REVIEW ARTICLE

Melatonin, oxidative stress and ageing

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Melatonin, the hormone secreted from the pineal gland, has an important role in regulating a number of physiological functions in the body. Of these, the antioxidative function has attained much importance because of its close association with the ageing process. The involvement of oxidative stress in ageing has been supported by genetic and dietary restriction studies. As melatonin has direct genomic actions and also forms a part of antioxidative defence system, the level of this hormone in the body fluids is the crucial factor that determines the healthy state of cells and tissues in the body. The progressive decline in melatonin secretion with increase in age has been suggested to be one of the main factors that enhance the effects of oxidative stress causing much cellular damage resulting in senescence and age-associated degenerative diseases.

AGEING has been defined as the 'sum total of all changes that occur in living organisms with passage of time that lead to functional impairment, increased pathology and death'[1]. A general decline in various biochemical and physiological functions is noted in most of the organs during ageing and this results in increased susceptibility to age-associated diseases. Theories based upon molecular aspects of cellular function like gene regulation, mitochondrial DNA mutations, error catastrophy, oxidative stress have all been suggested as the possible causative factors that contribute to the mechanism of ageing. A special section devoted to these under the title 'Molecular biology of ageing' has been published in 25 May 1998 issue of *Current Science*. Of the various factors, the free radical theory proposed by Harman[2] in 1956 has received considerable attention. According to this theory, ageing is due to accumulated effect of oxidative stress on cells and tissues that results in damage to lipids, protein, nuclear and mitochondrial DNA molecules[3]. The increased incidence of somatic mutations in the mtDNA molecules has been suggested to be an important contributory factor to human ageing and age-related degenerative diseases[4]. Though cells are also equipped with well-organized antioxidative defence system (ADS) comprising many substances like enzymes and vitamins, an imbalance between generation of free radicals and their disposal by antioxidative substances occurs as age advances, resulting in degenerative changes and senescence[5]. Increased production of superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) with increase in age has been documented[6], but a general decrease in the functional activity of ADS of cells and tissues is under intense study. In this context, it is worthwhile to discuss the role of melatonin, the major hormone secreted by the pineal gland, as it has significant antioxidative potential and the level of this hormone falls as the age advances[7]. An attempt has been made in this review to discuss the role of melatonin in the ageing process keeping in view the antioxidative potential of this hormone, as the main background. A brief account of nature of melatonins, biosynthesis and regulation of its secretion has been presented to understand the functional importance of melatonin in the ageing process.

Melatonin: Its nature and properties

Melatonin, or N-acetyl-5-methoxy tryptamine, was first identified by Lerner *et al.*[8]. It was first tried as a therapeutic agent for treating irregular skin pigmentations, but later found to posses anti-gonadotrophic activity[9,10]. The duration of melatonin secretion has been shown to signal the length of photoperiod in some animals and in these species it is said to regulate reproduction on seasonal basis[11]. The involvement of melatonin in sexual maturation, control of puberty[12] and thermo regulation[12a] has been studied. Melatonin acts as a hypnotic substance even when present at physiological concentrations and seems to participate in sleep regulatory mechanisms[13]. Its effect on 'T' cell function and lymphokine activated killer (LAK) cell activity suggests that this hormone has an immunoregulatory role[14]. Study of melatonin rhythm in depressives and its response to antidepressant treatment points out that melatonin has a suggestive role in the control of human mood and behaviour[15,16]. The hormone inhibits tumour growth and low melatonin levels have been detected in various categories of cancer patients[17,18]. As a rhythm regulator melatonin controls the phase and amplitude of circadian rhythm by acting both on supra chiasmatic nucleus (SCN), the biological clock that resides in the hypothalamus as well as on different cells and tissues of the body[19,19a]. Because of this action the hormone has been named as 'chronobiotic' or 'Internal Zeitgeber' by Armstrong[20]. Experimental studies conducted on these lines in animals have led to the understanding of melatonin's role in various biological rhythm disorders like delayed sleep phase syndrome (DSPS), jet lag, shift-work disorder, and mood disorders, etc. and use of this

hormone has been found beneficial in treating such disorders successfully[21]. According to Massion[22], 'melatonin is not only photosensitive but also psychosensitive'. Various types of meditation and yoga have been shown to enhance endogenous melatonin levels. Since both meditation and melatonin share several physiological effects like decrease in anxiety, stress, heart rate and blood pressure, the beneficial effects of meditation have been attributed to stimulation of pineal gland function and melatonin secretion[22].

Melatonin: Biosynthesis and regulation of secretion

Melatonin is synthesized mainly in the pineal gland. Synthesis also occurs in retina, GI tract, platelets and erythrocytes[23]. Pineal gland which forms the main source of melatonin in the body is deeply situated at the center of the brain, behind the third ventricle and weighs only 150 mg. The gland is composed of clusters of parenchymal cells known as pinealocytes and few glial cells. Calcification of the pineal gland does not affect its functional activity very much and the gland performs its secretory function throughout the life of an individual[24]. The biosynthetic scheme of melatonin is shown in Figure 1. Tryptophan serves as the precursor and is taken up actively from the blood by the pinealocytes and is then converted into serotonin by 5 hydroxylation and subsequent decarboxylation. Serotonin is then acetylated to form N acetyl serotonin by the enzyme aryl alkylamine N acetyl transferase (NAT) whose activity is highest during dark hours of the day–night cycle. N acetyl serotonin is subsequently methylated by the enzyme hydroxy indole O methyl transferase (HIOMT) to form melatonin. Both NAT and HIOMT are found in high concentrations in the pineal gland. Melatonin once formed is not stored in the gland but is secreted immediately into the blood stream. High secretory activity is found only at night. In human beings, plasma melatonin level begins to increase steadily after 9.00 PM to 11.00 PM and reaches the peak value around 2.00 AM to 4.00 AM. The level of this hormone then declines reaching a low value in the afternoon. Study of plasma melatonin among subjects of different age groups reveals a consistent decrease with increase in age. The nocturnal plasma melatonin values which are very high during the first year of life, 210 ± 35 pg/ml, decline to about 46.06 ± 4.0 pg/ml, in adults. The day time value remains more or less the same around 20.0 pg/ml (ref. 25). The decreased melatonin values are attributed mainly to the ageing process[26]. During childhood (1–10 yrs), melatonin rhythm is robust with largest day versus night differences. Even after reaching adulthood, circadian melatonin rhythm is maintained in most individuals. The melatonin rhythm then gradually wanes with the consequence that in old age, the day versus night difference in melatonin is barely discernible[7,27]. Older the individual, greater will be the reduction in amplitude of nocturnal melatonin secretion[5]. Urinary 6 hydroxy melatonin secretion measured in individuals of different age groups (expressed as µg/17 h) reveals the following data: 11.7 ± 5.0 µg in 20–39 yrs age group; 8.4 ± 5.8 µg in 40–59 yrs age group; 5.7 ± 3.0 µg in 60–79 yrs age group and 6.0 ± 3.7 µg in 80 yrs and above[28]. A progressive decline in urinary melatonin concentration is noted in this study. Reduced 6-sulphatoxy melatonin excretion was also noted in elderly post-menopausal women when compared to healthy control adults and in this study, disturbance in the timing of melatonin secretion associated with ageing has been supported[29]. The urinary 6-sulphatoxy or 6-hydroxy melatonin value closely follow the plasma melatonin values[23]. The insomnia seen in old age people has been suggested to be due to low nocturnal melatonin secretion[30]. Recently Wurtman *et al.* showed that treating insomniacs with melatonin capsules of 0.1 to 0.3 mg/day, which is sufficient to maintain the physiological level of this hormone during night, improved the sleep quality dramatically[31,32], showing that high nocturnal melatonin is essential for sleep induction and its decrease in old age affects sleep quality. However, the results of Kripke *et al.*[29] do not support the view that melatonin deficiency *per se* might lead to insomnia in aged individuals.

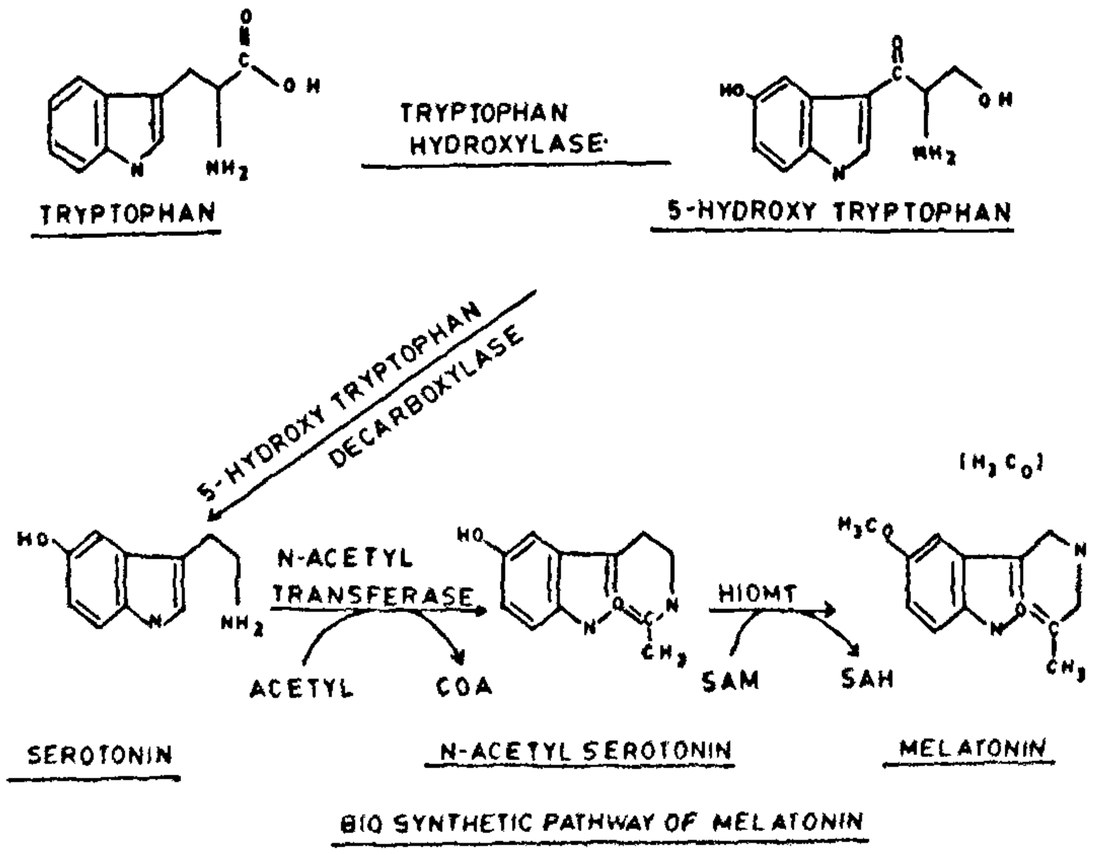

Figure 1. Melatonin biosynthesis.

Regulation of melatonin secretion

The rhythmic pattern of melatonin secretion with high nocturnal and low diurnal values is governed by the SCN of the hypothalamus, and this circadian rhythm of melatonin secretion in man is close to 25 h. Light

entrains or adjusts this rhythm by acting through the retino-hypothalamic tract[33]. Impulses from SCN pass to superior cervical ganglia from where they relayed to the pineal gland through post-ganglionic sympathetic nor-adrenergic fibers. Nor-epinephrine released from these nerve fibers activate β receptors mediated adenyl cyclase–cyclic AMP system (Figure 2) and promote melatonin synthesis[30]. The magnitude of melatonin secretion induced by nor-epinephrine has shown to be modulated by neuro peptide Y present as a co-transmitter in the post-ganglionic sympathetic fibers and this neuro peptide Y is essential even for regulating seasonal rhythmical pattern of melatonin secretion[34]. Recently the retinal photoreceptors that transmit light information to SCN have been identified and these receptors contain either rhodopsin or short wavelength blue cone receptors that are distinct from the receptors that mediate visual information[35]. These observations have been inferred from studies under taken on blind people, in whom light suppressed melatonin secretion completely[35]. Light-induced melatonin suppression occurred in people with colour blindness like protanopics and deuteranopics, substantiating thereby that either blue cone receptors or rod-like photo receptors mediate phototransduction to SCN[36].

Melatonin receptors

Melatonin regulates various physiological functions like control of circadian rhythm, sleep induction, reproductive functions by acting through specific receptors present in the brain and peripheral tissues[37]. Using 2 I(125) iodomelatonin and by employing autoradiographic and radio receptor assays different subtypes of melatonin receptors like Mel Ia, Mel Ib, Mel 2 have all been identified in the plasma membrane of neural and peripheral tissues[37]. Melatonin membrane receptor shares general features with other G-protein-linked receptors. By binding to these membrane receptors melatonin alters the conformation of specific subunits of intracellular G-protein which then bind to adenyl cyclase that either activate or inhibit it. Melatonin receptors present in the SCN are involved in circadian rhythm regulation while those that are found in the medio basal region are involved in the control of reproduction[38]. Melatonin receptors present in the granulosa cell membrane of ovarian follicles seem to be involved in the intra-ovarian regulation of steroidogenesis[39]. Melatonin being lipophilic in nature enters through the plasma membrane of cells and binds with cytosolic and nuclear membrane receptors[40]. Binding with calmodulin, melatonin alters calcium signalling and thereby affects the activity of intracellular enzymes like adenyl cyclase and phosphodiesterase[39].

Melatonin has been shown to bind specifically with nuclear orphan receptors α and β, RZR α and RZR β[41], and the retinoic orphan receptor[42]. These receptors mediate the transcriptional effects of melatonin. The mRNA that encodes RZR β has been located in regions like the retina, superior collicules, SCN with highest activity being found in the pineal gland[38,43,44]. Like other steroid hormone receptors, RZR/ROR α receptors also exhibit four domains: (i) an activated functional domain (AF1) located at the N terminal, (ii) an internal DNA binding domain (DBD), (iii) a hinge domain and (iv) a C-terminal, a hormone binding domain (HBD)[45]. Upon binding of ligand, the receptor unfolds and specific sites are made available for phosphorylation leading to activation of AF2 domain, dimerization and DNA binding[45]. Transcriptional activation of RZR/ROR α by melatonin occurs at nanomolar range[41,43], and all isoforms of RZR and ROR α's with the exception of ROR α 1 bind melatonin and are transcriptionally activated by it[41]. Functionally RZR α, ROR have been shown to be biologically responsive to melatonin treatment in human B lymphocytes modulating the transcription of 5 lipoxygenase gene[46]. These findings reveal that melatonin has direct genomic actions[44], and according to Steinhilber *et al.*[46] *melatonin is the natural ligand for RZRtγ and RZRβ* nuclear receptors. The free radical disposal action of melatonin is also said to be partly dependent upon its nuclear-binding capacity[47]. Antioxidant action of melatonin protects cells and tissues and prevents the occurrence of degenerative changes that can result in senescence[48]. A brief account of the role of melatonin as an antioxidant and the oxidative stress as a precipitating factor for ageing is given here.

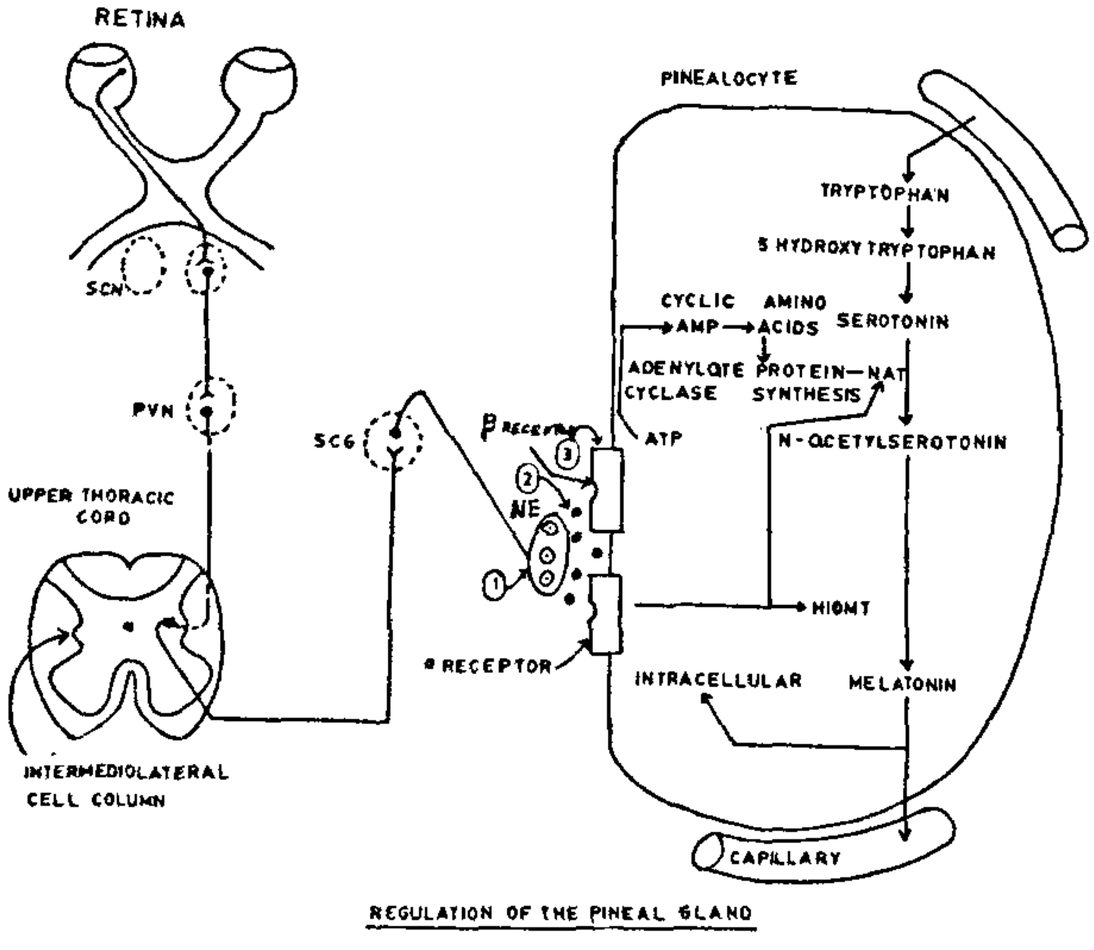

Figure 2. Regulation of melatonin secretion.

Free radicals and ageing

Reduction of molecular oxygen during metabolic reactions in the mitochondria releases large amounts of energy which is trapped and stored. However, nearly 1

to 5% of molecular oxygen is oxidized into superoxide radicals (O_2^-), reactive oxygen substrates (ROS) like hydrogen peroxide (H_2O_2)[49]. Superoxide radicals react with H_2O_2 to generate highly reactive hydroxyl ($^{\bullet}OH$) radicals through Fenton reaction, that requires the presence of either ferrous (Fe^{2+}) or cuprous ions (Cu^{2+}). But iron seems to be the commonest metal triggering this reaction as it forms the essential component of various proteins in the biological systems[50]. Hydroxyl radicals are highly reactive and once they are generated they can destroy virtually all cellular components including proteins, lipids and DNA molecules. They can also abstract hydrogen atoms from fatty acids and can generate peroxyl radicals and this process of lipid peroxidation is a highly destructive self-perpetuating event. The effects of these hydroxyl and peroxyl radicals lead to extensive damage of proteins resulting in ageing and age-related degenerative diseases[24,51]. Increased production of superoxide (O_2^-) and H_2O_2 with increase in age also leads to oxidative damage and mutation of nearby mitochondrial DNA molecules. These damaged molecules transcribe and translate to form dysfunctional protein subunits that give rise to defective respiratory enzymes[52]. The impaired respiratory chain not only curtails the formation of ATP molecules but also generates more ROS which further enhances oxidative damage to macromolecules. According to Wei *et al.*[52] this 'vicious cycle' is responsible for age-associated decline in the bioenergetic functions and for accelerating the ageing process. Biochemical studies of cellular and sub-cellular components have shown that concentration of oxidatively damaged proteins, lipids and DNA molecules increase with age[53]. The substance 8-hydroxy-deoxy-guanosine (8.OH.dG), a specific product of oxidative damage of DNA, increases in all tissues during the ageing process, substantiating thereby the occurrence of oxidative damage to DNA molecules[54]. Oxidative damage to DNA molecules causes depurination, depyrimidination, single-strand breaks, double-strand breaks, and cell apoptosis[55]. In proteins, oxidative damage results in loss of sulphydryl groups and selective decrease in enzymatic activity[56]. The phrase used to describe the damage done by free radicals is 'oxidative stress'. The degree of oxidative stress to which the cell is subjected determines whether the cell remains healthy or not[57]. The life span and variations in life span among different species and among individuals of the same species are attributed to the level of oxidative stress[58]. Enzymatic studies carried out in insects, birds and mammals have shown that species which generate superoxide radicals and hydrogen peroxide at lower rates have longer life spans[59]. Dietary restrictions which reduce the quantity of free radical generation, increases the life span correspondingly[56]. It was shown that dietary restrictions by 30% to 60% increased life span by the same level[56,60].

Normally the free radicals that are generated in our body are disposed off quickly by ADS present in cells and tissues. The ADS consists of free radical scavenging enzymes like manganese superoxide dismutase (Mn SOD), copper–zinc SOD, catalase, glutathione peroxidase (GSH-PX), reduced glutathione (GSH), vitamin C, vitamin E, etc. An imbalance caused by increased generation of free radicals and decreased functional efficiency of this ADS has been suggested to be one of the primary factors that contributes to the ageing process[7]. Though increased generation of free radicals has been demonstrated with increase in age, decrease in functional capacity of ADS is still under investigation. But melatonin, the hormone with antioxidative function, declines significantly with increase in age[5,7,23,26,28]. This will give rise to increased oxidative stress that can cause much cellular damage resulting in senescence. Recently, genetic studies have shown the presence of ADS genes which are important for regulating the synthesis of antioxidative enzymes[61]. According to Arking *et al.*[61], in L strain *Drosophila melanogaster* enhanced expression of ADS genes increased the synthesis of antioxidative enzymes like Mn SOD, Cu–Zn SOD and catalase. These enzymes reduced the intensity of oxidative stress and prolonged the life span of these organisms[61]. In addition to these longevity enhancing genes (ADS genes), the rate of ageing is also influenced by other genes that control the formation of cellular enzymes. Decreased transcription of these various genes results in decreased synthesis of cellular enzymes[62]. No single master gene that controls the activity of all enzymes has been identified, instead a number of genes that are involved in the control of different cellular enzymes determine the ageing process. These genes have facilitating effects during the early phase of life when they are engaged in promoting the synthesis of cellular enzymes[62,63]. The rate of expression of all these genes is influenced by hormonal factors and it is possible to modify the activities of these genes by modulating the hormonal levels. Increasing steroid levels have been found successful in enhancing genetic expression and promoting the synthesis of cellular enzymes[62].

Melatonin as an antioxidant

Melatonin is implicated in ageing because it has well-demonstrated antioxidant functions[5]. The hormone has been shown to participate in the ADS both as a radical scavenging antioxidant and as a preventive antioxidant[64]. It was Reiter[65], who first suggested that melatonin may have a role in scavenging free radicals. The capacity of melatonin as an antioxidant was first tested in a simple cell-free *in vitro* system wherein H_2O_2 was exposed to 254 nm ultra violet light to generate hydroxyl ($^{\bullet}OH$) radicals. Since $^{\bullet}OH$ radicals have very short half life, the rate of generation of these radicals

was measured by adducting them with a spin trapping agent 5,5-dimethyl-pyrroline-*N*-oxide or DMPO and these adducts have longer half life. The adducts (DMPO–$^{\bullet}$OH) were then quantitatively and qualitatively evaluated by using high pressure liquid chromatography (HPLC) with electrochemical deduction and electron spin resonance spectroscopy. By adding melatonin to this mixture, Tan *et al.*[66] were able to demonstrate that the hydroxyl ($^{\bullet}$OH) radical scavenging ability of melatonin was found to be far superior than the known intracellular antioxidants like GSH or mannitol. The concentration of melatonin needed to scavenge $^{\bullet}$OH radicals in this study was only 21 μg compared to GSH (121 μg) or mannitol (183 μg). Following this observation, another group of investigators tested the peroxyl (LOO$^{\bullet}$) scavenging ability of melatonin by using *in vitro* systems. In this study, it was found that the efficiency of LOO$^{\bullet}$ scavenging ability of melatonin was compared with trolox (a water soluble vitamin E), vitamin C and GSH. The potency was melatonin > trolox > vitamin C > GSH. Melatonin was found to be twice as efficient as vitamin E in scavenging peroxyl radicals[67].

Soon *in vivo* studies were conducted to demonstrate the efficiency of melatonin as an antioxidant. In these studies on rats, Tan *et al.*[68] injected chemical carcinogen safrole, a known free radical generating compound, to rats with a dose of 300 mg/kg with or without melatonin (0.2 to 04 mg/kg). The amount of melatonin was nearly 1500 to 750 times less than that of the safrole. Twenty four hours later it was noticed that the rats which received only safrole exhibited extensive hepatic nuclear DNA damage as revealed by the study of DNA adduct formation. But, the rats that received both safrole and melatonin showed only DNA damage that was very much lower, >40% and 90% less for 0.2 mg and 0.4 mg melatonin. Since this study demonstrates action of melatonin in pharmacological concentrations, the same workers designed another study to demonstrate the antioxidative potential of endogenous melatonin secreted by the pineal gland. In this study, safrole (100 mg/kg) was injected into a group of rats during day time when endogenous melatonin secretion is expected to be very low. The same dose of safrole was injected into another group of rats at night time early in the dark phase, when melatonin secretion would be rising to a peak level. When examined 8 h after the injection of safrole, it was found that the liver of the animals that received safrole at night exhibited less extensive DNA damage than those that received the injection at day time. Since no exogenous melatonin was given in this study, it was concluded that endogenous melatonin secreted by the pineal gland at night time was sufficient enough to resist the oxidative damage induced by safrole[69].

The observation that melatonin circulating in the body fluids give protection to cells and tissues against oxidative attack was further evaluated by another group of investigators[70]. Exposure to ionizing radiation induces DNA damage both by direct as well as indirect mechanisms and release of hydroxyl ($^{\bullet}$OH) radicals is one of the major indirect means by which ionizing radiation induces nuclear DNA damage. Human volunteers were administered with pharmacological doses of melatonin (300 mg) so as to increase the concentration of melatonin in their body fluids. Blood samples were then obtained from the volunteers at 0 h, 1 h, and 2 h after melatonin ingestion and the lymphocytes obtained from their blood samples were exposed to 150 C$G\gamma$ radiation. The genetic damage induced by ionizing radiation on lymphocytes was assessed by several parameters like the presence of chromosomal aberrations and micro nuclei. In this study it was noticed that lymphocytes collected either 1 h or 2 h after melatonin intake, exhibited significant decrease in the incidence of chromosomal aberrations and micro nuclei than those similarly irradiated by 150 C$G\gamma$ radiation at 0 h after melatonin administration. Exogenously administered melatonin or endogenously secreted melatonin crosses the cell membrane and gets concentrated more in the nucleus than in the cytosol[40]. The tendency of melatonin to get concentrated in the nucleus, coupled with its ability to scavenge $^{\bullet}$OH radicals provided the effective means by which the lymphocytes were protected from radiation-induced genetic damage. Thus DNA protection by melatonin revealed its high level diffusibility. By being very close to DNA molecules, melatonin was able to prevent genomic damage induced by gamma radiation[47].

The ability of melatonin in protecting cytosolic proteins from oxidative attack has been well demonstrated in the following study. Cataractogenesis is known to be caused by the damage inflicted on lenticular proteins by free radicals, where the lens becomes cloudy following oxidative attack. The presence of GSH in the lens normally protects the lenticular proteins from oxidative attack. Buthionine sulphoximine (BSO), an inhibitor of the enzyme γ glutamyl cysteine synthetase, will deplete GSH stores by reducing its synthesis. When BSO is given shortly after birth to new born rats, they develop cataract at the time their eyes are open (around two weeks of age), when melatonin secretion is very low. To test whether melatonin has the ability to protect the lenticular proteins from oxidative attack in the absence of GSH, Abe *et al.*[71] administered melatonin during the first two weeks to BSO-injected rats. It was found that melatonin treatment reduced the incidence of cataract to < 10% when compared to 90% in rats that received only BSO, showing that melatonin protected the lenticualr proteins from oxidative attack.

Oxidative stress has been implicated in neuro degenerative disorders like Parkinsonism and Alzheimer's diseases[48]. Parkinsonism can be produced in rapid and

dramatic form by injection of MPTP (1-methyl-4-phenyl-1,2,5,6-tetra-hydro-pyridine) to experimental animals. MPTP is metabolized by the enzyme mono amine oxidase B (MAO B) present in the astrocytes into MPP+(1-methyl-4-phenyl-pyridinium) which causes selective destruction of dopaminergic neurons of substantia nigra resulting in Parkinsonism. The process involves generation of a number of free radicals like superoxide, hydroxyl ($^{\bullet}OH$) and peroxyl ($LOO^{\bullet}$) radicals[72]. Epidemiological studies have shown that people who have been consuming cereals and grains from crops grown in the soil infested with paraquat, a toxic herbicide, developed symptoms of Parkinsonism[73]. Paraquat, an analogue of MPP^+, when administered into rats caused extensive lipid peroxidation in the lung tissues[73], and also in different regions of the brain particularly the corpus striatum[74].

To study the *in vivo* effect of melatonin on MPTP-induced neurotoxicity, Acuna Castroviego *et al.*[74] injected MPTP (20 mg/kg) to different groups of mice followed by either saline or melatonin (10 mg/kg) injections. Within 4 h after MPTP injection, there was significant increase in lipid peroxidation products like malondi aldehyde (MDA) and 4-hydroxy alkenals (4-HDA) in both striatum and hippocampus. MDA and 4-HDA are degraded lipid peroxidation products in cell membranes that are taken as an index of oxidative damage. In these animals, cloudy swelling of cells and hyperchromatic nuclei indicative of necrotic conditions were seen in the hippocampus and striatal area. The melatonin treatment along with MPTP not only reduced or eliminated the morphological changes but also abolished the MPTP-induced increase in lipid peroxidation products. The neuroprotective effects of melatonin against MPTP-induced lipid peroxidation have been proved in this study. The implication of this study is that melatonin as an endogenous antioxidant and non-toxic compound has a potential, beneficial value in treating Parkinsonism. Maintaining a high level of melatonin in old age may be helpful in resisting the development of Parkinsonism and other neuro degenerative disorders[48].

Kainic acid is a non degradable analogue of glutamate and its neuro toxic effects are attributed to the interaction of free radicals with macromolecules. Melchiorri *et al.*[75] incubated rat brain homogenates from cerebral cortex, cerebellum, hippocampus, hypothalamus and striatum with kainic acid in the presence or absence of melatonin. Kainic acid induced accumulation of lipid peroxidation products like MDA and HDA in all the brain areas studied. But addition of melatonin to the incubation medium significantly reduced kainate-induced accumulation of lipid peroxidation products. From this, the authors concluded that melatonin in sufficient concentrations even when present at physiological levels can reduce oxidative damage to macromolecules such as lipids or proteins[75]. In a condition known as infantile lipofuscinosis, accumulation of lipofuscin, the product of lipid peroxidation occurs in greater concentration. Melatonin rhythm is completely absent in this condition, showing that low levels of melatonin in these children only contribute to the increased lipid peroxidation[27].

Alzheimer's disease (AD) is yet another neuro degenerative disorder that occurs in aged individuals and is reported to be caused by deposition of senile plaques made up of amyloid β protein in cerebral and meningeal blood vessels. Release of free radicals has been suggested to be one of the reasons for amyloid β protein-induced neuro toxicity that results in AD. Exposure of cultured neuroblastoma (N2 A) cells to amyloid β protein resulted in marked cell damage characterized by diffuse membrane blebbing, cell retraction, abnormal distribution of chromatin towards the nuclear membrane, and karyorrhexis (fragmentation and condensation of nuclear material). Addition of melatonin to the culture media effectively prevented all the above-mentioned toxic effects induced by amyloid β protein. Melatonin prevented these changes by inhibiting lipid peroxidation as well as Aβ induced reduction of superoxide dismutase (SOD)[76]. The close association between ageing and AD and the similarities observed in the neuropathology of both conditions have prompted Pappolla *et al.*[76] to postulate that oxidative stress plays a major role in the pathogenesis of AD lesions. The study also points out that melatonin has a preventive role in ageing and neuro degenerative processes.

Melatonin: Its anti-oxidant action

Melatonin acts both as a radical scavenging and preventive antioxidant. The presence of an acetyl group in the side chain and a methoxy group at position 5 of the indole nucleus seems to be essential for its radical scavenging action[77]. When melatonin reacts with hydroxyl ($^{\bullet}OH$) radicals it contributes an electron and renders the hydroxyl radicals non toxic. In this process melatonin itself is converted into a weak radical known as indolyl cation radical which then scavenges superoxide radicals[66]. The indolylcation radical is then converted into *N*-acetyl-*N*-formyl-5-methoxy kynuramine. From this reaction, it is presumed that the likelihood of additional $^{\bullet}OH$ radicals being produced is reduced because of O_2^- (superoxide radical) is a necessary precursor of hydroxyl radicals[47].

Besides participating directly in the free radical scavenging action, melatonin disposes free radicals by acting as a preventive antioxidant. Preventive antioxidants arrest the formation of free radicals by enzymatic decomposition of their precursor molecules[64]. Glutathione peroxidase (GSH-Px) is the major antioxidative enzyme present in the brain which decomposes H_2O_2 to H_2O molecules. By doing so, it reduces the formation of hydroxyl

radicals. Melatonin has been shown to stimulate GSH-Px concentration when administered in physiological or pharmacological doses[64,78,79]. Melatonin also enhances the activity of glucose-6 phosphate dehydrogenase (G-6 PD) which is an antioxidative enzyme[79]. The probable mechanism by which melatonin increases the antioxidative enzyme activity is not known at present. As enhanced expression of ADS genes can increase the synthesis of antioxidative enzymes like SOD and catalase[61], and since melatonin has direct genomic actions[41,42,46], it is likely that melatonin increases the activity of ADS genes and thereby promotes the synthesis of antioxidative enzymes. Modulation of hormonal levels has been shown to modify the activity of genes that controls the level of various cellular enzymes[62]. Figure 3 is a schematic diagram showing the probable actions of melatonin for reducing the level of oxidative stress and for retarding the ageing process. Melatonin also reduces the activity of nitric oxide synthetase (NOS) activity[80]. NOS is considered as a pro-oxidant enzyme. It belongs to a family of enzymes that are homologous to cytochrome 450 reductase and help in the synthesis of nitric oxide (NO) from L-arginine. NO is free radical having only one paired electron. It reacts with superoxide radicals to form peroxynitrate anion (ONOO–) which can induce DNA damage by its own and also by generating fresh hydroxyl radicals. At physiological concentration, melatonin has been shown to reduce NOS activity in rat cerebellum thereby reducing the chance of peroxy nitrate and hydroxyl radical generation[80].

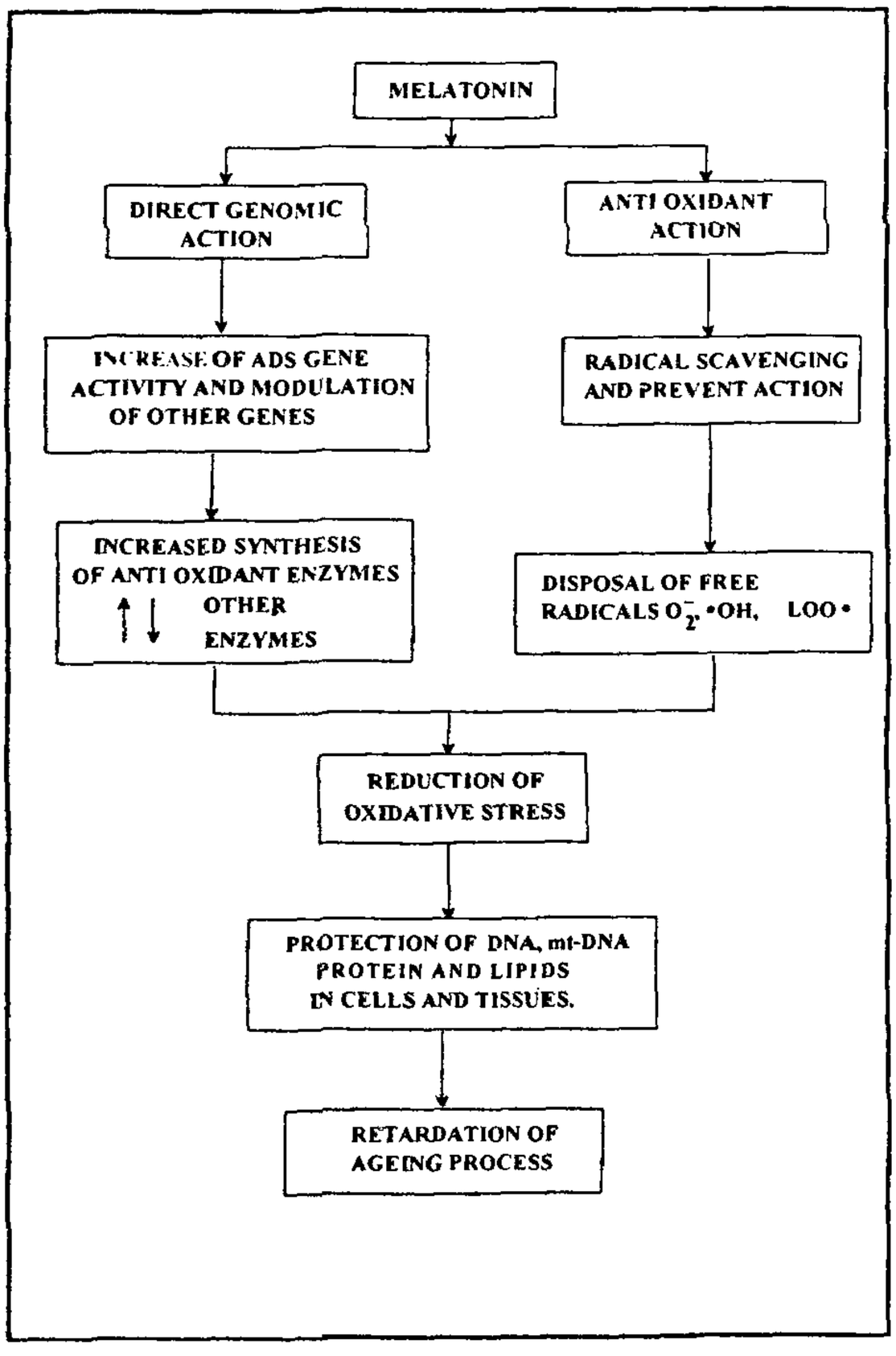

Figure 3. Role of melatonin in the ageing process.

Melatonin: Anti-ageing effects

The phenomenon of ageing has been suggested to be due to increased DNA damage, decreased capacity of DNA repair or both[55]. The antioxidative actions of melatonin reviewed in this paper strongly support the fact that melatonin protects DNA molecules, lipids and proteins from oxidative attack and it is this action that is responsible for its anti-ageing effects. Once secreted melatonin permeates through all cells and tissues with ease because of its high diffusibility and being ubiquitous in distribution it is able to act as an effective antioxidative molecule throughout the body, particularly in the brain region[7]. Armstrong and Redman[81] have given evidences to support that melatonin's anti-ageing effect reflects its physiological role rather than its pharmacological artefact. Maestroni *et al.*[82] showed that administration of melatonin in drinking water (10 µg/ml) increased survival rate of rats by 20% (931 versus 752 days) showing that melatonin has the capacity to increase life span. Since melatonin is non toxic and is freely available, it can be administered to aged persons regularly for prevention of degenerative changes. In this context it is worthwhile to consider the administration of this hormone in neuro degenerative disorders like Parkin-sonism and AD. In both these conditions, oxidative stress plays a predominant role.

Conclusion

Melatonin, the major hormone secreted from the pineal gland has been shown to participate in a number of physiological functions like regulation of reproduction, sleep and circadian rhythms. Its involvement in the control of ageing process has gained much importance in recent years because of its well-demonstrated antioxidative functions. Melatonin acts both as a radical scavenging and preventive antioxidant[7,48]. Since melatonin disposes free radicals and the level of this hormone falls significantly with increase in age, it has been suggested that the decline in the antioxidative capacity of the body is the main reason for ageing[5]. Since melatonin has direct genomic actions[41,42,46] and changes in the hormonal level can affect genetic expression[62], it

is likely that the decline in melatonin concentration with increase in age can reduce the activity of ADS genes resulting in decreased concentration of antioxidative enzymes causing much cellular damage and senescence. As melatonin has been able to counteract the effect of MPP+ induced neuro-toxicity and amyloid β protein-induced neuronal damage, it has a potential value in treating neuro-degenerative disorders like Parkinsonism and AD. Since both these disorders occur mainly in aged persons, there is every reason to believe that melatonin has a role in the ageing process.

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