

Melatonin as Antioxidant Under Pathological Processes

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Abstract: Many are the diseases which course with free radical formation. These disorders cover a great range of fields such as neurodegenerative, immune, inflammatory and mitochondrial-related diseases. Melatonin is the main pineal gland product and it functions as “time-giver” in the regulation of circadian rhythms, among others. But the actions of melatonin are not only restricted to the neuroendocrine physiology. In fact, it has been known as a radical scavenger, a role that has been deeply studied in all those conditions where free radicals are generated. Furthermore, melatonin has been shown to act as an indirect antioxidant, since it is able to increase the activity and expression of the main antioxidant enzymes, the machinery for the glutathione synthesis, and many others direct or indirectly implicated in the free radical removal. Melatonin can also diminish the activity or expression of enzymes or factors that are considered as prooxidants. Thus, researchers have paid attention to the possible actions of melatonin in the attenuation of those processes where free radical overproduction is implicated. This review summarizes some of the proposed melatonin mechanisms for different free radical-dependent pathological situations, as well as some patents on melatonin significance recently reported for the treatment of attention deficit, hyperactivity disorders, stress-related diseases, Chronic fatigue syndrome, diabetes, Parkinson’s disease, Alzheimer’s disease, age associated cognitive dysfunction and cancer.

Keywords: Melatonin, oxidative stress, free radicals, antioxidant, prooxidant, inflammation, immune system, neurodegeneration, aging, mitochondrial-related diseases.

INTRODUCTION

Many active intermediaries, such as electrophiles (tend to accept electrons) and free radicals (with the ability to damage cellular components) are produced during physiological and pathological processes (Fig. 1). The consequences of the damage initiated by these metabolic byproducts affect to a large range of biological reactions, like increases in the mutation rate and alteration of cellular membranes composition, structural proteins, metabolic and detoxifying enzymes, cellular signalling proteins. In many cases, the reactive intermediaries produced are able to convert cellular constituents in second generation reactive intermediaries, which are able to induce more damage [1].

Cellular Damage as Oxidative Stress Consequence

Free radicals are highly reactive molecules since they present an unpaired electron in their outer orbital. Thus, these radicals are prone to oxidize intracellular molecules, such as lipids, DNA and proteins giving rise to alterations in cell structures.

Tissue lipid peroxidation (LPO) is a degradative phenomenon as a consequence of free radical chain production and propagation which affects mainly to polyunsaturated fatty acids and that is strongly implied in the pathogenesis of several diseases, such as arteriosclerosis, diabetes, cancer and rheumatoid arthritis, as well as toxicity associated to drugs and aging [2]. Endogenous aldehydes formed during LPO process are implied in most of the pathophysiological

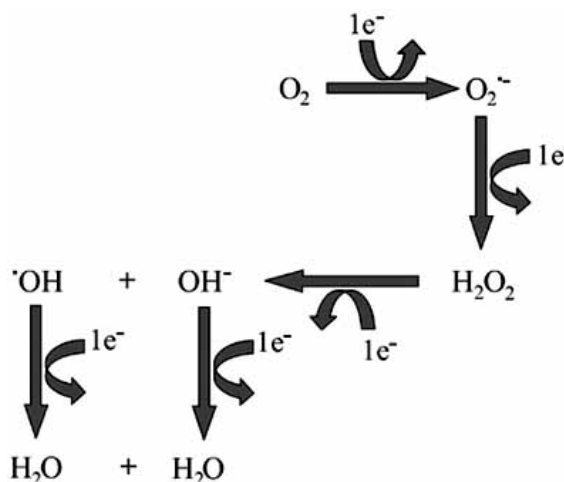


Fig. (1). Electron leakage within the mitochondrial respiratory chain. Free radical production in organisms with aerobic metabolism is a continuous and unavoidable process since molecular oxygen (O_2) reduction to water (H_2O) within the mitochondrial respiratory chain is not 100% efficient. In this way, the mitochondria is the main source of free radicals, due to electron leakage in the respiratory chain, with the resulting formation of reactive species, such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$), the most reactive. These free radicals are called reactive oxygen species (ROS), which are prone to oxidized intracellular molecules. Apart from the respiratory chain, there are other ROS-producing systems, such as cytochrome P450 oxidase family, NADPH oxidase, xanthine oxidase and autooxidation reactions of endogenous or exogenous substances.

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effects related to oxidative stress in cells and tissues [3]. Unlike free radicals, aldehydes derived from LPO are generally stable and they can diffuse within or outside the cells and attack targets far from their formation sites. Thus, these aldehydes are not only final products that are able to maintain the LPO process, but they can also act as mediators for primary free radicals that initiate LPO. Among the aldehydes formed during LPO, it is worthy to mention malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), which could be formed from arachidonic acid, linoleic acid or its hydroperoxides [3]. 4-HNE presents a large range of biological activities, including protein and DNA synthesis inhibition, enzyme inactivation, phospholipase C stimulation, expression of various genes and even it can react with DNA and proteins to generate several kinds of adducts [3] (Fig. 2).

The exposure to endogenous oxidants and electrophiles gives rise to an increase in the damage to cellular macromolecules as important as DNA. DNA damage can be a result of a series of reactions with the nitrogen bases of nucleic acids, the deoxyribose residues or the phosphodiester backbone (Fig. 2). DNA lesions, that are not properly repaired, accumulate through time and can contribute to the development of diseases associated with aging. $\cdot\text{OH}$ radical is able to add double bonds to nitrogen bases or to subtract hydrogen atoms from both methyl groups and deoxyribose residues [4]. $\cdot\text{OH}$ radical reactions with purines and pyrimidines can even be mutagenic. Furthermore, oxidative damage to other important molecules, such as lipids, can also affect to DNA, since LPO byproducts, MDA and 4-HNE, are able to react with the exocyclic amino groups of guanine, adenine and cytosine (Fig. 2).

Reactive species derived from polypeptide modification can occur in the peptide backbone, in nucleophilic lateral chains and in redox sensitive lateral chains (Fig. 2). In many cases, the chemistry associated to protein damage is dynamic and reversible, although many kind of protein adducts are accumulated during aging and/or aging related diseases. Free radicals produced throughout oxidative stress are able to damage the peptide backbone, generating carbonylated proteins. Such process is initiated by hydrogen subtraction in the carbon of the polipeptidic chain. If two protein radicals are close, they can cross-link [5]. Alternatively, they are able to attack carbon radical to form peroxide intermediaries which finally favour the formation of carbonyl groups within the peptides [5]. Carbonylated proteins are accumulated during aging and associated diseases [6]. Also, it is possible to find modified proteins by LPO byproducts in the case of cardiovascular and neuro-degenerative diseases [7, 8].

Antioxidant Systems

To counteract the harmful effects of free radicals aerobic organisms have developed a series of specialized mechanisms. There are two kinds of antioxidant defense to counterbalance the oxidative stress: antioxidant enzymes and non enzymatic antioxidants [9] (Fig. 3).

Apart from the antioxidant systems described in Fig. (3), in the last decade the molecule of pineal origin, melatonin, has attracted the researchers attention due to its antioxidant

ability, since it is able to scavenge free radicals and to increase activity and expression of the main antioxidant enzymes. This review mainly centers on the new melatonin advances and how this indolamine counteracts free radical production in aging and several pathologies. In recent patents, a dietary supplement of mitochondrial nutrients including melatonin is clinically and experimentally used for the preventing and cure of age and stress related disorders, chronic fatigue syndrome, diabetes, age-associated cognitive dysfunction, Parkinson's & Alzheimer's diseases [10]. Method for controlling the alertness of human volunteers were studied via light radiation specified by an output fraction of melatonin suppressive radiation [11].

Melatonin: Concepts

Melatonin, N-acetyl-5-methoxytryptamine, is a ubiquitous molecule with many different functions. Initially, it has been involved in the neuroendocrine system, mainly in reproductive physiology [12-15] DNA encoding high affinity melatonin receptor is used to characterize receptor agonist or antagonists for regulating circadian rhythm disorders or reproductive cycles. Later, it has been associated with circadian rhythm control and sleep processes in diurnal species [16-19]. The composition as functional food, a food or a beverage comprising astaxanthin or its ester are used for normalizing circadian rhythm of melatonin by protecting melatonin. More recently, its antioxidant properties have been discovered [20] as well as its actions in the immune system [21, 22] and in the tumor growth [23]. Metastatic colorectal cancer and breast cancer are treated by melatonin and its analogue is disclosed in recent patent reported [24].

In mammals, the main melatonin source is pineal gland. Melatonin presents a circadian rhythm, with low serum levels during the day and higher at night. The length of nocturnal melatonin secretion is proportional to the dark phase extension and, thus, depending on the latitude, the dark melatonin signal may change twelve hours or more throughout the year. In photoperiodic species, these annual changes provide the neuroendocrine signal which facilitates a large number of physiologic responses synchronized with the season change [25].

Melatonin Synthesis in the Pineal Gland

Melatonin belongs to a group of molecules characterized by the presence of an amino group, biogenic amines, and, more specifically, to the indolamine group, since it derives from the amino acid tryptophan (Fig. 4). Melatonin synthesis implies serotonin formation and the sequential action of the enzymes arylalkylamine *N*-acetyltransferase (AANAT or NAT) and hydroxyindole-*O*-methyltransferase (HIOMT). NAT has been considered as the limiting step in melatonin synthesis [26, 27], although there are some studies that rule out such possibility, giving more importance to HIOMT activity [28, 29].

Melatonin synthesis within rat pineal gland is seriously affected by light signals received through the eyes. During the night phase NAT activity is increased, showing values 10 to 100 times higher than those observed during the day. HIOMT activity is also increased and, with it, pineal melatonin levels. Light decreases very quickly pineal enzymes activities. If the external light conditions are

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place of production [3]; **DNA oxidation.** 1) The deoxyribose can react with an oxidizing agent giving rise to the pentose break and forming a base propenal. This modified nucleotide is able to react with other adjacent nucleotides, such as deoxyguanylate, forming the M₁G compound (Dedon *et al.*, Proc Natl Acad Sci USA. 1998). 2) On the other hand, LPO products, such as MDA and 4-HNE, are able to react with the exocyclic amine groups from guanine, adenine and cytosine (Luczaj *et al.*, Cell Mol Biol Lett. 2003). **Protein oxidation.** Side-chain (A) and α -carbon (B) oxidation is the previous step to protein carbonyl formation, which can be initiated from oxidation mediated by metals, such in the case of histidine, or due to a high ROS environment, such in the case of cysteine. LPO products are also able to interact with side-chains (C), giving rise to adducts which can even to inactivate the protein function [5, 7].

reversed, enzymatic activity is also reversed and, thus, pineal melatonin synthesis. Hence, there is a change in the pineal biosynthetic rhythm that is controlled by natural daily changes in illumination and it may be blocked by light pulses of a particular intensity during the night phase [30, 31].

The light information collected by the eyes leads the brain suprachiasmatic nucleus (NSC) through the retinohypothalamic pathway [32,33]. The pineal gland is, essentially, the intermediary between the external photoperiod and the internal media, the place where light/dark information is translated into a chemical messenger [17,34,35].

Melatonin Antioxidant Function

As mentioned before, melatonin has been also associated with the cellular antioxidant defense. It can develop its action at two levels: as a direct antioxidant, due its ability to act as a free radical scavenger, and as an indirect antioxidant, since it is able to induce the expression and/or the activity of the main antioxidant enzymes.

Direct Antioxidant Function

A good antioxidant must fulfil a series of characteristics: large distribution within tissues, cells and subcellular compartments, ability to cross morphophysiological barriers and quick transport into the cells [36]. Physical chemistry properties of melatonin allow this molecule to penetrate into all cells and cellular compartments in the organism, since it is highly soluble in lipids and partially in water [37-40].

Melatonin is a powerful free radical scavenger. It is able to remove H₂O₂, \cdot OH, peroxynitrite anion (ONOO⁻), singlet oxygen (¹O₂), O₂⁻ and peroxy radical (LOO \cdot). Melatonin antioxidant mechanism differs from mechanisms followed by the other antioxidants, since all of them need a redox cycle after electron donation (Fig. 3). Thus, classic antioxidants are able to promote oxidation as well as to prevent it. However, melatonin, as an electron-rich molecule, is able to interact with free radicals through consecutive reactions giving rise to many stable compounds that can be excreted by urine [41]. In fact, the melatonin antioxidant mechanism implied a free radical scavengers cascade, since secondary, and even tertiary, metabolites are also efficient free radicals scavengers, like *N*-acetyl-*N*-formyl-5-methoxykynuramine (AFMK) and *N*-acetyl-methoxykynuramine (AMK) (Fig. 4) [42-44]. The formation of such metabolites from melatonin implies that, unlike classic antioxidants, melatonin does not produce prooxidant reactions [45,46] and, even more, AMK and AFMK, in all the mitochondrial studies where comparisons were made, were more potent than melatonin itself [47].

It was thought that most of the melatonin was metabolized in the liver to 6-hydroxymelatonin and then excreted by

urine. However, melatonin may oxidize giving rise to kynuramines derivatives in a 30 percent of the cases, producing mainly AFMK and AMK, being the latter the major melatonin metabolite in the central nervous system [44]. AFMK is able to reduce 8-hydroxy-2-desoxyguanosine [48] and LPO. Furthermore, it can interact with OH, the most reactive free radical [44, 49]

On the other hand, the large subcellular distribution of melatonin allows its interaction with almost any kind of molecule, diminishing oxidative damage in both lipid and aqueous environments. This is supported experimentally by numerous data that show that melatonin is able to protect lipids in the cellular membranes, proteins in the cytosol and DNA in the nucleus from free radical damage [50].

Indirect Antioxidant Function

One of the main characteristic of melatonin is that its antioxidant function is developed also through the antioxidant enzymes activation. Thus, the indolamine is an ideal molecule to fight against oxidative stress.

Many are the works which show melatonin treatment is able to increase the activity and/or the expression of the main antioxidant enzymes, such as Superoxide dismutase (SODs), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione reductase (GRd) (Fig. 3). The studies developed with regard to antioxidant enzyme activation started with the administration of exogenous melatonin to rats, showing an increase in the GPx activity. Pablos *et al.* [51] observed that, depending on the tissue, the ability to recruit exogenous melatonin varied, so that tissues which accumulated more melatonin presented higher GPx increase. In the same way, SOD and CAT activities are also incremented after melatonin administration [52-57].

The effects of melatonin on antioxidant enzymes expression were also studied. It has been shown, in rat brain cortex, that melatonin administration increases MnSOD Cu/Zn SOD, GPx and CAT mRNA levels [58-61], being such increment more pronounced when melatonin is chronically rather than acute administered. Exogenous melatonin administration does not follow a definite criterion with regard the proper administration, being this always upper than serum melatonin level (1nM). However, it has been observed 1mM melatonin has any effect neither in GSH levels nor in antioxidant enzymes expression, arguing the authors that, at this concentration, melatonin role is merely as a direct antioxidant. Nevertheless, melatonin nanomolar concentrations are able to increase both GPx expression and activity. Even, it has been shown that such increment is GSH-dependent, possible due to GSH is the GPx cofactor [62]. Antioxidant enzymes expression increase by melatonin is an effect that is regulated by the novo protein synthesis,

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cytosol and in the mitochondrial intermembrane space, while Mn SOD is localized in the mitochondrial matrix. Both enzymes are crucial for the prevention of ROS-induced toxicity. Catalase (CAT) and the tandem glutathione peroxidase/reductase (GPx and GRd, respectively) contribute to the elimination of H₂O₂ and similar molecules. CAT contains heme as prosthetic group and is found mainly in peroxisomes but also in other cellular compartments, such as nucleus, sarcoplasm and, in certain cellular types, mitochondria. GPx/GRd system is formed by various components, including the enzymes GPx and GRd and the cofactors glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH). Although CAT and GPx have both the same purpose, they act at different substrate concentration: CAT presents low substrate affinity, working at high H₂O₂ concentrations, while GPx shows high substrate affinity, acting at low H₂O₂ concentrations. **Non enzymatic antioxidants.** They are a group of compounds that are able to remove reactive species directly, since they have the ability to donate an electron to a free radical. To considerer a molecule as radical scavenger, once it donates an electron it must form a non-toxic radical, because, in other way, the system would not be useful. In this category, molecules such as α -tocopherol (vitamin E), ascorbic acid (vitamin C), and glutathione (GSH) are included. α -Tocopherol is a liposoluble antioxidant that is able to scavenger free radicals in hydrophobic environments, such as cellular membranes. The main α -tocopherol store bound to the membrane is the mitochondrial inner membrane, where the electron transport chain is localized. Its antioxidant mechanism includes the inactivation of one free radical for each α -tocopherol molecule. This mechanism is operative when free radicals are rapidly formed, such as under strong oxidative conditions. The α -tocopherol radical removal by recycling represents an important action mechanism for a large group of compounds called α -tocopherol co-antioxidants. An example of this kind of substances is ascorbic acid and bilirubin, since they are the most important at the physiological level due to both present high plasma levels. An important ascorbic acid characteristic is its solubility in water, being found in cells cytosol. The reaction between ascorbic acid and α -tocopherol may be the responsible of the α -tocopherol antioxidant behaviour restoration under low oxidative conditions, taking place at high rates *in vitro* under physiological conditions. In turn, ascorbate must be recycled to maintain the adequate vitamin levels within tissue (Bowry *et al.*, J Biol Chem 1995). Another non enzymatic compound is γ -glutamylcysteinylglycine, glutathione (GSH). Its reducing properties are due to the presence of a sulphhydryl group (-SH) in the central cysteine. Once it is oxidized, two GSH molecules cross-link to form oxidized glutathione (GSSG) through a disulphide bridge between both cysteines. Due to the multiple functions developed by GSH, this compound is probably the main low molecular weight antioxidant molecule in the organisms. Most intracellular GSH is localized in the cytosol and the other part within the organelles, like mitochondria, nuclear matrix and peroxisomes. Thanks to its cysteine residue, GSH is able to oxidize to GSSG by electrophilic substances like free radicals and ROS/RNS, giving rise to a loss in the intracellular GSH content. In turn, GSH is able to remove free radicals and other ROS such as \cdot OH, lipid peroxides (LOO \cdot), (ONOO \cdot) and H₂O₂ in a direct or indirect manner through enzymatic reactions. It is important to point out that those variations in the GSH/GSSG proportion towards the oxidative state active many cell signalling pathways, such as Akt, calcineurin, NF- κ B, c-Jun. (Sen, Curr Top Cell Regul 2000).

probably involving melatonin receptors [63-65], although this fact remains unknown.

Melatonin Receptors

There are many factors that contribute to the diversity in response to melatonin stimulus and, in fact, there are increasingly evidences that show the complex role of melatonin in the modulation of several physiologic processes. Due to its small size, its lipophilic nature and/or an active cell import mechanism, melatonin is able to activate or inhibit signal transduction cascades in a receptor-independent fashion or through receptors. Since melatonin levels fluctuate through the day, the year and the life span, such fluctuations, *in vivo*, may impact significantly in receptor actions [66]. Actually, several specific melatonin receptors have been described in a large variety of mammalian and non-mammalian cells types.

Membrane Receptors

Melatonin membrane receptors have been characterized and identified in a large number of tissues by means of autoradiography and binding *ex vivo* studies, using ¹²⁵I iodomelatonin as ligand [25].

Three melatonin membrane receptor isoforms have been cloned. Mel1a gene encodes MT₁ receptor, Mel1b gene encodes MT₂ receptor [67] and Mel1c gene has been cloned from *Xenopus laevis*, although it is not expressed in mammals [25].

MT₁ and MT₂ are G-coupled receptors which share similar pharmacologic patterns [68] with picomolar melato-

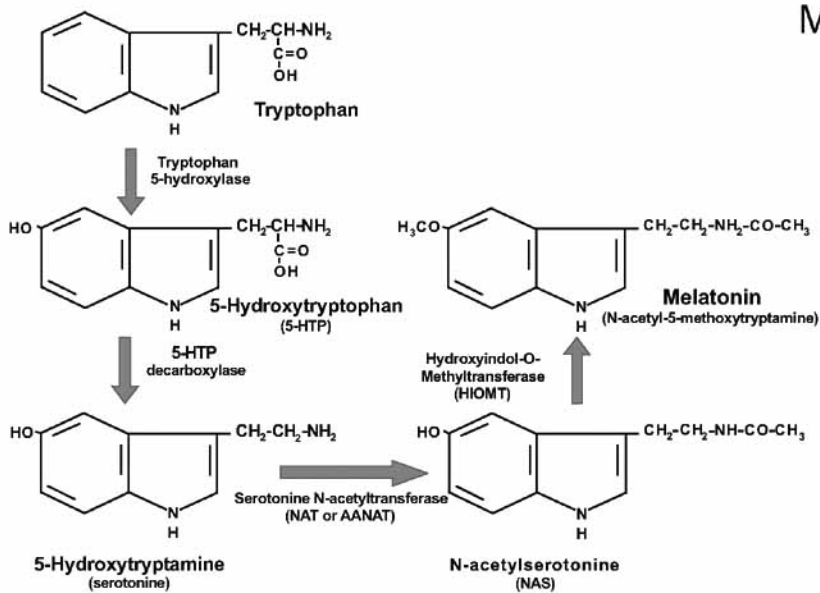
nin and ¹²⁵I iodomelatonin affinity. Apart from these high-affinity receptors, there are evidences that point out to a nanomolar binding site in hamster brain and kidney [68-70] named MT₃. This receptor shows rapid kinetics of ligand association/dissociation [69-70] due to its enzymatic nature, since such binding site has been identified as the enzyme quinone reductase 2 [71].

Since these receptors are localized within cell membrane, when melatonin binds, cellular functions are regulated by means of second messengers. Melatonin is able to mobilize many of them, considering that the three receptor subtypes describe above are possibly coupled to different effectors.

Apart from second messenger, melatonin signals through membrane receptors may be also regulated by cytoplasmic environment in the signal moment, exposure time and ligand concentrations [72]. In recombinant systems, short MT₁ receptor exposure to supraphysiological melatonin concentrations (100nM) decreases specific ¹²⁵I iodomelatonin binding sites. However, this treatment did not induce desensitization or receptor internalization [73]. On the other hand, MacKenzie *et al.* [74] have shown that exposition to 1 μ M melatonin for 5 hours resulted in a complete loss of melatonin-mediated stimulation of phosphoinositide hydrolysis, and an attenuation of melatonin (1nM)-mediated inhibition of forskolin-induced cAMP accumulation.

Treatment of circadian and sleep disorders with melatonin is often able to increase serum indoleamine levels at supraphysiological concentrations, something that may alter MT₁ receptor sensitivity. This is an important fact, since

MELATONIN SYNTHESIS



MELATONIN OXIDATION

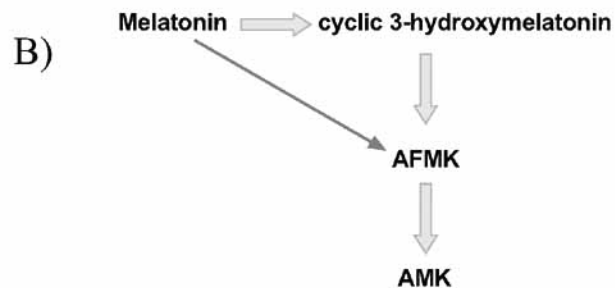
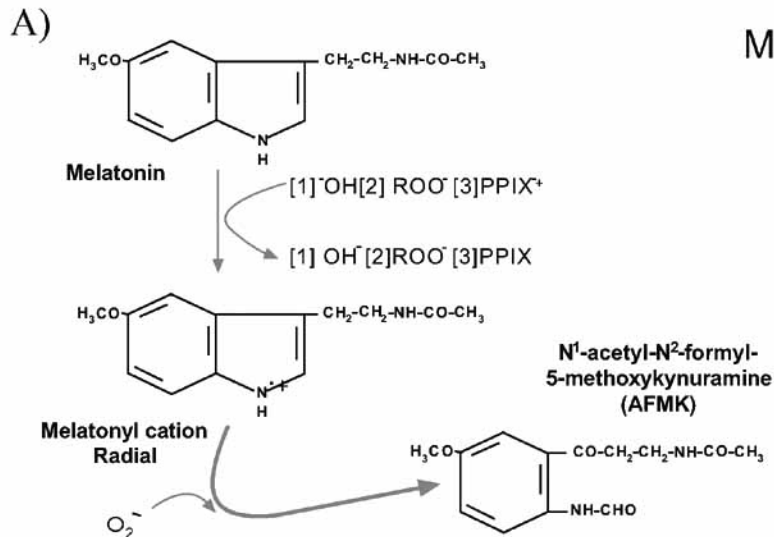


Fig. (4). Melatonin synthesis: For more details, see the text. **Melatonin oxidation.** A) **AFMK formation from melatonin.** After electron donating to hydroxyl radical [1], peroxy radical [2] or protoporphyrinyl radical [3], the melatonin cation radical formed is able to combine, in a non enzymatic mechanism, with superoxide anion, giving rise to AFMK. B) **AMK production.** AMK is a free radical scavenger more reactive than AFMK, since it is involved in one-electron transfer reactions whereas AFMK transfers, preferably, two electrons. Adapted from Hardeband and Pandi-Perumal [38].

this receptor is involved in suprachiasmatic nucleus regulation, vasoconstriction and neuroendocrine functions [75].

Nuclear Receptors

As seen above, melatonin can develop its actions through two mechanisms: receptor independent, as direct antioxidant, and membrane receptor dependent. However, since melatonin presents nuclear localization in different mammal cell types, this indolamine may also have a role within nucleus [65, 76-81].

Orphan nuclear receptors are members of the ligand-dependent transcription factors superfamily. Retinoic acid Z receptor subfamily (RZR) or orphan receptors (ROR) include three genes products: ROR splicing variants (ROR₁, ROR₂, ROR₃ and RZR), which differ in the N-terminal domain, RZR₃ and RZR. Members of this subfamily possess constitutive transcriptional activity, positive or negative depending on promoter and cellular type, and such activity could be intensified by ligand [82].

Becker-Andre *et al.* [83] have described melatonin as possible ligand for these receptors, although such results could not be reproduced [83]. Nevertheless, there is no doubt that ROR has to be in some way regulated by melatonin [84-88]. Thus, in bovine fetal serum cell cultures, melatonin treatment inhibits significantly ROR transcriptional activity in a dose-dependent manner. Melatonin effects in such activity are seen at low doses (10⁻¹¹M) and saturated at 10⁻⁵M. Other compounds, biological and structurally related to melatonin (biosynthetic precursors, serotonin and acetylserotonin), are not able to modulate ROR activity when used at the same melatonin doses [86].

On the other hand, melatonin does not suppress ROR at the receptor expression level. However, it seems that melatonin affects ROR DNA binding after 24-48 hours treatments, suggesting that the indoleamine acts directly, modifying receptor DNA binding, or regulating particular cofactors expression, essential for ROR DNA binding and the receptor transcriptional activity activation [86].

In summary, melatonin has several mechanisms to modulate cell physiology [75,82,88]. Due to melatonin characteristics with regard its antioxidant ability, the indoleamine has implied both in the treatment and development of numerous processes where oxidative stress has critical importance.

Free Radicals in Disease

As seen above, cells possess multiple sites for ROS/RNS production and mechanisms for their detoxification. Small fluctuations in the steady-state concentrations of ROS/RNS may play a role in intracellular signalling [89, 90]; however, uncontrolled increases in these highly reactive molecules lead to free radical-mediated chain reactions which indiscriminately damage proteins, lipids and DNA resulting, at last resort, in cell death [91] and being the primary or secondary cause of great number of diseases.

The main consequence of an increased ROS production is the subsequent decreased availability of intracellular antioxidants such as GSH, leading to an imbalance in the redox status. This, in turn, results in damage to the

mitochondrial electron transport chain (ETC) and a further elevation of free radical generation [92]. In fact, most available data indicate that the origin of excessive ROS generation is consequence of an impairment of the ETC [93]. Another important consequence of the reduction in the mitochondrial GSH content is the mitochondrial transition pore (MTP) opening due to oxidation of critical sulfhydryl groups present in the channel [94]. Furthermore, mitochondrial ROS can be discharged into the cytoplasm, where they induce calcium release from the endoplasmic reticulum, leading to mitochondrial calcium uptake, which in turn can also induce opening of the MTP [95].

Mitochondrial dysfunction associated with the loss of calcium homeostasis and enhanced cellular oxidative stress has long been recognized to play a major role in several pathologies which may have the mitochondria as the initial point or well this organelle is implied in a secondary manner.

SEVERE MITOCHONDRIAL DYSFUNCTION IN AGING AND DIVERSE PATHOLOGIES

Mitochondrial metabolism abnormalities, which cause human disease, have been recognized for more than 30 years. They include defects in mitochondrial fatty acid -oxidation, in Krebs cycle enzymes, in the oxidative phosphorylation system and in the ETC. These mitochondrial dysfunctions are present in aging and several diseases, such as neurodegenerative diseases, ischemia-reperfusion and sepsis [96].

In a healthy organism, aging can be considered as a consequence of gradual diminution in the individual abilities and functions which allow maintaining homeostasis. For a long time, many have been the theories proposed to explain the aging process: evolutionary theory [97], stochastic theories [98, 99], telomere shortening, participation theory [100] and multiple hormone deficiency theory [101], among others. But, nowadays, the free radical and the mitochondrial theories of aging are the most popular [102-104]. Free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death. Free radical theory of aging can integrate the other theories, since free radical production explains many of the facts stated for the other theories [104].

Aging is accompanied by structural changes in mitochondria and by a decrease in complex IV (cytochrome oxidase complex) and complex V (mitochondrial adenosine triphosphate synthetase complex) activities which result in a bioenergetic decline in memory and other brain functions [105]. In old organisms, mutations in mtDNA, alterations in respiratory chain enzymes activity and in membrane potential decrease are common characteristics of aged mitochondria [106]. Oxidative stress represents an early intrinsic component of any MTP-induced apoptotic-cascade [107]. Unifying programmed and stochastic theories of aging claim that cells are first programmed to differentiate and then they suffer progressive disorganization because of injury as the result of chronic oxidative stress [96].

A number of pathological and pharmacological studies on sporadic neurodegenerative diseases have hypothesized that mitochondrial dysfunction, inflammation, oxidative stress, and ubiquitin-proteasome system dysfunction play all important roles in the pathogenesis and progression of these

Table 1. Some Diseases which Course with Free Radical Formation Not Described in the Text

Cardiovascular diseases <i>Acute Myocardial Infarction</i> <i>Atherosclerosis</i> <i>Coronary Artery Disease</i>	Singh <i>et al.</i> , Biomed Pharmacother 2006 Cook, Swiss Med Wkly 2006 Schulman <i>et al.</i> , J Hypertens Suppl 2006 Cook, Swiss Med Wkly 2006
Cancer <i>Hepatocellular carcinoma</i> <i>Melanoma</i> <i>Extravasation of circulating cancer cells</i> <i>Gastric cancer</i>	Seitz and Stickel, Biol Chem 2006 Levrero, Oncogene 2006 Kadekaro <i>et al.</i> , Front Biosci 2006 Houle and Huot, Mol Carcinog 2006 Correa, Biol Chem 2006
Glaucoma and Cataracts	Izzoti <i>et al.</i> , Mut Res 2006 Kyselova <i>et al.</i> , J Diabetes Complications Siu <i>et al.</i> , J Pineal Res 2006
Respiratory tract diseases <i>Asthma</i> <i>Chronic Obstructive Pulmonary Diseases</i> <i>Cystic Fibrosis</i> <i>Acute Distress Syndrome</i>	Fujisawa, Curr Drug Targets Inflamm Allergy 2005 Mak and Chan-Yeung, Curr Opin Pulm Med 2006 Riccidrolo <i>et al.</i> , Eur J Pharmacol 2006
Chronic Kidney Disease	Lastra <i>et al.</i> , 2006
Lupus Erythematosus	Alves and Grima, Curr Rheumatol Rep 2003

pathologies [108]. Over the last two decades, tremendous strides toward acquiring a better knowledge of mitochondrial deficiencies in neurodegenerative diseases have been achieved. Thus, Alzheimer's disease shows important mitochondrial dysfunction and downregulation of mitochondrial ETC, being β -amyloid protein a severe oxidative stress inductor. Likewise, Friedreich's ataxia exhibits oxidative phosphorylation deficiencies in skeletal muscle [109], Parkinson's disease presents complex I (NADH dehydrogenase complex) deficiency and Huntington's disease, that displays the highest mitochondrial affectation, shows deficiencies in complex II (succinate dehydrogenase complex), complex III (cytochrome c reductase complex) and complex IV [110].

Ischemia is the condition suffered by tissues and organs when deprived of blood flow and it can be followed by flow reestablishment in the process known as reperfusion. During ischemia/reperfusion, damage induced by anoxia seems to be related to subsequent reoxygenation, which induces $\cdot\text{OH}$, responsible for the initiation of an apoptotic opening of the MTP [111]. NO generated during reperfusion due to nitric oxide synthase (NOS) activation, and the ONOO \cdot produced, seems to be the primary cause of damage to complex I and complex II during reperfusion [96].

Sepsis is a severe toxic state due to an abnormal immune response against bacterial infection in an organism, leading to iNOS induction and massive tissue damage [96]. The mechanisms of NO-induced toxicity depend in part on the reversible inhibition of complex IV [112] and in increases of

O $_2\cdot^-$ and H $_2\text{O}_2$ production by diaphragm mitochondria. A parallel increase in ONOO \cdot also impairs mitochondrial function and muscle contractility [113].

The partial oxidative stress dependence and the common mitochondrial damage observed in these diseases suggest the use of antioxidants in their treatments. Thus, the role of melatonin as antioxidant on mitochondria has been profusely studied.

MELATONIN ACTIONS ON MITOCHONDRIAL DYSFUNCTIONS

In vitro and *in vivo* experiments have shown that melatonin can influence mitochondrial homeostasis at different levels. Thus, melatonin effects in mitochondrial respiratory complexes have been largely described in oxidative stress experimental models. For instance, it has been shown, in a rat *t*-butyl hydroperoxide-induced oxidative stress model, that complexes I and IV within the mitochondrial respiratory chain present a significantly improvement in their activities when melatonin is administered [114], an observation in accordance with the results obtained after the administration of ruthenium red, a compound that significantly diminishes GPx activity [115]. As a result of the interaction of melatonin with the cited complexes, and the subsequent promotion of electron flux through the ETC, melatonin increases ATP production under basal conditions and counteracts cyanide-induced depletion of ATP associated with complex IV inhibition [114, 115]. These

melatonin effects have been also described in aging models, such as in senescence accelerated mice [116-118], in *in vitro* models of Alzheimer's disease [119], in *in vivo* models of Parkinson's disease [120] and in sepsis models [121], where the indolamine was able to restore the activity of mitochondrial complexes and ATP synthetase. Melatonin action on these complexes may be due, at least in part, to an effect on the expression of mtDNA, since the indolamine increases the expression of polypeptide subunits I, II and III of complex IV, encoded in mtDNA, from rat liver in a time-dependent manner in correlation with the increase in complex IV activity [122]. Furthermore, melatonin interaction with lipid bilayers, reducing lipid peroxidation and stabilizing mitochondrial inner membranes [123], may improve ETC activity [96]. Besides, it has been proposed a model of mitochondrial radical avoidance and support of electron flux by melatonin and its metabolite, AMK, which, due to their physical-chemical characteristics, are able to stabilize the ETC since they can compete with to capture the electron that leakage [44].

Mitochondria participate actively in the apoptotic process. In this way, melatonin has been shown to inhibit DNA fragmentation and cytochrome C release in mouse thymocytes treated with dexamethasone and glucocorticoids in a melatonin receptor independent manner [124]. In this case, melatonin may act, presumably, through the regulation of Bax protein levels [125].

Vitamins C and E, also endogenous antioxidants, have no such effects under these conditions [127]. Thus, melatonin role within mitochondria is not only important from the merely antioxidant point of view but also since it is able to regulate different pathways within this organelle.

Melatonin actions against ROS/RNS damage are not only restricted to mitochondria and to its role as antioxidant. In fact, either free radical overproduction could be directly related with the disease or disruption in the melatonin secretion pattern assists in disease precipitation, as we will see next.

MELATONIN AND AGING-ASSOCIATED DISEASES

The consequences of aging are also seen in melatonin production. In fact, the mean nighttime serum melatonin concentration is low during the first six months of life, increasing to a peak value at 1-3 years of age and diminishing on individuals age 15-20 years, with a moderated declination until old age [127]. Thus, if serum melatonin levels contribute to the antioxidant capacity [128, 129], such ability would be reduced in advanced age. Furthermore, alterations in the indolamine circadian rhythm may have severe effects. For instance, Blask and coworkers [130, 131] have described an increase breast cancer risk in female night shift workers due to melatonin suppression by light exposure at night.

In physiological aging, it has been described that the number and density of MT₁-expressing neurons in the suprachiasmatic nucleus were decreased in aged compared to young individuals [132]. This diminution in melatonin signaling could partly explain the prevalence of insomnia in older people. Melatonin, melatonin agonists and antagonists and other melatonergic agents are reported for the treatment

of insomnia sleep abnormality [133-135]. Linardakis patented non-addictive compositions for the sleep disorders and problems including melatonin [136]. Elderly persons have more fragmented sleep and a shorter duration of stage 3 and stage 4 sleep than that occurring in young adults [137]. Melatonin treatment promotes slow-wave sleep in the elderly and could be beneficial by augmenting the restorative phases of sleep [138-139], confirming its uses as chronobiotic. The phase advance of circadian melatonin rhythm during middle years of life is similar to the observed in "tau" hamsters and in the *Drosophila* short-period mutant *per^S*, two experimental models where increase levels of oxidative stress have been detected [140, 142]. Thus, shortening of the circadian period, as happen in elderly persons, may cause an increase of the oxidative stress that accelerates those processes associated with aging, for instance, neurodegenerative diseases [143, 144].

In fact, brain is severely affected by aging, since it suffers morphological and functional alterations that disturb most of the brain processes, such as synapses, neurotransmission, metabolism and, as last resort, this is translated into alterations in motor and sensory systems, sleep, memory and learning [144]. As reviewed by Floyd and Hensley [143], brain is extremely susceptible to oxidative stress, since this organ present a series of characteristics that make it vulnerable to free radical actions: it has a high content of unsaturated fatty acids, prone to oxidize; it requires high amounts of oxygen, almost 20% of the total amount used in humans; it presents high content of iron and ascorbate, crucial for membrane lipid peroxidation; and its antioxidant defenses are low.

The physiological consequences of aging may affect severely to the function of one or several organs. Neurodegenerative diseases, diabetes, autoimmune diseases, inflammatory diseases are by all known, where aging plays an important role. Recently, methods for the treatment of anxiety disorders, affective disorders, intracranial injury, spinalcord injury neurodegenerative diseases sclerosis, migraine, fibromyalgia and cerebrovascular disease by melatonin derivatives are discussed [145, 146]. The novel imidazopyridinyl-amides are used for treating disorders of the melatonergic system, sleep disorders, depression, schizophrenia, anxiety, Alzheimer's disease, cancer and cardiovascular disease [147]. Substituted melatonin derivatives are disclosed for inducing general anesthesia, sedation and hypnotic effects for prevention of jet-leg depression, epilepsy [148]. Melatonin is also used for the preparation of a formulation for the short term potentiating effect of non-barbiturate and non-benzodiazepine hypnotics [149]. Melatonin and its analogue are used in the treatment of attention deficit hyperactive disorder (ADHD) [150]. Recent patent discloses the use of deuterated N-[2-(5-methoxy-1H-indol-3-yl)ethyl] acetamides for the prevention, treatment or controlling disorder, disease or biological function that is associated to a relative melatonin deficiency [151].

Neurodegenerative Diseases

Neurodegenerative diseases are, most of them, characterized by protein-conformation disorders which affect brain function. Protein aggregation can result from a mutation in the sequence of the disease-related protein; a genetic altera-

tion that causes an elevation in the amounts of a normal protein; or can occur in the absence of genetic alterations, perhaps triggered by environmental stress or aging. In aggregation diseases, large intracellular or extracellular accumulations of aggregated proteins, known as inclusion bodies, are often formed [152].

Alzheimer's disease

Alzheimer's disease (AD) is actually the main cause of dementia. It is characterized by the deposition of the amyloid peptide (A β) outside of nerve cells forming extracellular plaques (senile plaques). Furthermore, intraneuronal accumulation of tau protein also occurs, due to abnormal tau phosphorylation [153-155]. The majority of cases (90-95%) are sporadic and the remainders are familiar, with differences in time the disease onsets (> 60 years old in the sporadic form, 40-60 in the familiar one) [156].

It is known that A β is neurotoxic and there are evidences suggesting a role for oxidative stress, since A β aggregation process is accelerated by A β peptide metal-catalyzed oxidation, giving rise to H₂O₂ production, which is able to react further with transition metals [157, 158] (Fig. 3)). Thus, an increase in H₂O₂ levels is observed in AD [159, 160]. Free radical production by A β aggregation has important consequences to brain physiology since it implies the necessity of a protection system, such as antioxidants. Brain has not a strong antioxidant defense, which makes it vulnerable to oxidative processes. In fact, alterations in enzymatic antioxidant activity may precipitate neurodegeneration, as seen in Down's syndrome, where almost all patients develop AD. A β gene is situated on chromosome 21 [161], which implies a higher risk to develop oxidative processes as state above. Furthermore, there is an overexpression of Cu/Zn SOD, also localized in chromosome 21, [162] with the concomitant increase of H₂O₂ production. Thus, oxidative stress processes are really important in AD pathophysiology.

On the other hand, in AD the microtubule associated protein tau is excessively phosphorylated in degenerating neurons [155, 163]. As mentioned before, LPO products, mainly MDA and 4-HNE, are able to react with different cell macromolecules, such as lipids, DNA and proteins (Fig. 2). 4-HNE increases tau phosphorylation and diminishes its dephosphorylation by direct binding to tau [163] confirming, once more, that oxidative stress byproducts participate in AD. The effects of 4-HNE presence are beyond a covalent protein binding. In fact, Camandola and coworkers [164] have demonstrated that this LPO byproduct is able to suppress selectively basal and inducible NF- κ B DNA binding activity in cultured rat cortical neurons. NF- κ B is a transcription factor implicated in the transcriptional upregulation of inflammatory genes in response to changes in cellular oxidation-reduction status, as well as antioxidant enzymes genes [165-168]. Thus, inhibition of the NF- κ B pathway may have harmful effects for cell survival [169-170].

Therefore, AD patients present increased levels of oxidized proteins, lipids and DNA and these factors are strongly related with aging [143]. For this reason, antioxidant therapy was considered for AD prevention and treatment.

Melatonin has been deeply studied in AD. Firstly, melatonin levels decrease during aging and AD patients present even lowest melatonin levels [171]. This melatonin diminution affects to the regulation of the circadian rhythms, such as sleep-wake cycle. Melatonin treatment has been shown to improve sleep quality and suppressed sundowning, both characteristic of AD patients [138, 139, 172]. In this regard, melatonin deficiency may appear as a consequence rather than one of the causes of AD, although melatonin loss may aggravate the disease [173]. In second place, due to free radical implication in AD, melatonin has been proposed as a good candidate to ameliorate the AD effects. DNA, lipid and protein oxidation are part of the AD pathology and melatonin has by far demonstrated to be able to counteract or to diminish the harmful effects of oxidative damage [174]. Furthermore, recent works have demonstrated that melatonin is able to attenuate the detrimental effects of both A β aggregation and tau phosphorylation. Feng *et al.* [175, 176] have shown that early melatonin treatment in AD is able to reduce thiobarbituric acid-reactive substances (TBARS) levels, increase GSH content and SOD activity and prevent those processes that lead to cell death in a amyloid precursor protein (APP) transgenic mouse model. In the case of tau phosphorylation, melatonin effectiveness is not only due to its antioxidant properties but also its modulation of the phosphorylation system [177] which may imply various mechanisms, such as suppression of protein kinase A overactivation [178] and members of the p38 MAPK family [179].

Parkinson's disease

Another very well-known neurodegenerative disease is Parkinson's disease (PD). PD is a common progressive neurodegenerative disease characterized by the loss of dopaminergic neurons of substantia nigra and the presence of the fibrillar cytoplasmic aggregates of α -synuclein (α -Syn) in multiple brain regions. Mutations in the α -Syn gene at codons 30 (A30P) and 53 (A53T) in the α -Syn gene have been shown to segregate with autosomal dominant forms of Familial Parkinson's disease. Furthermore, it is also possible an abnormal aggregation of wild-type α -Syn, implicated in the pathogenesis of PD, and other related diseases that are classified as α -synucleinopathies [180]. Normal α -Syn and its mutated forms (A30P and A53T) are prone to self-aggregate, producing amyloid fibrils, although some differences exist with regard the protofibrilization rate, being this one higher in the mutant proteins than that of wild-type protein. This protein is closely related to dopamine metabolism, since it participates in the modulation of dopamine amounts at nerve terminals. This α -Syn role is really important, since dopamine is prone to autoxidize, and an enhanced oxidative stress accelerates the formation of α -Syn aggregates [181, 182]. In fact, oxidation of dopamine gives rise to an excessive formation of H₂O₂, O₂⁻, NO and \cdot OH [183]. This oxidation may be a result both from autoxidation and monoamine oxidase-mediated oxidation. Furthermore, the high iron content within the brain, even higher in PD patients [184], promotes free radical formation via Fenton's reactions (Fig. 3).

L-DOPA is the drug used in PD therapy, since it can be converted enzymatically to dopamine, restoring its levels

within the surviving neurons. But L-DOPA can undergo autooxidation, increasing, even more, the oxidative stress situation at nigro-striatal sites and being L-DOPA treated patients for long time at risk [185].

With regard to antioxidant treatment, Rocchitta *et al.* [186] have recently shown that L-DOPA administration in rats induces formation of L-DOPA-semiquinone, which can act as oxidant, decreasing dopamine and ascorbic acid levels. However, melatonin coadministration significantly inhibits L-DOPA autooxidation and fully restored dopamine and ascorbic acid levels. In a similar way, the antioxidant N-acetylcysteine is able to protect L-DOPA and dopamine from autooxidation, although it needs the ascorbic acid cooperation. Melatonin not only succeeds in L-DOPA and dopamine protection, but also maintains ascorbic acid levels [186]. The catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) produces a similar loss in nigro-striatal dopaminergic neurons as seen in PD. Melatonin treatment immediately post-surgery attenuates dopaminergic denervation in the 6-OHDA-lesioned rat striatum when administered at physiological concentrations [187]. Ascorbic acid, -tocopherol and N-acetylcysteine treatment did not show the same results [126]. Lastly, only high doses of melatonin are able to suppress iron-induced neurodegeneration of the nigrostriatal dopaminergic system when administered locally or after 7 days repetitive systemic injection [188], showing that, depending on the challenge, melatonin doses may vary.

In fact, some authors consider that melatonin treatment in PD has little to do to improve the situation, since dopamine autooxidation is a process initiated many years before first symptoms are discovered and an antioxidant therapy has little to do at this level. Furthermore, in some PD patients, melatonin administration is able to exacerbate the motor impairment observed during the disease process [189, 190]. Thus, melatonin may prevent oxidative stress in the early stages of the disease but exacerbate various aspects dopamine degeneration at the final ones [191].

Huntington's disease

Huntington's disease (HD), unlike AD and PD, both with sporadic forms, is an autosomal dominant neurodegenerative disease, caused by a CAG triplet-repeat expansion coding for a polyglutamine (polyQ) sequence in the N-terminal region of the huntingtin (htt) protein [192]. Many works have suggested the possible mechanisms underlying the disease development and they include oxidative stress, mitochondrial defects, excitotoxicity, and activation of death effector proteases. Despite these accumulating data, the precise contribution of these cell death mechanisms in HD pathogenesis is still unknown [193]. Thanks to experimental animal models, it was possible to elucidate the pathological mechanisms underlying the disease process. For instance, kainic or quinolinic acid intrastriatal injections produced lesions similar to those seen in HD, suggesting that glutaminergic overactivity, or excitotoxicity, plays a central role in HD pathogenesis. Quinolinic acid (2,3-pyridine dicarboxylic acid, QUIN) is a metabolite of the tryptophan-kynurenine pathway and it is present in human and rat brain [194, 195]. The concentration of this metabolite is known to increase with age [196]. It is an endogenous glutamate agonist with relative selectivity for N-methyl-D-aspartate (NM-

DA) receptors [194, 197, 198]. It produces oxidative damage in neurons and glutamate-type excitotoxicity [199] (Fig. 5).

On the other hand, chronic inhibition of succinate dehydrogenase (mitochondrial complex II) by systemic injection of the selective inhibitor 3-nitropropionic acid (3NP) has been used also as an animal model of HD, since the impairment of the complex II activity is a prominent metabolic alteration in HD [200]. Calabresi and coworkers [201] have demonstrated that 3NP significantly enhanced basal intracellular Ca^{2+} levels, even more dramatic after the co-application of NMDA. This large, lasting increase in intracellular Ca^{2+} levels after combined inhibition of mitochondrial complex II, above cited, and activation of NMDA receptors might initiate the cascade of metabolic events leading to selective neuronal death in the striatum [201,202]. Recently, Shin *et al.* [203] have described that the accumulation of mutant huntingtin in glial nuclei in HD brains leads to a decrease in glutamate transporters. This event implies that striatal medium-sized spiny neurons will receive an excessive glutamatergic input. Excitotoxic events observed after the activation of NMDA receptors are related to elevated cytoplasmic concentrations of Ca^{2+} , which can initiate a number of pathological processes [204], such as ATP depletion and secondary effects resulting from these changes. Increase of the intracellular Ca^{2+} levels in both QUIN and 3NP models may account for other processes that further increase the deleterious consequences of such treatments (Fig. 5).

Although less studied, melatonin administration in HD models demonstrated, once more, that its antioxidant ability may account in the reduction of the oxidative stress processes induced by QUIN and 3NP treatments. Furthermore, it has been shown that melatonin not only act as direct antioxidant, especially in QUIN models where this metabolite acts both through NMDA receptors and QUIN-iron complexes (Fig. 5), but also it modulates the activity/expression of certain proteins closely related to the pathology development. In fact, melatonin is able to downregulate the activity of NOS [205], leading in a decrease of NO production.

Diabetes and its Relation to Melatonin at Two Levels

Diabetes mellitus is one of the most studied diseases since the number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity [206]. Diabetes is characterized by a constant hyperglycemia that can be the result of an inappropriate insulin production, lack in the response of insulin targets or both. There are two types of diabetes: type 1 is defined by the loss of the insulin-producing beta cells of the islets of Langerhans of the pancreas, commonly by an autoimmune destruction; type 2 is characterized by defective insulin secretion and reduced insulin sensitivity. Type 2 is the most common, with a strong association with obesity and aging.

Melatonin may have two main roles in diabetes. For instance, it has been reported that individuals with nocturnal lifestyle presented a reduced night peak plasma melatonin and their glucose concentration was higher than in control group [207]. Thus, Ha *et al.* [208] hypothesized that decrea-

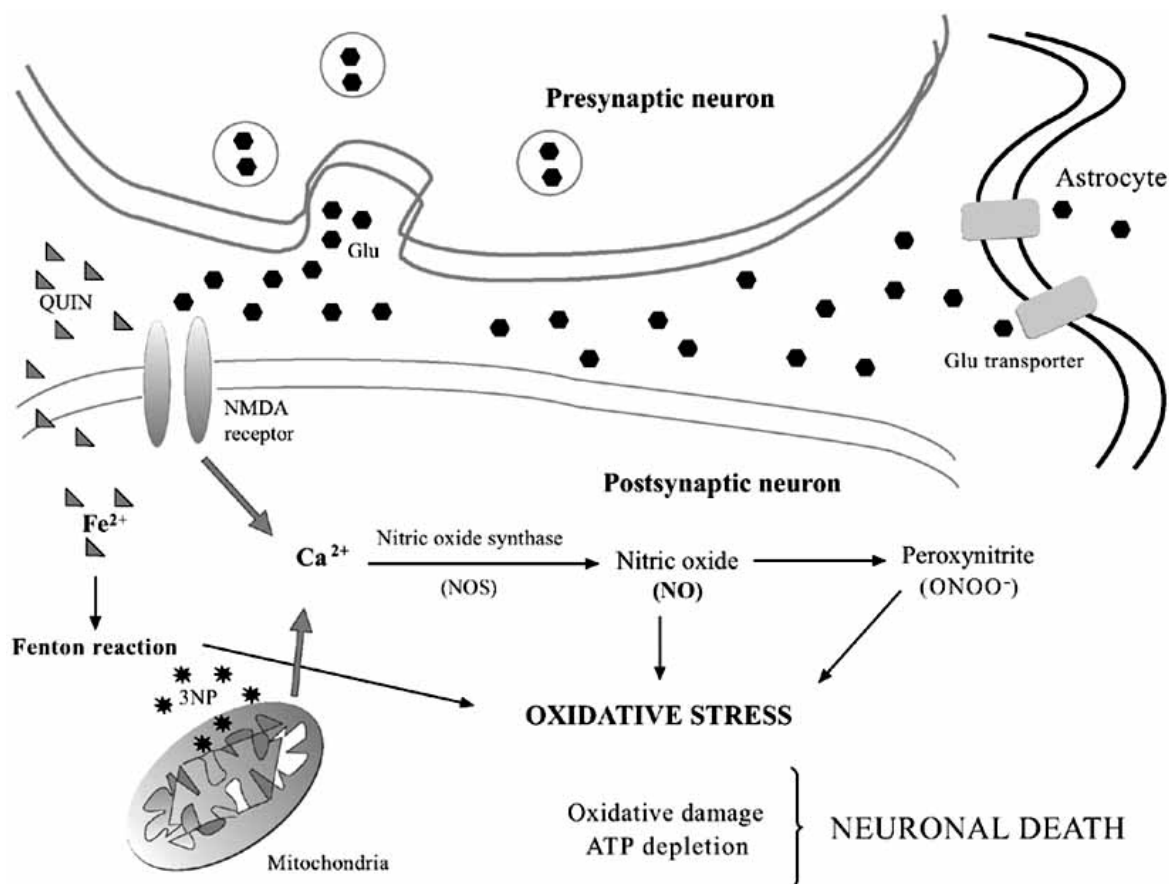


Fig. (5). Huntington's disease models. In the presynaptic neuron, glutamate (Glu) is secreted into the synaptic space and interacts with glutamate receptors (NMDA receptor) in the postsynaptic neuron. The signal received implies the release of intracellular Ca^{2+} , with the consequent increase of nitric oxide synthase (NOS) activity. To avoid an overstimulation, synaptic space glutamate is uptaken by the astrocytes through a Glu transporter. Excitotoxic events observed after sustained activation of NMDA receptors by **quinolinic acid** (QUIN) are related to elevated cytosolic concentrations of free Ca^{2+} and the processes thereafter. Nitric oxide (NO) is implicated in this mechanism. NO can contribute to the oxidative damage via the peroxynitrite pathway ($ONOO^-$) and Glu release and radicals formed secondarily as a consequence of excitotoxicity. Moreover, Glu reuptake is inhibited by QUIN, another effect promoting excitotoxicity. Several reports have suggested that the toxic effects of QUIN are primarily mediated by NMDA receptor overactivation through a typical process of excitotoxicity. However, more recently, several other studies have shown that QUIN is also capable of inducing oxidative injury directly, potentially involving a pattern of toxicity that might be partially independent of NMDA receptor activation. **3-Nitropropionic acid** (3NP) treatment affects directly to mitochondria, at complex II level. Furthermore, this compound is able to induce an increase in intracellular calcium levels that finally results in increase $ONOO^-$ levels. Adapted from Vega-Naredo *et al.* [51].

sed melatonin levels, as happens in nocturnal lifestyle and aging, are related, at least partially, to the increased prevalence of diabetes. These authors demonstrate that melatonin stimulates glucose transport via melatonin membrane receptor, a study that confirms previous observations which related glucose homeostasis with melatonin levels [209, 210]. The other role played by melatonin in diabetes may be as an antioxidant. In fact, increased LPO is accepted to be one of the main causes of diabetic complications [211], since the possible sources of oxidative stress and damage to proteins in diabetes include free radicals generated by autoxidation reactions of sugars and sugar adducts to proteins and by autoxidation of unsaturated lipids in plasma and membrane proteins [212, 213]. Furthermore, hyperglycemia also favours the formation of highly reactive free radicals such as $O_2^{\cdot-}$ and $ONOO^-$, and all of them are able to activate other pathways implicated in the pathogenesis of

diabetic complications [214] and induces reduction in the levels of antioxidant vitamins A, E and C, GSH and in the activity of antioxidant enzymes such as GSH-Px [211]. Antioxidant treatment has been studied and, as shown by Baydas *et al.* [211], both melatonin and vitamin E are able to reduce MDA concentrations in diabetic rats to similar levels, although the necessary melatonin concentrations to reach these results are smaller than vitamin E.

AUTOIMMUNE DISEASES

Rheumatoid Arthritis and its Dependence on Melatonin Circadian Pattern

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder of unknown etiology characterized by non-specific inflammation of the peripheral joints with joint swelling, morning stiffness, destruction of articular tissues

and joint deformities. The development of RA is partly related to the excess production of ROS and a lowered ability to remove oxidative stress, since RA bloodstream neutrophils and monocytes overproduce these highly reactive species [215-217]. There are studies indicating that pro-inflammatory cytokines, such as IL-1 and TNF- α , are involved in the formation of toxic ONOO \cdot by increasing the activity of NOS [218], and there is also an increase of NOS and NADPH oxidase activities [217]. With these premises, antioxidant therapy seems to be one of the best to counterbalance the overproduction of free radicals. But, contrary to the disease mentioned before, the use of melatonin to neutralize RA oxidative stress is not recommended. Serum melatonin levels in RA patients are higher than the observed in controls, since RA individuals present the highest melatonin production two hours earlier than in controls and decreases similarly in both groups [219]. Melatonin circadian rhythm synchronizes some of the main parameters of the immune system, with daily and seasonal rhythms. In fact, Th1 cytokines present their maximum peak at the same time as melatonin, when cortisol levels are the lowest [219]. *In vivo*, melatonin is able to increase the immune system actions when it is depressed or when melatonin is administered in the afternoon or the evening [220]. For its ability to increase the immune response, melatonin might promote autoimmune diseases by acting directly on immature and mature immunocompetent cells [221], a fact that makes melatonin not always beneficial.

RA geographical distribution follows a striking pattern, since in studies developed in northern and southern population it was shown that RA prevalence is much higher in north Europe than in Mediterranean countries and the severity of the disease is also elevated in northern patients [219]. Thus, in the case of RA, it is necessary melatonin synthesis and/or action inhibition and oxidative stress neutralization must be achieved through other antioxidants.

MASSIVE INFLAMMATORY RESPONSE

Sepsis

Bacterial infection might elicit a massive inflammatory response that comprises the function of multiple tissues, with serious consequences when vital organs are affected. The implication of oxidative stress in sepsis has been largely described. In fact, free radical formation affects specially to mitochondrial function. Callahan *et al.* [222] have shown that the administration of either an inhibitor of nitric oxide synthesis or an O $_2^{\cdot-}$ scavenger effectively prevented the mitochondrial dysfunction observed in sepsis. These authors described that the production of ONOO \cdot , by NO and O $_2^{\cdot-}$ interaction, alters mitochondrial function following endotoxin administration, since ONOO \cdot is able to produce State 3 mitochondrial dysfunction, but without causing uncoupling of oxidative phosphorylation [222]. Furthermore, after 48 hours it is observed a selective depletion of several mitochondrial proteins, running in parallel with the physiologic alterations observed in O $_2$ consumption after that period [222]. This protein depletion could be related to oxidative damage derived from ONOO \cdot overproduction [223]. A study done in skeletal muscle cells from critical ill septic patients have shown the existence of a bioenergetic abnormality which involves antioxidant depletion, reduced

complex I activity, low ATP levels and increased NO production [224]. In the case of sepsis, NO production is really important since it has been described that the expression and activity of the inducible form of nitric oxide synthase (iNOS) is increased in septic process, with the subsequent increment of NO. This compound competes with oxygen for its binding site at complex IV within mitochondria in a reversible way within physiological conditions. However, increments in NO concentration implicates complex inhibition, with the subsequent electron leakage and the formation of O $_2^{\cdot-}$. Thus, there would be an increase in ONOO \cdot formation, which is able to inhibit in an irreversible manner the four complex of the ETC, the ATP synthase and to oxidize membrane lipids [225]. Escames *et al.* [225] have studied the sepsis process in iNOS knockout mice. The analysis of these mice (iNOS $^{-/-}$) versus their controls (iNOS $^{+/+}$) in septic conditions have revealed that in the absence of iNOS, the oxidative stress found in iNOS $^{-/-}$ septic mice was not increased, as compared with septic iNOS $^{+/+}$ animals, showing that in $^{-/-}$ animals, the present antioxidant system is enough to maintain mitochondrial redox status. Furthermore, their results show a 40-50% inhibition of all respiratory complex activity in sepsis. This inhibition is related to NO production in this condition in the case of $^{+/+}$ animals, since no changes were observed in iNOS $^{-/-}$ mice. Melatonin treatment restore mitochondrial homeostasis in septic iNOS $^{+/+}$ mice. In fact, melatonin administration in septic conditions is able to restore complex activity above the basal values. And, even more, the indoleamine seems to be effective when mitochondrial damage and oxidative stress is present, since it did not affect to iNOS $^{-/-}$ mice, without effects in the case of mitochondrial dysfunction [225]. Melatonin administration in septic situations has been also described in the treatment of septic newborns with excellent results [226].

MELATONIN ANALOGS AND/OR METABOLITES

Due to melatonin antioxidant properties, many studies have been made to discover compounds with improve antioxidant abilities. Thus, the first to be considered were tryptophan derivates and some of them have been determined as free radical scavengers, such as the melatonin precursor, N-acetylserotonine (Fig. (4)), as well as melatonin byproducts, like AFMK and AMK [227].

Moreover, apart from its free radical scavenger properties, researches have also analyzed possible melatonin receptor antagonists, for a more controlled regulation. As seen above, in the case of PD, melatonin is effective in the early stages of the disease, although it enhances motor dysfunction in advance ones. Thus, Willis and Robertson [228] have studied two known melatonin analogues, ML-23 and S-20928, in a PD model. Both have shown to be able to reverse the PD-like deficits seen in the more chronic models of PD, since they can facilitate motor recovery. These results suggest that the use of melatonin analogues can favour recovery from dopamine depleting lesions after dopamine degeneration has started and this recovery is not attributable to the antioxidant properties of this hormone, avoiding the effects observed in some PD cases after melatonin administration [228, 229].

Apart from its excellent antioxidant characteristics, AMK has also demonstrated its ability to regulate neural nitric oxide synthase (nNOS). Leon *et al.* [230] have analyzed the effects of melatonin and its byproducts, AFMK and AMK, on nNOS activity. Only melatonin and AMK were able to inhibit nNOS activity in a dose-response manner. But the IC₅₀ for AMK was significantly lower than melatonin both *in vivo* and *in vitro* studies, and this metabolite is able to bind to Ca²⁺-Calmodulin, thus competing with nNOS.

CURRENT & FUTURE DEVELOPMENTS

As seen throughout this review, melatonin presents several mechanisms of action to develop its large number of functions. It can act as a direct free radical scavenger, as indirect antioxidant through the regulation of antioxidant enzymes and it is able to modulate several physiological pathways through membrane and nuclear receptor, with the implication of many second messengers.

Its first known function was in the neuroendocrine axis, as the hormone of darkness. Melatonin regulation of the circadian rhythm has been proved to be extremely important, since deviations from the normal pattern imply the deregulation of important rhythms. Thus, in those situations where melatonin rhythm is disturbed, such as aging and some diseases, the indolamine administration could be considered as preventive, since many are the situations where the melatonin pattern maintenance delays the disease apparition.

Another important component that has to be taken into account is the implication of melatonin in those situations where free radical production is enhanced. In such situations, melatonin has demonstrated to be more effective than other common antioxidants, with the advantage that lower doses are needed.

In the future studies to be than with regard to melatonin and analogs applications, many things need to be taken into consideration. Depending on the pathology, it is important to strengthen or inhibit one or other melatonin function. Furthermore, along the disease progresses, melatonin effects can be opposite, so a narrow study of the disease situation must be developed.

Many things remain to be done with regard to melatonin applications. The better knowledge of its mechanisms of action would help in the understanding of the indolamine implication in a broad spectrum of diseases, which further would aid in their treatments.

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