in this field of research. Besides the data from sheep, there are also findings in the human suggesting a CSF route of secretion of melatonin (Seifman *et al.* 2008; Leston *et al.* 2010). For ethical reasons, these studies are not as refined as those performed in sheep.

Maurizi (2003; 2010) has been a long-time advocate of the release of melatonin, directly or indirectly, into the CSF. He recently reviewed the morphological evidence of the human brain which supports his supposition (Maurizi 2010). While it is an interesting speculation, Maurizi (2010) does not provide any measurements of CSF melatonin levels; for this, he depends on the work of others (Reiter & Tan 2002; Tan *et al.* 2010).
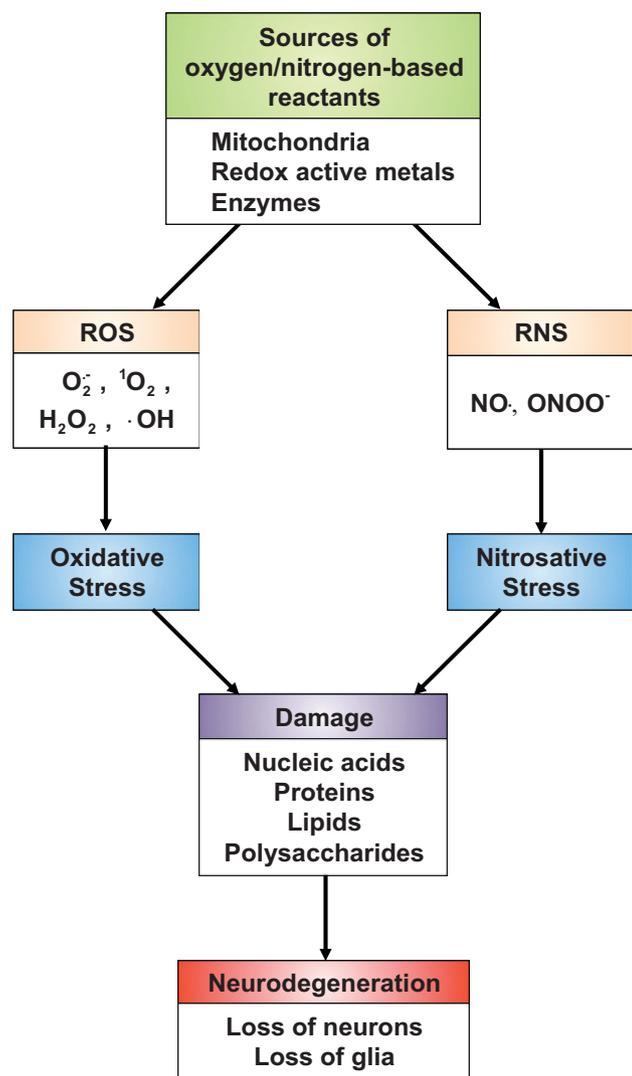
Given that melatonin has actions within the central nervous system (Cardinali *et al.* 2002a; Wu & Swaab 2005; Cervantes *et al.* 2008; Manda & Reiter 2010), the release of this indoleamine directly into the CSF of the ventricular system would have obvious advantages (Reiter & Tan 2002; Tan *et al.* 2010; Arendt *et al.* 1985). The volume of CSF is small compared to that of the blood and, thus, the dilution factor in the former fluid would be much less than in the plasma; this could well explain the higher CSF concentrations as reported in the sheep (Skinner & Malpaux 1999; Tricoire *et al.* 2002). Normally, systemically-released melatonin is quickly metabolized in the liver to yield primarily 6-hydroxymelatonin sulfate (Arendt *et al.* 1985). This fate would be avoided by CSF-released melatonin. Melatonin in the ventricles would have ready access to neurons and glia, especially presumably those cells located relatively near to the ventricular walls. Once taken up by neurons, melatonin is metabolized to kynuramines which have essential functions in neuronal physiology (Hardeland *et al.* 2009). Also, some of the basic actions of melatonin, e.g., the mediation of seasonal reproduction in photoperiodic species (Hoffman & Reiter 1965; Reiter 1973), involve interactions of melatonin with neurons in the medial basal hypothalamus and in the pars tuberalis of the anterior pituitary gland. Melatonin in the CSF that is taken up by tanycytes lining the infundibular recess of the third ventricle would readily access these sites (Reiter *et al.* 2010a). Finally, considering the ability of melatonin to protect neurons and glia from free radical damage (Jou *et al.* 2004; Das *et al.* 2010; Manda *et al.* 2009; Dong *et al.* 2010), the discharge of melatonin into the ventricles would put it in immediate contact with the CNS cells it normally protects.

The markedly different concentrations of melatonin in the CSF and blood raises another important issue. So-called physiological concentrations of melatonin have classically been based on blood levels as a reference value, which even at night are in the low nanomolar range. It is now clear, however, that blood concentrations of melatonin are not representative of levels in the CSF (Skinner & Malpaux 1999; Tricoire *et al.* 2002) or for that matter in other fluids/tissues (Bubenik 2001; Jimenez-Jorge *et al.* 2007; Tan *et al.* 1999a; Carrillo-Vico *et al.* 2004; Tan *et al.* 1999b). Thus, concentrations of melatonin that may be considered pharmacological in the blood are in fact physiological in other fluids/cells (Reiter & Tan 2003). Moreover, it has been speculated, based on limited data, that concentrations of melatonin in subcellular organelles, e.g., mitochondria (Martin *et al.* 2000) and the nucleus (Menendez-Pelaez & Reiter 1993; Mennenga *et al.* 1991), may exceed those in extracellular space. Thus, when the phrase "physiological concentration of melatonin" is used, it is judicious to define these values in the context of a specific fluid or subcellular organelle (Reiter & Tan 2003).

Melatonin: Preserving Neurons

The amount of oxidative abuse and tissue loss inflicted by free radicals in the CNS is generally greater than the quantity of damage that occurs in other organs under similar conditions. This relates, in part, to the fact that the brain normally uses a disproportionately larger amount of the inhaled O_2, the precursor of ROS/RNS. Thus, despite its small size, relative to the total body mass, even in the so-called "inactive" state the brain extracts up to 20% of the total O_2 carried by the blood for its exclusive use; this percentage increases as neural tissue becomes progressively more active. In addition

Sources of oxygen/nitrogen-based reactants

Mitochondria
Redox active metals
Enzymes

ROS

$O_2^{\cdot-}$, $^1O_2$, H_2O_2 , $\cdot OH$

RNS

$NO^{\cdot}$, $ONOO^-$

Oxidative Stress

Nitrosative Stress

Damage

Nucleic acids
Proteins
Lipids
Polysaccharides

Neurodegeneration

Loss of neurons
Loss of glia

FIGURE 1. A general scheme illustrating the destructive role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the central nervous system that results in the loss of cellular elements (neurons and glia) which culminate in neurodegeneration. There are multiple sources of ROS [superoxide anion radial ($O_2^{\cdot-}$); singlet oxygen ($^1O_2$); hydrogen peroxide (H_2O_2); hydroxyl radical ($\cdot OH$)]. The peroxynitrite anion ($ONOO^-$) is formed when $O_2^{\cdot-}$ couples with nitric oxide ($NO^{\cdot}$). The two most damaging reactants are the $\cdot OH$ and $ONOO^-$. Melatonin, which readily enters neurons and glia, increases the efficiency of electron transfer through the mitochondrial respiratory chain thereby reducing electron leakage and free radical generation (a process referred to as radical avoidance) and it directly scavenges ROS/RNS when they are formed. Additionally, melatonin stimulates antioxidative enzymes which metabolize ROS to harmless products (not shown in this scheme). Melatonin also suppresses the formation of $NO^{\cdot}$ by inhibiting the prooxidative enzyme, nitric oxide synthesis (not shown in this scheme). The resulting neurodegeneration contributes to disorders such as Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, etc., as well as neurobehavioral and psychological disorders.

to its high utilization of O_2, neurons have a reduced capacity to protect themselves from intrinsically generate radicals and related products because of a relative deficiency of the enzymes required to metabolize radicals to inactive products.

The problem of molecular damage and death of neurons is compounded by the fact that neurons, once lost, generally do not replenish themselves because they are terminally differentiated and postmitotic. In recent years, however, this rule has been shown not to apply to all areas of the brain since neural progenitor cells allow some neurons to be replaced on a regular basis, e.g., neurons in the dentate gyrus of the hippocampal formation (Manda *et al.* 2009; Ramirez-Rodriguez *et al.* 2009; Kim *et al.* 2010; Uban *et al.* 2010; Ohira *et al.* 2010). What is additionally of interest is that the neuronal precursors that are capable of mitosis respond to melatonin with elevated proliferative capacity (Manda *et al.* 2009; Kim *et al.* 2004). Thus, melatonin not only preserves neurons from destruction during an oxidative challenge, but it also promotes the replacement of cells by stimulating neuron progenitor cell proliferation.

The loss of neurons either as a function of aging, genetically-based destructive processes or accidental damage, often has dire consequences in terms of dementia, neurodegenerative diseases and psychological disturbances (Fig. 1). The effects of age on brain deterioration may be in part related to the loss of endogenously-generated melatonin which naturally occurs in older individuals of at least the species in which studies have been performed, including the human (Reiter *et al.* 1980, 1981; King *et al.* 1981; Sack *et al.* 1986; Pang *et al.* 1990).

That diminished melatonin production may be consequential in terms of contributing to deteriorative changes associated with growing old are observations which show that surgical removal of the pineal gland early in life in rats accelerates the aging process in many organs as shown by the more rapid buildup of oxidatively-damaged molecules within cells (Reiter *et al.* 1999a). A major explanation of the aging process is that it occurs because of accumulated free radical damage (Harman 2003). If this theory has validity, then the loss of melatonin during aging may be one factor that promotes cellular deterioration not only in the brain but in other tissues as well (Reiter *et al.* 1996; Reiter *et al.* 2008). This may also have implications for individuals who are genetically relatively deficient in melatonin at an early age. The question is, does the amplitude of the nocturnal melatonin peak (as an index of the amount of melatonin being produced) have any influence on the rate of aging? While an answer to this question is not available, the early pinealectomy study mentioned above (Reiter *et al.* 1999a) indicates this could be the case.

Dementia, motor and sensory deficits, difficulties in remembering as well as extrapyramidal motor dysfunction often become manifested in aged humans. These degenerative processes are accompanied by the loss

of neurons and are often manifested as serious neurodegenerative diseases. Moreover, the actual loss of neurons in these individuals is often considered to be a result of the consistent free radical bludgeoning that occurs within the neurons which eventually succumb to apoptosis or necrosis. Some names of these neurodegenerative diseases are known even to the lay person and are under intensive investigation by the scientific community.

One such condition is Alzheimer disease (AD). The clinical signs of AD (persistent anterograde memory loss, language deterioration, dementia and associated neurobehavioral abnormalities) are well known and, unfortunately, experienced by many elderly individuals. Considering the larger number of individuals that are surviving into advanced age, AD is expected to become more common (Cummings & Cole 2002). Some of the major neuropathological indicators of this disease include extraneuronal senile plagues, which contain β-amyloid deposits, and intracellular neurofibrillary tangles, which include paired helical filaments (Mancuso *et al.* 2010). These abnormalities contribute to extensive diffuse neuronal loss, particularly in the hippocampus and neocortex and a reduction in actual brain size (Teter 2004). While early onset AD has been reported, well over 90% of the cases are what is referred to as a late-onset disease. Obviously aging, along with the presence of apolipoprotein (Apo) E4 polymorphisms are clearly risk factors for AD (Teter 2004).

That free radical damage contributes to neuronal loss in AD is supported by data showing that the brain of these individuals contains large numbers of oxidatively-damaged molecules including modified DNA, lipids and proteins (Perry *et al.* 1998). Since oxidative stress is a component of AD, antioxidants are of interest as potential treatments for this condition (Lee *et al.* 2010a). Because of its high efficacy as an antioxidant, melatonin has also been tested for its ability to forestall neurodegenerative signs in several experimental animal models of AD (Reiter *et al.* 1999b; Pappolla *et al.* 2000; Lahiri *et al.* 2005) and the neurobehavioral deficits that occur in humans (Brusco *et al.* 1998; Lauterbach *et al.* 2010; Sanchez-Barcelo *et al.* 2010; Cardinali *et al.* 2002b).

The results garnered from experimental models of AD strongly support the likelihood that melatonin may well be beneficial in alleviating neuronal loss resulting from β-amyloid deposition and the development of neurofibrillary tangles (Matsubara *et al.* 2003; Deng *et al.* 2006; Feng *et al.* 2006). In the study by Matsubara and colleagues (Matsubara *et al.* 2003), providing melatonin in the drinking water of transgenic mice who normally accumulate β-amyloid in their brain as they age, actually reduced the neural β-amyloid burden, limited oxidative damage and lower the death rate of the animals. The suppressive effect of melatonin on β-amyloid was also observed by Olcese *et al.* (Olcese *et al.* 2009) who used a similar

transgenic mouse model. The results of this complete study also showed that orally administered melatonin (in the drinking water) also abated the inflammatory response which also contributes to AD (Rosales-Corral *et al.* 2010). When the transgenic AD-prone mice were given melatonin, their performance in a battery of cognitive tests was also improved compared to that in non-melatonin supplement mice (Olcese *et al.* 2009). The behavioral tests that were performed included tasks to examine effects on working memory, spatial reference learning/memory and basic mnemonic function. Although only a single experiment has examined whether the protective effects of melatonin on experimental AD pathology are membrane receptor mediated, that report indicated these receptors are not involved (Pappolla *et al.* 2002). This finding is consistent with melatonin's protective actions being receptor independent (at least independent of membrane receptors for the indoleamine); if this is confirmed, it will suggest that the direct free radical scavenging actions of melatonin contribute to the ability of this indoleamine to alleviate the signs of AD.

Compared to the relatively large amount of information regarding the ability of melatonin to modulate processes resulting from β-amyloid toxicity, information regarding the actions of this indoleamine on tau hyperphosphorylation and neurofibrillary tangle formation is scant. Neurofibrillary tangles in neurons include paired helical filaments which are rich in the microtubule-associated protein, tau. The tangles are formed as a consequence of the hyperphosphorylation of the tau protein; this cytoskeletal modification contributes to the pathology of AD.

Benitez-King *et al.* (2004, 2006) have been leading researchers in defining the actions of melatonin on cytoskeletal organization and function. They have shown that melatonin preserves the integrity of cytoskeletal organization thereby improving cell function including neurite outgrowth. Given that neurofibrillary tangles represent a distorted cytoskeleton, melatonin has been assessed for its ability to modulate tau hyperphosphorylation.

The β-adrenergic agonist, isoproterenol, induces tau hyperphosphorylation in hippocampal cells when it is injected directly into the temporal lobe. Using this model, Wang *et al.* (2005) found that the intraperitoneal administration of melatonin arrested isoproterenol-mediated tau hyperphosphorylation. They also deduced from their findings that melatonin prevented the phosphorylation of tau at both the Tau-1 and PHF-1 sites. Based on these findings, the authors concluded that melatonin may well be beneficial in reducing Alzheimer-like tau hyperphosphorylation.

The hyperphosphorylation of tau protein involves specific kinases and phosphatases. One kinase known to phosphorylate the tau protein is GSK-3β (Crespo-Biel *et al.* 2007). Using organotypic hippocampal slices, Hoppe and co-workers (Hoppe *et al.* 2010) reported

that melatonin reduced activation of GSK-3β and tau phosphorylation that were induced by the addition of AB_{25-35} to the culture medium. The authors speculated that, because of their observations, melatonin may have utility in protecting against β-amyloid phosphorylation of tau in the AD brain and that this may be a result of the ability of melatonin to repress GSK-3β activity.

Mitochondria are a major source of free radicals even under normal conditions and, in AD, neural mitochondria are believed to be a major site of excessive free radical generation (Reddy & Beal 2005). The actions of melatonin at the mitochondrial level in terms of its ability to scavenge radicals and reduce processes that culminate in cellular apoptosis are well defined (Acuna-Castroviejo et al. 2003; Leon et al. 2004, 2005; Paradies et al. 2010); this suggests that this site of action of melatonin could contribute to its efficacy in reducing AD pathophysiology. It is likely, however, the free radical scavenging alone does not account for the ability of this indoleamine to so effectively reduce oxidative damage and neuronal loss via apoptosis in models of AD. Melatonin's high efficacy in these situations coupled with its favorable safety profile should encourage clinicians to consider its use to combat this very debilitating neurodegenerative disease.

Parkinson disease (PD) is another common neurodegenerative disorder that is believed to involve free radical destruction of dopamine-containing neurons. The neurons that are pulverized in PD are located in the pars compacta of the substantia nigra in the mesencephalon. The dopaminergic neurons undergo apoptosis due to mitochondrial dysfunction which results in excessive free radical generation. While many of the signs of PD are attributed to loss of dopaminergic neurons, it is in fact a multisystem disorder with a variety of etiologies and it presents with a number of different clinical phenotypes (Inzelberg et al. 2002; Savica et al. 2010).

Several drugs are used to induce PD-like signs in experimental animals; these include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine and rotenone. Melatonin has been shown, in most studies, to reduce the damaging actions of each of these toxins at the level of the dopaminergic neurons and to attenuate neurobehavioral consequences. The most widely used of these toxins for the purpose of inducing PD-like signs in animals is MPTP (Chiueh et al 1993; Schapira 2010).

When injected into an animal, MPTP successfully crosses the blood-brain barrier after which it enters astrocytes where it is metabolized to its neurotoxic metabolite, 1-methyl-4-phenylpyridinium ion (MPP+). MPP+ escapes the astrocyte and enters the dopaminergic neuron via the dopamine transporter; once in the neuron, MPP+ interferes with mitochondrial complex I function allowing for the formation of a massive number of free radicals which eventually kill the neuron via apoptosis (Chetsawang et al 2007).

The initial study reporting on the ability of melatonin to attenuate the toxicity of MPTP was published by Acuna-Castroviejo et al (1997). Using mice, they observed that MPTP administration caused elevations in products of lipid peroxidation, indicative of heightened oxidative stress, in the corpus striatum and hippocampus and a reduced striatal tyrosine hydroxylase activity; this enzyme is rate-limiting in dopamine production. When melatonin was given in conjunction with MPTP, none of the changes that followed MPTP administration alone appeared. Follow-up studies from the same laboratory have established that melatonin also counteracts MPTP-mediated DNA fragmentation in cells of the striatum and midbrain and synergizes with deprenyl to ameliorate MPTP-induced mitochondrial damage and dopamine depletion (Khaldy et al 2003; Ortiz et al 2001). Also, they found that not only melatonin, but a metabolite of melatonin that also possesses antioxidant activity, N1-acetyl-5-methoxykynuramine (AMK), was neuroprotective against MPTP toxicity. In this case, AMK prevented MPTP-mediated elevation in mitochondrial inducible nitric oxide synthase (iNOS) which follows compromised mitochondrial function (Tapias et al 2009). PD has been shown to be associated with rises in iNOS which results in the generation of nitric oxide. The latter molecule couples with the superoxide anion to form the highly toxic peroxynitrite anion. Thus, by reducing iNOS AMK likely reduced peroxynitrite formation and the induction of neuronal apoptosis. All other consequences of MPTP toxicity that lead to neuronal apoptosis have also been shown to be prevented or markedly reduced by the concurrent administration of melatonin (Wang 2009). Thus, mitochondrial calcium overload, mitochondrial membrane depolarization and opening of the mitochondrial transition pore all of which follow the treatment of animals with MPTP, have been found to be stymied by melatonin (Wang 2009). Additionally, melatonin induces what is referred to as radical avoidance (Hardeland 2005). Collectively, the studies using MPTP toxicity as a model of parkinsonism have documented that melatonin is an effective inhibitor of dopaminergic cell loss and could, therefore, be beneficial in the treatment of human PD.

Other models used to test the efficacy of melatonin in reducing signs of PD in experimental animals have been less frequently used than MPTP. 6-hydroxydopamine (6-OHDA) is a catecholaminergic neurotoxin that is injected directly into the substantia nigra; it damages and eventually kills the adjacent dopaminergic neurons resulting in degeneration of the ascending nigrostriatal pathway (Shah et al 2010). Uniformly, melatonin successfully counteracted 6-OHDA toxicity either when the indoleamine was injected peripherally daily or administered via the drinking water (Reiter et al 2010b). Of interest are also the observations that melatonin promoted the growth and function of stem cells that had been implanted into the 6-OHDA-lesioned substantia nigra (Sharma et al

2007). Given that stem cell therapy is attracting a great deal of attention as a means of potentially restoring neural function in individuals with neurodegenerative diseases including PD, these findings indicate that when stem cells are implanted an appropriate co-treatment may be melatonin therapy.

Rotenone is a pesticide which, when administered to animals, duplicates many of the neuropathological changes normally found in PD (Betarbet & Greenamyre 2007). While there is a single study which claims that melatonin did not protect against rotenone toxicity (Tapias *et al* 2010), all others showed the indoleamine was capable of doing so (Reiter *et al* 2010b). In the successful investigations, animal models from fruit flies to rats were used. Why the investigation of Tapias *et al* (2010) is such an obvious exception is not apparent.

Because of the frequency with which AD and PD occur in humans, most of the studies that have examined melatonin's efficacy in inhibiting neurodegeneration have used models for these conditions. There are other neural diseases of the aged where free radical damage also appears to be a conspicuous feature. Huntington's disease (or chorea) and amyotrophic lateral sclerosis (ALS) are fatal conditions in humans. The etiology of Huntington's disease (HD) involves oxidative stress-mediated neuronal loss initially in the striatum and cerebral cortex with eventual spread to other areas of the brain (Browne & Beal 2006). ALS is a consequence primarily of the death of motor neurons in the ventral horn of the spinal cord; in this condition as well, oxidative stress is accepted as a major detrimental process (Wood-Allum & Shaw 2010). In experimental models of HD (Cabrera *et al* 2000), melatonin was found to reduce oxidative damage in the CNS and in both HD and ALS patients the indoleamine had some beneficial effects although much more data is required before the suggestion that melatonin may be an effective treatment for these conditions is accepted (Wang 2009).

Melatonin: Preserving Glia

Supporting elements of the CNS, i.e., glial cells, also benefit from the presence of melatonin. Oligodendrocytes, which from myelin in the CNS, are protected from damage and stimulated to preserve myelin formation in the hypoxic rats given melatonin (Olivier *et al* 2009); the benefits provided by melatonin in this case relates to its antioxidant properties (Kaur *et al* 2010).

Microglia are equivalent to macrophages and exist throughout the CNS; they are quickly activated during virtually any pathological change in the brain (Jellinger 2008; Watkins *et al* 2007; Hulsebosch 2008). There is some evidence that microglia may produce melatonin (Fukuda *et al* 2010), although this finding is far from definitive. Methamphetamine (METH), a common drug of abuse, is neurotoxic and promotes oxidative stress and inflammation in the CNS. In cultured

microglial cells, METH induces the generation of both ROS and RNS and stimulates the production of toxic pro-inflammatory cytokines. These METH-mediated actions are inhibited by melatonin (Tocharus *et al* 2010).

There are a variety of interactions of melatonin with astrocytes but the most complete studies documenting the ability of the indoleamine to scavenge ROS in astrocytic mitochondria are provided in two publications by Jou and co-workers (2010; 2004; 2008) which are summarized in a recent review article (Tocharus *et al* 2010). These well-illustrated articles describe and document conclusively the ability of melatonin to scavenge ROS at the level of the mitochondria in astrocytes. In doing so, melatonin prevents apoptosis of these cells. These findings are perhaps the best to date which confirm melatonin's actions at the mitochondrial level. Reactive astrocytosis, which occurs as a consequence of constant light exposure in rats, is reduced by melatonin administration (Baydas *et al* 2002). Since constant light exposure depresses pineal melatonin production and secretion, these findings suggest that endogenous melatonin levels are adequate to hold reactive gliosis in check (Stankov *et al* 1991).

The actions of melatonin in the regulation of glial cell physiology is no less important than the influence of this indoleamine on neuronal function (Fig. 1). Hence, more extensive investigation of melatonin/glial cell interactions seem warranted.

Concluding Remarks

In this brief review we have emphasized the direct free radical scavenging, receptor-independent actions of melatonin in the CNS. Certainly, membrane melatonin receptors exist on brain cells (Stankov *et al* 1991; Zawilska *et al* 2009; Adi *et al* 2010) and it seems likely that they also participate in the protective actions of melatonin on neurons and glia. Moreover, besides the specific neurodegenerative diseases discussed herein, melatonin use has also been shown to be beneficial in other pathophysiological disorders that involve the brain (Molina-Carballo *et al* 1997; Milano *et al* 2008; Bendz & Scales 2010; Rahman *et al* 2010). Research related to the neural benefits of melatonin is still in its infancy and it is anticipated that this area of research will continue to attract enthusiasts over the next decade.

The reports summarized in this brief review demonstrate unequivocally the ability of an endogenously-produced molecule, melatonin, to reduce oxidative damage in the central nervous system. The importance of these findings lie in the fact that free radical damage to neurons and glia contributes to a variety of neurodegenerative diseases that are common in the aged. As reviewed herein, in models of Alzheimer disease, Parkinson disease, Huntington disease and amyotrophic lateral sclerosis, melatonin has proven effective in reducing the molecular deterioration and cellular

death that results from the excessive generation of both ROS and RNS in the diseased brain. Given the high safety profile of melatonin (Sanchez-Barcelo *et al* 2010; Korkmaz *et al* 2009), its utility in treating these highly debilitating neural disorders, which essentially have no or limited treatments, should be seriously considered. Preliminary and limited clinical trials have already shown that melatonin could prove effective in reducing neural damage associated with the oxidative and nitrosative destruction that inevitably occurs in the central nervous system as a function of aging.

REFERENCES

1 Acuna-Castroviejo D, Coto-Montes A, Monti MG, Ortiz GG, Reiter RJ (1997). Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life Sci.* **60**: PL23–29.

2 Acuna-Castroviejo D, Escames G, Leon J, Carayo A, Khaldy H (2003). Mitochondrial regulation by melatonin and it metabolites. *Adv Exp Biol Med.* **527**: 549–557.

3 Adi N, Mash DC, Ali Y, Singer C, Shehadeh L, Papapetropoulos S (2010). Melatonin MT1 and MT2 receptor expression in Parkinson's disease. *Med Sci Monit.* **16**: BR 61–7.

4 Arendt J, Bojkowski C, Franey C, Wright J, Marks V (1985). Immunoassay of 6-hydroxy melatonin sulfate in human plasma and urine: abolition of the urinary 24-hour rhythm with atenolol. *J Clin Endocrinol Metab.* **60**: 1166–1173.

5 Arendt J, Wirz-Justice A, Bradtke J (1978). Annual rhythm of serum melatonin in man. *Neurosci Lett.* **7**: 327–330.

6 Bandyopadhyay D, Biswas K, Bandyopadhyay U, Reiter RJ, Banerjee AK (2000). Melatonin protects against stress-induced gastric lesions by scavenging hydroxyl radicals. *J Pineal Res.* **29**: 143–151.

7 Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD *et al.* (1995). Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int.* **26**: 497–502.

8 Baydas G, Reiter RJ, Nedzvetski VS, Nerush PA, Kirichenko SV (2002). Altered glial fibrillary acidic protein content and its degradation in the hippocampus, cortex and cerebellum of rats exposed to constant light: reversal by melatonin. *J Pineal Res.* **33**: 134–139.

9 Bendz LM & Scales AC (2010). Melatonin treatment for insomnia in pediatric patients with attention-deficit/hyperactivity disorder. *Ann Pharmacother.* 44: 185–191.

10 Benitez-King G (2006). Melatonin as a cytoskeletal modulator: implications for cell physiology and disease. *J Pineal Res.* **40**: 1–9.

11 Benitez-King G, Ramirez-Rodriguez G, Ortiz L, Meza I (2004). The neuronal cytoskeleton as a potential therapeutical target in neurodegenerative diseases and schizophrenia. *Curr Drug Targets CNS Neurol Disord.* 3: 515–533.

12 Betarbet R & Greenamyre JT (2007). Parkinson's disease: animal models. *Handb Clin Neurol.* **83**: 265–287.

13 Blanchard B, Pompon D, Ducrocq C (2000). Nitrosation of melatonin by nitric oxide and peroxynitrite. *J Pineal Res.* **29**: 184–192.

14 Brömme HJ, Morke W, Peschke E, Ebelt H, Peschke, D (2000). Scavenging effect of melatonin on hydroxyl radicals generated by alloxan. *J Pineal Res.* **29**: 201–208.

15 Browne SE & Beal MF (2006). Oxidative damage in Huntington's disease pathogenesis. *Antioxid Redox Signal.* **8**: 2061–2073.

16 Brusco LI, Marquez M, Cardinali DP (1998). Monozygotic twins with Alzheimer's disease treated with melatonin: case report. *J Pineal Res.* **25**: 260–263.

17 Bubenik GA (2001). Localization, physiological significance and possible clinical implication of gastrointestinal melatonin. *Biol Signals Recept.* **10**: 350–366.

18 Cabrera J, Reiter RJ, Tan DX, Qi W, Sainz RM, Mayo JC *et al.* (2000). Melatonin reduces oxidative neurotoxicity due to quinobinic acid: in vitro and in vivo findings. *Neuropharmacology.* **39**: 507–514.

19 Cardinali DP, Brusco LI, Liberczuk C, Furio AM (2002b). The use of melatonin in Alzheimer's disease. *Neuroendocrinol Lett.* **23**(Suppl 1): 20–23.

20 Cardinali DP, Brusco LI, Lloret SP, Furio AM (2002a). Melatonin in sleep disorders and jet-lag. *Neuroendocrinol Lett.* **23**(Suppl 1): 9–13.

21 Carrillo-Vico A, Calvo JR, Abreu P, Lardone PJ, García-Maurino S, Reiter RJ *et al.* (2004). Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine and/or paracrine substance. *FASEB J.***18**(3): 537–539.

22 Ceraulo L, Ferrugia M, Tesoriere L, Segreto S, Livrea MA, Turco Liveri V (1999). Interactions of melatonin with membrane models: portioning of melatonin in AOT and lecithin reversed micelles. *J Pineal Res*. **26**: 108–112.

23 Cervantes M, Morali G, Letechipia-Vallejo G (2008). Melatonin and ischemia-reperfusion injury of the brain. *J Pineal Res.* **45**: 1–7.

24 Chetsawang J, Govitrapong P, Chetsawang B (2007). Melatonin inhibits MPP+-induced caspase-mediated death pathway and DNA fragmentation factor-45 cleavage in SK-N-SH cultured cells. *J Pineal Res.* **43**: 115–120.

25 Chiueh C, Miyake H, Peng MT (1993). Role of dopamine autoxidation, hydroxyl radical generation, and calcium overload in underlying mechanisms involved in MPTP-induced parkinsonism. *Adv Neurol.* **60**: 251–258.

26 Crespo-Biel N, Canudas A, Pallas M (2007). Kainate induces AKT, ERK and cdk5/GSK-3β pathway deregulation, phosphorylates tau protein in the mouse hippocampus. *Neurochem Int.* **50**: 435–442.

27 Cummings JL & Cole G (2002). Alzheimer disease. *J Am Med Assoc.* **287**: 2335–2338.

28 Das A, McDowell M, Pava MJ, Smith JA, Reiter RJ, Woodward JJ *et al.* (2010). The inhibition of apoptosis by melatonin in VSC4.1 motoneurons exposed to oxidative stress, glutamate excitotoxicity, or TNF-α toxicity involves membrane melatonin receptors. *J Pineal Res.* **48**: 157–169.

29 Deng YQ, Xu GG, Duan P, Zhang Q, Wang JZ (2006). Effects of melatonin on wortmannin-induced tau hyperphosphorylation. *Acta Pharmacol Sin.* **26**: 519–526.

30 Dong W, Huang F, Fan W, Cheng S, Chen Y, Zhang W et al. Differential effects of melatonin on amyloid-β peptide$_{25-35}$ induced mitochondrial dysfunction in hippocampal neurons at different states of culture. *J Pineal Res.* **48**(2): 117–125.

31 Feng Z, Qin C, Chang Y, Zhang JT (2006). Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. *Free Rad Biol Med.* **40**: 101–109.

32 Fukuda T, Akiyama N, Ikeganir M, Takahashi H, Sasaki A, Oka H *et al.* (2010). Expression of hydroxyindole-O-methyltransferase enzyme in the human central nervous system and in pineal parenchymal tumor cells. *J Neuropathol Exp Neurol.* **69**: 498–510.

33 Hardeland R (2005). Antioxidant protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine.* **27**: 119–130.

34 Hardeland R, Tan DX, Reiter RJ (2009). Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J Pineal Res*. **47**: 109–126.

35 Harman D (2003). The free radical theory of aging. *Antioxd Redox Signal.* **5**: 557–561.

36 Hoffman RA & Reiter RJ (1965). Pineal gland: influence on gonads of male hamsters. *Science.* **148**: 1609–1611.

37 Hoppe JB, Frozza RL, Horn AP, Comiran RA, Bernardi A, Campos MM *et al.* (2010). Amyloid-β neurotoxicity in organotypic culture is attenuated by melatonin: involvement of GSK-3β, tau and neuroinflammation. *J Pineal Res.* **48**: 230–238.

38 Hulsebosch CE (2008). Gliopathy ensures persistent inflammation and chronic pain after spinal cord injury. *Exp Neurol.* **214**: 6–9.

39 Hülsemann M (1968). Vergleichende histologische untersuchungen über das vorkommen von Gliafasern in de epiphysis cerebri von Säugetieren. *Acta Anat.* **66**: 249–278.

40 Inzelberg R, Schechtman E, Paleacu D (2002). Onset age of Parkinson disease. *Am J Med Genet.* **111**: 459–460.

41 Jellinger KA (2008). Neuropathological aspects of Alzheimer disease, Parkinson disease and frontotemporal dementia. *Neurodegen Disord.* **5**: 118–121.

42 Jimenez-Jorge S, Guerrero JM, Jimenez-Caliani AJ, Naranjo MC, Lardone PJ, Carrillo-Vico A *et al.* (2007). Evidence for melatonin synthesis in rat brain during development. *J Pineal Res.* **42**(3): 240–246.

43 Jou MJ (2008). Pathophysiological and pharmacological implications of mitochondria-targeted reactive oxygen species generation in astrocytes. *Adv Drug Deliv Rev.* **60**: 1512–1526.

44 Jou MJ, Peng TH, Hsu LF, Jou B, Reiter RJ, Yang CM *et al.* (2010). Visualization of melatonin's multiple mitochondrial levels of protection against mitochondrial $Ca^{2\pm}$ mediated permeability transition and beyond in rat brain astrocytes. *J Pineal Res.* **48**: 20–38.

45 Jou MJ, Peng TI, Reiter RJ, Jou SB, Wu HY, Wen ST (2004). Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. *J Pineal Res.* **37**: 55–70.

46 Kappers JA (1960). The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. *Z Zellforsch.* **52**: 163–215.

47 Kaur C, Sivakumar V, Ling EA (2010). Melatonin protects periventricular white matter from damage due to hypoxia. *J Pineal Res.* **48**: 185–193.

48 Khaldy H, Escames G, Leon J, Bikjdaouene L, Acuna-Castroviejo D (2003). Synergistic effects of melatonin and deprenyl against MPTP-induced mitochondrial damage and DNA depletion. *Neurobiol Aging.* **24**: 491–500.

49 Kim DH, Kim JM, Park SJ, Lee S, Yoon BH, Ryu JH (2010). Early activated microglia play a role in transient forebrain ischemia-induced neural precursor proliferation in the dentate gyrus of mice. *Neurosci Lett.* **475**: 74–79.

50 Kim MJ, Kim HK, Kim BS, Yim SV (2004). Melatonin increases cell proliferation in the dentate gyrus of maternally separated rats. *J Pineal Res.* **37**: 193–197.

51 King TS, Richardson BA, Reiter RJ (1981). Age-associated changes in pineal serotonin N-acetyltransferase activity and melatonin content in the male gerbil. *Endocr Res Commun.* **8**: 253–262.

52 Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX (2009). Melatonin: an established antioxidant worthy of use in clinical trials. *Mol Med.* **15**: 43–50.

53 Lahiri DK, Chen DM, Lahiri P, Bondy S, Greig NH (2005). Amyloid, cholinesterase, melatonin and their roles in aging and neurodegenerative conditions. *Ann NY Acad Sci.* **1056**: 430–449.

54 Lambeth JD (2007). Nox enzymes, ROS and chronic disease: an example of antagonistic pleiotropy. *Free Rad Biol Med*. **43**: 332–347.

55 Lauterbach EC, Victoroff J, Coburn KL, Shillcult SD, Doonan SM, Mendez MF (2010). Psychopharmacological neuroprotection in neurodegenerative disease: assessing the preclinical data. *J Neuropsychiatry Clin Neurosci.* **22**: 8–18.

56 Lee HP, Zhu XW, Casadesus RJ, Castellani RJ, Nunomura A, Smith MA *et al.* (2010a). Antioxidant approaches for the treatment of Alzheimer's disease. *Expert Rev Neurother.* **10**: 1201–1208.

57 Lee SI, Jeong SR, Kang YM, Han DX, Jin BK, Namgung U *et al.* (2010b). Endogenous expression of interleukin-4 regulates macrophage activation and confines cavity formation after traumatic spinal cord injury. *J Neurosci Res*. **88**: 2409–2419.

58 Leon J, Acuna-Castroviejo D, Escames, G, Tan DX, Reiter RJ (2005). Melatonin mitigates mitochondrial malfunction. *J Pineal Res.* **38**: 1–9.

59 Leon J, Acuna-Castroviejo D, Sainz RM, Mayo JC, Tan DX, Reiter RJ (2004). Melatonin and mitochondrial function. *Life Sci.* **75**: 765–790.

60 Leston J, Harthe C, Brun J, Mottolese C, Mertens P, Sindou M *et al.* (2010). Melatonin is released in the third ventricle in humans: a study of movement disorders. *Neurosci Lett.* **469**: 294–297.

61 Maldonado MD, Murillo F, Terron MP, Flores LJ, Tan DX, Manchester LC *et al.* (2007). The potential of melatonin in reducing morbidity/mortality after craniocerebral trauma. *J Pineal Res.* **42**: 1–11.

62 Mancuso M, Orsucci D, Lo Gerfo A, Calsolaro V, Siciliano G (2010). Clinical features and pathogenesis of Alzheimer's disease: involvement of mitochondria and mitochondrial DNA. *Adv Exp Biol Med.* **685**: 34–44.

63 Manda K & Reiter RJ (2010). Melatonin maintains adult neurogenesis and cognitive functions after irradiation. *Progr Neurobiol.* **90**: 60–68.

64 Manda K, Ueno M, Anzai K (2009). Cranial irradiation-induced inhibition of neurogenesis in hippocampal dentate gyrus of adult mice: attenuation by melatonin pretreatment. *J Pineal Res.* **46**: 71–78.

65 Martin M, Macias M, Escames G, Leon J, Acuna-Castroviejo D (2000). Melatonin but not vitamins C and E maintains glutathione homeostasis in t-butyl hydroperoxide-induced mitochondrial oxidative stress. *FASEB J.* **14**: 1677–1679.

66 Matsubara E, Bryant-Thomas T, Pacheco Quinto J, Henry TL, Poeggeler B, Herbert D *et al.* (2003). Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. *J Neurochem.* **85**: 1101–1108.

67 Maurizi CP (2003). The puzzle of where cerebrospinal fluid is absorbed: new pieces. *Med Hypotheses.* **60**: 102–103.

68 Maurizi CP (2010). Choroid plexus portals and a deficiency of melatonin can explain the neuropathology of Alzheimer's disease. *Med Hypotheses.* **74**: 1059–1066.

69 Mayo JM, Sainz RM, Tan DX, Antolin I, Rodriguez C, Reiter RJ (2005). Melatonin and Parkinson's disease. *Endocrine.* **27**: 169–179.

70 Menendez-Pelaez A & Reiter RJ (1993). Distribution of melatonin in mammalian tissues: relative importance of nuclear verses cytosolic localization. *J Pineal Res.* **15**: 59–69.

71 Mennenga K, Ueck M, Reiter RJ (1991). Immunohistological localization of melatonin in the pineal gland and retina of the rat. *J Pineal Res.* **10**: 159–164.

72 Mess B & Trentini GP (1974). Melatonin level in cerebrospinal fluid and choroid plexus following intravenous administration of the labeled compound. *Acta Physiol Acad Sci Hung.* **45**: 25–31.

73 Milano S, Parisi P, Pelliccia A, Luchetti A, Paolino MC, Villa MP (2008). Melatonin to prevents migraine or tension-type headache in children. *Neurol Sci.* **29**: 285–287.

74 Molina-Carballo A, Munoz-Hoyos A, Reiter RJ, Sanchez-Forte M, Moreno-Madrid F, Rufo-Campos M *et al.* (1997). Utility of high doses of melatonin as adjunctive anticonvulsant therapy in a child with severe myoclonic epilepsy: two years' experience. *J Pineal Res.* **23**: 97–105.

75 Ohira K, Hagihara H, Toyama K, Takao K, Kanai M, Funakoshi H *et al.* (2010). Expression of tryptophan 2, 3-dioxygenase in mature granule cells of the adult mouse dentate gyrus. *Mol Brain.* **3**(1): 26.

76 Olcese JM, Cao C, Mori T, Mamcarz MB, Maxwell A, Runfeldt MJ *et al.* (2009). Protection against cognitive deficits and markers of neurodegeneration by long-term oral administration of melatonin in a transgenic model of Alzheimer disease. *J Pineal Res.* **47**: 82–96.

77 Olivier P, Fontaine RH, Loron G, Van Steenwinckel J, Biran V, Massonneau V *et al.* (2009). Melatonin promotes oligodendroglial maturation of injured white matter in neonatal rats. *PLoS One.* **4**: e7128.

78 Ortiz GG, Crespo-Lopez ME, Moran-Moguel C, Garcia JJ, Reiter RJ, Acuna-Castroviejo D (2001). Protective role of melatonin against MPTP-induced mouse brain cell DNA fragmentation and apoptosis in vivo. *Neuroendocrinol Lett.* **22**: 101–108.

79 Pablos MI, Agapito MT, Gutierrez R, Recio JM, Reiter RJ, Barlow-Walden L et al. (1995). Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. J Pineal Res. **19**: 111–115.

80 Pablos MI, Reiter RJ, Ortiz GG, Guerrero JM, Agapito MT, Chuang JI et al. (1998). Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. Neurochem Int. **32**: 69–75.

81 Pang SF, Tsang CW, Hong GX, Yip PC, Tang PL, Brown GM (1990). Fluctuation of blood melatonin concentrations with age: result of changes in pineal melatonin secretion. J Pineal Res. **8**: 179–192.

82 Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J et al. (2000). An assessment of the antioxidant and antiamyloidogenic properties of melatonin: implications for Alzheimer's disease. J Neural Transm. **107**: 203–221.

83 Pappolla MA, Simovich MJ, Bryant-Thomas T, Chyan YJ, Poeggeler B, Dubocovich M et al. (2002). The neuroprotective activities of melatonin against Alzheimer beta-protein are not mediated by melatonin membrane receptors. J Pineal Res. **32**: 135–142.

84 Paradies G, Petrosillo G, Paradies V, Reiter RJ, Ruggiero FM (2010). Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. J Pineal Res. **48**: 297–310.

85 Pelham R (1975). A serum melatonin-rhythm in chickens and its abolition by pinealectomy. Endocrinology. **96**: 543–546.

86 Pelham R, Vaughan GM, Sandock KL, Vaughan MK (1973). Twenty-four-hour cycle of a melatonin-like substance in the plasma of human males. J Clin Endocrinol Metab. **37**: 341–344.

87 Perry G, Castellani RJ, Hirai K, Smith MA (1998). Reactive oxygen species mediate cellular damage in Alzheimer's disease. J Alzheimer's Dis. **1**(1): 45–55.

88 Quay WB (1965). Histological structure and cytology of the pineal organ in birds and mammals. Progr Brain Res. **10**: 49–86.

89 Quay WB (1970a). Endocrine effects of the mammalian pineal. Am Zool. **10**: 237–46.

90 Quay WB (1970b). Pineal structure and composition of the orangutan (Pongo pygmaeus). Anat Rec. **168**: 93–104.

91 Rahman SA, Kayumov L, Shapiro CM (2010). Antidepressant action of melatonin in the treatment of delayed sleep phase syndrome. Sleep Med. **11**: 131–136.

92 Ramirez-Rodriguez G, Klempin F, Babu H, Benitez-King G, Kempermann G (2009). Melatonin modulates cell survival of new neurons in the hippocampus of adult mice. Neuropsychopharmacology. **46**: 71–78.

93 Reddy PH & Beal MF (2005). Are mitochondria critical in the pathogenesis of Alzheimer's disease? Brain Res Brain Res Rev. **49**: 618–623.

94 Reiter RJ (1973). Pineal control of a seasonal reproductive rhythm in male golden hamsters exposed to natural daylight and temperature. Endocrinology. **92**: 423–430.

95 Reiter RJ, Cabrera J, Sainz RM, Mayo JC, Manchester LC, Tan DX (1999b). Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and Parkinsonism. Ann NY Acad Sci. **890**: 471–485.

96 Reiter RJ, Croft CM, Johnson JE Jr, King TS, Richardson BA, Vaughan GM et al. (1981). Age-associated reduction in nocturnal melatonin levels in female rats. Endocrinology. **109**: 1295–1297.

97 Reiter RJ, Manchester LC, Tan DX (2010b). Neurotoxins: free radical mechanisms and melatonin protection. Curr Neuropharmacol. **8**: 194–210.

98 Reiter RJ, Pablos MI, Agapito TT, Guerrero JM (1996). Melatonin in the context of the free radical theory of aging. Ann NY Acad Sci. 786: 362–378.

99 Reiter RJ, Paredes SD, Korkmaz A, Manchester LC, Tan DX (2008). Melatonin in relation to the "strong" and "weak" versions of the free radical theory of aging. Adv Med Res. **53**: 119–129.

100 Reiter RJ, Paredes SD, Manchester LC, Tan DX (2009). Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol. **44**: 175–200.

101 Reiter RJ, Richardson BA, Johnson LY, Ferguson BM, Dinh DT (1980). Pineal melatonin rhythm: reduction in aging Syrian hamsters. Science. **210**: 1372–1373.

102 Reiter RJ & Tan DX (2002). Role of CSF in the transport of melatonin. J Pineal Res. **33**: 61.

103 Reiter RJ & Tan DX (2003). What constitutes a physiological level of melatonin? J Pineal Res. **34**: 79–80.

104 Reiter RJ, Tan DX, Fuentes-Broto L (2010a). Melatonin: a multi-tasking molecule. Progr Brain Res. **181**: 127–151.

105 Reiter RJ, Tan DX, Kim SJ, Manchester LC, Qi W, Garcia JJ et al. (1999a). Augmentation of indices of oxidative damage in life-long melatonin deficient rats. Mech Ageing Dev. 110: 157–173.

106 Reiter RJ, Tan DX, Osuna C, Gitto E (2000). Actions of melatonin in the reduction of oxidative stress: a review. J Biomed Sci. **7**: 444–458.

107 Reiter RJ, Vaughan MK, Blask DE (1975). Possible role of the cerebrospinal fluid in the transport of pineal hormones in mammals. In: Knigge KM, Scott DE, Kobayashi H, Ishii S, editors. Brain-Endocrine Interaction II. The Ventricular System., Karger, Basel, pp 337–354.

108 Ressmeyer AR, Mayo JC, Zelosko V, Sáinz RM, Tan DX, Poeggeler B et al. (2003). Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxy-kynuramine. Redox Rep. **8**: 205–213.

109 Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V et al. (2004). Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res. **36**: 1–9.

110 Rosales-Corral S, Reiter RJ, Tan DX, Ortiz GG, Lopez-Armas G (2010). Functional aspects of redox control during neuroinflammation. Antioxid Redox Signal. **13**: 193–247.

111 Rosen J, Than NN, Koch D, Poeggeler B, Laatsch H, Hardeland R (2006). Interactions of melatonin and its metabolites with ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res. **4**: 374–381.

112 Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM (1986). Human melatonin production decreases with age. J Pineal Res. **3**: 379–388.

113 Samantaray S, Das A, Thakore NP, Matzelle DD, Reiter RJ, Ray SK et al. (2009). Therapeutic potential of melatonin in traumatic central nervous system injury. J Pineal Res. **47**: 134–142.

114 Sanchez-Barcelo EJ, Mediavilla MD, Tan DX, Reiter RJ (2010). Clinical uses of melatonin: evaluation of clinical trials. Curr Med Chem. **17**: 2070–2096.

115 Savica R, Rocca WA, Ahlskog JE (2010). Where does Parkinson disease start? Arch Neurol. **67**: 798–801.

116 Schapira AH (2010). Complex I: inhibitors, inhibition and neurodegeneration. Exp Neurol. **224**: 331–335.

117 Schmid M (2009). Iron chelation therapy in MDS: what have we learnt recently? Blood Rev. **23**(Suppl 1): S21–25.

118 Seifman MA, Adamides AA, Nguyen PN, Vallance SA, Cooper DJ, Kossmann T et al. (2008). Endogenous melatonin increases in cerebrospinal fluid of patients after severe traumatic brain injury and correlates with oxidative stress and metabolic disarray. J Cerebr Blood Flow Metab. **28**: 684–696.

119 Shah A, Garzon-Mundi T, Mahajan R, Duenas VJ, Quinones-Hinojosa A (2010). Animal models of neurological disease. Adv Exp Med Biol. **671**: 23–40.

120 Sharma R, McMillan CR, Niles LP (2007). Neural stem cell transplantation and melatonin treatment in a 6-hydroxydopamine model of Parkinson's disease. J Pineal Res. **43**: 245–254.

121 Sheridan MN & Reiter RJ (1970). Observations on the pineal system in the hamster. I. Relations of the superficial and deep pineal to the epithalamus. J Morphol. **131**: 153–161.

122 Sheridan MN, Reiter RJ, Jacobs JJ (1969). An interesting anatomical relationship between the hamster pineal gland and the ventricular system of the brain. J Endocrinol. **45**: 131–132.

123 Skinner DC & Malpaux B (1999). High melatonin concentrations in ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology. **140**: 4399–4405.

124 Smith JA, Mee TJX, Barnes ND, Thornburn RJ, Barnes JLC (1976). Melatonin in serum and cerebrospinal fluid. Lancet. **2**: 425.

125 Stankov B, Fraschini F, Reiter RJ (1991). Melatonin binding sites in the central nervous system. *Brain Res Rev*. **16**: 245–256.

126 Stascia P, Paneth P, Rosiak JM (2000). Hydroxyl radical reaction with melatonin molecule: a computational study. *J Pineal Res*. **29**: 125–127.

127 Stascia P, Wanski P, Rosiak JM (1998). Melatonin as a hydroxyl radical scavenger. *J Pineal Res*. **25**: 65–66.

128 Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ (1993). Melatonin: a potent, hydroxyl radical scavenger. *Endocr J*. **1**: 57–60.

129 Tan DX, Manchester LC, Reiter RJ, Plummer BF, Limson J, Weintraub ST *et al.* (2000). Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radic Biol Med*. **29**: 1177–1185.

130 Tan DX, Manchester LC, Reiter RJ, Qi W, Hanes MA, Farley NJ (1999a). High physiological levels of melatonin in bile of mammals. *Life Sci*. **65**: 2523–2529.

131 Tan DX, Manchester LC, Reiter RJ, Qi WB, Zhang M, Weintraub ST *et al.* (1999b). Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochem Biophys Acta*. **1472**: 206–214.

132 Tan DX, Manchester LC, Sanchez-Barcelo E, Mediavilla MD, Reiter RJ (2010). Significance of high levels of endogenous melatonin in mammalian cerebrospinal fluid and in the central nervous system. *Curr Neuropharmacol*. **8**: 162–168.

133 Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007). One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species. *J Pineal Res*. **42**: 28–42.

134 Tapias V, Cannon JR, Greenamyre JT (2010). Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson's disease model. *J Neurosci Res*. **88**: 420–427.

135 Tapias V, Escames G, Lopez LC, Lopez A, Camacho E, Carrion MD *et al.* (2009). Melatonin and its brain metabolite N(1)-acetyl-5-methoxykynuramine prevent mitochondrial nitric oxide synthase induction in Parkinson mice. *J Neurosci Res*. **87**(13): 3002–3010.

136 Teter B (2004). ApoE-dependent plasticity in Alzheimer's disease. *J Mol Neurosci*. **25**: 167–175.

137 Tocharus J, Khonthun C, Chongthammakun S, Gonitrapong P (2010). Melatonin attenuates methamphetamine-induced overexpression of pro-inflammatory cytokines in microglial cell lines. *J Pineal Res*. **48**: 347–352.

138 Tricoire H, Locatelli A, Chemineau P, Malpaux B (2002). Melatonin enters cerebrospinal fluid through the pineal recess. *Endocrinology*. **143**(1): 84–90.

139 Turjanski AG, Rosenstein RE, Estrin DA (1998). Reactions of melatonin and related indoles with free radicals: a computational study. *J Med Chem*. **41**: 3684–3689.

140 Turjanski AG, Saenz DA, Doctorovich F, Estrin DA, Rosenstien RE (2001). Nitrosation of melatonin by nitric oxide: a computational study. *J Pineal Res*. **33**: 31–36.

141 Uban KA, Sliwowska JL, Lieblich S, Ellis LA, Yu WL, Weinberg J *et al.* (2010). Prenatal alcohol exposure reduces the proportion of newly-produced neurons and glia in the dentate gyrus of the hippocampus in female rats. *Horm Behav*, in press.

142 Vaughan GM, Pelham RW, Pang SF, Loughlin LL, Wilson KM, Sandock KL *et al.* (1976). Nocturnal elevation of plasma melatonin and urinary 5-hydroxyindole acetic acid in young men; attempts at modification by brief changes in environmental lighting and sleep and by autonomic drugs. *J Clin Endocrinol Metab*. **42**: 752–764.

143 Velkov ZA, Velkov YZh, Galunska BT, Paskalev DN, Tadjer AV (2009). Melatonin: quantum-chemical and biochemical investigation of antioxidant activity. *Eur J Med Chem*. **44**: 2834–2839.

144 Vidrio E, Jung H, Anastasio C (2008). Generation of hydroxyl radicals from dissolved transition metals in surrogate lung fluid solutions. *Atmos Environ*. **42**: 4369–4379.

145 Vigh B & Vigh-Teichman I (1988). Comparative neurohistology and immunocytochemistry of the pineal complex with special reference to the CSF-contacting neuronal structures. *Pineal Res Rev*. **6**: 1–65.

146 Wang X (2009). The anti-apoptotic activity of melatonin in neurodegenerative diseases. *CNS Neurosci Ther*. **15**: 345–357.

147 Wang XC, Zhang J, Yu X, Han L, Zhou ZT, Zhang Y *et al.* (2005). Prevention of isoproterenol-induced tau hyperphosphorylation by melatonin in the rat. *Sheng Li Xue Bao*. **57**: 7–12.

148 Watkins LR, Hutchinson MR, Ledeboer A, Wieseler-Frank J, Milligan ED, Maier SF (2007). Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opoids. *Brain Behav Immun*. **21**: 131–146.

149 Wojcik M, Burzynska-Pedziwiatr I, Wozniak LA (2010). A review of natural and synthetic antioxidants important for health and longevity. *Curr Med Chem*, in press.

150 Wood-Allum C & Shaw PJ (2010). Motor neurone disease: a practical update on diagnosis and management. *Clin Med*. **10**: 252–258.

151 Wu YH & Swaab DF (2005). The human pineal gland and melatonin in aging and Alzheimer's disease. *J Pineal Res*. **38**: 145–152.

152 Yap LP, Garcia JV, Han D, Cadenas E (2009). The energy-redox axis in aging and age-related neurodegeneration. *Adv Drug Deliv Rev*. **61**: 1283–1298.

153 Zavodnik IB, Domanski AV, Lapshina EA, Bryszewska M, Reiter RJ (2006). Melatonin directly scavenges free radicals generated in red blood cells and a cell-free system: chemiluminescence measurements and theoretical calculations. *Life Sci*. **79**: 391–400.

154 Zawilska JB, Skene DJ, Arendt J (2009). Physiology and pharmacology of melatonin in relation to biological rhythms. *Pharmacol Rep*. **61**: 383–410.

155 Zhang H, Squadrito GL, Uppu R, Pryor WA (1999). Reaction of peroxynitrite and melatonin: a mechanistic study. *Chem Res Toxicol*. **12**: 526–534.