REVIEW ARTICLE

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 (•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 •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

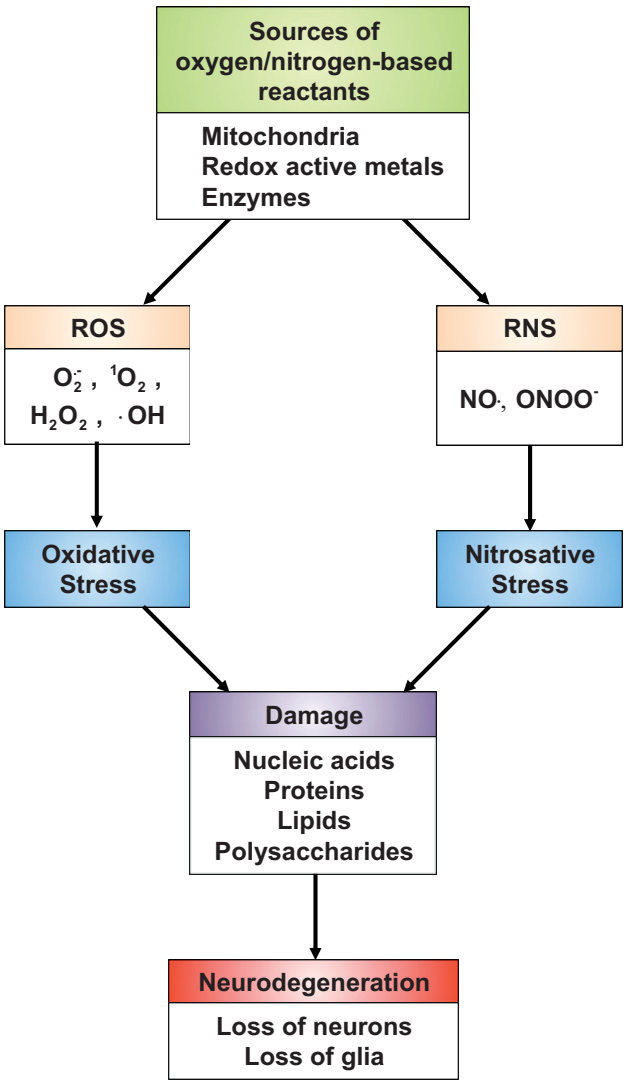

FIGURE 1. A general scheme illustrating the destructive role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the central nervous system that results in the loss of cellular elements (neurons and glia) which culminate in neurodegeneration. There are multiple sources of ROS [superoxide 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.

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