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Melatonin antioxidative defense: therapeutical implications for aging and neurodegenerative processes

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Abstract. The pineal product melatonin has remarkable antioxidant properties. It is secreted during darkness and plays a key role in various physiological responses including regulation of circadian rhythms, sleep homeostasis, retinal neuromodulation and vasomotor responses. It scavenges hydroxyl, carbonate and various organic radicals as well as a number of reactive nitrogen species. Melatonin also enhances the antioxidant potential of the cell by stimulating the synthesis of antioxidant enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase, and by augmenting glutathione levels. Melatonin preserves mitochondrial homeostasis, reduces free radical generation and protects mitochondrial ATP synthesis by stimulating Complex I and IV activities. The decline in melatonin production in aged individuals has been suggested as one of the primary contributing factors for the development of age-associated neurodegenerative diseases. The efficacy of melatonin in preventing oxidative damage in either cultured neuronal cells or in the brains of animals treated with various neurotoxic agents, suggests that melatonin has a potential therapeutic value as a neuroprotective drug in treatment of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), stroke and brain trauma. Therapeutic trials with melatonin indicate that it has a potential therapeutic value as a neuroprotective drug in treatment of AD, ALS and HD. In the case of other neurological conditions, like PD, the evidence is less compelling. Melatonin's efficacy in combating free radical damage in the brain suggests that it can be a valuable therapeutic agent in the treatment of cerebral edema following traumatic brain injury or stroke. Clinical trials employing melatonin doses in the range of 50–100 mg/day are warranted before its relative merits as a neuroprotective agent is definitively established.

Key words:

Melatonin; mitochondria; free radicals; oxidative stress; aging; Parkinson's disease; Alzheimer's disease; Huntington's disease; amyotrophic lateral sclerosis, stroke.

List of Abbreviations

3xTg-AD: triple-Tg mouse model of Alzheimer's disease
6-OHDA: 6-hydroxydopamine
AANAT: arylalkylamine N-acetyltransferase
AD: Alzheimer's disease
AFMK: *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine
AMK: *N*¹-acetyl-5-methoxykynuramine
ALS: amyotrophic lateral sclerosis
apoE4: apolipoprotein E4
APP: amyloid protein precursor
ASMT: acetylserotonin O-methyltransferase
AVP: arginine vasopressin
A β : amyloid beta
BBB: blood brain barrier

Bcl-2: B cell lymphoma proto-oncogene protein
c3OHM: cyclic 3-hydroxymelatonin
CaM: calmodulin
CSF: cerebrospinal fluid
DA: dopamine
ETC: electron transport chain
GABA: γ -aminobutyric acid
GH: growth hormone
GPx: glutathione peroxidase
GR: glutathione reductase
GSH: glutathione
GSK-3: glycogen synthase kinase 3
HD: Huntington's disease
HIOMT: hydroxyindole-O-methyl transferase
IL-1 β : interleukin-1 β
IL-R₁: interleukin-1 receptor1
iNOS: inducible nitric oxide synthase
KA: kainic acid
MAO: monoamine oxidase
MAP: microtubule-associated protein
MCI: mild cognitive impairment
mHtt: mutated huntingtin gene
MPTP: 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine
MT₁: melatonin receptor 1
MT₂: melatonin receptor 2
mtNOS: mitochondrial nitric oxide synthase
mtPTP: mitochondrial permeability transition pore
NMDA: N-methyl-D-aspartate
nNOS: neuronal nitric oxide synthase
NOS: nitric oxide synthase
PD: Parkinson's disease
PP: protein phosphatase
PS1: presenilin 1
QR2: quinone reductase
RBD: rapid eye movement-associated sleep behavior disorder
RNS: reactive nitrogen species
ROS: reactive oxygen species
SCN: suprachiasmatic nuclei
SOD: superoxide dismutase
Tg: transgenic
TNF-R₁: tumor necrosis factor receptor 1
TNF- α : tumor necrosis factor- α
VEGF: vascular endothelial growth factor
VIP: vasoactive intestinal polypeptide

Basic Melatonin Physiology

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous substance secreted by the pineal gland of all mammals, including man. Additionally, its presence has been confirmed in many plants (Dubbels et al., 1995), Chinese herbs (Chen et al., 2003), and unicellular organisms (Balzer and Hardeland, 1991; Hardeland et al., 1995). Melatonin participates in diverse functions of the body including sleep and circadian rhythm regulation, immunoregulation and may have anti-cancer actions (Pandi-Perumal et al., 2006). Melatonin is a potent free radical scavenger and regulator of redox-active enzymes (for a recent review see Galano et al., 2011).

Besides the pineal gland or related structures, such as the retina, a quite a number of different organs or cells have the capability to synthesize melatonin. These include the gastrointestinal tract, bone marrow, leukocytes, membranous cochlea, Harderian gland, and, perhaps, also skin and other brain areas (Hardeland, 2008, 2009a; Hardeland and Poeggeler, 2007, 2008; Jiménez-Jorge et al., 2007; Tan et al., 2003). From these other sites of formation, melatonin is either poorly released or only in response to specific stimuli, e.g., as a post-prandial surge from the gastrointestinal tract (Bubenik, 2002; Hardeland and Pandi-Perumal, 2005; Huether et al., 1992; Huether, 1993, 1994). Relative to the amounts present in the pineal gland and the circulation, the quantities of melatonin in extrapineal tissues are by no means negligible (Bubenik, 2002; Huether, 1993).

Melatonin is synthesized from serotonin through two enzymatic steps. A first step is the N-acetylation by arylalkylamine N-acetyltransferase (AANAT) to yield N-acetylserotonin. The physiological regulation of AANAT, with its sharp increase in activity at night and very rapid decrease at light onset, has received considerable attention as the major regulatory phenomenon controlling the onset and offset of melatonin synthesis (Klein, 2007). The second step in melatonin synthesis is the transfer of a methyl group from S-adenosylmethionine to the 5-hydroxy group of N-acetylserotonin to yield melatonin. This reaction is catalyzed by the enzyme hydroxyindole-O-methyl transferase (HIOMT), more recently called acetylserotonin O-methyltransferase (ASMT) in human genetic databases. Although the day/night changes of HIOMT are less prominent (Cardinali and Pévet, 1998; Claustrat et al., 2005), it is now known to be responsible for the amplitude of peak levels of melatonin reached during darkness (Liu and Borjigin, 2005; Ribelayga et al., 2000). Other details of melatonin biosynthetic pathway are discussed in the legend to Fig. 1.

Environmental lighting, acting through the eye in adult mammals and in part directly on the pineal in lower vertebrates and birds, has profound effects on rhythms in pineal melatonin biosynthesis. Exposure of animals to light at night rapidly depresses pineal melatonin synthesis. Based on denervation or nerve stimulation studies, a simple model of pineal regulation was envisioned, comprising two premises: (i) the neural route for environmental lighting control of melatonin secretion is the neuronal circuit "retina - retinohypothalamic tract - suprachiasmatic nuclei (SCN) - periventricular hypothalamus - intermediolateral column of the thoracic cord gray - superior cervical ganglion - internal carotid nerves - pineal gland"; (ii) norepinephrine released from sympathetic terminals at night activates postsynaptic β -adrenoceptors

coupled to the adenylate cyclase-cAMP system, with a contribution of α_{1B} -adrenergic activation of phospholipase C β that leads to rises in Ca²⁺, protein kinase C and calmodulin (CaM) kinases (Cardinali, 1983; Cardinali and Pévet, 1998; Maronde and Stehle, 2007). These processes jointly stimulate melatonin synthesis and release. The additional presence of central peptidergic pinealopetal pathways indicates that regulation of melatonin biosynthesis is more complex and multifactorial than commonly inferred (Cardinali, 1983; Cardinali and Pévet, 1998; Moller and Baeres, 2002; Mukda et al., 2009; Simonneaux and Ribelayga, 2003).

Once formed melatonin is not stored within the pineal gland but diffuses out into capillary blood and cerebrospinal fluid (CSF) (Tan et al., 2010). The delicate connective tissue capsule of the pineal gland does not prevent diffusion of melatonin into CSF. Inasmuch as melatonin arrives early in CSF at the 3rd ventricle as compared to the lateral ventricles. As melatonin passes through all biological membranes with ease, brain tissue may have higher melatonin levels than other tissues in the body (Tan et al., 2010). Indeed melatonin levels in the CSF entering the 3rd ventricle from the pineal recess have been found to be 5 to 10 times higher than simultaneous blood levels (Tricoire et al., 2002). However, in most parts of the ventricular system and in the spinal canal, melatonin concentrations are much lower. Whether melatonin is taken up by the brain tissue and/or rapidly metabolized, is unknown, since determinations of melatonin levels in the CNS and brain areas have yielded highly divergent results (Hardeland, 2010b).

Melatonin is involved in the control of various physiological functions in the body such as seasonal reproduction (Dardente, 2012; Reiter, 1980), sleep regulation (Cardinali et al., 2012; Monti and Cardinali, 2000; Wurtman and Zhdanova, 1995), immune function (Carrillo-Vico et al., 2006; Esquifino et al., 2004; Radogna et al., 2010), inhibition of tumor growth (Blask et al., 2011; Mediavilla et al., 2010), blood pressure regulation (Domínguez-Rodríguez et al., 2010; Scheer et al., 2004), retinal physiology (Guido et al., 2010; Rosenstein et al., 2010; Scher et al., 2003), control of circadian rhythms (Dawson and Armstrong, 1996), modulation of human mood and behavior (Brown et al., 2010) and free radical scavenging (Galano et al., 2011; Hardeland et al., 2010).

Melatonin participates in many of these mechanisms by acting through two G-protein-coupled membrane receptors (MT₁ and MT₂) (Dubocovich et al., 2010). MT₁ and MT₂ receptors are found in the cell membrane as dimers and heterodimers. GPR50, a G-protein coupled melatonin receptor ortholog that does not bind melatonin itself, dimerizes with MT₁ receptors and can block melatonin binding (Levoye et al., 2006). As representatives of the G-protein coupled receptor family, MT₁ and MT₂ melatonin receptors act through a number of signal transduction mechanisms that ultimately result in specific physiological responses (Dubocovich et al., 2010). Figure 2 depicts an overview of major signaling pathways of the melatonin membrane receptors MT₁ and MT₂.

In addition to binding to MT₁ and MT₂ receptors, melatonin has been shown to display affinity for another binding site, originally considered to represent a membrane-bound receptor (MT₃), but ultimately confirmed to be an enzyme, quinone

reductase 2 (QR2 or NQO2) (Nosjean et al., 2000). Quinone reductases are generally believed to protect against oxidative stress resulting from electron transfer reactions of quinones, but the specific role of subform QR2 is still poorly understood. Polymorphisms in the promoter of the human *QR2* gene are associated with Parkinson's disease (PD) (Harada et al., 2001) and a decline in cognitive ability over time (Payton et al., 2010).

Melatonin also binds to transcription factors belonging to the retinoic acid receptor superfamily, in particular, splice variants of ROR α (ROR α 1, ROR α 2 and ROR α isoform d) and RZR β (Lardone et al., 2011; Wiesenberg et al., 1995). Retinoic acid receptor subforms are ubiquitously expressed in mammalian tissues and relatively high levels were detected especially in T- and B-lymphocytes, neutrophils and monocytes (Lardone et al., 2011). Although the activity of these nuclear binding proteins has for quite some time been a matter of debate and although their affinity to melatonin is lower, compared to MT₁, their classification as nuclear receptors now seems to be justified (Hardeland et al., 2010).

Melatonin may also act directly on cells through its binding to CaM (Benítez-King et al., 1996), tubulin (Cardinali and Freire, 1975), calreticulin and, perhaps, other Ca²⁺-binding proteins (Macias et al., 2003). The relevance of these findings to mammalian cells largely depends on the affinity of these binding sites to melatonin, which seems to be modulated, in the case of CaM, by its interaction with CaM-activated enzymes (Landau and Zisapel, 2007). Activations of protein kinase C and CaM kinases can now be explained by G α_q - and G $\beta\gamma$ -dependent activations of phospholipase C β subforms and, perhaps, phospholipase C η (Hardeland, 2009a).

Based on what is known at present, melatonin's action as an antioxidant agent appears to be independent of the above mentioned receptors (León-Blanco et al., 2004). However, upregulation of the antioxidant enzyme γ -glutamylcysteine synthase involves nuclear transcription; electrophoretic mobility shift assay analysis has shown melatonin-dependent increases in DNA binding of AP-1 and RZR/ROR α (Urata et al., 1999), indicating the involvement of a nuclear receptor. In addition findings on protection of liver and heart by the MT₁/MT₂-selective melatonergic agonist ramelteon under conditions leading to oxidative stress points to the inference that these membrane receptors may have antioxidant activity, mainly because ramelteon has no substantial radical scavenging properties (Mathes et al., 2008).

Melatonin Effects on Mitochondria

Free radical generation

The synthesis of ATP via the mitochondrial respiratory chain is the result of a proton potential generated by the electron transport chain (ETC) (for rev. see Acuña-Castroviejo et al., 2011). Although ideally all the oxygen should be reduced to water via a 4-electron reduction reaction driven by Complex IV, under normal conditions a certain, but relevant percentage of oxygen can be reduced by dissipating single electrons yielding free radicals. Approximately 3-5 % of oxygen is converted to reactive oxygen species (ROS).

The primary and quantitatively most abundant ROS produced by the ETC is the superoxide anion radical ($O_2^{\bullet-}$), which is formed by electron leakage from molecular oxygen (O_2) (Fig. 3) (Acuña-Castroviejo et al., 2011; Boveris and Boveris, 2007). Multiple electron transfer leading to other ROS is negligible, but, secondarily, peroxyxynitrite anion ($ONOO^-$, formed from $O_2^{\bullet-}$ and nitric oxide $\bullet NO$), hydroxyl radical ($\bullet OH$, formed via H_2O_2 from superoxide dismutation, or from homolysis of peroxyxynitrous acid, $ONOOH$), carbonate radical ($CO_3^{\bullet-}$, formed from $\bullet OH$ and HCO_3^- , or from $ONOOCO_2^-$ decomposition) and nitrogen dioxide ($\bullet NO_2$, formed from $ONOOH$ or $ONOOCO_2^-$) are generated (Fig. 3). These other ROS and RNS (reactive nitrogen species) have a much higher reactivity than $O_2^{\bullet-}$. Hence, the rates at which single-electron transfer reactions generate the low-reactivity superoxide anion radical as a precursor, and, additionally, the rates at which the secondary toxic reactants are produced, are crucial to mitochondrial and cellular activity (Acuña-Castroviejo et al., 2011; Boveris and Boveris, 2007; Genova et al., 2004).

Mitochondria are not only the primary site of ROS generation but also the primary target of attack for ROS and RNS (Acuña-Castroviejo et al., 2011). Damage to the mitochondrial ETC can cause breakdown of the proton potential, apoptosis or lead to further generation of free radicals maintaining a vicious cycle, which may also ultimately result in cell death either of the necrotic or the apoptotic type.

$O_2^{\bullet-}$, once formed, quickly undergoes dismutation to H_2O_2 and O_2 by superoxide dismutases (SODs) in mitochondrial matrix by a subform carrying manganese in the active center (Mn-SOD). However, the affinity of $O_2^{\bullet-}$ to $\bullet NO$ is similarly high as that to SODs. Therefore, the availability of $\bullet NO$ determines the rates at which the adduct, peroxyxynitrite, and the decomposition products from $ONOOH$ or $ONOOCO_2^-$, i.e., $\bullet NO_2$, $CO_3^{\bullet-}$ and/or $\bullet OH$ are generated. High rates of $\bullet NO$ synthesis, which are typical for calcium-dependent excited states of neurons and for inflammatory conditions, can contribute to oxidative and nitrosative stress. H_2O_2 is converted into $\bullet OH$ in the presence of transition metals such as Fe^{2+} and is disposed of by peroxidases, such as hemoperoxidase ("catalase") and glutathione (GSH) peroxidase (GPx) (Chance et al., 1979).

In the brain, GPx plays the dominant role in metabolizing H_2O_2 . GPx uses H_2O_2 and other hydroxyperoxides as substrates in the process of conversion of GSH into its oxidized form GSSG. Because GSH is required in sufficient quantities for GPx activity, auxiliary enzymes providing the reduced cosubstrate are also important, including GSH reductase (GR) and glucose-6-phosphate dehydrogenase (which synthesize reducing equivalents for the action of GR) as well as the rate-limiting enzyme of GSH formation, γ -glutamylcysteine synthase (Ghanta and Chattopadhyay, 2011). All these enzymes are upregulated by melatonin, either through direct or indirect mechanisms (Hardeland, 2005).

Among other sources of ROS, formation of H_2O_2 , $O_2^{\bullet-}$ and OCl^- by microglia or invading myeloperoxidase-expressing leukocytes becomes relevant in inflammatory situations. This is not restricted to acute brain inflammation, but also plays a role in some neurodegenerative diseases (Cunningham, 2011). Alzheimer's disease (AD) is an example of an atypical, lingering form of inflammation, in which some classical

hallmarks such as neutrophil infiltration and edema are usually absent, whereas other characteristics including acute-phase proteins and cytokines are clearly demonstrable (Cunningham, 2011). In the brain, inflammatory ROS production is intertwined with secondary excitotoxicity and RNS formation that is, e.g., demonstrable by enhanced protein nitration (for discussion see Hardeland, 2009b). Various flavin enzymes also generate ROS, usually H_2O_2 , sometimes accompanied by $O_2^{\bullet-}$ production. In dopaminergic neurons, the oxidation of dopamine (DA) by monoamine oxidase (MAO) generates hydrogen peroxide. The increased destruction of DA by MAO has been postulated to be the reason for degeneration of dopaminergic neurons causing PD (Olanow, 1990).

The free radical $\bullet NO$ is produced by several subforms of nitric oxide synthase (NOS) (Pacher et al., 2007). Since $\bullet NO$ is a gaseous compound, it can cross membranes with ease and, therefore, it enters mitochondria, regardless of its neuronal, glial or vascular origin. $\bullet NO$ strongly interferes with components of the respiratory chain, in particular cytochrome c oxidase (Pacher et al., 2007). Moreover, its metabolite ONOO⁻ and radicals derived from this can damage proteins of the respiratory complexes. The existence of a separate mitochondrial NOS (mtNOS) has been described but many of the published data are debatable (Chuang, 2010). In the adult mouse brain, no mtNOS was demonstrated under carefully controlled conditions (Lacza et al., 2004). $\bullet NO$ entering neuronal mitochondria and peroxynitrite formed there by combination with $O_2^{\bullet-}$ from electron leakage do not only interfere with the respiratory chain, at already moderate concentrations, but, at elevated levels, lead to free radical-mediated chain reactions that destroy protein, lipid and DNA molecules.

Melatonin and mitochondrial homeostasis

Several studies have suggested that the neuroprotective role of melatonin in aging and in many neurodegenerative conditions such as AD and PD can be due to its direct antioxidant role in mitochondrial homeostasis (Acuña-Castroviejo et al., 2011; Srinivasan et al., 2011). When acutely added in vitro, melatonin diminished the increase in respiration caused by addition of Krebs' cycle substrates and ADP to mitochondrial preparations (Reyes Toso et al., 2003). This finding demonstrates melatonin's ability to interact with the ETC (Hardeland, 2005), but the possibility of pharmacological effects of electron interception by elevated melatonin levels has to be considered, which may partially block the electron flux by alternate electron acceptance. Melatonin does not affect basal mitochondrial respiration (Reyes Toso et al., 2003).

In one study the daily administration of physiological amounts of melatonin to senescence-accelerated or senescence-resistant mice over a period of 5 months was reported to increase state 3 respiration and respiratory control index in liver mitochondria (Okatani et al., 2002). These results were taken as evidence of melatonin's ability to normalize cellular respiration, to attenuate oxidant formation and, thus, to protect cells against oxidative damage.

Although mild uncoupling is usually expected to reduce electron leakage, additionally observed rises in dinitrophenol-dependent uncoupled respiration may reflect melatonin-induced changes in respiratory capacity. A long-term improvement

of mitochondrial function in vivo can be explained in different ways, either in terms of interactions with the electron flux or by induction of antioxidant enzymes which prevent damage to the respiratory chain (Acuña-Castroviejo et al., 2011). Moreover, increases in gene expression of components of Complexes I and IV were observed, which may have also contributed to normalized respiration.

A low-affinity mitochondrial binding site for melatonin ($IC_{50} = 0.8 \mu M$) has been associated with an inhibition of the mitochondrial permeability transition pore (mtPTP) and reported to mediate an anti-apoptotic action of melatonin at pharmacological concentrations (Andrabi et al., 2004). Under physiological conditions, this binding site would be only relevant if melatonin accumulated in sufficiently high concentrations, a fact suggested by the intramitochondrial concentrations of melatonin observed in some experiments (Venegas et al., 2011). Indeed melatonin, which possesses both hydrophilic and lipophilic properties, crosses cell membranes with ease and is capable of concentrating within subcellular compartments (Menéndez-Pelaez and Reiter, 1993) including mitochondria (Martin et al., 2000). Melatonin at millimolar concentrations has been reported to stabilize the fluidity of mitochondrial inner membranes (García et al., 1999).

The binding of melatonin to mitochondrial membranes was first reported in studies using 2-[^{125}I]-iodomelatonin (Yuan and Pang, 1991). Administration of melatonin increases the activities of mitochondrial respiratory Complexes I and IV in a time-dependent manner in brain and liver tissues (Martin et al., 2002). It has been suggested that melatonin interacts with complexes of the ETC and may donate electrons and, as an oxidized intermediate, may also accept electrons, thereby increasing the electron flow. This property, which is not shared by most antioxidants previously investigated (with the exception of ubiquinone), can enable melatonin both to participate in electron transport and to act as an antioxidant (Acuña-Castroviejo et al., 2011).

The phenomenon of electron donation by melatonin and electron acceptance by the melatonyl cation radical was used as the basis of a model proposing that melatonin could act at low, quasi-catalytic concentrations to reduce electron leakage (Hardeland, 2005). Similar effects were predicted for the melatonin metabolite *N*¹-acetyl-5-methoxykynuramine (AMK), with regard to its single-electron transfer reactions, reactivity and amphiphilicity (Hardeland, 2005). In fact, mitochondrial protection by AMK was confirmed by the findings of Acuña-Castroviejo et al. (2002). However, melatonin-mediated modulation of electron flux can be explained by other mechanisms, including direct flux control at Complex I, prevention of ROS- and RNS-induced interruptions of electron transport, and de novo synthesis of respirasomal proteins (Acuña-Castroviejo et al., 2011).

Melatonin increases the mitochondrial GSH pool and reduces hydroxyperoxide levels. The latter effect can be regarded as the sum of indirect antioxidant effects via GSH levels and of reduction of electron leakage. By safeguarding electron flow through the transport chain melatonin increases ATP production (Acuña-Castroviejo et al., 2011). Melatonin prevents membrane lipid peroxidation and mitochondrial and

nuclear DNA oxidation induced by enhanced oxidative stress and has been shown to be antiapoptotic in several experimental model systems (Sainz et al., 2003).

Melatonin administration has been found to effectively counteract oxidative mitochondrial DNA damage and to restore the mitochondrial respiratory control system, by preventing the decreases in Complex I and IV activities (Zhang et al., 2009). Since reduced Complex I activity is associated with and is a sign of enhanced electron leakage, the resulting increase in oxidative stress is sufficient to induce apoptosis. Melatonin's action in restoring Complex I activity back to normal levels thus assumes significance for overall health in its prevention of age associated degenerative changes (Acuña-Castroviejo et al., 2011).

Melatonin and Neuronal Damage

Many acute conditions, e.g. hypoxia, stroke, physical trauma, hypoglycemia, drug neurotoxicity, viruses, radiation or noxious stimuli, are sufficient to produce neuronal damage. Similar mechanisms are also likely to be involved in neurodegenerative disorders, a group of chronic and progressive diseases that are characterized by selective and symmetric losses of neurons in motor, sensory, or cognitive systems. Clinically relevant examples of these disorders are AD, PD, amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) (Aliev et al., 2011; Green et al., 2011).

Although the origin of neurodegenerative diseases has remained mostly undefined, three major and frequently interrelated processes, i.e., glutamate excitotoxicity, free radical-mediated damage and mitochondrial dysfunction, have been identified as common pathophysiological mechanisms leading to neuronal death (Reiter, 1998). Because melatonin is a direct and indirect antioxidant it has been proposed as a neuroprotective agent (Reiter et al., 2010). Melatonin's neuroprotective properties, as well as regulatory effects on circadian disturbances, validate melatonin's benefits as a therapeutic substance in the symptomatic treatment of neurodegenerative diseases. Moreover, melatonin exerts anti-excitatory, and at sufficient dosage, sedating effects (Caumo et al., 2009; Golombek et al., 1996) so that a second neuroprotective mode of action may exist involving the γ -aminobutyric acid (GABA)-ergic system as a mediator. This view is supported by studies indicating that melatonin protects neurons from the toxicity of the amyloid- β ($A\beta$) peptide (the main neurotoxin involved in AD) via activation of GABA receptors (Louzada et al., 2004).

Melatonin also has anti-excitotoxic actions. Early studies in this regard employed kainate, an agonist of ionotropic glutamate receptors, and gave support to the hypothesis that melatonin prevents neuronal death induced by glutamate (Giusti et al., 1996b; Manev et al., 1996a). It has also been reported that administration of melatonin reduces the injury of hippocampal CA1 neurons caused by transient forebrain ischemia (Cho et al., 1997; Kilic et al., 1999) or high glucocorticoid doses (Furio et al., 2008). Following a hypoxic injury in rats, melatonin administration reduced glutamate levels and structural damage caused by hypoxia to neurons, axons and dendrites in the brainstem suggesting that it is capable of ameliorating excitotoxic damage (Kaur et al., 2011). A further demonstration that melatonin deficiency can

potentiate neuronal damage is the finding that more severe brain damage and neurodegeneration occurs after stroke or excitotoxic seizures in melatonin-deficient rats (Manev et al., 1996b). Pineal melatonin concentration decreased after hypoxic injuries in rats (Kaur et al., 2007).

Melatonin has often demonstrated superiority to vitamin C and E in protection against oxidative damage and in scavenging free radicals (Galano et al., 2011). Additionally, melatonin potentiates effects by other antioxidants, such as vitamin C, Trolox (a water soluble vitamin E analog) and NADH. The antioxidative efficiency of melatonin is high because the metabolites formed by free radical scavenging also act as free radical scavengers. This holds, in particular, for cyclic 3-hydroxymelatonin, *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine (AFMK) (Tan et al., 2003) and, with highest potency, AMK (Behrends et al., 2004; Silva et al., 2004). Thus, the interaction of melatonin with free radicals initiates an antioxidant cascade, which may allow the elimination of, in the extreme, up to 10 oxidizing free radicals.

Nevertheless, these effects are not sufficient for explaining melatonin's protective potency. Various secondary antioxidant effects have been described, which are based on upregulation of antioxidant and downregulation of prooxidant enzymes. In several studies some of these effects were highly variable, depending on the tissue and species involved, such as induction of SODs and catalase (for details see Hardeland, 2005). Other effects were consistently observed; in particular, this holds for GPx and for GR, presumably in response to GPx-dependent increases in GSSG, the oxidized form of GSH. Notably, enzyme inductions were more pronounced in the CNS of avian and other non-mammalian species.

Melatonin contributes to maintain normal GSH levels (Subramanian et al., 2007) by stimulating GSH biosynthesis via γ -glutamylcysteine synthase and glucose-6-phosphate dehydrogenase (Kilanczyk and Bryszewska, 2003; Rodríguez et al., 2004). Melatonin treatment enhanced brain GSH levels which were depressed following a hypoxic injury of developing rats (Kaur et al., 2010).

Antioxidative signaling is of particular significance because blood concentrations of melatonin are low, even at night, as compared to other antioxidants such as vitamin C or GSH (Galano et al., 2011). Although melatonin levels are several times higher in certain body fluids and tissues than in blood this may not be sufficient to fully explain the protective effects observed. Nevertheless, the much higher melatonin concentrations, e.g., in the bile (Tan et al., 1999), gastrointestinal tract (Bubenik, 2002; Kvetnoy et al., 2002) or bone marrow (Conti et al., 2000) should be of significance. According to Reiter and Tan (2003) melatonin concentrations in the blood should not be taken as an index to judge its concentration in other body fluids and intracellular compartments of the cell. Inasmuch as many cells of the body have the potential to synthesize melatonin, local melatonin production could increase under high free radical generating conditions. Assuming these conditions are met, melatonin could fulfill all the requirements for designation as an effective physiological antioxidant (Galano et al., 2011).

In addition to stroke, the efficacy of melatonin in inhibiting oxidative damage has also been tested in a variety of neurological disease models where free radicals have

been implicated as being at least partial causal agents of the condition. Thus, melatonin has been shown to reduce A β protein toxicity in AD animal models (Dragicevic et al., 2011; Matsubara et al., 2003; Olcese et al., 2009; Pappolla et al., 1997), to reduce oxidative damage in several models of PD (Acuña-Castroviejo-Castroviejo et al., 1997; Chuang and Chen, 2004; Dabbeni-Sala et al., 2001; Jin et al., 1998; Saravanan et al., 2007; Singhal et al., 2010), to protect against glutamate excitotoxicity (Das et al., 2010; Giusti et al., 1996a) and to lower neural damage due to cadmium toxicity (Jiménez-Ortega et al., 2011; Poliandri et al., 2006), δ -aminolevulinic acid toxicity (porphyria) (Carneiro and Reiter, 1998; Onuki et al., 2005; Princ et al., 1997), hyperbaric hyperoxia (Pablos et al., 1997; Shaikh et al., 1997), brain trauma (Beni et al., 2004; Kabadi and Maher, 2010; Tsai et al., 2011), γ radiation (Erol et al., 2004; Shirazi et al., 2011; Taysi et al., 2008), focal ischemia (Kilic et al., 2011; Koh, 2012; Lee et al., 2004; Tai et al., 2011) and a variety of neural toxins (Reiter et al., 2010).

The various types of toxicities listed above can result in cell death by necrosis or apoptosis. Apoptotic neuronal death requires RNA and protein synthesis, and depletion of trophic factors. Apoptosis also involves single-strand breaks of DNA. Neurotrophic factors have been found to rescue neurons from this type of death (Green et al., 2011). They may act via cellular anti-apoptotic components, such as the B cell lymphoma proto-oncogene protein (Bcl-2). The neuroprotective function of Bcl-2 is particularly well demonstrated in naturally occurring or experimentally induced neuronal death, which can be prevented by overexpression of Bcl-2 (Chuang, 2010; Green et al., 2011). In the highly complex context of different roles of members of the Bcl family, Bcl-2 is capable of blocking the apoptotic pathway by preventing the formation of a functional mtPTP and, thus, the release of the mitochondrial enzyme cytochrome c, which represents the final and no-return signal of the apoptotic program (Green et al., 2011; Khandelwal et al., 2011; Kluck et al., 1997). Studies in vitro indicate that melatonin enhances expression of Bcl-2 and prevents apoptosis (Jiao et al., 2004; Koh, 2011; Radogna et al., 2010; Wang, 2009). More recent studies have gone beyond the functions of Bcl. Melatonin was shown to directly inhibit the opening of the mtPTP, thereby rescuing cells (Jou, 2011; Peng et al., 2012). In addition to its antioxidant actions, melatonin directly diminished mtPTP currents, with an IC₅₀ of 0.8 μ M (Andrabi et al., 2004), a concentration which seems physiologically possible if mitochondrial accumulation of melatonin occurs.

Besides glutamate toxicity and oxidative stress, inflammation has been reported as another mechanism leading to damage/death of neurons in several brain pathologies including hypoxic-ischemic injuries. Activated microglia and endothelial cells under such conditions release proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), which cause damage to neurons (Floden et al., 2005). TNF- α has been shown to mediate neuronal death through binding with its receptor TNF-R₁ which has an intracellular death domain and its activation results in mitochondrial dysfunction, oxidative damage and silencing of survival signals (Nakazawa et al., 2006). IL-1 β damages neurons by binding to its receptor IL-R₁ and by initiating mechanisms such as excitotoxicity and increased inducible NOS (iNOS) production through IL-R₁ signaling. Melatonin is known to exert anti-inflammatory

actions and has been shown to reverse the inflammatory response in several brain pathologies by suppressing the production of inflammatory cytokines. Experimental studies have shown that melatonin is neuroprotective in ischemia/reperfusion injury as it inhibits the inflammatory response (Pei and Cheung, 2004). Melatonin treatment has also been demonstrated to reduce the levels of proinflammatory cytokines TNF- α and IL-1 β induced by A β in rat brain (Rosales-Corral et al., 2003) and to suppress the release of these cytokines by microglia (Kaur et al, unpublished data).

Therefore, data have accumulated indicating that melatonin may curtail all major processes in neuronal damage, i.e. glutamate excitotoxicity, free radical-mediated injury, neuroinflammation and apoptosis. In addition, melatonin, in acting as an endocrine arm of the circadian clock, promotes the restorative phases of sleep, a situation associated with neurotrophic effects. Thus melatonin may function as a unique chronobiotic-cytoprotective agent (Cardinali and Scacchi, 2010).

Neurodegenerative diseases have become a major health problem and a growing recognition exists that efforts to prevent these diseases must be undertaken by both governmental and non-governmental organizations. Regular intake of antioxidants by the elderly has been recommended for prevention of age-associated neurodegenerative diseases, although the efficacy of this treatment is still discussed (Hausman et al., 2011). In this context, the pineal product melatonin may have major significance since one of the features of advancing age is the gradual decrease in the endogenous synthesis of this important antioxidant (Bubenik and Konturek, 2011).

Melatonin and Aging

In humans pineal melatonin production is higher in younger age groups (18-54 years) as compared to older individuals (Karasek and Reiter, 2002; Sack et al., 1986; Skene and Swaab, 2003). The highest peak secretion of melatonin is found in children at 3-5 years of age (Cavallo, 1993). With some exceptions (Fourtillan et al., 2001; Zeitzer et al., 1999) the decline of melatonin production with age has been consistently reported (Brown et al., 1979; Dori et al., 1994; Ferrari et al., 2008; Girotti et al., 2000; Iguchi et al., 1982; Lieveise et al., 2011; Luboshitzky et al., 2001; Mazzoccoli et al., 2010b; Mishima et al., 2000, 2001; Rosen et al., 2009; Siegrist et al., 2001; Waldhauser and Steger, 1986).

Age-associated changes in the day/night rhythm of melatonin production have been found, with phase advances being encountered more frequently in the elderly as compared to young women (Skene and Swaab, 2003). It has also been shown that SCN function declines with age, particularly in patients with ageing-associated neurodegenerative disorders, a major cause of dementia and other poor health condition in the elderly population (Pandi-Perumal et al., 2002; Wu and Swaab, 2007). As shown in non human primates, in addition to the age-associated attenuation of hormone levels and reduction of humoral circadian signaling, there are also significant age-related changes in intracrine processing enzymes and hormone receptors which may further affect the functional efficacy of these hormones (Urbanski and Sorwell, 2011). In addition to degenerative processes in the SCN or its

input and output pathways, pineal calcification can be another reason for age-related declines in melatonin secretion (Mahlberg et al., 2009).

The decline in melatonin production and altered melatonin rhythms can be major contributing factors to the increased levels of oxidative stress and the associated degenerative changes that are seen in the elderly. Nevertheless, individuals of the same chronological age can exhibit dissimilar degrees of senescence-associated functional impairment, differences which may be attributable to the well documented inter-individual variations in melatonin levels (Bergiannaki et al., 1995; Ferrari et al., 2008; Grof et al., 1985; Travis et al., 2003). Variations in the degenerative changes of cells and tissues have been attributed to variations in melatonin production, changes which are more often determined by the physiological age of an individual rather than his chronological age (Barzilai et al., 2010). Recently it has been shown that there are genetic variations in the enzyme ASMT (HIOMT), a metabolic step that determines the amount of melatonin produced. These variations have been linked to autism spectrum disorder (Jonsson et al., 2010; Melke et al., 2008), recurrent depression (Galecki et al., 2010) and intellectual disability (Pagan et al., 2011).

In terms of aging, the immunostimulatory actions of melatonin are of particularly high relevance (Cardinali et al., 2008; Guerrero and Reiter, 2002; Mazzoccoli et al., 2010a). Although the importance of the age-dependent decreases in melatonin production as contributors to immunosenescence is not yet fully established, this issue merits further research attention. Age-associated changes in the average melatonin concentration, as well as in the amplitude of its circadian rhythm, can have profound effects on the entire circadian system. These changes can be expected to produce a host of multiple, pleiotropic effects and, presumably, dysfunctions that could be efficiently antagonized by melatonin (Cardinali et al., 2008).

Melatonin in AD

AD is an age-associated neurodegenerative disease that is characterized by progressive loss of cognitive function, loss of memory and other neurobehavioral manifestations. In spite of the number of studies undertaken, its etiology remains an enigma. Many mechanisms such as genetic factors, chronic inflammation associated with cytokine release, oxidative stress, and trace element neurotoxicity have been suggested as possible underlying causes (Ehrnhoefer et al., 2011; Jucker and Walker, 2011). The pathological manifestations of AD include amyloid plaques and neurofibrillary tangles. The free A β molecule is Fenton-reactive due to bound copper and, therefore, leads to cell death through induction of oxidative stress. Additionally, A β initiates flavoenzyme-dependent increases in intracellular H₂O₂ and lipid peroxides, which also promote free radical generation.

AD is related to mitochondrial dysfunction and can now be viewed as having characteristics reminiscent of other mitochondrial diseases associated with pathological oxidant formation (Swerdlow, 2011). Attenuation or prevention by administration of suitable antioxidants should be the basis for a strategic treatment of

AD. Though vitamin E and C have been used for treatment of patients (Devore et al., 2010), the neurohormone melatonin has assumed a significant role in view of the fact that it has been shown to be an effective antioxidant in a number of transgenic mouse models of AD, as discussed below. In one of these models, 4-month-old transgenic mice exhibited increases in levels of brain thiobarbituric acid-reactive substances, and reductions in SOD and GSH content, changes that were attenuated by administration of melatonin (Feng et al., 2006). Moreover, melatonin has been shown to exhibit antifibrillogenic activities, even when fibrillogenesis was enhanced by apolipoprotein E4 (apoE4), effects which were not seen to this extent when antioxidant vitamins were applied (Poeggeler et al., 2001).

Experimentally it has been shown that suppression of serum melatonin levels in rats through exposure to constant illumination results in a number of AD-like behavioral effects and neurochemical changes (Ling et al., 2009). These included spatial memory deficits, tau hyperphosphorylation at multiple sites, activation of glycogen synthase kinase-3 and protein kinase A, as well as suppression of protein phosphatase-1. An increased expression of endoplasmic reticulum stress-related proteins including BiP/GRP78 and CHOP/GADD153 was also taken as evidence of prominent oxidative damage and organelle lesions. All these impairments were partially attenuated by the simultaneous administration of melatonin (Ling et al., 2009). Endogenous melatonin deficiency can, therefore, contribute to the disease progression, and the beneficial effects of replacement therapy may well be warranted in AD.

Melatonin prevents the death of neuroblastoma cells exposed to A β polypeptide (Pappolla et al., 1997, 1999, 2000). Using murine neuroblastoma cells (N2a) (Pappolla et al., 1997) demonstrated that co-incubation of neuroblastoma cells with A β polypeptide and melatonin significantly reduced several features of apoptosis such as cellular shrinkage or formation of membrane blebs. Melatonin also reduced the levels of lipid peroxidation in the cultured neuroblastoma cells by scavenging free radicals released by A β (He et al., 2010). The neurofibrillary tangles of AD patients are composed of abnormally bundled cytoskeletal fibers, due to hyperphosphorylation of tau, a microtubule-associated protein, and of neurofilament H/M subunits, processes which lead to misfolding and accumulation of these proteins, along with disruption of microtubules. Thus, inhibition or reversal of hyperphosphorylation may be effective in preventing tauopathies.

Okadaic acid, a potent protein phosphatase inhibitor, induced cell death in neuroblastoma cells, accompanied by a striking decrease in mitochondrial metabolic activity (Benítez-King et al., 2003). Okadaic acid induces physiological and biochemical changes similar to those seen in AD. It increased the levels of 4-hydroxynonenal and decreased antioxidant enzyme activities in cultured neuronal cells (Perez et al., 2002). Either melatonin or vitamin C administration prevented the effects of okadaic acid in NIE 115 cells (Benítez-King et al., 2003). While both vitamin C and melatonin reduced free radical damage, the reduction was greater with melatonin. Although vitamin C failed to increase the levels of glutathione S-transferase

and GR, melatonin increased the levels of both enzymes significantly. Melatonin also inhibited the phosphorylation and accumulation of neurofilaments.

Similar results were obtained, in neuroblastoma N2a cells, with calyculin A, an inhibitor of protein phosphatases 2A and 1, but in this study an additional activation of GSK-3 (glycogen synthase kinase 3, a redox-controlled enzyme involved in various cell regulatory mechanisms) was observed (Li et al., 2005). Apart from hyperphosphorylation and lethal oxidative stress, including decreases in SOD, melatonin also reversed GSK-3 activation, thus supporting the conclusion that the methoxyindole action was not only based on its antioxidant properties, but also on interference with protein phosphorylation, especially by stress kinases.

Inhibition of protein phosphatase (PP)-2A and PP-1 by calyculin A induced AD-like hyperphosphorylation of tau and spatial memory retention impairment. The administration of melatonin before injection of calyculin A could prevent calyculin A-induced synaptophysin loss, memory retention deficits, as well as hyperphosphorylation of tau and neurofilaments. Furthermore, melatonin partially reversed the phosphorylation of the catalytic subunit of PP-2A at Tyrosine 307 (Y307), a crucial site negatively regulating the activity of PP-2A, and reduced the levels of malondialdehyde, a marker of oxidative stress, induced by calyculin A (Yang et al., 2011). Melatonin's antioxidant action may be of additional importance with regard to inflammatory responses of chronically activated microglia in AD (Kitazawa et al., 2004). For a recent review on the effect of melatonin on several neurological diseases with inflammatory components see (Esposito and Cuzzocrea, 2010).

In several experiments transgenic (Tg) murine models of AD have been used to assess a possible therapeutic beneficial effect of melatonin (Bedrosian et al., 2011; Dragicevic et al., 2011; Feng et al., 2006; Feng and Zhang, 2004; García et al., 2010; García-Mesa et al., 2012b; Matsubara et al., 2003; Olcese et al., 2009; Spuch et al., 2010). The administration of melatonin partially inhibited the expected time-dependent elevation of A β , reduced abnormal nitration of proteins, and increased survival in the treated amyloid protein precursor (APP) Tg mice (Matsubara et al., 2003). Melatonin was found to be effective in inhibiting A β deposition in APP 695 Tg mice, a model in which senile plaques appear in the cortex as early as 8 months of age (Feng et al., 2006; Feng and Zhang, 2004). These mice display behavioral impairments and memory deficits, deficiencies that were alleviated following long term administration of melatonin at a daily dose of 10 mg/kg. The treatment also reduced the number of apoptotic neurons (Feng et al., 2006; Feng and Zhang, 2004). AAP Tg mice were also used to explore a "sundowning"-like behavior and the efficacy of melatonin to treat it (Bedrosian et al., 2011). A temporal pattern of anxiety-like behavior emerged with elevated locomotor activity relative to adult mice near the end of the dark phase which was refractory to melatonin treatment (Bedrosian et al., 2011).

In other experiments APP + presenilin 1 (PS1) double Tg mice were used. In one of these experiments APP/PS1 Tg mice receiving melatonin from 2-2.5 months of age to their killing at age 7.5 months exhibited reduced cognitive impairments. Decreased A β deposition and inflammatory cytokines in hippocampus and entorhinal cortex

were also found. Cortical mRNA expression of three antioxidant enzymes (SOD-1, GPx, catalase) was also significantly reduced (Olcese et al., 2009). In a similar group of animals treated for 1 month with melatonin the analysis of isolated brain mitochondria indicated that melatonin treatment decreased mitochondrial A β levels by two- to fourfold in different brain regions (Dragicevic et al., 2011). This was accompanied by a near complete restoration of mitochondrial respiratory rates, membrane potential, and ATP levels in isolated mitochondria. In APP-expressing neuroblastoma cells in culture, mitochondrial function was restored by melatonin or by the structurally related compounds indole-3-propionic acid or AFMK, an effect blocked by melatonin receptor antagonists indicating melatonin receptor signaling is required for the full effect (Dragicevic et al., 2011). APP/PS1 Tg mice were also used to assess the efficacy of a tacrine-melatonin hybrid to inhibit amyloid-induced cell death and amyloid burden in brain parenchyma. The efficacy of the tacrine-melatonin hybrid to reduce A β toxicity suggested the possibility for a new potential therapeutic strategy in AD (Spuch et al., 2010).

In APP Tg 2576 mice fed with aluminium lactate melatonin co-administration prevented the prooxidant effect of the toxic in the hippocampus (García et al., 2010) but not its behavioral effects (García et al., 2009). The triple-Tg mouse model of AD (3xTg-AD) is the only model to exhibit both A β and tau pathology that is characteristic of the human form (Sterniczuk et al., 2010). Melatonin was highly effective against the immunosenescence and cognitive loss that 3xTg-AD mice show (García-Mesa et al., 2012a).

From all these studies it is clear that melatonin treatment gives neuroprotection against oxidative injury by maintaining the survival of both neuronal cells and glial cells. Studies carried out on cultured neuroblastoma cells in transgenic models of AD reveal that melatonin can attenuate the oxidative damage induced by A β . Overall, they explain why clinical studies on melatonin efficacy at the early stages of AD showed significant regression of disease.

Melatonin secretion in AD patients

Circulating melatonin levels are lower in AD patients than in age-matched controls (Ferrari et al., 2000; Liu et al., 1999; Mishima et al., 1999; Ohashi et al., 1999; Skene et al., 1990; Uchida et al., 1996). Decreased CSF melatonin levels observed in AD patients have been linked to a reduced pineal melatonin synthesis rather than to dilution of the CSF (Tan et al., 2010). It is interesting to note that CSF melatonin levels are decreased even in the preclinical stages when the patients have no cognitive impairment (Braak stages I-II), thus suggesting that reduced melatonin levels may be an early marker for the very first stages of the disease (Wu et al., 2003; Zhou et al., 2003). The decreased nocturnal melatonin levels with loss of melatonin diurnal rhythmicity may be the consequences of SCN clock gene dysfunction with altered noradrenergic regulation and depletion of the melatonin precursor serotonin by increased MAO A activity (Wu et al., 2006b). According to one study (Skene and Swaab, 2003), reduced light transmission through the ocular lens or a defective retina-retinohypothalamic tract-SCN pathway are possible causes of disturbed circadian rhythmicity in AD patients.

Treatment of AD patients with melatonin

There are two reasons why it is quite convenient the use of melatonin or melatonin analogs in AD patients. AD patients show a greater breakdown of the circadian sleep/wake cycle compared to similarly aged, non demented controls. Demented patients spend their nights in a state of frequent restlessness and their days in a state of frequent sleepiness. These sleep/wake disturbances become increasingly more marked with progression of the disease (Zhong et al., 2011). Hence, replacement of melatonin levels in brain can be highly convenient in these patients. On the other hand, the bulk of information on the neuroprotective properties of melatonin derived from experimental studies turns highly desirable to employ pharmacological doses in AD patients with the aim of arresting or slowing disease's progression.

In AD patients with disturbed sleep/wake rhythms there is a greater degree of irregularities in melatonin secretion than in patients who do not exhibit such disturbances (Mishima et al., 1999). Impairments in melatonin secretion are also related to both age and severity of mental dysfunction. Other factors that are related to mental impairment are suppressed levels of nocturnal growth hormone (GH) and increases in both the mean levels and nadir values of plasma cortisol (Magri et al., 2004; Mazzoccoli et al., 2010b). Interestingly, melatonin augments GH secretion as well as prevents in animals models of glucocorticoid-induced neuronal loss in the hippocampus (Furio et al., 2008).

In several studies loss or damage of neurons in the hypothalamic SCN and other parts of the circadian timing system have been implicated in the circadian disturbances of demented patients (Hu et al., 2009; Skene and Swaab, 2003; Swabb et al., 1985; van Someren, 2000). The SCN of AD patients have tangles (Stopa et al., 1999). Wu and coworkers (2007) have shown by immunocytochemistry that both MT₁-expressing neurons and arginine vasopressin (AVP)/vasoactive intestinal polypeptide (VIP)-expressing neurons in the SCN are strongly diminished in the advanced neuropathological stages of AD.

A chronobiological approach using melatonin, bright-light therapy, restricted time in bed and diurnal activity has been proposed as a therapeutic alternative for the management of sleep/wake disorders in AD patients. The aim of these therapies is to improve sleep and diurnal activity and consequently to increase the quality of life in patients. The safety of melatonin treatment is high, with very few or no adverse effects. In the case of bright light therapy, however, there is a very significant risk of retinal damage from repeated exposure to the high intensities of visible light (Beatty et al., 2000; Hall and Gale, 2002; Wu et al., 2006a).

Table 1 summarizes the clinical studies in AD patients published so far. An initial, preliminary examination of the sleep-promoting action of melatonin (3 mg p.o. for 21 days) was carried out in a small non-homogenous group of elderly patients with primary insomnia and with insomnia associated with dementia or depression. The investigation found that 7 out of 10 dementia patients who had sleep disorders and who were treated with melatonin (3 mg p.o. at bed time) showed decreased sundowning and reduced variability of sleep onset time (Fainstein et al., 1997). In another study, 10 individuals with mild cognitive impairment (MCI) were given 6 mg

of melatonin before bedtime. Improvement was found in sleep, mood and memory (Jean-Louis et al., 1998b).

Another study in this line of investigation evaluated the effect on AD patients with sleep disorders and sundowning agitation of daily administration of 6-9 mg melatonin for longer periods of time (2-3 years). The retrospective account of 14 AD patients after the extended period of therapy with melatonin indicated that all experienced improvements in sleep quality (Brusco et al., 2000). Sundowning, diagnosed clinically in all patients examined, was no longer detectable in 12 of them, with attenuated symptoms in the other 2 patients. Another significant observation in this study was the arrest of the cognitive and amnesic alterations expected in comparable populations of patients not receiving melatonin. This should be contrasted with the significant deterioration of clinical conditions of the disease found in patients after 1-3 years of progression of the disease. Further support for the hypothesis that melatonin could be useful to slow cognitive decay in AD was provided by a case report study which included two 79 years old male monozygotic twins diagnosed 8 years earlier. One of them was treated with melatonin whereas his brother did not receive melatonin treatment (Brusco et al., 1998a).

The potential efficacy of melatonin for treatment of AD patients is also supported by other studies. Mishima and co-workers (2000) administered a 6 mg dose of melatonin for 4 weeks to 7 inpatients with AD who exhibited an irregular sleep-waking cycle. Melatonin significantly reduced the percentage of nighttime activity compared to placebo. Cohen-Mansfield et al. (2000) reported the efficacy of melatonin (3 mg/day at bed time) for improving sleep and alleviating sundowning in 11 elderly AD patients. Analysis revealed a significant decrease in agitated behavior and a significant decrease in daytime sleepiness. A remarkable effect of a 3 mg-dose of melatonin to reduce sundowning was reported in an open study using actigraphy to define sleep patterns in seven AD patients (Mahlberg et al., 2004). In another study including 45 AD patients having sleep disturbances and treated for 4 months with 6-9 mg melatonin/day, a positive effect of melatonin was documented when used as an adjuvant medication to treat cognitive or behavioral consequences of AD (Cardinali et al., 2002). In a double blind study 3 mg of melatonin given for 4 weeks significantly prolonged actigraphically evaluated sleep time, decreased activity at night and improved cognitive function in AD type of dementia (Asayama et al., 2003). In a multicenter, randomized, placebo-controlled clinical trial of two dose formulations of oral melatonin, 157 subjects with AD and nighttime sleep disturbance were randomly assigned to 1 of 3 treatment groups: placebo, 2.5-mg slow-release melatonin, or 10-mg melatonin given daily for 2 months (Singer et al., 2003). When sleep was defined by actigraphy, trends for increased nocturnal total sleep time and a decreased number of awakenings after sleep onset in the melatonin groups were observed. On subjective measures, caregiver ratings of sleep quality showed significant improvements in the 2.5-mg sustained-release melatonin group relative to placebo (Singer et al., 2003).

In a recent publication, published data concerning melatonin treatment in AD were analyzed (Cardinali et al., 2010). As summarized in Table 1, eight reports (5 open label studies, 2 case reports) (N = 89 patients) supported a possible efficacy of

melatonin: sleep quality improved and in patients with AD sundowning was reduced and the progression of cognitive decay was slowed. In 6 double blind, randomized placebo-controlled trials 210 AD patients were examined. Sleep was objectively measured by wrist actigraphy (N = 5) and additionally neuropsychological assessment and sleep quality were subjectively evaluated (N= 6). In 4 studies (N= 143) sleep quality increased, sundowning decreased significantly and cognitive performance improved whereas there was absence of effects in 2 studies (N = 67) (Cardinali et al., 2010).

In another paper de Jonghe and co-workers performed a systematic search of studies published between 1985 and April 2009 on melatonin and sundowning in AD patients (de Jonghe et al., 2010). All papers on melatonin treatment in dementia were retrieved. The effects of melatonin on circadian rhythm disturbances were scored by means of scoring sundowning/agitated behavior, sleep quality and daytime functioning. A total of 9 papers, including four randomized controlled trials (n = 243), and five case series (n = 87) were reviewed. Two of the randomized controlled trials found a significant improvement in sundowning/agitated behavior. All five case series found an improvement. The results on sleep quality and daytime functioning were inconclusive.

Therefore, the question whether melatonin has any value in preventing or treating AD, affecting disease progression of the neuropathology and the driving mechanisms, remains unanswered. One of the problems with AD patients in fully developed pathology is the heterogeneity of the group examined. Moreover, a reduced hippocampal expression of MT₂ melatonin receptors has been reported in AD patients, heralding a possible lack of response to exogenous melatonin (Savaskan et al., 2005). In addition, the diminution of MT₁ receptors in the circadian apparatus at late stages of the disease may explain why melatonin treatment is less effective at this stage (Wu and Swaab, 2007).

Thus, early initiation of treatment can be decisive for therapeutic success (Quinn et al., 2005). MCI is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia (Gauthier et al., 2006). Some of these patients develop AD. As shown in Table 2, 5 double blind, randomized placebo-controlled trials and 1 open-label retrospective study (N = 651) consistently show that the administration of daily evening melatonin improves sleep quality and cognitive performance in MCI (Cardinali et al., 2010; Furio et al., 2007; Jean-Louis et al., 1998b; Peck et al., 2004; Riemersma-van der Lek et al., 2008). Therefore, melatonin treatment could be very effective at early stages of the disease.

In any event, double-blind multicenter studies are needed to further explore and investigate the potential and usefulness of melatonin as an antidementia drug. Its apparent usefulness in symptomatic treatment, enhancing sleep quality, reducing sundowning, etc., even in a progressed state, further underlines the need for such decisive studies.

The mechanisms accounting for the therapeutic effect of melatonin in AD patients and MCI remain unknown. Melatonin treatment mainly promotes slow wave

sleep in the elderly (Monti et al., 1999), and can be beneficial in AD by augmenting the restorative phases of sleep, including the augmented secretion of GH and neurotrophins. Melatonin was found to protect against the circadian changes produced by β -amyloid₂₅₋₃₅ microinjection in SCN of golden hamsters (Furio et al., 2002). Demonstration of the utility of melatonin treatment in correcting the biochemical pathology of AD was established in the Tg murine models, as discussed above.

Melatonin in PD

PD is a major neurodegenerative disease characterized, in its clinically relevant stages, by the progressive degeneration of DA-containing neurons in the substantia nigra pars compacta. However, PD does not start in the nigrostriatum, but rather in the brainstem or even the spinal cord of subjects who remain asymptomatic for a long period of time (Braak et al., 2004; Grinberg et al., 2010). Although the appearance of Lewy bodies (clumps of α -synuclein and ubiquitin proteins in neurons which are detectable in post-mortem brain histology) can be traced back to the earlier stages, the primary etiology remains unknown.

Key symptoms, such as tremor, rigidity, bradykinesia and postural instability, develop when about 3/4 of dopaminergic cells are lost in the substantia nigra pars compacta, and consequently the smooth, coordinated regulation of striatal motor circuits is lost (Maguire-Zeiss and Federoff, 2010; Tansey et al., 2007). Other, non-motor symptoms are seen in PD, and some of them, such as hyposmia, depression or rapid eye movement (REM)-associated sleep behavior disorder (RBD), can precede the onset of disease. Non-motor symptoms are often misdiagnosed and untreated although their appearance is an index of a worse prognosis and lower quality of life.

At this time, there is no treatment that will delay or stop the progression of PD, and medications currently available are mostly symptomatic. The increased incidence of age-associated neurodegenerative diseases such as PD has been attributed to the augmented generation of free radicals and the associated oxidative stress, which is enhanced in certain regions of the aging brain (Fahn and Cohen, 1992a; Gibson et al., 2010; Olanow, 1992). Increased lipid peroxidation, decreased levels of GSH and increased iron levels occur in the brains of PD patients (Dexter et al., 1989). As the increased iron levels can promote the Fenton reaction, it seems feasible that an increased hydroxyl radical formation induces free radical damage. Free radical damage of lipids, proteins and nucleic acids have all been reported in the substantia nigra of Parkinsonian patients (Alam et al., 1997). Oxidative stress has been suggested to be the major cause of dopaminergic neuronal cell death. Exposure to high concentrations of H₂O₂ that are formed during oxidation of DA by MAO may also be a major cause for destruction of dopaminergic neurons in PD (Fahn and Cohen, 1992b).

What is the role of melatonin in prevention and treatment of PD? The studies designed to answer this question have produced controversial findings. Some studies suggest that melatonin has beneficial effects in that it arrests the neurodegenerative changes induced in the experimental models of Parkinsonism. Others have reported

adverse effects, demonstrating in experimental models of PD that melatonin exacerbates motor deficits.

Acuña-Castroviejo et al. used the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) model of PD in rats to show that melatonin could counteract MPTP-induced lipid peroxidation in striatum, hippocampal, and midbrain regions (Acuña-Castroviejo et al., 1997). Mayo et al. showed that when added to incubation medium containing the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), melatonin significantly prevented the increased lipid peroxidation which normally would have occurred in cultured DA PC 12 cells (Mayo et al., 1998). Melatonin also increased the levels of antioxidant enzymes (Mayo et al., 1998). Additionally, melatonin reduced pyramidal cell loss in the hippocampus, a cellular area which undergoes degeneration in the brains of PD patients, and which presumably causes memory deficits in patients. Thomas and Mohanakumar (2004) similarly demonstrated in vitro and ex vivo models, as well as in an in vivo MPTP rodent model, that melatonin had potent hydroxyl radical scavenger activity in the mouse striatum and in isolated mitochondria. In addition to these primary effects the investigators also found secondary increases in SOD activity.

The attenuation of MPTP-induced superoxide formation indicates an additional neuroprotective mechanism by melatonin. Intra-median forebrain bundle infusion of a ferrous-ascorbate-DA hydroxyl radical generating system, which causes significant depletion of striatal DA, could be significantly attenuated by melatonin administration (Borah and Mohanakumar, 2009). In another study, Antolín et al. (2002) used the MPTP model and found that melatonin was effective in preventing neuronal cell death in the nigrostriatal pathway as indicated by the number of preserved DA cells, of tyrosine hydroxylase levels, and other ultrastructural features. These findings thus demonstrated that melatonin clearly prevents nigral dopaminergic cell death induced by chronic treatment with MPTP.

MPTP elicits its neurotoxic effects by increasing the amount of •NO derived from iNOS. This action mainly affects DA neurons while •NO derived from neuronal NOS (nNOS) has a damaging effect on dopaminergic fibers and terminals in the striatum. A future therapy for PD may require agents that inhibit the degenerative effects of iNOS in the substantia nigra pars compacta (Zhang et al., 2000). Since melatonin can effectively downregulate iNOS and prevent •NO formation in the brain (Cuzzocrea et al., 1997; Escames et al., 2004) it should be regarded as a drug of potential choice for arresting the neuronal degeneration associated with PD.

MPTP, through its metabolite methyl 1-4 phenyl pyridinium, causes direct inhibition of Complex I of the mitochondrial electron transport chain. Such an inhibition of Complex I has been reported in the substantia nigra of patients suffering from PD. By increasing Complex I and IV activities of the mitochondrial electron transport chain, melatonin may exert a beneficial effect on MPTP-induced lesions (Acuña-Castroviejo et al., 2011). Melatonin also stimulates the gene expression of three antioxidant enzymes Cu/Zn-SOD, Mn-SOD, and GPx in cultured dopaminergic cells (Mayo et al., 1998).

It has been said that an abnormal assembly of the cytoskeleton is involved in the pathogenesis of neurodegenerative diseases. Lewy bodies, which are considered to be cytopathologic markers of Parkinsonism, comprise abnormal arrangements of tubulin, ubiquitin, microtubule-associated protein (MAP) 1 and MAP 2 (Vekrellis et al., 2011). Melatonin is very effective in promoting cytoskeletal rearrangements and thus may have a potential therapeutic value in the treatment of neurodegenerative diseases including Parkinsonism (Benítez-King et al., 2004).

However, other studies do not support the hypothesis that melatonin is of therapeutic benefit in Parkinsonism. For instance, reduction of melatonin by pinealectomy, or by exposure of rats to bright light, have been found to enhance recovery from experimental Parkinsonism in rats, i.e., spontaneous remission of symptoms following 6-OHDA or MPTP have been observed, whereas melatonin administration aggravated them (Willis and Armstrong, 1999). The mechanism by which melatonin exacerbated motor impairments appeared to be similar to that seen caused by excess DA production and release, processes which occur in degenerating indoleamine and catecholamine containing neurons in experimental PD. (Tapias et al., 2010), using a rotenone model of PD in rats, found that melatonin administration led to striatal catecholamine depletion, striatal terminal loss, and nigral DA cell loss, thus aggravating the disease.

Based on this, Willis and Armstrong (1999) suggested that a possible treatment for PD could involve the reduction of melatonin production or the pharmacological blockade of melatonin action on its receptors. Indeed, reduction of rigidity and bradykinesia through inhibition of melatonin synthesis (through the strategic application of bright light) has been found in pilot studies to reduce the incidence of insomnia as well as fragmented sleep (Willis and Armstrong, 1999). These observations thus contradict published reports of melatonin's beneficial effects on Parkinsonian symptoms, and the use of melatonin as an adjunct therapy to either halt progressive degeneration or for providing symptomatic relief in PD patients has been questioned (Willis and Robertson, 2004). In a study in rats using the 6-OHDA model of Parkinsonism, the administration of the melatonin receptor antagonist ML-23 totally abolished 6-OHDA-induced mortality, thus suggesting that blockade of melatonin's functions or decreasing its bioavailability may offer a novel approach for the treatment of Parkinsonism (Willis and Robertson, 2004).

A possible hampering role of melatonin in PD is also suggested by epidemiological studies. Because of the lower rates of cancer mortality/incidence in patients with PD, speculations about risk or preventive factors common to both diseases, including life-style factors (such as smoking) and genetic susceptibility have been entertained (Rod et al., 2010). Longer intervals of working night shifts is associated with reduced melatonin levels and reduced risk of PD among whereas longer hours of sleep appear to increase their risk (Schernhammer et al., 2006). While lower melatonin concentrations may predict a higher cancer risk (Stevens et al., 2011), there is also some evidence that they may be associated with a lower risk of PD.

The study of melatonin secretion in PD has revealed some interesting findings. A phase advance in nocturnal melatonin levels in levodopa-treated Parkinsonian patients was noted but this was not observed in untreated patients when compared to control subjects (Fertl et al., 1993). Similarly a phase advance of about 2 h in plasma melatonin secretion was seen in PD patients receiving dopaminergic treatment when compared to untreated patients (Bordet et al., 2003). An increase in daytime melatonin secretion was also noted in levodopa-treated patients and was suggested to be an adaptive response to neurodegeneration that could play a neuroprotective role through an antioxidant effect (Bordet et al., 2003).

The occurrence of motor fluctuations in PD was related to fluctuations in serum melatonin levels, a finding that was attributed to interactions of monoamines with melatonin in the striatal complex (Escames et al., 1996). Melatonin may exert direct motor effects through its interactions with DA and serotonin. Changes in levodopa-related motor complications may be related to changes in melatonin secretion pattern. Levodopa-related motor complications occur in nearly half of the patients with PD on completion of first 5 years of treatment (Koller, 1996).

The hypothesis that melatonin has an inhibitory motor effect which is probably involved in wearing-off episode (i.e. the progressively shorter intervals during which symptoms remain adequately controlled as if the effects of medication would start to "wear off") has been supported by some therapeutic studies. Stimulation of globus pallidus inhibited an increase in daytime plasma melatonin levels seen in PD patients as compared to healthy subjects (Catala et al., 1997) and was also reported to improve motor symptoms and complications in patients with PD (Olanow et al., 2000).

The finding that a reduced expression of melatonin MT₁ and MT₂ receptors occurs in amygdala and substantia nigra in patients with PD (Adi et al., 2010) suggests the involvement of the melatonergic system in the abnormal sleep mechanisms seen in PD. Indeed, melatonin has been used for treating sleep problems, insomnia, and daytime sleepiness in PD patients. The published data are summarized in Table 3.

In a study undertaken on 40 patients (11 women, 29 men; range 43-76 yrs) melatonin was administered for a treatment period of 2 weeks, in doses ranging from 5 mg to 50 mg/day (Dowling et al., 2005). To avoid the possibility of producing a circadian shift melatonin was administered 30 min before bedtime (circadian shifts can occur if administered melatonin is administered at any other time). All subjects were taking stable doses of antiparkinsonian medications during the course of the study. Relative to placebo, treatment with 50 mg of melatonin significantly increased night time sleep, as revealed by actigraphy. As compared to 50 mg or placebo, administration of 5 mg of melatonin was associated with significant improvement of sleep in the subjective reports. The study also found that the high dose of melatonin (50 mg) was well tolerated (Dowling et al., 2005).

In another study 18 PD patients were randomized after performing a basal polysomnography to receive melatonin (3 mg) or placebo one hour before bedtime for four weeks (Medeiros et al., 2007). Subjective sleep quality was assessed by the Pittsburgh Sleep Quality Index and daytime somnolence by the Epworth Sleepiness Scale. All measures were repeated at the end of treatment. On initial assessment, 14

patients (70%) showed poor quality sleep and eight (40%) excessive diurnal somnolence. Increased sleep latency (50%), REM sleep without atonia (66%), and reduced sleep efficiency (72%) were found in polysomnography (PSG). Sleep fragmentation tended to be more severe in patients on lower doses of levodopa although melatonin significantly improved subjective quality of sleep. The objective abnormalities remained unchanged. Motor dysfunction was not improved by the use of melatonin (Medeiros et al., 2007).

Administration of melatonin 3-12 mg at bedtime has been shown to be effective in the treatment of RBD (Table 3). This benefit has been reported in one case report (Kunz and Bes, 1997), two open-label prospective case series of patients with RBD (Kunz and Bes, 1999; Takeuchi et al., 2001) and two retrospective case series (Anderson and Shneerson, 2009b; Boeve et al., 2003). Taken together, these reports include a total of 38 patients. Thirty-one were noted to experience improvement with melatonin, 2 more experienced transient improvements and 1 seemed to worsen. Follow-up as far as 25 months was reported. PSG showed statistically significant decreases in number of R epochs without atonia and in movement time in R. This contrasted with the persistence of tonic muscle tone in R sleep seen with patients treated with clonazepam. Because of these data a recent clinical consensus recommended melatonin use in RBD at Level B, i.e. "assessment supported by sparse high grade data or a substantial amount of low-grade data and/or clinical consensus by the task force" (Aurora et al., 2010).

As mentioned above, not all the clinical studies are in the vein to support a beneficial role of melatonin in PD. Exposure to light of 1000 to 1500 lux intensity for 1 to 1.5 h, one h prior to bedtime for two to five weeks has been found to improve the bradykinesia and rigidity observed in twelve PD patients (Willis and Turner, 2007). A reduction in agitation and psychiatric side effects were also reported in this study. The authors suggested that activation of the circadian system by antagonizing melatonin secretion with bright light has a therapeutic value for treating the symptoms of PD (Willis, 2008).

It must be noted, however, that bright light has been employed in a number of studies for treating depressive symptoms (Rosenthal et al., 1984) on the basis that it could reset the phase of abnormal circadian rhythms seen in depressed patients (Lewy et al., 1984). Indeed, although evening bright light exposure produces a momentary suppression of melatonin, it actually causes a rebound increase in melatonin secretion late in the night (Beck-Friis et al., 1985). Hence, the bright light exposure may ultimately facilitate melatonin secretion rather than suppress it. The conclusion that PD is a "melatonin hyperplasia" disorder (Willis, 2008) awaits further demonstration. Indeed, the decrease in the amplitude of circulating melatonin rhythm (Bordet et al., 2003) and the reduced melatonin receptor expression in the substantia nigra reported in PD patients (Adi et al., 2010) indicate a weakened melatonin signaling in PD.

Bright light effects may be indicative of circadian changes in PD. This is supported by the reduced expression of the circadian clock gene *Bmal1* in leukocytes (Cai et al., 2010b), although effects on peripheral oscillators do not necessarily allow

conclusions on changes in the hypothalamic master clock. The recent finding that the mouse striatal DA receptors D1R and D2R are under circadian control (Cai et al., 2010a), can be seen as an interesting facet in this context, although circadian variations in receptor expression are by no means exceptional features.

Melatonin in HD

Sleep disturbances are very prevalent in HD patients and can substantially impair their quality of life. They coexist with abnormal melatonin secretion as shown by examining the 24 h secretion profiles in early stage HD patients and normal controls (Aziz et al., 2009b). Although mean diurnal melatonin levels were not different between the two groups, the timing of the evening rise in melatonin levels was significantly delayed by about 90 min in HD patients. Moreover, diurnal melatonin levels strongly correlated with both motor and functional impairment, indicating that melatonin levels in HD may progressively decline with advancing disease (Alders et al., 2009; Aziz et al., 2009a).

Mitochondrial dysfunction occurs in HD (Chen, 2011). However, although current evidence from genetic models of HD indicated the link between mutation of the huntingtin gene (mHtt) and mitochondrial dysfunction, impairment of ETC appears to be a late secondary event in disease's evolution (Oliveira, 2010b). Upstream events include defective mitochondrial calcium handling and impaired ATP production. Also, transcription abnormalities affecting mitochondria composition, reduced mitochondria trafficking to synapses, and direct interference with mitochondrial structures enriched in striatal neurons, are possible mechanisms by which mHtt amplifies striatal vulnerability (Oliveira, 2010a).

An animal model was developed by using 3-nitropropionic acid, an inhibitor of mitochondrial Complex II. In this model, that replicated the neurochemical, histological and clinical features of HD, melatonin administration was reported to defer the clinical signs of the disease (Tunéz et al., 2004). The data confirmed an early observation in rat brain homogenate treated with quinolinic acid (Southgate and Daya, 1999).

Recently, a detailed examination of the neuroprotective properties of melatonin in the genetic model of HD was published (Wang et al., 2011a). Melatonin delayed disease onset and prolongs lifespan in the R6/2 transgenic mouse HD model. In addition, melatonin MT₁ receptor levels decrease in cultured striatal cells, mouse brain, and human striatum associated with mHtt-mediated toxicity, receptor depletion becoming greater as the disease progresses. Further, MT₁ receptor knockdown made cells more vulnerable to cell death, whereas MT₁ receptor overexpression increases resistance to cell death (Wang et al., 2011b). The administration of melatonin counteracted the MT₁ receptor depletion attributable to mHtt in vitro and in vivo. The authors concluded that functional MT₁ receptor depletion contributed to cell death in the genetic model of HD (Wang et al., 2011c). Melatonin had little or no inhibitory effect on huntingtin fibrillogenesis (Heiser et al., 2000).

Melatonin in ALS

ALS is a neurodegenerative disease in which motoneurons in the anterior horn of the spinal cord and motor neurons of cerebral cortex gradually degenerate. Dysfunction and premature death of these neurons causes spasticity, hyperreflexia, muscular atrophy, and generalized paralysis (Blackhall, 2012). The disorder produces muscle weakness and muscular atrophy as both the upper and lower motoneurons deteriorate. In contrast, mental function remains normal. Respiratory failure is the common cause of death, occurring within 2-5 years after diagnosis. Most ALS cases occur sporadic. Among those individuals with the familial form, roughly 20% have a mutation in the gene for the antioxidant enzyme SOD (Synofzik et al., 2012).

Different pathological mechanisms have been suggested to contribute to cell death in ALS, independent of the underlying molecular/genetic defect. These include impaired axonal transport, mitochondrial dysfunction, neurofilament disorganization, protein aggregation, and impaired proteasome function. Also excitotoxic mechanisms contribute to ALS. Indeed, the only therapeutic drug with a marginal effect on patient survival is riluzole, an antiexcitotoxin (Cifra et al., 2012).

Since there is no known cure for ALS a number of strategies have been tested. Melatonin is one of them. However a limited number of studies are available. Given that oxidative stress has been associated with ALS, Weishaupt et al. (2006) examined the ability of melatonin to attenuate neural damage in a genetic mouse model of ALS (SOD1G93A-transgenic mouse) and in 31 patients with sporadic ALS. In the mouse model, orally administered melatonin delayed disease progression and extended survival. In this report, disease progression was defined as the time span between the onset of hind limb tremor and premature death; this was delayed by 25% in the melatonin-treated mice. In the ALS patients, melatonin (5 mg/kg) was applied nightly as a suppository; duration of treatment varied among the patients and ranged from 2-24 months. Melatonin was well tolerated with some patients reporting improved sleep quality (Weishaupt et al., 2006). Circulating protein carbonyls were reduced in the blood after more than 4 months of treatment compared to levels before treatment onset. Therefore, combining melatonin with the conventional drugs used for treatment, due to their presumed synergistic actions, may improve the outcome of ALS patients (Weishaupt et al., 2006). Additional trials with melatonin, alone and in combination with other drugs, are needed to clarify the potential benefit of melatonin in subjects with ALS.

Melatonin and Brain Trauma

The neuropathological changes observed during neuronal injury, trauma or stroke, have all been ascribed to an enhancement of oxidative stress which in turn induces lipid peroxidation, protein and DNA oxidation (Hall et al., 2010; Hall, 2011; Lin and Lee, 2009). When administered to rats kainic acid (KA), an agonist of the ionotropic glutamate receptor, causes excessive release of glutamate and consequently produces severe brain damage via N-methyl-D-aspartate (NMDA) receptor activation. The KA model of excitotoxicity is used as a model for studying

oxidative stress during neuronal degeneration induced by brain trauma. KA ionotropic glutamate receptor activation results in increased Ca^{2+} influx via NMDA-controlled channels causing $\bullet\text{NO}$ production through activation of nNOS. Melatonin inhibits either glutamate- or NMDA-induced excitation (Tan et al., 1998). Melatonin iontophoreses have been found to counteract excitation induced by tris (2-carboxyethyl) phosphine hydrochloride, showing that the redox site of the NMDA receptor may be target of melatonin action (Escames et al., 2004). Both *in vivo* and *in vitro* studies have shown that melatonin prevents the neurodegeneration induced by kainate. Melatonin greatly reduces kainate induced oxidative damage in homogenates of cerebral cortex, cerebellum, hippocampus, hypothalamus and striatum (Melchiorri et al., 1996).

Oxidative apoptotic cell death induced directly by glutamate in cultured hippocampal cells (HT-22) and hippocampal brain cells have been found to be reduced after melatonin administration (Lezoualc'h et al., 1996). MT_2 receptors are present in the human hippocampus and presumably are involved in the mediation of these effects of melatonin (Savaskan et al., 2005). Bouslama et al. reported that melatonin administration could prevent learning disorders in brain-lesioned newborn mice (Bouslama et al., 2007). Injections of the glutamate analog ibotenate into the brains of 5-day-old mice were used to produce excitotoxic lesions resembling those of white-matter lesions that are seen in hypoxic preterm newborns. Behavioral conditioning and learning responses were also abolished by the ibotenate treatment, the administration of melatonin protecting against the development of these deficits. Melatonin also reduced ibotenate-induced lesions in white matter as confirmed by histological analysis (Bouslama et al., 2007). A recent study has shown that melatonin treatment reduced the apoptosis of oligodendrocytes, axon degeneration and vascular endothelial damage in the white matter of neonatal rats following a hypoxic injury (Kaur et al., 2010).

The neuroprotective effect of melatonin in cerebral edema has also been investigated. Edema plays a major contributing role in neurotrauma that follows traumatic brain injury (Fraser, 2011). Trauma causes neuronal death by abnormal production of ROS such as superoxide radical, hydroxyl radical and hydrogen peroxide leading to excessive lipid peroxidation. Pinealectomy or melatonin deficiency has been found to aggravate brain damage caused by stroke (Manev et al., 1996c) or focal ischemia (Kilic et al., 1999). In a study of vasogenic brain edema caused by cold-induced lesions in rats, Gorgulu et al. (2001) evaluated the effect of melatonin in the development of edema and the destruction of blood brain barrier (BBB) that become manifest 24 h after injury. Melatonin was found to be effective in decreasing brain edema and restoring BBB permeability at the penumbra zone of cold injury, and in reducing the infarct area (Kabadi and Maher, 2010). Ultrastructural studies showed that melatonin treatment decreased nuclear and mitochondrial damage, axonal and myelin changes. Recently evidence that this effect was independent on membrane melatonin receptors was provided in MT_1/MT_2 knockout mice (Kilic et al., 2011).

The pathophysiology of traumatic cerebral injury and cerebral edema has been attributed to excessive $\bullet\text{NO}$ release (Wada et al., 1998), a process which can induce

the formation of peroxynitrite anions by reacting with superoxide anions. Melatonin prevents the toxic effects of peroxynitrite both by inhibiting \bullet NO synthesis as well as by scavenging peroxynitrite (Cuzzocrea et al., 1997; Lin and Lee, 2009; Mesenge et al., 1998). In a study of children with epilepsy under carbamazepine therapy, administration of melatonin (6-9 mg/day for 14 days) increased the activity of the antioxidant enzyme GPx in plasma, thus suggesting that melatonin exerts antioxidant activity in these patients (Gupta et al., 2004).

Besides \bullet NO, vascular endothelial growth factor (VEGF) has been suggested as a factor underlying disruption of the BBB and subsequent edema formation (Nag et al., 2011). Following a hypoxic injury, disruption of the BBB was evidenced in many parts of the brain by an increased permeability of the blood vessels which resulted in swelling of the astrocyte processes in close association with the blood vessels. This was attributed to increased VEGF and \bullet NO and production following the hypoxic injury (Kaur et al., 2006). The swelling of astrocytes or their processes was reduced significantly following melatonin administration. The concomitant reduction in VEGF production was thought to be responsible for a decrease in edema formation through decreased vascular permeability and reduced astrocytic swelling (Kaur et al., 2006).

A number of animal models are being used to investigate the effects of melatonin on brain injury caused by ischemia followed by reperfusion (Lin and Lee, 2009). One of these models is the Mongolian gerbil which has an incomplete circle of Willis and in which bilateral ligation of the carotid arteries totally interrupts the blood supply to the forebrain. By using this model it was demonstrated that a 10-minute ischemic period caused by occlusion of carotid arteries and followed by 5 minutes reperfusion resulted in significant increases in nitrite/nitrate levels an effect prevented by the administration of melatonin prior to occlusion inhibited \bullet NO production (Guerrero et al., 1997). In another study melatonin reduced the cortical and striatal infarct volume by 60% and 30%, respectively ((Borlongan et al., 2000). In addition to reducing infarct volume, melatonin also decreased DNA double and single strand breaks and enhanced cell viability in the penumbral area of the infarct (Cheung, 2003). In a meta-analysis of 14 studies involving 432 animals examined in different models of focal cerebral ischemia, melatonin was found equally effective in permanent or temporary ischemia, thus suggesting that melatonin should be considered as a candidate neuroprotective drug for the treatment of human stroke (Macleod et al., 2005). Indeed, a cross-sectional matched case-control analysis indicated an impaired nocturnal melatonin secretion in the acute phase of ischemic stroke in humans (Atanassova et al., 2009).

Melatonin vs. Melatonin Analogs

As melatonin exhibits both hypnotic and chronobiotic properties, it has been used for treatment of age-related insomnia as well as of other primary and secondary insomnia. A recent consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia and circadian rhythm sleep disorders concluded that melatonin is the first choice treatment when a hypnotic is indicated in patients over 55 yr (Wilson et al., 2010). Melatonin has also been

successfully used for treatment of sleep problems related to perturbations of the circadian time keeping system like those caused by jet-lag, shift-work disorder or delayed sleep phase syndrome (Arendt et al., 1997; Srinivasan et al., 2010).

Since melatonin has a short half life (less than 30 min) its efficacy in promoting and maintaining sleep has not been uniform in the studies undertaken so far. Thus the need for the development of prolonged release preparations of melatonin or of melatonin agonists with a longer duration of action on sleep regulatory structures in the brain arose (Turek and Gillette, 2004). Slow release forms of melatonin (e.g., Circadin®, a 2 mg- preparation developed by Neurim, Tel Aviv, Israel, and approved by the European Medicines Agency in 2007) and the melatonin analogs ramelteon, agomelatine, tasimelteon and TK-301 are examples of this strategy.

Ramelteon (Rozerem®, Takeda Pharmaceuticals, Japan) is a melatonergic hypnotic analog approved by the FDA for treatment of insomnia in 2005. It is a selective agonist for MT₁/MT₂ receptors without significant affinity for other receptor sites (Miyamoto, 2009). In vitro binding studies have shown that ramelteon affinity for MT₁ and MT₂ receptors is 3-16 times higher than that of melatonin.

Agomelatine (Valdoxan®, Servier, France) is a recently introduced melatonergic antidepressant, acts on both MT₁ and MT₂ melatonergic receptors with a similar affinity to that of melatonin (IC₅₀ 1.3x10⁻¹⁰ and 4.7x10⁻¹⁰ M, respectively) and also acts as an antagonist to 5-HT_{2C} receptors at a 3 orders of magnitude greater concentration (IC₅₀ 2.7x10⁻⁷ M) (Millan et al., 2003). Agomelatine has been licensed by EMEA for treatment of major depressive disorder at doses of 25 – 50 mg/day.

Tasimelteon, (VES-162) is a MT₁/MT₂ agonist developed by Vanda Pharmaceuticals that completed phase III trial in 2010. In animal studies, tasimelteon exhibited the circadian phase shifting properties of melatonin. In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10 to 100 mg/day (Rajaratnam et al., 2009). The FDA granted tasimelteon orphan drug designation status for blind individuals without light perception with non-24-hour sleep-wake disorder in 2010.

TIK-301 (formerly LY-156,735) has been in a phase II clinical trial in the USA since 2002. Originally it was developed by Eli Lilly and Company and called LY-156,735. In 2007 Tikvah Pharmaceuticals took over the development and named it TIK-301. It is a chlorinated derivative of melatonin with MT₁/MT₂ agonist activity and 5HT_{2C} antagonist activity. TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo controlled study using 20 to 100 mg/day doses in healthy volunteers (Mulchahey et al., 2004). The FDA granted TIK-301 orphan drug designation in 2004, to use as a treatment for circadian rhythm sleep disorder in blind individuals without light perception and individuals with tardive dyskinesia.

As shown by the binding affinities, half-life and relative potencies of the different melatonin agonists it is clear that studies using 2-5 mg melatonin/day are probably unsuitable to give appropriate comparison with the effect of ramelteon, agomelatine, tasimelteon or TIK-301, which in addition to being generally more potent than the native molecule are employed in considerably higher amounts (Cardinali et al., 2012).

Several other compounds are being investigated with relatively more selective activity on melatonin receptor subtypes, therefore heralding an interesting future era for melatonin agonist research (Hardeland, 2010a; Spadoni et al., 2010).

Conclusions

Oxidative stress has been implicated in the pathogenesis of a number of neurodegenerative diseases such as AD, PD, HD and ALS, and of pathologic neurological conditions such as stroke and brain trauma. A high oxygen consumption rate coupled with a low anti-antioxidant potential of the brain are the main causes for enhanced oxidative stress of the brain in aged individuals.

Melatonin is an effective antioxidant both in vitro and in vivo in experimental models of neurodegenerative diseases. Melatonin has been shown to arrest neuropathological changes not only by scavenging free radicals but also by increasing the antioxidant enzymes and by attenuating free radical formation by neuronal, astrocyte and microglial cells. At both physiological and pharmacological concentrations, melatonin increases gene expression and the activities of antioxidant enzymes, e.g. GPx, GR and SODs. Melatonin also increases the efficiency of mitochondrial ETC by enhancing Complex I and IV activity in brain tissues. Through all of these mechanisms melatonin prevents nuclear and mitochondrial DNA cleavage and apoptosis induced by neurotoxic agents such as A β , kainate or quinolinic acid. Melatonin also inhibits NMDA-induced neuronal excitation by inhibiting nNOS and by redox site modulation.

The efficacy of melatonin in preventing oxidative damage in either cultured neuronal cells or in the brains of animals treated with various neurotoxic agents, suggests that melatonin has a potential therapeutic value as a neuroprotective drug in treatment of AD, HD, ALS and brain trauma. In the case of other neurological conditions, like PD, the evidence is less compelling. Inasmuch as melatonin deficiency seems to be one of the major causes of the development of neurodegenerative diseases, intake of melatonin or its synthetic analogs (selected according to their chronobiotic and/ or antioxidant actions) can be recommended not only for arresting the progress of this disease but also for preventing its occurrence as well.

It must be noted that the doses of melatonin employed in humans may be unnecessarily low when one takes into consideration the binding affinities, half-life and relative potencies of the different melatonin agonists on the market. In addition to being generally more potent than the native molecule these analogs are employed in considerably higher amounts (Cardinali et al., 2011). Licensed doses of ramelteon vary from 8 to 32 mg/day while agomelatine has been licensed for treatment of major depressive disorder at doses of 25 – 50 mg/day. In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10 to 100 mg/day (Rajaratnam et al., 2009) and TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo controlled study using 20 to 100 mg/day (Mulchahey et al., 2004).

Melatonin has a high safety profile and it is usually remarkably well tolerated. In some studies melatonin has been administered at large doses to patients (Chahbouni et al., 2010; Voordouw et al., 1992; Waldhauser et al., 1984; Weishaupt et al., 2006). Therefore, further studies employing melatonin doses in the 100 mg/day are needed to clarify its potential therapeutical implications in humans. From animal studies it is clear that a number of preventive effects of melatonin, like those in neurodegenerative disorders, need high doses of melatonin to become apparent. If one expects melatonin to be an effective neuroprotector it is likely that the low doses of melatonin employed so far (2-5 mg/day) are not very beneficial.

Disclosures

S.R. Pandi-Perumal is a stockholder and the President and Chief Executive Officer of Somnogen Canada Inc., a Canadian Corporation. He declares that he has no competing interests that might be perceived to influence the content of this article.

All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any nature or kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this manuscript.

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Table 1. Studies including treatment of AD patients with melatonin.

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|--|---|---|------------------|--|---|--------------------------------|
| Open-label study | 10 demented patients | 3 mg melatonin p.o./daily at bed time | 3 weeks | Daily logs of sleep and wake quality completed by caretakers | 7 out of 10 dementia patients having sleep disorders treated with melatonin showed a significant decrease in sundowning and reduced variability of sleep onset time | (Fainstein et al., 1997) |
| Open-label study | 14 AD patients | 9 mg melatonin p.o./daily at bed time | 22 to 35 months | Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment. | Sundowning was not longer detectable in 12 patients and persisted, although attenuated in 2 patients. A significant improvement of sleep quality was found. Lack of progression of the cognitive and behavioral signs of the disease during the time they received melatonin. | (Brusco et al., 2000) |
| Case report | Monozygotic twins with AD of 8 years duration | One of the patients was treated with melatonin 9 mg p.o./daily at bed time. | 36 months | Neuropsychological assessment. Neuroimaging. | Sleep and cognitive function severely impaired in the twin not receiving melatonin as compared to the melatonin-treated twin. | (Brusco et al., 1998) |
| Open-label, placebo-controlled trial | 14 AD patients | 6 mg melatonin p.o./daily at bed time or placebo | 4 weeks | Daily logs of sleep and wake quality completed by caretakers. Actigraphy | AD patients receiving melatonin showed a significantly reduced percentage of nighttime activity compared to a placebo group | (Mishima et al., 2000). |
| Open-label study | 11 AD patients | 3 mg melatonin p.o./daily at bed time | 3 weeks | Daily logs of sleep and wake quality completed by the nurses. | Significant decrease in agitated behaviors in all three shifts; significant decrease in daytime sleepiness | (Cohen-Mansfield et al., 2000) |
| Open-label study | 45 AD patients | 6–9 mg melatonin p.o./daily at bed time | 4 months | Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment. | Melatonin improved sleep and suppressed sundowning, an effect seen regardless of the concomitant medication employed. | (Cardinali et al., 2002) |
| Randomized double blind placebo controlled | 25 AD patients | 6 mg of slow release melatonin p.o. or placebo at | 7 weeks | Actigraphy | Melatonin had no effect on median total time asleep, number of awakenings or sleep efficiency. | (Serfaty et al., 2002) |

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|---|-------------------------|--|------------------|---|--|-----------------------------|
| cross over study | | bed time | | | | |
| Double-blind, placebo-controlled study | 20 AD patients | Placebo or 3 mg melatonin p.o./daily at bed time | 4 weeks | Actigraphy. Neuropsychological assessment. | Melatonin significantly prolonged the sleep time and decreased activity in the night. Cognitive function was improved by melatonin. | (Asayama et al., 2003) |
| Randomized, placebo-controlled clinical trial | 157 AD patients | 2.5-mg slow-release melatonin, or 10-mg melatonin or placebo at bed time | 2 months | Actigraphy. Caregiver ratings of sleep quality | Non significant trends for increased nocturnal total sleep time and decreased wake after sleep onset were observed in the melatonin groups relative to placebo. On subjective measures, caregiver ratings of sleep quality showed a significant improvement in the 2.5-mg sustained-release melatonin group relative to placebo. | (Singer et al., 2003) |
| Open-label study | 7 AD patients | 3 mg melatonin p.o./daily at bed time | 3 weeks | Actigraphy. Neuropsychological assessment. | Complete remission of day night rhythm disturbances or sundowning was seen in 4 patients, with partial remission in other 2. | (Mahlberg et al., 2004) |
| Randomized, placebo-controlled study | 17 AD patients | 3 mg melatonin p.o./daily at bed time (7 patients). Placebo (10 patients) | 2 weeks | Actigraphy. Neuropsychological assessment. | In melatonin-treated group, autographic nocturnal activity and agitation showed significant reductions compared to baseline. | (Mahlberg and Walther 2007) |
| Randomized, placebo-controlled study | 50 AD patients | Morning light exposure (2,500 lux, 1 h) and 5 mg melatonin (n=16) or placebo (n=17) in the evening. Control subjects (n=17) received usual indoor light (150-200 lux). | 10 weeks | Nighttime sleep variables, day sleep time, day activity, day: night sleep ratio, and rest-activity parameters were determined using actigraphy. | Light treatment alone did not improve nighttime sleep, daytime wake, or rest-activity rhythm. Light treatment plus melatonin increased daytime wake time and activity levels and strengthened the rest-activity rhythm. | (Dowling et al., 2008) |
| Case report | 68-year-old man with AD | 5 – 10 mg melatonin | 20 months | Polysomnography | Melatonin was effective to suppress REM sleep behavior disorder | (Anderson et al., 2008) |

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|--------------------------------------|--|--|-------------------------|-----------------|---|------------------------|
| Randomized, placebo-controlled study | who developed rapid eye movement (REM) sleep behavior disorder 41 AD patients | p.o./daily at bed time. Melatonin (8.5 mg immediate release and 1.5 mg sustained release) (N = 24) or placebo (N = 17) administered at 10:00 P.M. | 10 days | Actigraphy. | There were no significant effects of melatonin, compared with placebo, on sleep, circadian rhythms, or agitation. | (Gehrman et al., 2009) |

Table 2. Studies including treatment of MCI patients with melatonin

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|---|--|--|------------------|--|---|---|
| Double-blind, placebo-controlled, crossover study | 10 patients with MCI | 6 mg melatonin p.o./daily at bed time | 10 days | Actigraphy. Neuropsychological assessment. | Melatonin enhanced the rest-activity rhythm and improved sleep quality. Total sleep time unaffected. The ability to remember previously learned items improved along with a significant reduction in depressed mood. | (Jean-Louis et al., 1998a) |
| Double-blind, placebo-controlled pilot study | 26 individuals with age-related MCI | 1 mg melatonin p.o. or placebo at bed time | 4 weeks | Sleep questionnaire and a battery of cognitive tests at baseline and at 4 weeks | Melatonin administration improved reported morning "restedness" and sleep latency after nocturnal awakening. It also improved scores on the California Verbal Learning Test-interference subtest. | (Peck et al., 2004) |
| Open-label, retrospective study | 50 MCI outpatients | 25 had received daily 3-9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication | 9-18 months | Daily logs of sleep and wake quality. Initial and final neuropsychological assessment. | Patients treated with melatonin showed significantly better performance in neuropsychological assessment. Abnormally high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality. | (Furio et al., 2007) |
| Randomized, double blind, placebo-controlled study | 354 individuals with age-related MCI | Prolonged release melatonin (Circadin, 2 mg) or placebo, 2 h before bedtime | 3 weeks | Leeds Sleep Evaluation and Pittsburgh Sleep Questionnaires, Clinical Global Improvement scale score and quality of life. | PR-melatonin resulted in significant and clinically meaningful improvements in sleep quality, morning alertness, sleep onset latency and quality of life | (Wade et al., 2007) |
| Long-term, double-blind, placebo-controlled, 2 x 2 factorial randomized | 189 individuals with age-related cognitive decay | Long-term daily treatment with whole-day bright (1000 lux) or dim (300 lux) light. | 1 to 3.5 years | Standardized scales for cognitive and noncognitive symptoms, limitations of activities of daily | Light attenuated cognitive deterioration and ameliorated depressive symptoms. Melatonin shortened sleep onset latency and increased sleep duration but adversely affected scores for depression. The combined treatment of bright light plus melatonin showed the best effects. | (Riemersma-van der Lek RF et al., 2008) |

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|--|---|--|------------------|--|---|--------------------------|
| study | | Evening melatonin (2.5 mg) or placebo administration | | living, and adverse effects assessed every 6 months. | | |
| Prospective, randomized, double-blind, placebo-controlled, study | 22 individuals with age-related cognitive decay | Participants received 2 months of melatonin (5 mg p.o. /day) and 2 months of placebo | 2 months | Sleep disorders were evaluated with the Northside Hospital Sleep Medicine Institute (NHSMI) test. Behavioral disorders were evaluated with the Yesavage Geriatric Depression Scale and Goldberg Anxiety Scale. | Melatonin treatment significantly improved sleep quality scores. Depression also improved significantly after melatonin administration. | (Garzon et al., 2009) |
| Open-label, retrospective study | 60 MCI outpatients | 35 had received daily 3-9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication | 9-24 months | Daily logs of sleep and wake quality. Initial and final neuropsychological assessment. | Abnormally high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality. Patients treated with melatonin showed significantly better performance in neuropsychological assessment. | (Cardinali et al., 2010) |

Table 3. Studies including treatment of PD and RBD patients with melatonin

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|--------------------------------------|----------------------------|--|------------------|--|--|-------------------------------|
| Open-label, placebo-controlled trial | 40 PD patients | 5 - 50 mg melatonin p.o./daily at bed time. All subjects were taking stable doses of antiparkinsonian medications. | 2 weeks | Actigraphy | Relative to placebo, treatment with 50 mg of melatonin significantly increased night time sleep, as revealed by actigraphy. As compared to 50 mg or placebo, administration of 5 mg of melatonin was associated with significant improvement of sleep in the subjective reports | (Dowling et al., 2005) |
| Open-label, placebo-controlled trial | 18 PD patients | 3 mg melatonin p.o./daily at bed time | 4 weeks | Polysomnography (PSG). Subjective evaluation by the Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale. | On initial assessment, 14 patients showed poor quality sleep EDS. Increased sleep latency (50%), REM sleep without atonia (66%), and reduced sleep efficiency (72%) were found in PSG. Melatonin significantly improved subjective quality of sleep. Motor dysfunction was not improved by the use of melatonin. | (Medeiros et al., 2007) |
| Case report | 1 RBD patient | 3 mg melatonin p.o./daily at bed time | 5 months | Actigraphy, PSG | Significant reduction of motor activity during sleep, as measured by actigraphy. After 2 months' treatment, PSG showed no major changes except an increase of REM sleep. | (Kunz and Bes 1997) |
| Open-label prospective case series | 6 consecutive RBD patients | 3 mg melatonin p.o./daily at bed time | 6 weeks | PSG | Significant PSG improvement in 5 patients within a week which extended beyond the end of treatment for weeks or months. | (Kunz and Bes 1999) |
| Open-label prospective case series | 14 RBD patients | 3-9 mg melatonin p.o./daily at bed time | variable | PSG | Thirteen patients and their partners noticed a suppressing effect on problem sleep behaviors after melatonin administration. % tonic REM activity in PSG findings was decreased after melatonin administration. Melatonin concentrations in 10 RBD patients were under 30 pg/mL at maximal values, their mean 33.5 pg/mL RBD patients with low melatonin secretion tended to respond to melatonin therapy. | (Takeuchi et al., 2001) |
| Retrospective case series. | 39 RBD | All initially treated with clonazepam. When melatonin was | | | 21 patients continued to take clonazepam, 8 used another medication, and 4 required a combination of medications to control symptoms adequately. Zopiclone was used in 11 patients either alone or in combination. Two patients used melatonin (10 mg) and both found it effective. Combination therapy | (Anderson and Shneerson 2009) |

| Design | Subjects | Treatment | Study's duration | Measured | Results | Reference(s) |
|----------------------------|-----------------|---|------------------|----------|---|----------------------|
| Retrospective case series. | 14 RBD patients | used, it was given at a 10 mg p.o./daily at bed time. 3-12 mg melatonin p.o./daily at bed time | 14 months | PSG | (lonazepam/gabapentin/melatonin) was used in one patient. 8 patients experienced continued benefit with melatonin beyond 12 months of therapy. | (Boeve et al., 2003) |

Figure Legends

FIGURE 1 Biosynthetic pathway of melatonin in the pineal gland. * In various non-mammalian species, tryptophan 5-hydroxylase can act as a rate-limiting enzyme. This may be also discussed for some extrapineal sources of melatonin. At some extrapineal sites, AANAT seems to be replaced by other, less specific *N*-acetyltransferases (NATs). In rodents, the circadian increase of AANAT activity is instead caused by strong upregulation of gene expression. The complex of a pAANAT dimer with 14-3-3 proteins is only moderately stable. Upon its dissociation, pAANAT is readily dephosphorylated. The photic shutoff is initiated by decreases in cAMP and Ca^{2+} . In humans, this leads to a lack of re-phosphorylation of AANAT subunits, in rodents to decreases pCREB (cAMP/ Ca^{2+} response element-binding protein)-dependent AANAT transcription. ** Alternate name (also denomination of gene): acetylserotonin methyltransferase (ASMT). Again, the possibility of *O*-methylation by other, less specific *O*-methyltransferases has been discussed for some extrapineal sites.

FIGURE 2 Overview of major signaling pathways of the melatonin membrane receptors MT_1 and MT_2 . The figure combines various pathways, which are not collectively present in every target cell. Moreover, MT_1 - and MT_2 -dependent signaling pathways, which are only partially identical, are combined, because of possible heterodimerization and uncertainties concerning several cell types. Additional routes or interconnections have been reported. Up- and downregulations of cGMP, by decreases of phosphodiesterase and decreased NO, respectively, require further elucidation. Abbreviations: AC: adenylyl cyclase; Akt: homolog of kinase from retrovirus AKT8; Ca^{2+}_i : intracellular calcium; CaM: calmodulin; cAMP: cyclic adenosine 3',5'-monophosphate; DAG: diacyl glycerol; ERK: extracellular signal-regulated kinase; IP_3 : inositol 1,4,5-trisphosphate; Kir3.1/2: subtypes 3.1/2 of inward rectifier K^+ channels; MAP kinase: mitogen-activated protein kinase; MEK: MAP ERK kinase; pCREB: phosphorylated cAMP/ Ca^{2+} response element-binding protein; PI3K: phosphoinositide 3-kinase; PLC: phospholipase C; PK: protein kinase; Raf: homolog of retroviral kinase, the product of oncogene *v-raf*; \uparrow : upregulation or rise; \downarrow : downregulation or decrease.

FIGURE 3 Formation of most damaging free radicals: overview of the main routes leading from the primary, low-reactivity radical, the superoxide anion, to hydroxyl and peroxyxynitrite-derived radicals. Abbreviation: mPTP: mitochondrial permeability transition pore. The question mark in this field indicates a debate on the existence of superoxide flashes. Various additional routes of uncertain quantitative relevance have been omitted, e.g., on formation and reactions of the peroxyxynitrite radical and on the formation of other nitrosating intermediates. Moreover, hydroxyl and carbonate radicals can lead, by interaction with organic compounds, to numerous organic radicals. Well-known examples are peroxyxyl and alkyl radicals formed by reactions with hydroxyl radicals.

Figure 1

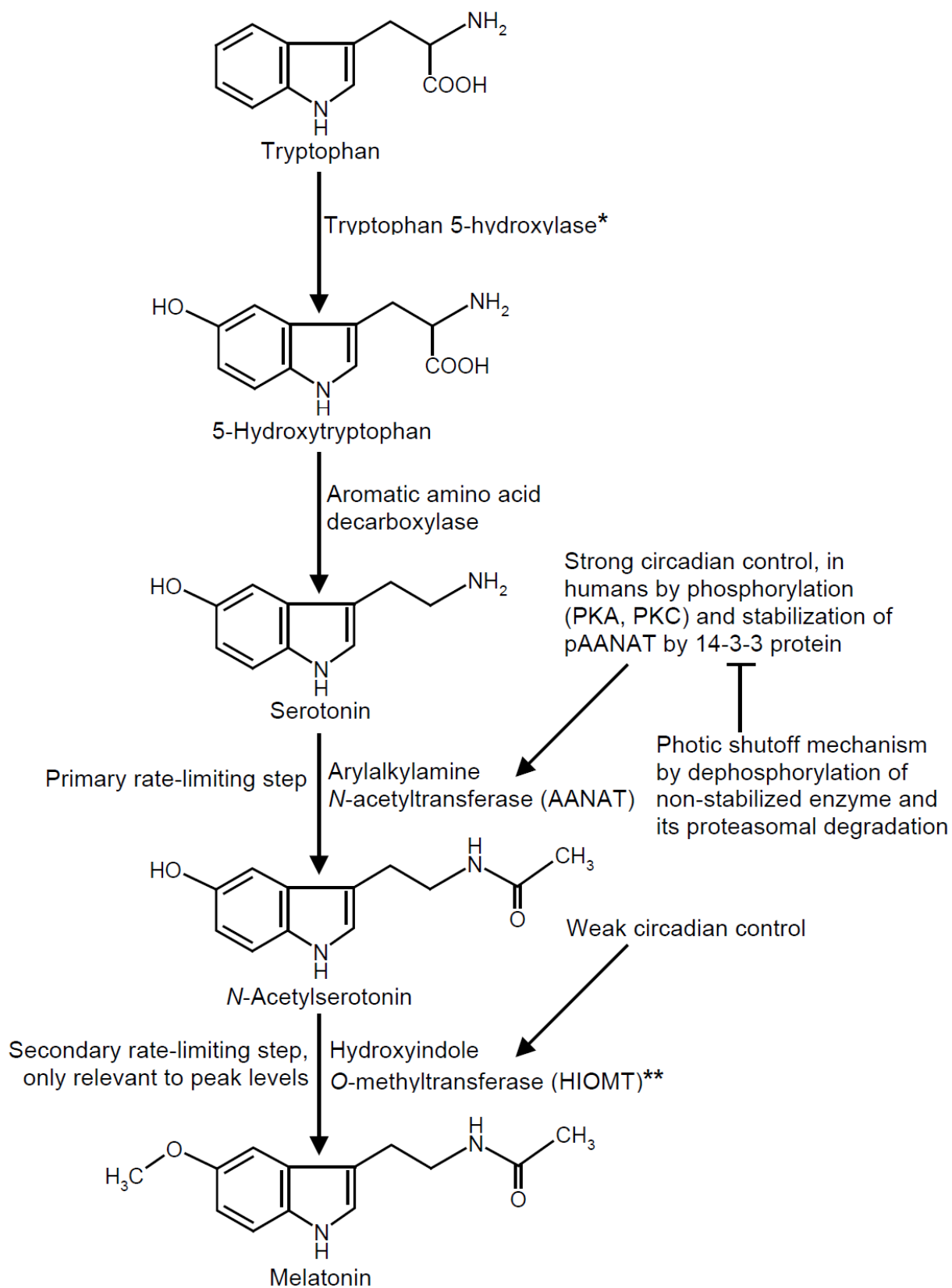


Figure 2

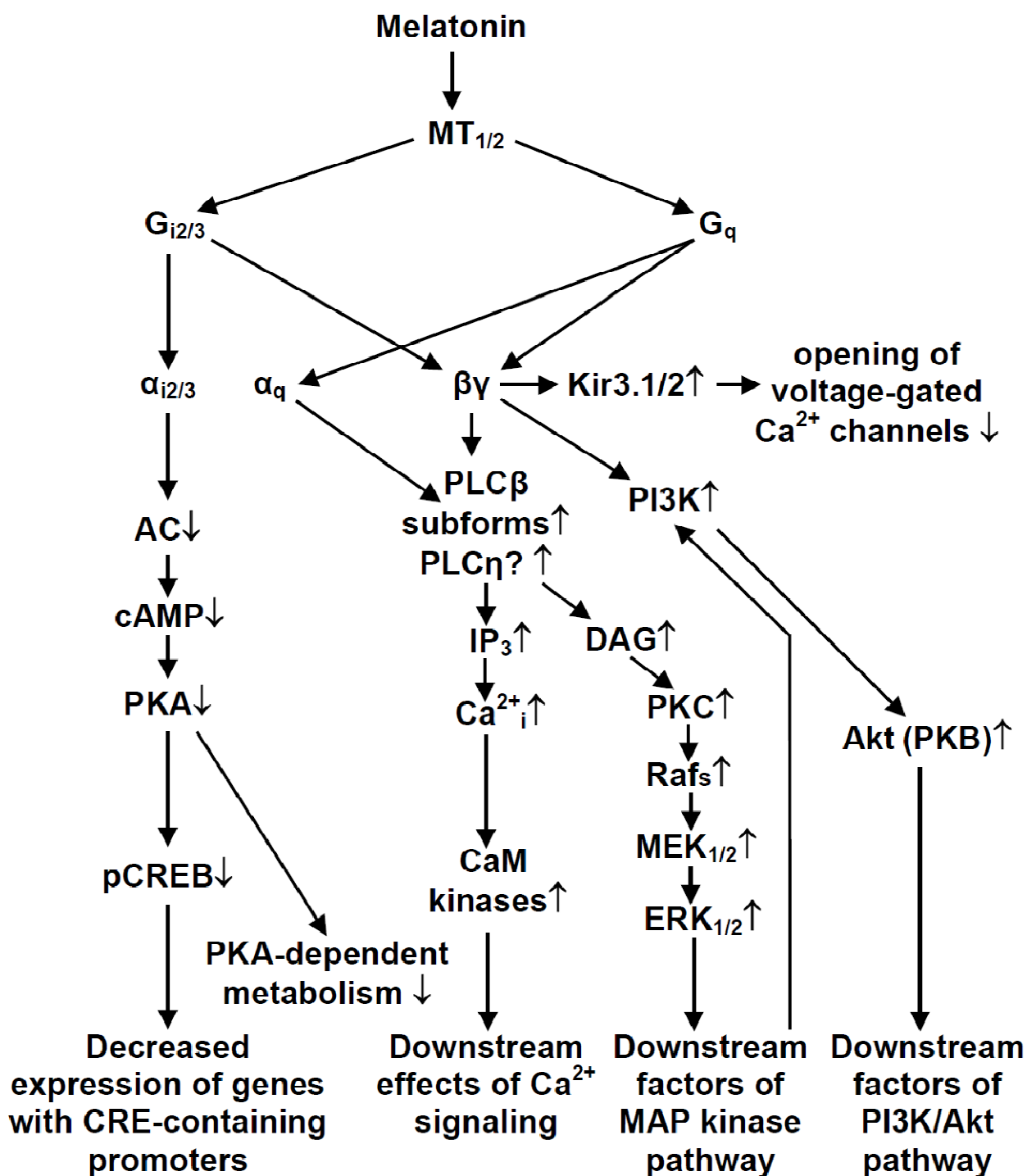


Figure 3

