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Pineal function during aging: attenuation of the melatonin rhythm and its neurobiological consequences

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Abstract. The pineal hormone melatonin is a potent free radical scavenger. In particular, it quenches what is generally considered the most toxic and damaging free radical produced in the organism, the hydroxyl radical (•OH). Melatonin production in the pineal gland declines progressively with age such that in old animals and elderly humans the levels of melatonin available to the organism are a fraction of that of young individuals. A prominent theory of aging claims that the anatomical and functional degeneration that organs undergo during aging is a consequence of accumulated free radical damage. This being so, melatonin may well play a significant role in aging processes. If the drop in melatonin which normally occurs as animals age could be prevented, perhaps the aging process would also be delayed. Also, supplemental administration of melatonin may be beneficial in delaying age-related degenerative conditions. Certainly, free radical damage has been implicated in a number of neurodegenerative disorders. Theoretically, melatonin administration may forestall these as well.

Key words: pineal gland, melatonin, aging, free radical scavengers, hydroxyl radical, neurodegenerative disorders

Like the function of many organs, that of the pineal gland regresses with advancing age (Reiter, 1992). This degeneration is most obviously reflected in the gradual reduction in the amplitude of the nocturnal melatonin rise (Iguchi et al. 1982). The consequences of the attenuated melatonin cyclic during aging may be widespread and, in fact, it has been speculated that the loss of the melatonin cycle may lead to other age-related changes that are generally detrimental to the organism (Armstrong and Redman 1991, Grad and Rozencwaig 1993, Poeggeler et al. 1993, Reiter et al. 1993, Pierpaoli and Regelson 1994). Indeed, the association of the pineal gland and melatonin with aging generally and with a variety of age-related diseases in particular is receiving increased attention. As a result, the potential beneficial effects of exogenously administered melatonin in age-related neurodegenerative conditions is of special interest (Poeggeler et al. 1993, Reiter et al. 1993, 1994). This brief review summarizes the changes in the circadian melatonin rhythm that animals, including man, experience with age and it also considers some of the potential neurobiological consequences of the gradual depression of the melatonin cycle.

CHANGES IN THE BIOSYNTHETIC CAPABILITIES OF THE PINEAL GLAND DURING AGING

The chief pineal hormone, melatonin, is produced in a circadian manner in the pineal gland of all mammals. Typically during the day, pineal melatonin production and secretion remains uniformly low while at night the synthesis and discharge of this important hormone increases markedly; this unique nighttime secretion of melatonin has led to the concept that melatonin is the chemical expression of darkness (Reiter 1991a). The intracellular mechanisms governing the nocturnal production and release of melatonin by the mammalian pineal gland are well understood (Reiter 1991b). Light, which is a natural consequence of day, synchronizes the melatonin rhythm to 24 hours and the imposition of light at night, provided it is of sufficient intensity, rapidly and completely depresses high nighttime melatonin production (Illnerova et al. 1985, Lewy et al. 1980, Stokkan and Reiter 1994).

Throughout life the pineal gland of all animals produces melatonin more abundantly at night than during the day. Since melatonin is rapidly released from the pineal gland once it is produced, the blood melatonin rhythm reflects the amount being produced in the pineal at virtually the same time. Thus melatonin concentrations in the blood are also higher at night than they are during the day. However, especially in the human the amplitude of the nocturnal rise varies greatly among individuals (Arendt 1988). Thus, persons of roughly the same age may exhibit widely divergent nocturnal blood melatonin concentrations (Fig. 1). Since the rhythm is genetically determined, the cycle within an individual is highly stable.

As advanced age approaches, the ability of the pineal gland to produce melatonin gradually dim-

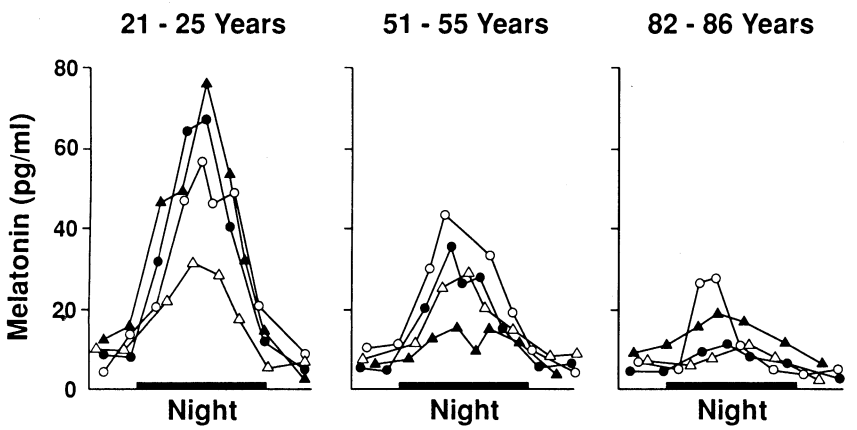

Fig. 1. Blood melatonin rhythms in four human males in each of the age groups indicated. As shown, during aging the nocturnal increase in blood melatonin concentrations wanes.

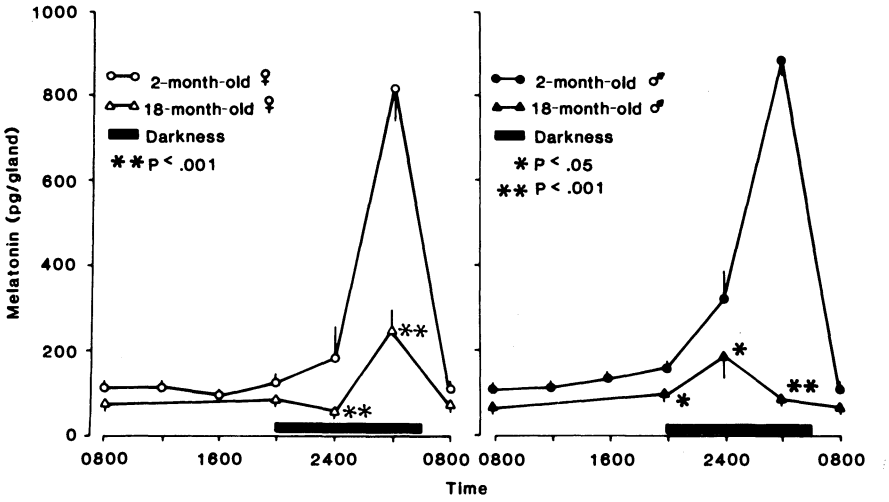

Fig. 2. Pineal melatonin levels in 2-month-old and 18-month-old male and female Syrian hamsters. The reduced melatonin rhythm seen in the pineal gland of hamsters is typical of all mammals studied to date. From Reiter et al. (1990).

inishes such that, in old age, the pineal melatonin rhythm is only a vestige of that in younger animals (Reiter et al. 1980, 1981) (Fig. 2). The attenuated rhythm in the pineal gland is also reflected in a gradual loss of melatonin from the blood (Fig. 1) and reduced melatonin metabolites in the urine (Sack et al. 1986). Since melatonin enters cells with ease (Menendez-Pelaez and Reiter 1993), the gradual reduction of blood melatonin concentrations is likely accompanied by lower intracellular levels of this important hormone; since the cell nuclei particularly accumulate melatonin (Menendez-Pelaez et al. 1993), presumably this portion of the cell suffers most from the reduction in pineal melatonin production.

The mechanisms which account for the depressed melatonin rhythm in aged mammals probably involves a reduced number of β-adrenergic receptors on the pinealocyte membranes (Henden et al. 1992) and damage to cells in the suprachiasmatic nuclei (SCN) of the hypothalamus (Poeggeler et al. 1993) which are involved in transfer of photoperiodic information from the retinas to the pineal gland. Normally, norepinephrine released from sympathetic nerve terminals within the pineal gland interact with β-adenoreceptors leading to stimulation of melatonin synthesis (Reiter 1991b). Damage to neurons in the SCN occurs primarily because glutamate, an excitatory amino acid neurotransmitter released from retinohypothalamic neurons onto SCN cells, induces the formation of a vary of toxic oxygen radicals eventually leading to destruction of the post synaptic neurons (Poeggeler et al. 1993). Interestingly, melatonin is a potent oxygen radical scavenger (Tan et al. 1993a), and therefore it may assist in delaying degeneration of these cells.

CONSEQUENCES OF THE ATTENUATED MELATONIN RHYTHM DURING AGING

The physiology of the pineal hormone melatonin was initially defined in terms of its regulation of the neuroendocrine-reproductive axis in photoperiodic species (Reiter and Fraschini 1969). In a wide variety of mammals whose reproductive state changes seasonally with the photoperiod, it is the changing melatonin rhythm that signals the neuroendocrine-reproductive axis as to the prevailing photoperiodic conditions. Besides it effects on reproductive physiology, the pineal gland, via its hormone melatonin, regulates a variety of other neuroendocrine functions as well.

Melatonin's actions, however, far transcend its regulatory influence on the hypothamo-pituitary axis. Indeed, its most important function may well be manifested within every cell in the organism. Recently, Tan and colleagues (1993a) demonstrated that melatonin is a very potent scavenger of

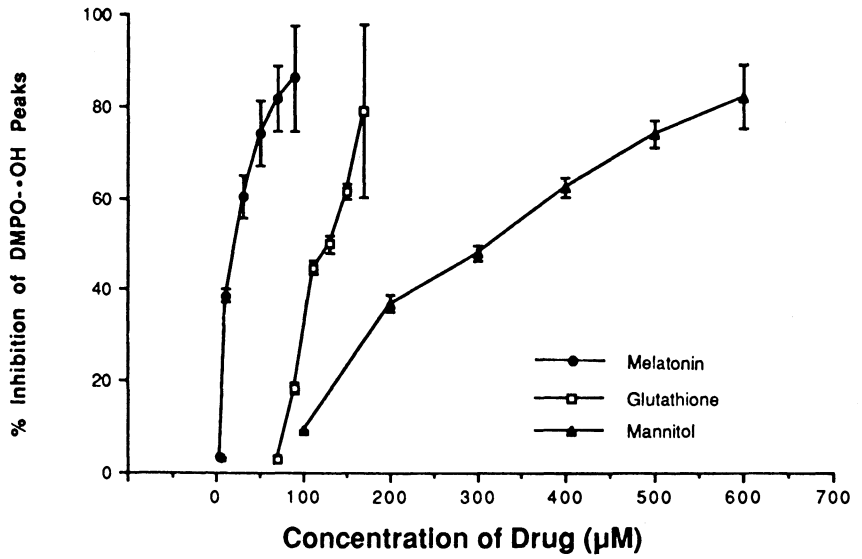

Fig. 3. Compared to glutathione and mannitol, two well known oxygen radical scavengers, melatonin proved to be significantly more effective in quenching the highly toxic •OH. The concentrations of each compound (IC_{50}) required to scavenge 50% of the •OH in a defined *in vitro* system were 21 μM, 123 μM and 283 μM for melatonin, glutathione and mannitol, respectively. Adapted from Tan et al. (1993).

the highly toxic hydroxyl radical (•OH). Remarkably, relative to other well known free radical scavengers, i.e., glutathione and mannitol, melatonin proved to be 5X and 14X more effective, respectively (Fig. 3). The •OH is widely considered to be the most highly reactive and damaging free radical produced in the organism (Halliwell and Aruoma 1991) (Fig. 4).

•OH are formed from molecular oxygen (O_2). More than 95% of the oxygen taken into an organism is efficiently used for the production of energy at the level of cellular mitochondria. However, up to 5% of the O_2 inhaled is reduced by a single electron to the superoxide anion (Fig. 5). The superoxide anion is weakly toxic and can damage some molecules; it is also quickly converted to hydrogen peroxide (H_2O_2) in the presence of the enzyme superoxide dismutase (SOD) (Fridovich 1978). H_2O_2, at concentrations normally produced intercellularly, is relatively non-toxic and it is quickly metabolized by catalase or glutathione peroxidase to water and O_2. However, in the presence of transition metals, e.g., iron or copper, H_2O_2 is converted to the •OH via what is referred to as the Fenton reaction (Fig. 6).

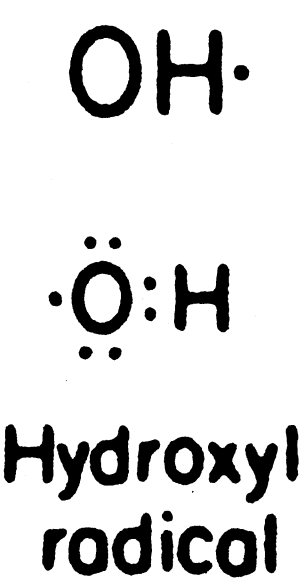

Fig. 4. The highly toxic hydroxyl radical is a result of the 3 electron reduction of molecular oxygen. This radical is highly reactive and readily damages macromolecules in the vicinity of where it is produced.

The high toxicity of the •OH leads to damage of macromolecules such as lipids, proteins and DNA (Kehrer 1993). The brain is especially vulnerable to oxidative attack because (1) the central nervous system contains high concentrations of non-heme iron, (2) the brain, relative to its size, utilizes large quantities of oxygen, (3) ascorbic acid, which is generally considered an antioxidant also possess prooxidant actions, is in high concentrations in the brain, (4) the brain contains large amounts of 22:6 and 20:4 unsaturated fatty acids which are easily oxidized, and (5) neurons, because they do not divide, once damaged may continue dysfunctioning for life (Harman et al. 1976, Sadrzadek and Eaton 1988).

That melatonin can prevent oxidative changes *in vivo* is inferred from a number of studies. Normally,

$$O_2 + 1e^- \longrightarrow O_2^-$$

$$O_2 + 2e^- + 2H^+ \longrightarrow H_2O_2$$

$$O_2 + 3e^- + 3H^+ \longrightarrow \cdot OH + H_2O$$

Fig. 5. A small percentage of the molecular oxygen (O_2) inhaled is initially reduced to the superoxide anion (O_2); the one electron reduction of the superoxide anion, a step that also requires the enzyme superoxide dismutase (SOD), leads to the production of hydrogen perioxide (H_2O_2). H_2O_2 is further reduced to the hydroxyl radical (•OH).

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + \cdot OH$$

$$H_2O_2 + Cu^+ \longrightarrow Cu^{2+} + OH^- + \cdot OH$$

Fig. 6. In the presence of either iron (Fe^{2+}) or copper (Cu^{1+}), H_2O_2 is quickly converted to the hydroxyl radical which is highly toxic to macromolecules. This reaction of transition metals with H_2O_2 to form the hydroxyl radical is known as the Fenton reaction.

Ca^{2+}-stimulated + Mg^{2+}-dependent ATPase extrudes excess calcium from cells. In cardiomyocytes the activity of this enzyme is regulated by the number of free radicals produced in the tissue (Kaneko et al. 1989). Generally, increased production of oxyradicals reduces the activity of the Ca^{2+} pumping enzyme. Thus Chen et al (1993) reasoned that high blood melatonin levels *in vivo* would lead to a commensurate elevated activity of the Ca^{2+} pump. They showed in fact that cardiac ATPase activity is highest in the late night/early morning immediately following the nocturnal peak of melatonin; this nocturnal rise in Ca^{2+} pump activity was obliterated by the elimination of the circadian melatonin rhythm following surgical removal of the pineal gland. Chen and colleagues (1993, 1994) further showed that the activity of Ca^{2+} pump activity rose in cardiomyocyte membranes treated with melatonin. The actions of melatonin on the activity of the Ca^{2+} pump were believed to be due to melatonin's antioxidant actions. Using other systems, the work of Ianas et al. (1994) and Pierrefiche et al. (1993) also has provided evidence of the antioxidant capacity of melatonin.

Other indirect, but very compelling, evidence for melatonin's free radical scavenging activity *in vivo* is provided by the work of Tan and associates (1993b, 1994). In these studies rats were treated with a chemical carcinogen, safrole, which is highly toxic and damages intracellular macromolecules because it induces the formation of oxygen-based free radicals. The damage to DNA is very marked in safrole-treated animals and the altered DNA can be quantified using a variety of techniques. Tan and

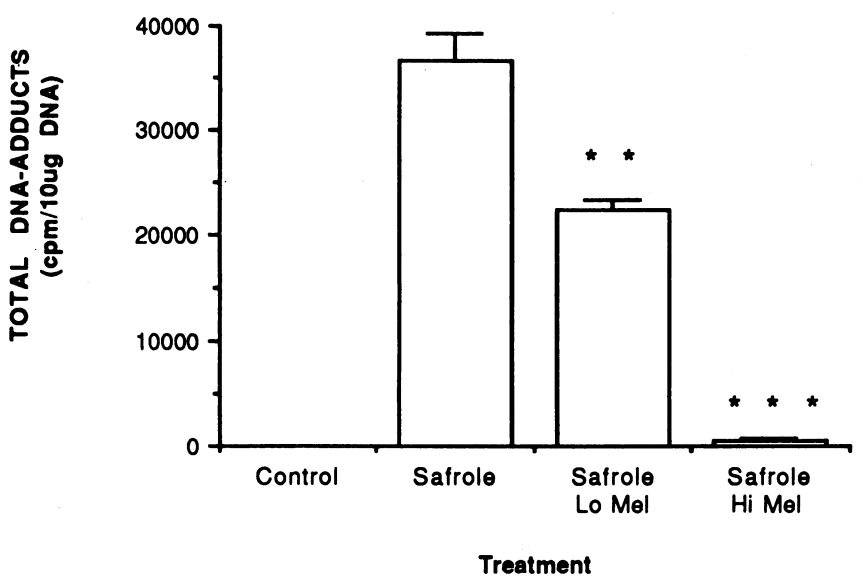

Fig. 7. Mean (±SEM) levels of DNA adducts, representing DNA damage, in the liver of rats treated with the chemical carcinogen safrole without or with either a low (0.2 mg/kg) or a high (0.4 mg/kg) dose of melatonin. Control rats received neither safrole nor melatonin. From Tan et al. (1993b).

colleagues (1993b, 1994) utilized a ^{32}P-post labeling method of DNA followed by chromatography and quantitative autoradiography (Reddy and Randerrath 1986) to estimate the cellular damage induced by safrole with and without melatonin pretreatment. When rats were treated with a 300 mg/kg dose of safrole, 24 h later massive damage to hepatic DNA was apparent. On the other hand, if the animals were simultaneously treated with either a 0.2 mg/kg or 0.4 mg/kg dose of melatonin, DNA damage was reduced by more than 40% and 90%, respectively (Fig. 7). Thus with doses of melatonin 1500X and 750X less than safrole, melatonin afforded very significant protection for DNA against the highly toxic carcinogen safrole. Even though these doses of melatonin were very much lower than that of safrole, they nevertheless produced supraphysiological levels of melatonin in the blood. Hence, to verify that even physiological levels of melatonin provided protection against safrole-mediated DNA damage, Tan and co-workers (1994) went on to show that the nighttime rise in endogenous melatonin production was sufficient to counteract a portion of the damage normally inflicted by safrole administration. This indicates that melatonin is not only an effective pharmacological agent in free radical protection, but that at physiological concentrations it is also a relevant and potent antioxidant *in vivo*.

An important feature of any antioxidant is that it be near the cellular component it is destined to protect. Hence, to be important as an intracellular free radical scavenger, the molecule must get into the cell and be present in sufficiently high concentrations to be effective. It has long been known that melatonin is highly lipophilic and, therefore, it passes through cellular membranes with ease. Furthermore, it was recently suggested that melatonin is not as hydrophobic as originally thought. Melatonin's solubility in H_2O is as high as 5×10^{-3} M (Shida et al. 1994) which allows it easy transport through the cytosol and into the nucleus. While some of melatonin's actions are undoubtedly exerted through membrane bound receptors (Morgan and Williams 1989, Weaver et al. 1991), other actions of this molecule may utilize the recently described nuclear binding sites (Acuña-Castroviejo et al. 1993, 1994). Finally the ability of melatonin to scavenge •OH clearly does not require either a receptor or binding molecule (Tan et al. 1993a).

That melatonin is not only capable of but in fact does get into subcellular compartments was recently demonstrated (Menendez-Pelaez and Reiter 1993, Menendez-Pelaez et al. 1993). For years, immunocytochemists had claimed that within the cell the reaction product for melatonin was located primarily in the cytosol (Bubenik et al. 1974, Vivien-Roels et al. 1981). However, more recent studies seem to indicate that this is not the case. Mennenga et al. (1991) proposed a nuclear localization for melatonin. This concept was recently supported by the observations of Menendez-Pelaez and co-workers (1993) who used both immunocytochemistry and radioimmunoassay, after cellular fractionation, to prove the highest concentrations of melatonin are located in the nuclear fraction rather than in the cytosol. Interestingly, a re-examination of earlier published immunocytochemical and immunofluorescent photomicrographs (Menendez-Pelaez and Reiter 1993) reveals that in at least some cases the figures were misread and indeed even in some of the published photomicrographs the concentration of the melatonin reaction product is higher in the nucleus than in the cytosol. This misinterpretation is exemplified by a paper related to the immunofluorescence of melatonin wherein the published micrographs clearly show the fluorescent product in the nuclei although the authors conclude that it is the cytosol that exhibits the highest melatonin levels (Tillet et al. 1989). In general, it seems that some authors may have relied too heavily on the early publications and have misinterpreted their own findings as a result. The nuclear melatonin binding sites described by Acuña-Castroviejo et al. (1993, 1994) may account in part for the higher levels of melatonin in the nucleus. Additionally, melatonin may intercalate with and possibly bind to DNA, further increasing its intranuclear concentration (D.X. Tan, R.J. Reiter, L.D. Chen, B. Poeggeler, unpublished observations). Within the nucleus, melatonin

levels can reach concentrations of micromolar levels (Menendez-Pelaez et al. 1993).

Brain levels of melatonin are highly reliant on melatonin derived from the blood. Within 30 min after the administration of exogenous melatonin, when blood levels are high, concentrations of the indole in neural tissue are also elevated (Menendez--Pelaez et al. 1993). Likewise, melatonin levels are higher in brains of rats collected at night, when endogenous blood melatonin levels are also elevated, than in those collected during the day. Finally, pinealectomy, which reduces circulating melatonin levels, causes a commensurate large drop in brain melatonin concentrations. Considering melatonin's potent •OH radical scavenging activity (Tan et al. 1993a), the loss of melatonin would leave the brain highly vulnerable to oxidative attack. Thus, aging which is associated with a marked drop in endogenous melatonin availability would leave neural tissue progressively more exposed to highly damaging free radicals (Poeggeler et al. 1993, Reiter et al. 1993). Besides its ability to directly scavenge the •OH, we also have found that its exogenous administration induces a rise in neural glutathione peroxidase, an important antioxidative enzyme. Thus, melatonin may not only reduce oxidative damage by directly scavenging free radicals but it may indirectly do so by stimulating other antioxidative processes (L.R. Barlow-Walden, B. Poeggeler, R.J. Reiter, unpublished observations).

It is very likely that free radical attack on neurons or neural tissue is involved with a number of neurodegenerative conditions in the aged. Degeneration of the nervous system during aging does not occur uniformly in the brain. Excitatory amino acid neurotransmitters, because they induce the formation of free radicals, are especially destructive to neurons (Murphy et al. 1989, Poeggeler et al. 1993). Hence areas of the brain which contain nerve endings that release excitatory amino acids degenerate more quickly than do other portions of the brain. The reduction of melatonin with age would seemingly accelerate the damage produced by reactive oxygen species. Also, protection against free radical generation which follows stress or exposure to toxic agents would be partially lost with aging as melatonin levels fall.

Specific neurodegenerative conditions that have been linked to oxygen-based radicals include Parkinson's and Alzheimer's diseases. Although in neither of these conditions is the cause (or causes) of the disease understood *in toto*, there is evidence that neuronal degeneration occurs in part because of unchecked free radical attack. For example, Parkinsonism has been associated with exposure to neurotoxic agents (Langston et al. 1983), a generalized increase in the production of free radicals (Adams and Odunze 1991), an accelerated dopamine turnover (Spina and Cohen 1989) and higher than normal levels of lipid peroxidation (Dexter et al. 1986). Each of these conditions would normally be associated with increased free radical damage. Finally, a role for iron in the etiopathology of Parkinson's disease has been proposed (Youdim et al. 1993), this transition metal, as indicated above, is directly involved with the production of •OH. Other findings also implicate free radical damage in the etiology of Parkinson's disease (Reiter et al. 1994).

Although less apparent than in Parkinson's disease, Alzheimer's dementia may also involve free radical damage to key central nervous system structures (Hajimohammadreza and Brammer 1990, Subborao et al. 1990). Without question, neurodegenerative conditions are most frequently observed in the elderly and the progression of these diseases often accelerate as an individual ages. Also, in advanced age melatonin production and secretion by the pineal gland is at its lowest level (Reiter 1992). The question thus arises, are the depressed melatonin levels consequential in reference to neurodegeneration? Interestingly, there is even evidence that Alzheimer's subjects have lower melatonin levels than non-Alzheimer's subjects (Nair et al. 1986, Skene et al. 1990). However, whether melatonin production is lower because these individuals suffer from dementia, whether it is related to the condition, or whether it is merely coincidental with Alzheimer's disease remains to be established.

CONCLUDING REMARKS

For a number of years, the pineal gland and melatonin have been implicated in the processes of aging. A number of theories has arisen and each implies a very different function for melatonin in delaying aging (Armstrong and Redman 1991, Grad and Rozencwaig, 1993, Pierpaoli and Regelson 1994). However, a basic conclusion of each of these theories is that the age-related decline in melatonin promotes organismal degeneration and, if melatonin could be maintained at levels seen in young individuals, aging and age-related diseases may be delayed. The most recent theory which promotes the beneficial effects of melatonin is directly linked to the free radical scavenging activity of the indole (Poeggeler et al. 1993, Reiter et al. 1993); a prominent theory of aging implicates free radicals as being causative (Harman 1992). If in fact free radicals play a major role in the degenerative processes of aging, which they seem to do, then exogenously administered melatonin may have some ameliorative effects on not only age-related neural degeneration but aging generally.

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