The Ageing Pineal Gland and Its Physiological Consequences

Russel J. Reiter

Summary

Melatonin, the chief hormone of the pineal gland, is produced and secreted into the blood in a circadian manner with maximal production always occurring during the dark phase of the light:dark cycle. Whereas the 24h rhythm of melatonin production is very robust in young animals including humans, the cycle deteriorates during ageing. The rhythm of melatonin can be substantially preserved during ageing by restricting the food intake of experimental animals; this same treatment increases the life span of the animals. The exogenous administration of melatonin to non-food restricted animals also reportedly increases their survival. Moreover, melatonin has been shown to have immunoenhancing effects and oncostatic properties. The implication of these studies is that melatonin may have both direct and indirect beneficial effects in delaying ageing processes or it may retard the development of processes (e.g., immunodeficiency and tumor growth) which contribute to a reduced life span.

Introduction

The pineal gland, a small endocrine organ located in the brain having shed a vestigiality complex which encumbered it for decades, has now been shown to be related to a wide variety of bodily functions. Although pineal physiology was initially defined in terms of its control of seasonal reproduction(‘), more recent investigations have linked this ubiquitously acting organ to such widely diverse functions as immunocompetence[2], circadian locomotor activity[3], oncogenesis[4], mood [5], and - in recent years - to the process of ageing[2].

The chief hormonal mediator of the pineal gland is N-acetyl-5-methoxytyrptamine, commonly known as melatonin. Melatonin’s synthesis within the pineal gland exhibits a marked circadian rhythm with maximal synthesis and secretion always occurring at night[6]. The cyclic production of melatonin within the pineal is generated by neuronal activity in the suprachiasmatic nuclei (SCN) of the hypothalamus with the rhythm being synchronized to 24h by the prevailing light; dark cycle[7,8]. The eyes, via the SCN, are anatomically and functionally linked to the pineal gland by way of neural connections in both the central and peripheral nervous systems[9]. Interruption of the neural connections at any point between the SCN and the pineal negates the ability of gland to produce and secrete its hormonal product melatonin. Likewise, either the extension of the light period into the night or the acute exposure of animals or man to light at night inhibits or interrupts, respectively, nocturnal melatonin production[8]. Because of its exclusive nighttime synthesis, melatonin has come to be known as the “chemical expression of darkness” and as the “Dracula of the endocrine system”.

Melatonin Synthesis and Its Rhythmic Nature

The nocturnal formation of melatonin is primarily a results of the release of norepinephrine (NE) from postganglionic sympathetic neurons that end in the gland. During the day, the SCN actively inhibit the release of NE from intrapineal neurons; conversely, at night the inhibitory message from the SCN to the pineal gland is suppressed and NE release proceeds unabated. Once released, NE interacts with β- and a-adrenergic receptors[10] on the membranes of the pinealocytes, the hormone producing cells of the pineal (Fig. 1.). The β-adrenergic receptors are linked via a stimulatory guanine nucleotide-binding regulatory protein to adenylate cyclase whose activation leads to intracellular increases in cyclic 3',5'-adenosine monophosphate (cAMP)[12]; cAMP is the intracellular second messenger which mediates the rise in the rate limiting enzyme in melatonin production, serotonin N-acetyltransferase (NAT)(“). The transcriptional and translational mechanisms intervening between the rise in cAMP and the nocturnal activation of NAT are poorly understood. The interaction of NE released from the intrapineal sympathetic nerves with a-adrenergic receptors leads to an amplification of the intrapinealocyte cAMP response; this translates into an augmented rise in NAT activity and also melatonin synthesis. About 85% of the melatonin formed each night within the pineal results from the activation of β-receptors by NE with 15% being a consequence of α-adrenergic receptor stimulation[11]. Besides NE and its adrenergic binding sites, there may be a variety of other neuromodulators and associated receptors which modify the nocturnal rise in melatonin production and release[14]. Once melatonin is synthesized, it is quickly released into the blood where, because of its highly lipophilic nature, it readily gains access to every fluid and presumably every cell in the organism[15]. As in the blood, in every fluid where it has been measured melatonin concentrations are higher at night than during the day. In almost all cases, pineal NAT activity and melatonin levels as well as melatonin concentrations in body fluids are well correlated, although under some circumstances these correlations do break down (see below).

At birth, the circadian melatonin rhythm in mammals is absent. In the rodent pineal the cycle becomes apparent postnatally shortly after the postganglionic sympathetic nerves grow into the developing gland[16]. In newborn humans, a

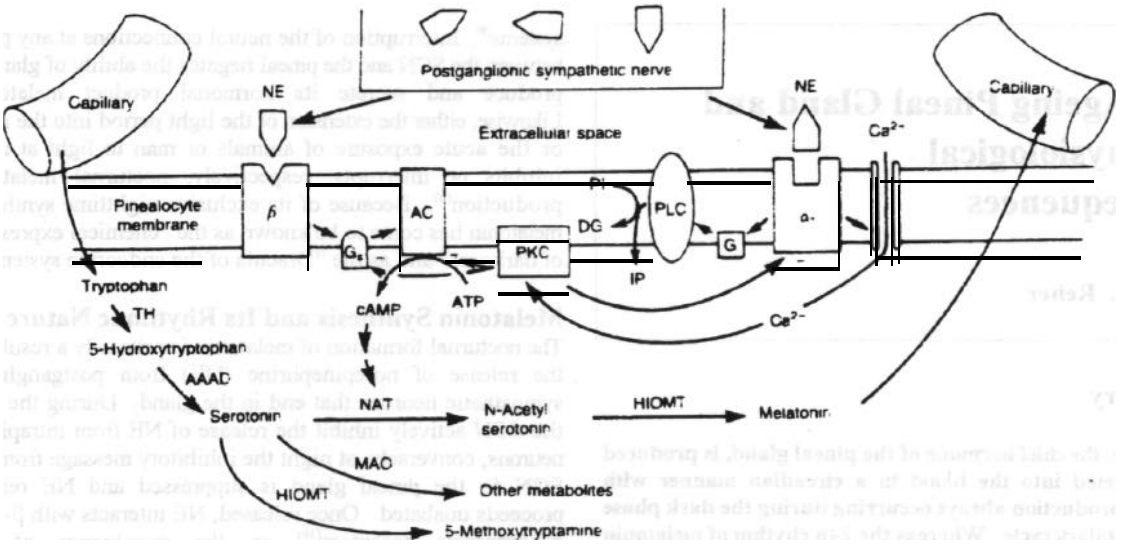

Fig. 1. Transmembrane signal mechanisms involved in mediating pineal melatonin production at the sympathetic neuron pinealocyte interface. Whereas most of the identified mechanisms are based on solid experimental evidence some of the presumptive signal transduction process remain to be clarified. AAAD l-aromatic amino acid decarboxylase. AC. adentiate cyciase: DG. diacylglycerol: G. guaninc nucieotide binding: protein: H1OMT. hydroxyinode-O-methyltransferase I mosito phosphate: PI phosphitidylinsoitol. PKC. protein kmase C: PLC. phosphoiipase C: al. o-adrenergic receptor. ßabrenergle recetor. Modified from Reiter[10].

melatonin rhythm in the blood is not discernible until 3-4 months of age after which it seems to develop rapidly until one year of age[17]. Prepubertally, mammals exhibit a robust circadian melatonin rhythm. As humans undergo sexual maturation there reportedly is a significant drop in nocturnal melatonin levels, a change that may be permissive to pubertal development[18]. After adulthood is achieved, virtually all mammals continue to exhibit a 24h melatonin rhythm with low and high values being associated with day and night, respectively[6,13].

Attenuation of the Melatonin Rhythm with Age

In advanced age, the melatonin rhythm deteriorates although there is evidence that the neuroendocrine system exhibits an increased sensitivity to the hormone[19]. The attenuation of the melatonin cycle with age seems to be a gradual process associated with the generalized decline in the functional capacity of many organs; thus, there seems to be no specific degenerative aging process which abruptly diminishes the ability of the pineal gland to either synthesize or secrete melatonin. Among mammals, reduced pineal melatonin synthesis with age has been studied most thoroughly in the Syrian hamster, rat, Mongolian gerbil and man.

When the 24h melatonin rhythm was compared in 2-month-old (young adults) and 18-month-old (old) male and female hamsters, the rhythm in the old animals of both sexes was found to be severely dampened[20]. Although a nocturnal peak of melatonin was still apparent compared to that in the 2-month-old hamsters, the old animals had melatonin levels that were reduced by about 75% (Fig. 2). Female hamsters at this age (18-months) are beyond their reproductive life span while the males may still be capable of siring young; Syrian hamsters rarely live beyond 24-month of age.

In a subsequent study, 14-month-old hamsters were found to display a melatonin rhythm with an amplitude that was intermediate between those of 2-month-old and 18-month-old animals[21], this suggests a gradual deterioration of pineal melatonin synthesis with aging. This is supported by similar observations in rats where 2-, 12- and 29-month-old animals were found to have high, intermediate and low peak pineal melatonin values at night[22]. Unexpectedly, the activity of the enzyme NAT, which is rate limiting in melatonin production, did not exhibit a similar age-associated drop. Subsequentstudies in hamsters and rats have shown that blood melatoninvalues are also significantly diminished in advanced age[23,24].

The gerbil pineal is a special case since, besides producing less melatonin later in life[21], the gland also develops numerous concretions (corpora arenacea) in early adulthood[25]. The development of concretions is also a common feature of the human pineal, especially in old individuals[26]. It has often been tacitly assumed that the degree of pineal calcium deposition is inversely related to the ability of the pineal gland to produce its hormone melatonin.

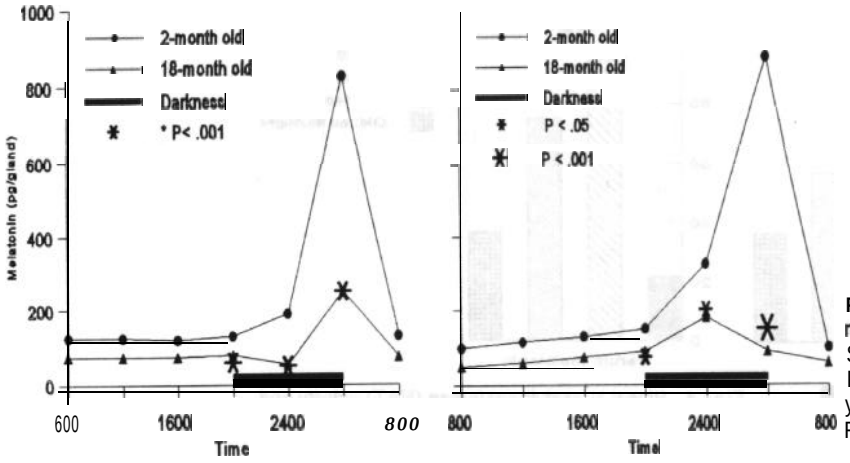

Fig. 2. Pineal melatonin contents of 2-mon-old and 1 8-mon-old female and male Syrian hamsters during **a 24h** period. The P values signify differences between the young and old animals at that time point. From Reiter et al.[20]

There is, however, no evidence to support this supposition. Interestingly, like melatonin synthesis, the formation of concretions in the gerbil pineal relies on the gland being sympathetically innervated[27] and, furthermore, once formed the concretions disappear if the animals are superior cervical ganglionectomized[28]. Thus, both the deposition and the maintenance of calcium deposits within the pineal are **active** processes which somehow relate to the NE activity at the level of the pinealocytes. To assume, however, that an increased number of calcium deposits in the gland directly impairs its ability to produce melatonin is unfounded experimentally.

The biosynthetic activity of the human pineal gland responds like that of other species in advanced age; thus, the general consensus is that the amplitude of the blood melatonin rhythm drops gradually as humans become old[29, 30]. Besides producing and secreting less melatonin at night, the pineal gland in elderly individuals exhibits a shorter duration of elevated nocturnal melatonin (Fig. 3)[31]. Finally, the chief urinary metabolite of melatonin, 6-hydroxymelatonin sulfate, shows a gradual age-related decline in humans between 20 and 95 years of age[32]. Although no specific studies have been conducted to correlate the degree of pineal calcification with circulating melatonin levels in humans, both morphological studies of the pinealocytes[33] and preliminary biochemical analyses of pineal enzymes[34] indicate no negative effect of concretions on the ability of the gland to produce melatonin.

While a reduction in the amplitude of the melatonin rhythm in advanced age is now well documented, this is not the only parameter of the rhythm that changes with age. Usually, as the melatonin peak diminishes, so does the duration of elevated melatonin. Both the amplitude as well as the duration of the melatonin peak may be important aspects of melatonin's ability to express its physiological activity. Finally, melatonin may only be active when its peak is internally coincident with a sensitive period for melatonin[35].

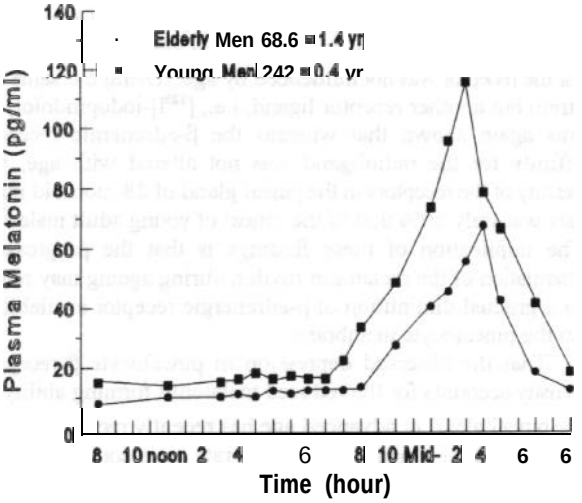

Fig. 3. Blood melatonin concentrations in young and elderly human females throughout **a 24h period.** Mean age of the subjects ± SEM is indicated. Modified from Nair et al.[31]

Thus, any test of pineal/melatonin/ageing interactions should take into account all of these aspects of the melatonin rhythm.

Mechanisms of Attenuation of the Melatonin Rhythm

The most frequently proposed mechanism invoked to explain the attenuation of the pineal and plasma melatonin rhythms in old age is a reduction in the number of β-adrenergic receptors on the pinealocyte membrane. As noted above, these receptors are primarily responsible for mediating the effects of nocturnally released NE from sympathetic neurons within the gland and initiating the intracellular events which lead to the augmented nighttime production of melatonin[12, 13].

Two publications have discussed the change in β-adrenergic receptors in the pineal of rats during ageing[36, 37].

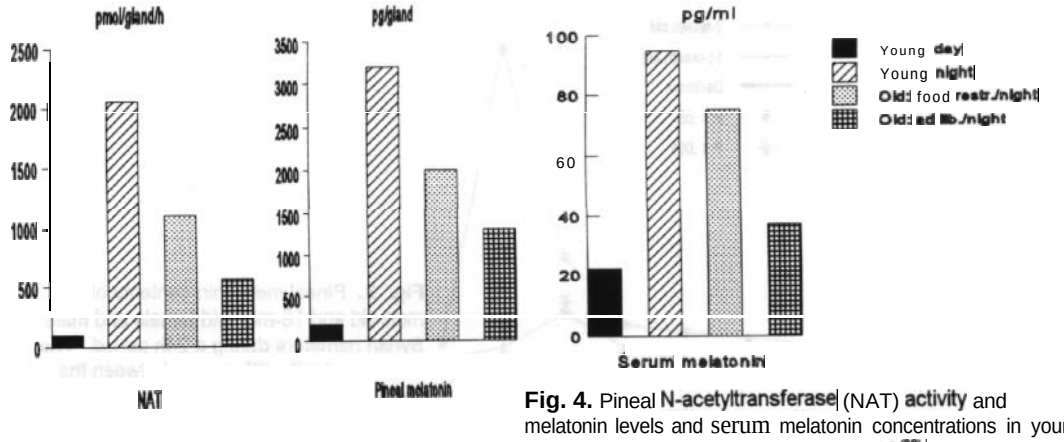

Fig. 4. Pineal N-acetyltransferase (NAT) activity and melatonin levels and serum melatonin concentrations in young and old rats. Modified from Stokkan et al.[38]

With the use of the radioligand [^{3}H]-dihydroalprenolol, 3-mon-old male Fisher 344 rats were found to exhibit a higher density of pineal β-adrenergic receptors than did the pineal gland in 24-mon-old rats, although the affinity of the ligand for the receptor was not influenced by age. Using the same rat strain but another receptor ligand, i.e., [^{125}I]-iodopindolol, it was again shown that whereas the β-adrenergic receptor affinity for the radioligand was not altered with age, the density of the receptors in the pineal gland of 28-mon-old male rats was only 50% that of the pineal of young adult males[37]. The implication of these fmdings is that the progressive attenuation of the melatonin rhythm during ageing may relate to a gradual diminution of β-adrenergic receptor availability on the pinealocyte membrane.

That the observed depression in pinealocyte p-receptor density accounts for the reduced melatonin-forming ability of the pineal gland in advanced age has recently received strong experimental support. Fisher 344 rats are a commonly used model to investigate ageing phenomena. Using this rat strain it has been shown that live can be dramatically prolonged by restricting food intake throughout life[38]. Thus, *ad libitum* fed Fisher 344 rats usually die at approximately 30 mon of age while food-restricted animals not uncommonly survive to **44** mon of age. When pineal biosynthetic activity and β-adrenergic receptor density *were* compared *in ad libitum* and food-restricted (by 40%) 28-mon-old rats there were obvious differences in both parameters. As seen in other studies, the *ad libitum* fed old rats (compared to 3-mon-old animals) had highly depressed nocturnal pineal NAT activity and pineal and serum melatonin levels[39]. All three parameters were significantly preserved in old rats that had experienced virtually life-long food restriction (Fig. 4). Preservation of pineal melatonin synthesis was accompanied by a similar maintenance of high β-adrenergic receptor density in the pineal gland (Fig. 5)[37]. Although a definitive causal relationship between the preserved melatonin rhythm and the number of P-receptors is not unequivocally proven by these

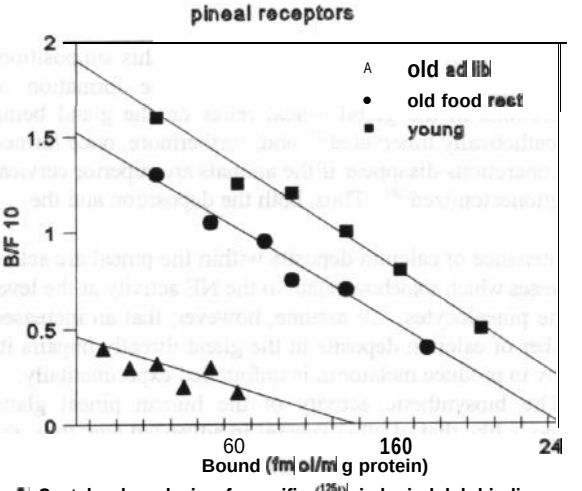

Fig. 5. Scatchard analysis of specific (^{125}I) iadopindolol binding data from 3 groups of rats. The data suggest a single class of β-adrenergic receptors. From Henden et al. [36]

observations, the findings are certainly consistent with this possibility.

There are obviously other potential explanations for the reduced ability of the pineal gland to metabolize serotonin (5HT) to melatonin in the pineal gland of old animals, but few have been seriously investigated. Presynaptically, the formation and or release of NE from the intrapineal sympathetic nerve endings could be reduced as a consequence of ageing; this would presumably result from a diminished nocturnal firing rate of the neurons in the SCN. Although these are ideas that need testing, it has not been done to date. Post β-adrenergic receptor mechanisms within the pineal gland may remain intact during ageing as suggested by the observation that if p-receptor density is preserved by restricting food intake, the synthetic mechanisms for melatonin production are virtually normal[39]. It is possible, however, that the intracellular synthetic mechanisms were independently

preserved in the old rats by life-long food restriction.

One factor that seems definitely not to account for the drop in melatonin with age is a lack of the precursor 5HT. In the pineal gland of old Fisher rats (either *ad libitum* or food-restricted), nocturnal 5HT levels are actually higher than those of 3-man-old animals; thus, there is ample precursor available for melatonin synthesis in rats of all ages[39].

Potential Consequences of the Attenuated Melatonin Rhythm during Aging

In recent years, melatonin has been at least theoretically defined as an anti-aging hormone([40]). Indeed, the hypothesis has been put forward that "aging is secondary to pineal failure**([41]). According to this hypothesis, aging is a syndrome of a relative melatonin deficiency accompanied by a diminished melatonin; 5HT ratio, which is detrimental to neurophysiology and causally related to the aging process. The findings discussed above could relate to the theory. Namely, dietary restriction clearly increases the life span of a variety of animals; likewise, the procedure also tends to preserve the pineal melatonin rhythm[39]. The conservation of the melatonin rhythm in food-restricted animals is particularly interesting since, typically, prolonged food restriction depresses the function of virtually every other endocrine organ([42]), yet the melatonin cycle responds in an opposite manner, i.e., it is maintained([39]). The question then arises, is the prolonged life span experienced by food-restricted animals related to greater melatonin availability? Only a few data relate to the ability of melatonin to influence the duration of survival, but the correlations are positive. When mice are given melatonin in their drinking water, they lived noticeably longer (20%), i.e., from a mean of 752+/-80 days in non-melatonin treated mice to a mean of 931+/-80 days in mice given melatonin every night([2]). The contrary expectation would be that pinealectomized animals, with the resulting melatonin deficiency, may die at an earlier age than pineal intact animals; such studies have not been performed and should have a high priority.

Whereas the observations in the previous paragraph are tantalizing and suggestive of a potential association between melatonin and aging, as yet there is no definitive evidence indicating that there is a causal link. There are key experiments that could be performed to either prove or disprove an interaction of aging processes and melatonin secretion. For example, the administration of melatonin prophylactically throughout life could be tested for its ability to retard any number or age-related processes. On the other hand, as noted above, surgical removal of the pineal gland, which removes the bulk of the circulating melatonin and certainly eliminates its circadian levels in the blood, would be expected to generally hasten the manifestations of aging. In these studies, however, the effects of the respective treatments on other bodily functions, e.g., reproduction, should be monitored since there are some correlations between reproductive activity and life expectancy[43] additionally, disturbances of circadian rhythms resulting from either excess or limited melatonin availability may lead to deterioration of physiological processes which result in premature death[44].

If melatonin-treated animals do survive longer, why do they? Is it merely a consequence of a generalized beneficial metabolic effect of melatonin, or does it relate to a specific function of the pineal hormone? The immunostimulatory effect of melatonin is one argument that could be used to explain the increased longevity in melatonin-treated animals. Although this is certainly a proposition posed by workers in this field, they are extremely careful not to conclude that melatonin's only beneficial effect in reference to survivability is due to its immunoenhancing properties[2, 40].

The alleged stimulatory effects of melatonin on the immune system are also interesting in the context of aging inasmuch as the neuropathology normally associated with advanced age may be related to an immunological disorder in which there are elevated serum levels of brain reactive autoantibodies (BRA)[45]. New Zealand Black (NAB) mice already have high circulating BRA levels early in life, and they rarely survive for more than 12 months([46]). However, if NZB mice are treated with melatonin, their life expectancy is increased and neuropathological signs normally associated with aging develop more slowly than in mice not given supplemental melatonin[47]. The implication is that melatonin may prolong life by suppressing BRA production.

Equally important and possibly related to melatonin's stimulatory role on the immune system is the ability of melatonin to suppress tumor growth[4]. This effect was demonstrated at least two decades ago and more recent findings are strongly supportive of the oncostatic properties of melatonin in given situations[48, 49]. If melatonin-treated animals do exhibit an increased duration of survival, it could relate to the increased resistance they exhibit to infection and/or to the reduced likelihood of the animals developing immunologically-related tumors. Even if melatonin were found not to promote increased life expectancy, because of its alleged immunoenhancing and oncostatic properties, it may improve the quality of life.

The ability of melatonin to function as a tumor-inhibiting agent may be directly applicable to the experiments summarized above which describe the prolonged life span of food-restricted rata. As mentioned, *ad libitum* fed Fisher rats typically die at about 30 months of age. At this time, they not uncommonly exhibit numerous tumors, e.g., mammary gland and epidermal tumors among others; these likely contribute to the death of these animals[38, 42]. When this same strain of rat is food-restricted, they survive much longer and at 30 months of age they display few of the *tumors* characteristic of *ad libitum*-fed animals and, as noted, their melatonin rhythm is partially preserved. It has been known for years that underfeeding delays the development of experimental cancers. Could this be due to the preservation of the cyclic production of melatonin, a natural oncostatic agent? Additionally, it was

shown many years ago that dietary restriction increases the sensitivity of the neuroendocrine-reproductive axis to melatonin[50]. If cancer cells also exhibit an increased sensitivity to melatonin when certain nutrients are limited, then the action of food restriction in reference to tumor growth could be two-fold, i.e., it not only preserves the melatonin rhythm but it also renders the tumorous cells more liable to inhibition by melatonin. This combination, because it would suppress tumor growth, could prolong life span especially when combined with the ability of melatonin to enhance the function of the immune system[2].

Mechanistically, it is not yet totally clear how and where melatonin intervenes to modulate aging and age-related processes, if in fact it does. The bulk of melatonin binding sites (receptors) that have been identified are in the central nervous system, although there are occasional reports of receptors distributed outside of the brain as we11([51-55]). Melatonin binding sites on immunologically active organs and cells as well as on tumor cells are almost undocumented, although this does not mean they do not exist at these locations. Even in organs where melatonin receptors are well studied, e.g., the pars tuberalis of the anterior pituitary gland, the molecular mechanisms of action of melatonin are only partially defined[56-58].

Besides the possible decreased longevity, an increased probability of cancer, and the relatively suppressed immune function that may accompany a relative melatonin deficiency in old age, there may be other age-associated phenomena which are related to the gradually deteriorating melatonin cycle during aging. Thus, sleep inefficiency[59,60], Alzheimer's disease[31], and psychiatric disorders[5] are only a few of the potentially associated conditions.

Final Comment

Numerous reports now suggest that the pineal gland, via its hormone melatonin may somehow either directly or indirectly delay aging itself or inhibit age-related disease processes. Because of these findings, melatonin has been classified as an anti-aging hormone and as a juvenile hormone. If, in fact, these well-based predictions are finally unequivocally verified the pineal gland could be known as the veritable "fountain of youth". The data accumulated to date certainly justify serious consideration of the possibility that supplemental melatonin may be beneficial during aging.

Acknowledgments

Research in this field by the author has been supported by grants from the National Science Foundation and the National Institute of Health.

References

1 **Reiter, R J. (1973). Comparative physiology; Pineal gland Annu.** *Rev. Physiol. 35,305328.*

2 **Maestroui, G. J. M.,** Conti, **A aud PierpaoIi, W. (1989). Melatonin,** *stress,* **and** *the* **immune** *system. Pineal Res. Rev. 7,203-226.*

3 **Armstrong, S. M. (1989). Melatonin: The intemal Zeigeber of mammals?** *Pineal Rex Rev. 7,157-202.*

4 **Blask, D. E (1984).** *The pineal: An oncostatk* **gland?** *In The Pineal Gland* **(ed. R. J. Reiter), pp. 253-284. Raven, New York.**

5 **Wetterberg, L., Beck-Friis, J. and Kjelhnau, B. F. (1990). Melatonin as a marker fix a subgroup of depression in adults.** In *Biological Rhythms, Mood Disorders, Light Therapy, and the Pineal Gland (eds.,* **M. Schatii and S. L, Schatii), pp. 69-95.**

6 **Arendt, J. (1988). Melatonin. Clan.** *Endocrinol, 29,205-229.*

7 **KIein, D. C aud Moore, R Y, (1979). Pineal N-acetyWerase and hydroxyindole-O-methyltransferaese Control by the retinohypothalamic tract and the suprachiasmatic nuclei.** *Brain Res. 174,245-262.*

8 **Reiter,R J. (1985). Action spectra, dose- relationships and temporal aspects of light's effects on the pineal gland.** *Ann. N. Y. Acad. Sci. 453,2* **15-230.**

9 **Reiter, R J. (1981). The mammalian pineal gland: Structure and function.** *Am. J. Anat.* **162,287-313.**

10 **Pangerl, B, Paugeri, A. aud Reiter, R J. (1990). Circadian variations of adrenergic receptors in the mammalian pineal gland: A** *review. J. Neural Transm.* **81,17-30.**

11 **Reiter, R J. (199 1). Pineal melatonin: Cell biology of its synthesis and of its physiological interactions.** *Endocrine Rev.* **12, 15 l-l 80.**

12 **Mikuni, M., Saito, Y., Koyama, T. and Yamashita, I. (1981). Circadian variation of cyclic AMP in the rat pineal gland.** *J. Neurochem.* **36, 1295-1297.**

13 **Chik, C. L. and Ho, A. K. (1989). Multiple regulation of cyclic nucleotides in rat pinealocytes.** *Prog. Biophys. Molec. Biol.* **53, 197-203.**

14 **Cardinali, D. P., Vacas, M. I., Rosensteiu, R E, Etchegoyen, G. S., Keller Sanuiento, M. I., Gonzalez Salveyra, C. aud Pereyra, E N. (1987). Multifactorial control of pineal melatonin synthesis: An analysis** *through* **binding sites.** *In Advances in Pineal Research,* **Vol. 2 (eds., R. J. Reiter and F. Fraschini), pp. 5 l-66. John Libbey, London.**

15 **Reiter, R J. (199 1). Pineal gland: Interface between the photoperiodic environment and the endocrine** *system. Trends Endocrinol. Metab. 2,* **13-19.**

16 **Rollage, M. D. and Stetson, M. H. (1981). Ontogenyofthepineal melatonin** *rhythm in* **golden hamsters.** *Biol. Reprod.* **24, 3 1 l-3 14.**

17 **Gupta, D. (1988). Pathophysiology of pineal function in health and disease in children.** *Pineal Res. Rev.* **6, 261-300.**

18 **Waldhauser, F., Lynch, H. J. aud Wurtman, R J. (1984). Melatonin** *in* **body fluids: Clinical significance.** *In The Pineal Gland* **(ed., R. J. Reiter), pp. 345-370. Raven, New York.**

19 **Reiter, R J., Vriend, J., Brainard, G. C., Matthews, S. A. aud Craft, C. M. (1982). Reduced pineal and plasma melatonin levels and gonadal atrophy in old hamsters kept under winter photoperiods.** *Exp. Aging Res.* **8, 27-30.**

20 **Reiter, R J., Richardson, B. A., Johnson, L. Y., Ferguson, B. N. and Dinh, D. T. (1990). Pineal melatonin rhythm: Reduction in aging Syrian hamsters.** *Science* **210, 1372-1373.**

21 **Reiter, R J., Johnson, L. Y., Steger, R W., Richardson, B. A. and Petterborg, L. J. (1980). Pineal biosynthetic activity and neuroendocrine physiology** *in the* **aging hamster and gerbil.** *Peptides* **(Suppl. 1) 1, 69-77.**

22 **Reiter, R. J., Craft, C. M., Johnson, J. E., Jr, King, T. S., Richardson, B. A., Vaughan, G. M. and Vaughan, M. K. (1981). Age-associated reduction in nocturnal melatonin levels in female rats,** *Endocrinology* **109, 1295-1297.**

23 **Pang, S. F., Taug F. and Taug, P. L. (1983). Decreased serum and pineal concentrations of melatonin and N-acetylserotonin in aged male hamsters.** *Horm. Res.* **17, 228-234.**

24 **Pang, S. F., Tang, F. and Tang, P. L. (1984). Negative correlation of age and the levels of pineal melatonin, pineal N-acetylserotonin and serum melatonin in male rats.** *J. Exp. Zool. 229,41-*

47.
25 Japha, J. L., Eder, T. J. and Goldsmith, E D. (1976). Calcified inclusions in the superficial pineal gland of the Mongolian gerbil, Meriones *ungutculatus. Acta Anat.* **95**, 533-544.
26 Welsh, M. G. (1985). Pineal calcification: Structural and functional *aspects. Pineal Res. Rev.* **3**, 41-68.
27 Reiter, R J., Welsh, M. G. and Vaughan, M. K. (1976). Age-related changes in the intact and sympathetically denervated gerbil pineal gland. *Am. J. Anat.* **146**, 427-432.
28 **Champney, T. H., Joshi, B. N., Vaughan, M. K. And Reiter, R J.** (1985). Superior cervical ganglionectomy results in the loss of pineal concretions *in the* adult male gerbil *Meriones unguiculatus. Anat. Rec.* **211**, 465-468.
29 **Touitou, Y., Fevre, M., Lagugvey, M., Carayon, A., Boydon, A. and Reinbert,** A. (1981). Age and mental health related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle stimulating hormone. *J. Endocrinol.* **91**, 467-475.
30 Iguchi, H., Kato, K. and Ibayashi, H. (1982). Age-dependent reduction in serum melatonin concentrations in healthy human subjects. *J. Clrn. Endocrinol. Metab.* **55**, 27-29.
31 Nair, N. P. V., Hariharasubramanian, N., Pilapil, C., Isaac, I. and Thavundayil, J. X. (1986). Plasma melatonin-An index of brain aging in humans? *Biol. Psychiat.* 21, 141-150.
32 Sack, R. L., Lewy, A. J., Erb, D. L., Vollmer, W. M. and Sir, C. M. (1986). Human melatonin production decreases with age. *J. Pineal Res.* **3**, 379-388.
33 Tapp, E and Huxley, M. (1972). The histological appearance of the human pineal gland from puberty to old age. *J. Pathol. 108*, 137-144.
34 **Wurtman, R J., Axelrod, J. and Barchas, J. D.** (1964). Age and *enzyme* activity *in the* human pineal. *J. Clin. Endocrinol. Metab. 24*, 299-301.
35 Reiter, R. **J.** (1987). The melatonin message: Duration versus coincidence hypotheses. *Life Sci.* **46**, 2 119-2 13 1.
36 Greenberg, L. H. and Weiss, B. (1978). β-Adrenergic receptors in aged rat brain: Reduced number and capacity of pineal gland to develop supersensitivity, *Science* **201**, 6 **l-63.**
37 Henden, T., Stokkan, K.-A., Reiter, R J., Nonaka, K. 0, Lerchl, A. and **Jones, D. J.** (1992). The age-associated reduction in pineal β-adrenergic receptor density is prevented by life-long food restriction *in* rats. *Biol. Signals* **1**, 34-39.
38 **Masoro, E J.** (1988). Food restriction in rodents: An evaluation of its role in aging. *J. Gerontol, 43*, B59-B64.
39 Stokkan, K. A., Reiter, R J., Nonaka, K. O., Lerchl, A., Yu, B. P. and Vaughan, M. K. (199 1). Food restriction retards aging of the pineal gland. *Brain Res.* **545**, 66-72.
40 Maestroni, G. J. M., Conti, A. and Pierpoali, W. (1988). Pineal melatonin, its fundamental immunoregulatory role in aging **and cancer.** *Ann. N. Y. Acad. Sci.* **521,** 140-148.
41 **Rozenchwaig, R, Grad, B. R and Ochoa, J.** (1987). The role of melatonin and *serotonin in* aging. *Med. Hypotheses* **23**, 337-352.
42 Meites, J. (1990). Aging: Hypothalamic catecholamines, neuroendocrine-immune interactions, and dietary restriction. *Proc. Soc. Exp. Biol. Med.* **195,3043** 11.
43 **Trentini, G. P., De Gaetani, C. and Criscuolo, M.** (1991). Pineal gland and aging *Aging 3*, 103-I 16.
44 **Armstrong, S. M. and Redman, J. R** (1991). Melatonin a chronobiotic with anti-aging properties, *Med. Hypotheses* **34**, 300-309.
45 **Nandy, K., Lal, H., Bennett., M. and Bennett, D.** (1983). Correlation between a learning disorder and elevated brain reactive antibodies *in* aged C57/B1/6 and young NZB mice. *Life Sci.* **33**, 1499-1503.
46 **Threatt, J., Nandy, K. and Fritz, R** (1977). Brain reactive antibodies in serum of old mice demonstrated by immunofluorescence. *J. Gerontol.* **26**, 3 16-323.
47 **Pierpaoli, W.** (1991). The pineal gland: A circadian or seasonal aging clock. *Aging* **3**, 99-1 0 1.
48 Reiter, R J. (1988). Pineal gland, cellular proliferation and neoplastic growth: *An historical* account. *In The Pineal Gland and Cancer*, (eds., D. Gupta and R. J. Reiter), pp. 41-64. Brain Research Promotions, Tubingen.
49 Blask, D. E, Hill, S. M. and Pelletier, D. B. (1988). Oncostatic signaling by the pineal gland and melatonin in the control of breast cancer. *In The Pineal Gland and Cancer, (eds.,* D. Gupta, A Attanasio, and RJ. Reiter). pp. 195-206. Brain Research Promotions, Tubingen.
50 **Sorrentino, S., Jr. Reiter, R J. and Schalch, D. S.** (1971). Interactions of the pineal gland, binding, and underfeeding on the reproductive organ size and radioimmunoassayable growth hormone. *Neuroendocrinology 7,105-l* 15.
51 **Dubocovich, M.** (1988). Pharmacology and function of melatonin receptors. *FASEB J.* **2**, 2765-2773.
52 **Morgan, P. J. and Williams,** L. **M.** (1989). Central melatonin receptors: Implications for a mode of **action.** *Experimentia* **45**, 955-965.
53 **Stankov, B.** and **Reiter, R J.** (1990). Melatonin receptors: Current *status,* facts and hypotheses. *Life Sci.* **46**, 971-982.
54 Viswanathan, M., Laitinen, J. T. and Saavedra. J. M. (1990). Expression of melatonin receptors in arteries involved in thermoregulation. *Proc. Natl. Acad. Sci.* **87**, 1165-1171.
55 Lopez-Gonzalez, M. A., Calvo, J. R, Rabio, A., Goberna, R and Guerrero, J. M. (199 1). Characterization of melatonin binding sites in the Harderian gland and median eminence of the rat. *Life Sci.* **48**, 1165-1171.
56 Carlson, L. L., Weaver, D. R and Reppert, S. M. (1990). Melatonin signal transduction in hamster brain: Inhibition of adenylyl cyclase *by* a pertussis toxin-sensitive *G* protein. *Endocrinology* **125**, 2670-2676.
57 **Weaver, D. R, Carlson, L. L. aud Reppert, S. M.** (1990). Melatonin receptors and signal transduction in melatonin-sensitive and melatonin-insensitive populations *in* white-footed *mice (Peromyscus Leucopus). Brain Res.* **506**, 353-358.
58 Morgan, P. J. (1991). Molecular signaling via the melatonin receptor. *Adv. Pineal Res. 5*, 19 l-l 97.
59 Reiter, R J. (1977). Pineal interaction with the central nervous *system. Waking Sleeping* **1**, 253-258.
60 Wnrtman, R. J. (1985). Melatonin Secretion as a mediator of circadian variations *in* sleep and sleepiness. *J. Pineal Res.* **2**, 30 I-303.

Russel J. Reiter is at the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762 USA