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REVIEW ARTICLE

MELATONIN, BIOLOGICAL RHYTHM DISORDERS AND PHOTOTHERAPY

VENKATRAMANUJAN SRINIVASAN

Department of Physiology,
PSG Institute of Medical Sciences and Research,
Peelamedu, Coimbatore – 641 004

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Abstract : Biological rhythms are endogenous in nature and are generated by self sustained oscillators present in the living organisms themselves. Of these, circadian rhythms are the most thoroughly studied and are driven by the suprachiasmatic (SCN) of hypothalamus. The recent discovery of high affinity melatonin receptors ML1, ML2 in SCN suggests that melatonin is involved in the control of circadian rhythm generation. The fact that biological rhythm disorders like delayed sleep phase syndrome (DSPS), Jet lag, shift-work disorders, seasonal effective disorder (SAD) respond well either to phototherapy or melatonin adds further support to the concept that melatonin is involved in the pathogenesis of these conditions. Indeed altered melatonin rhythms have bee documented in MDP, shift work disorder, endogenous depression etc. In addition to functioning as a rhythm regulator, melatonin is also involved in the control of sleep, regulation of body temperature, reproduction, and as a free radical scavanger and antioxidant protecting the cells and tissues of our body against oxidative damage. Low levels of melatonin in cancer patients and patients with coronary heart disease indicate that melatonin may be involved in these disorders also.

Key words : pineal gland, melatonin, jet lag, suprachiasmatic nucleus, circadian rhythm disorders, shift work antioxidant

INTRODUCTION

Living organisms exhibit certain kinds of biological periodicity that enable them to measure the passage of time. These rhythms known as biological rhythms are of many kinds that include circadian (24 hr; circa =about, dies = day), circannual (year), circaseptan (weekly), circatidal (matching with the cycle of daily high and low tides), circazygic (cycle of unusually high and low tides occurring each fortnight) etc. Certain plants exhibit rhythms which manifest once in several years. The classical example of such rhythms are "KURINJI" plants of strobilanthes genus grown in the hilly regions of South India. The flowers of these plants bloom once in twelve years and this fact has been documented even two thousand years ago in Tamil Literature (3). The author coins the word "CIRCADOD–ECANNUAL" to designate the biological rhythms which occur once in twelve years.

Circadian rhythms have been the most thoroughly studied among biological rhythms and their medical implications have been well understood in recent years (1-6). It was De Marian's early 18th century observations that leaves of mimosa plant open during daytime and close at night, even when the leaves are placed in complete darkness provided the first scientific evidence that circadian rhythms are generated by self-sustained oscillators known as "biological clocks" present in the organisms themselves (3,7). Ablation, transplantation, or electrophysiological studies undertaken in animals have revealed that circadian rhythms are generated by the suprachiasmatic nucleus (SCN) of the anterobasal hypothalamus (8, 9), and the finding has been confirmed in human subjects (10). The suprachiasmatic nucleus is involved in the generation of circadian rhythms of locomotor activity, food intake, water intake, sexual behaviour, core body temperature, sleep, ACTH secretion, prolactin, gonadotorophin and melatonin secretions (9, 59). Circadian rhythms are thus endogenous rhythms and differ from other daily rhythms in having a free running period and entrainment by a zeitgeber (time giver). In the absence of temporal cues like the light-dark cycle they free run and are readjusted to 24 hrs by light which acts through the retina and the retino-hypothalamic pathway (8-11). They also exhibit the properties of phase shift and temperature compensation (11).

Human beings have a free running period longer than 24 hrs but close to 25 hrs. If the circadian pacemaker were not reset, the timing of their endogenous rhythms would lose upto 1 hr per day with respect to clock time of each day (7). Man has been synchronizing his daily sleep-wakefulness rhythms and rest activity cycle with light-dark cycles of the external environment all these years and this physiological adaptation has enabled him to maintain his physical and mental health in perfect condition (12). But in recent years the rapid growth of industrialisation, introduction of fast transport systems, telecommunication networks have forced him to work either continuously or in rotating shifts during night hours causing severe disruptions of his circadian organisation giving rise to many biological rhythm disorders. (1-6, 72). The major biological rhythm disorders that afflict our modern industrialized society are :

(1) circadian rhythm sleep disorders,

(2) jet lag or intercontinental flight dysrhythmia,

(3) shift-work disorder and

(4) chronobiological mood disorders.

Severe disturbances of sleep-wakefulness rhythm and abnormal phase position of different circadian rhythm are the prominent features noted in these disorders. These disorders do not respond well to the conventional methods of treatment like the use of hypnotic drugs or sedatives but respond adequately to therapeutic designs based upon chronobiologic principles like phototherapy (13, 14) or use of pineal hormone melatonin (15) and hence the study of this hormone and the link between phototherapy - pineal and biological rhythm disorders has become necessary. A comprehensive account of pineal gland and

its involvement on various physiological functions has been brought out in the earlier review written by this author (41, 42) and the present review is restricted mainly to the role of melatonin in biological, rhythm disorders. A brief account of pineal gland, the major site of melatonin biosynthesis is however essential for understanding the therapeutic importance of melatonin and the role of phototherapy in biological rhythm disorders.

Melationin biosynthesis

Melatonin is synthesized primarily in the pineal gland of all species including man and an account of this gland has become necessary for understanding the regulatory mechanisms that govern melatonin production and release.

Pineal gland : Pineal gland forms an integral part of the photoneuro endocrine system in all species of animals including man and is closely related to optic pathway (16). In certain species, the presence of parapineal is referred as the "third eye". In human beings pineal gland is deeply situated in the midline of the brain and weighs about 150 mgm. About 80% of cells are pinealocytes that are specialized for the systhesis and secretion of melatonin. The presence of opsin-retinal S antigen in these cells suggests their close relationship to the retinal photoreceptor cells (17). Human pineal gland remains active throughout the life of the individual and it has been proved that calcification does not affect its function (18). In some individuals little calcification occurs even in the ninth or tenth decades of their life showing thereby that aging does not negatively affect the pineal enzyme activities (19). Melatonin biosynthesis also occurs in the retina, enterochromaffin cells of the gut, platelets of certain animals and erythrocytes of human beings (20). In the retina melatonin synthesis occurs within the photoreceptors and HIOMT activity has been demonstrated in the retina by Weichmann and his co-workers in 1985, cited in (87), and the retinal N-acetyl transferase (NAT) and melatonin rhythm exhibit rhythmicity that is similar to that of pineal melatonin rhythm (87).

It was Lerner and his co-workers in 1958, who first identified melatonin in the pineal gland (21). Tryptophan serves as the precursor for the biosynthesis and the pathway is shown in Fig.1. Most of the biosynthetic activity occurs only at night, and the key enzyme N-acetyl transferase (NAT) activity correlates closely with melatonin production (22). Melatonin production exhibits a circadian rhythm and this rhythm is regulated by the SCN (23). Once formed melatonin is not stored in the pineal gland but is released immediately into the blood stream (24). Melatonin from the blood rapidly passes through the choroid plexus and then into the CSF. However, direct release of melatonin into CSF also exists in certain species of animals (22).

Regulation of melatonin secretion

Pineal gland is innervated mainly by post-ganglionic sympathetic nerve fibres of the superior cervical ganglia and this innervation is very essential for the rhythmic production and release of melatonin (25). Norepinephrine, the major neurotransmitter released from the sympathetic post-ganglionic nerve fibres

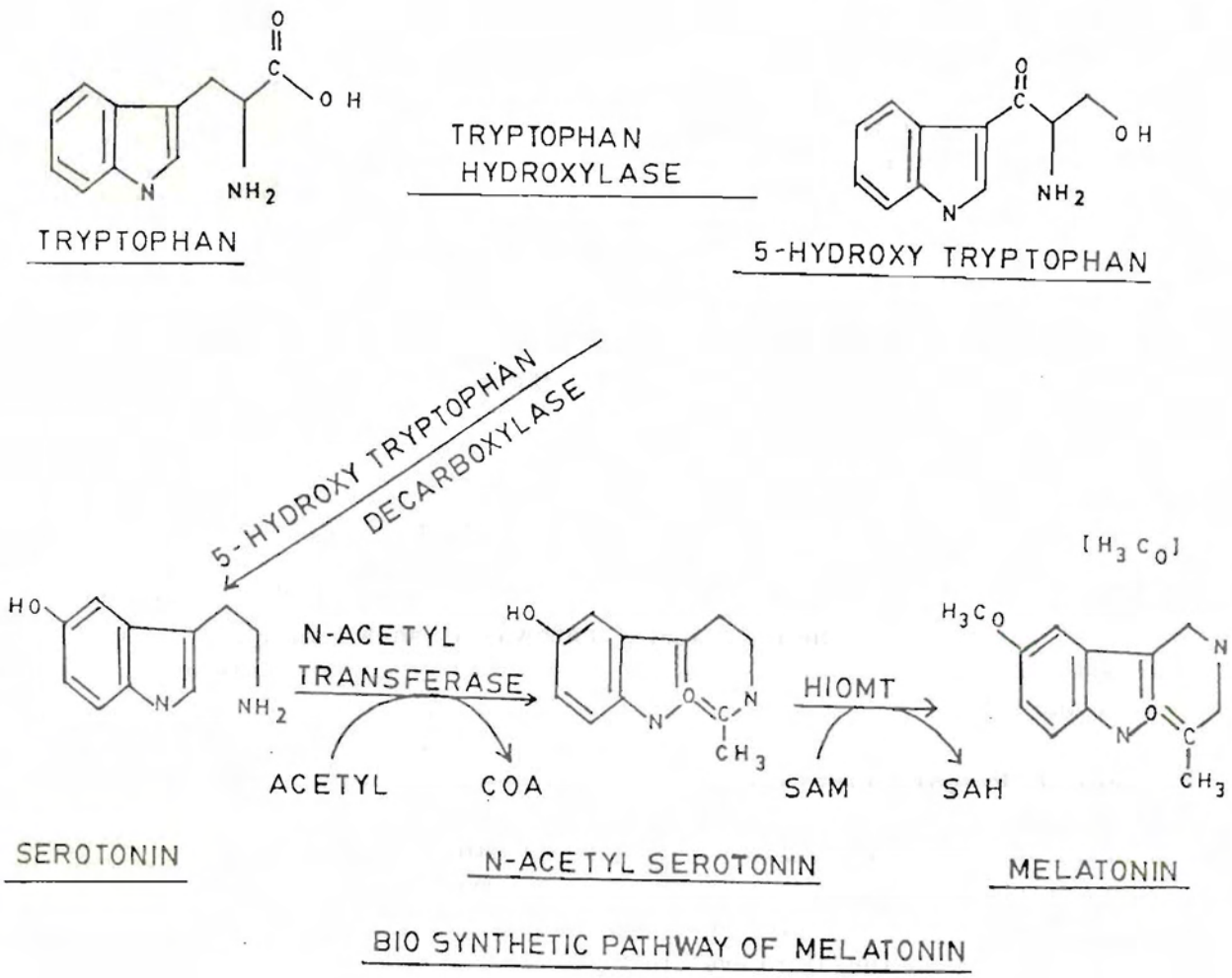
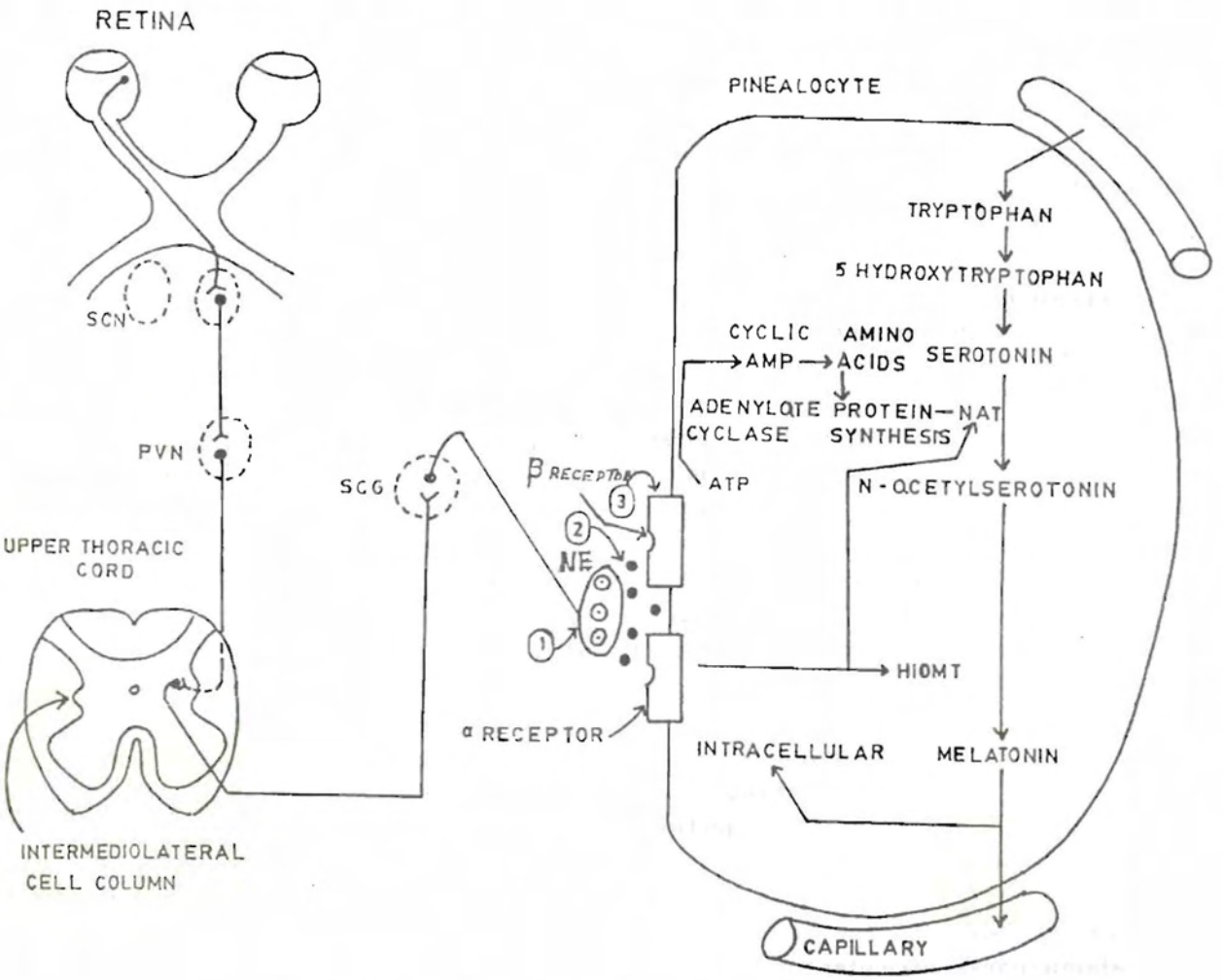


Fig. 1 : Melatonin Biosynthesis.

regulates melatonin production through the activation of beta-adrenergic receptors-cyclic AMP system present on the cell membrane of pinealocytes (26) and this is illustrated in Fig. 2. The intrinsic electrical discharge from the SCN increase during dark hours of the day that in turn determines the quantity of norepinephrine (NE) released from the post-ganglionic sympathetic nerve fibres which supply pinealocytes (22). Other neurotransmitter-receptor sites, such as serotonergic, D2-dopaminergic, GABAergic and benzodiazepinergic, have also been identified in the pineal gland of mammals and the interaction among these receptor sites also implicated in the regulation of the pineal gland secretion (27). Study of melatonin biosynthesis using chick pinealocytes have revealed that rhythmic production is governed through cyclic AMP or calcium ions by circadian oscillators located within pinealocytes themselves (28).



REGULATION OF THE PINEAL GLAND

Fig. 2 : Pathways showing the influence of Light–dark cycle and SCN on Melatonin synthesis by the pineal gland.

Light–dark cycle and melatonin production

The pattern of melatonin secretion, its duration, phase and amplitude are largely influenced by changes in light-dark cycles. Light is the primary environmental agent whose influence is not only to entrain but also to suppress melatonin production (30). A mono synaptic retino-hypothalamic pathway conveys information about the light-dark cycle to SCN (Fig. 2), which in turn determines the level of melatonin production. Non-visual cone like photoreceptors are the ones that are involved in the perception of light which regulates our circadian rhythms through SCN and pineal gland (31, 97).

Neurotransmitter in the SCN:

The neurotransmitter that is involved in conveying information about photoperiod length to suprachiasmatic nucleus is not precisely known. Acetylcholine has been first suggested, but in recent years the excitatory neurotransmitter glutamate has been suggested to play an active role in mediating the effect of light on the SCN (31). N-methyl-D-aspartic acid (NMDA) glutamate receptor antagonist has been shown to block the light induced phase shifts in rodents (29). Apart from the major retino-hypothalamic pathway, there are also two other afferent inputs to SCN. Though they are not essential for entertainment, they seem to modify the response of SCN to the light stimulus. The indirect visual pathway from lateral geniculate nucleus and intergeniculate nucleus to the hypothalamus is thorough the geniculo hypothalamic tract and appears to be mediated through the neuropeptide Y (29). Efferent projections from the SCN to paraventricular nucleus contain vasoactive intestinal polypeptide (VIP), vasopressin and neurophysin. This hypothalamic-paraventricular nucleus is the pathway through which light-dark cycle and the intrinsic electrical activity of SCN modulates the melatonin production and release from the pineal gland.

Light intensities as dim as 100 to 500 lux also has been shown to suppress melatonin production (32).

Melatonin is metabolized primarily in the liver by 6-hydroxylation followed by sulfate or glucuronide conjugation (20).

Melatonin rhythm in body fluids

Circadian variation is a characteristic feature of melatonin secretion. The plasma levels are low during day time and are high at night and this rhythm is generated by SCN and is entrained by light-dark cycle (30). RIA, HPLC, gas chromatography, mass spectrometry are some of the methods that are currently available for quantification of melatonin in body fluids (20). The secretory fashion of melatonin has been well studied by measuring plasma melatonin levels at hourly intervals (30), and the values are 10 pgm/ml to 30 pgm/ml during day time; 40 to 80 pgm/ml during night (19, 20). The onset of nocturnal melatonin elevation occurs between 9 PM to 11 PM (33), and this elevation lasts nearly for 9 hrs. The concentration of 6-sulfatoxy melatonin in plasma is also low during day time and is undetectable in the afternoon or early evening but its values range from 80-100 pgm/ml of plasma during night time (20). Pulsatile secretory pattern of melatonin has been documented by some (34) and the pulse frequency has been found to be one pulse per every 60 to 80 min and some are of the opinion that melatonin pulse frequency is not as regular as in the case of LH secretorypattern (33). However, human plasma melatonin rhythm is remarkable and it occurs faithfully from day to day, from week to week almost like a "hormonal finger print" (34). Melatonin present in the CSF also exhibits a similar rhythm but its functional importance is now known (22). Melatonin rhythm in the saliva is similar to that of the plasma (35).

Factors that affect melatonin levels:

Age : Melatonin rhythm appears around 6 to 8 weeks of life and plasma melatonin concentration steadily increases thereafter and reaches a lifetime peak around 3 to 5 years of age (33). The daytime melatonin secretion pattern does not show much difference between younger children or adults but remains generally less than 20 pgm/ml. But the nocturnal melatonin concentration exhibits a significant reduction from 210±35 pgm/ml in younger children to 46±4 pgm/ml of young adults. A significant reduction in urinary 6-hydroxy melatonin levels also has been noted in both males and females as age advances (19, 33, 36).

Sex : Sex does not seem to affect melatonin level significantly (5).

Body weight : A significant negative correlation has been found between body weight and serum melatonin levels with lower melatonin levels being found in taller or heavier persons (5).

Posture : Changes from standing to supine position has been shown to cause a marked fall both in plasma and salivary melatonin levels and hence care should be exercised when comparing the melatonin levels of healthy person with bed-ridden patients (37).

Melatonin receptors

The localization and characterization of putative melatonin receptors in neuronal and non-neuronal tissues of many vertebrate species including man has been made possible by the use of 2 (^{125}I) iodomelatonin (44), a highly specific radioligand. Pharmacological and kinetic studies of 2 (^{125}I) melatonin binding sites led to the identification of ML 1, ML 2 subtypes of melatonin receptors (38). The xenopus ML1 receptor has recently been cloned from cDNA library derived from dermal melanophore line (45), and melatonin receptors belong to the superfamily of seven transmembrane domain "G" protein coupled receptors that are functionally interrelated to the inhibition of adenyl cyclase (44, 46). By using *in vitro* autoradiography, high affinity melatonin receptors ML1 have been identified in the suprachiasmatic nucleus (SCN) of the hypothalamus and these receptors are involved in the circadian rhythm regulating function of melatonin (46, 47). Melatonin receptors present in the mediobasal hypothalamus may be involved in the control of reproduction (47), and receptors in the neural retina and superior colliculus seems to be involved in the regulation of visual function (38). High affinity melatonin receptors identified in the cerebral and caudal arteries are probably involved in cardiovascular and thermoregulatory functions (47).

Melatonin also binds directly with cytosolic and nuclear sites and induces a variety of physiological effects. This is in addition to their effects on membrane bound receptors (38). Recently a specific nuclear protein melatonin receptor, RZRβ related to retinoid receptor has been identified and this receptor is said to mediate the transcriptional effects of melatonin. The mRNA of RZRβ is found in the pineal gland, retina, superior colliculus and SCN (46). This exciting discovery points to the possibility that melatonin possess direct genomic action (38, 46, 47, 99, 103).

Physiological action of melatonin

In the early part of this Century, McCord and Allen (1917) first reported that extracts of bovine pineals induced blanching of the skin in amphibians (38). Since this compound caused aggregation of melanin granules within melanophores, Lerner and his co-workers (21) named this substance as melatonin. Early studies of melatonin identified this substance mainly as antigonadotrophic, since the extracts of pineals produced only inhibitory effects on gonads (39). However, progonadotrophic effects also have been found (90). The role of melatonin in the onset of puberty is gaining much importance currently and melatonin appears to have a direct and continuous regulatory actions on gonadotrophin secretory pattern from infancy to the onset of puberty (33, 40, 41). Melatonin is one of the few endogenous substances that has influence on LHRH pulse generator (33, 40). Since the purpose of this review paper is to focus the readers' attention on biological rhythm disorders, involvement of melatonin in sleep-wakefulness rhythm, sleep and biological rhythm and their disorders will be discussed in somewhat greater detail than the other functions of melatonin.

Melatonin and sleep

The casual relationship between melatonin production and the occurrence of sleep at night prompted many investigators to postulate that melatonin is involved in the physiological regulation of sleep mechanisms (41, 42, 47, 48, 81). When low doses of melatonin were applied intranasally it induced sleep in human volunteers (48). Similarly in studies involving administration of melatonin at doses ranging from 10 to 240 mg produced subjective feelings of sleep of and sleepiness (49). Recently administration of low physiological doses of melatonin varying from 0.3 mgm to 1.0 mgm to human volunteers induced sleep in all the subjects of the study.

Exogenous melatonin administration elevated the plasma melatonin levels significantly, irrespective of the existing phase of circadian rhythmicity (50). This low physiological doses of melatonin induced quiet restful sleep not only in normal healthy adults but also in elderly insomniacs (51, 54). Unlike other hypnotic drugs, melatonin did not cause any day time sleepiness that usually follows overnight sleep induced by the administration of hypnotic drug and in this aspect it is found for superior to the conventional hypnotics (51). Administration of melatonin to blind human subjects also during daytime induced sleep by elevating daytime plasma melatonin levels. A significant correlation was found between plasma melatonin levels and sleep, thereby substantiating the fact that rise of endogenous melatonin in the plasma is an important factor that determines the sleep timing pattern (52). In another recent study it was found that administration of melatonin in the dose of 5 mg/day late in the evening provided the correct sleep onset signal and induced sleep in insomniacs (53). All these recent clinical studies support the concept that melatonin acts as a physiological regulator of sleep mechanisms (105).

Melatonin as a biological rhythm regulator

Melatonin has been found to alter the timing of mammalian circadian rhythms and has been shown to function in concert with

light to hold the circadian rhythms in tune with prevailing environmental light-dark cycles. This has prompted many investigators to label this hormone as a circadian chemical messenger or "chronobiotic" (55, 56). The fact that melatonin is secreted in a circadian manner and this rhythm is governed by SCN and modified by light-dark cycle suggested to many that this hormone is concerned with regulation of circadian rhythmicity.

Melatonin has been termed as an internal zeitgeber regulating rhythms occurring in every cell, organ and tissues present in our body (55). Evidences for this have been obtained partly from animal studies but mostly from clinical studies undertaken in subjects suffering from various biological rhythm disorders.

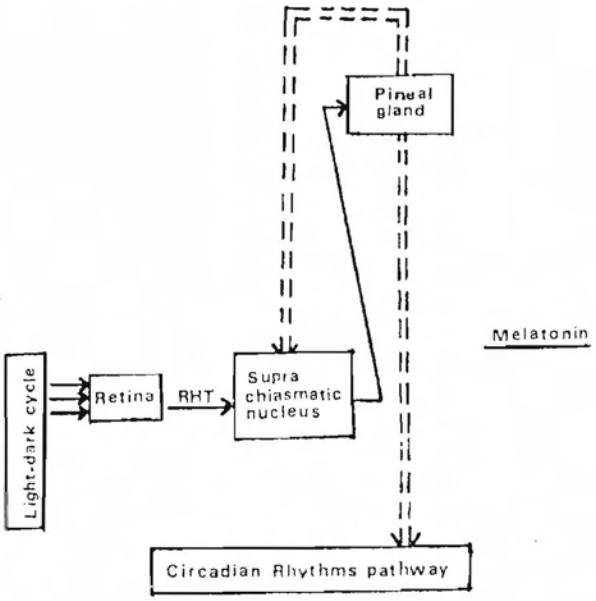
Experimental animal studies: In certain species of birds such as house sparrows, pineal body is the important structure generating circadian rhythmicity and this was demonstrated by Gaston and Menaker in 1968, cited in (55). Pinealectomy resulted complete arrhythmia in these birds. In certain other species of birds, pinealectomy alone had no effect on rhythmicity but pinealectomy along with opthalmic enucleation produced arrthmia showing there by that melatonin produced by the pineal gland and retina are important in the regulation of circadian rhythms (57). The study also indicated that pineal gland acts as the biological clock in birds (11). In contrast to these observations, in rodents neither pinealectomy not enucleation did not abolish the complete occurrence of circadian rhythms (58), but lesions of SCN abolished the circadian rhythms of locomotor activity (59).

Initial studies of this nature raised doubts about melatonin involvement in circadian rhythm regulation. However, later studies showed the importance of melatonin as a circadian rhythm regulator (55, 60, 61, 62, 63). Daily subcutaneous injection of melatonin in the dose of one mgm/kg of body weight has been shown to entrain free running locomotor rhythm in rats housed in completed darkness (60). A single melatonin administration regime has been shown to take over all the synchronizing properties of light-dark cycle (61, 62) and indeed single injection of melatonin 50 micro gm/kg of body weight, has been shown to phase advance the circadian locomotor rhythms for a period of about two weeks (63). Authors of these studies stated that melatonin entrains circadian rhythms by acting on the SCN itself. Recent discovery of high affinity melatonin binding sites in the SCN only confirms the earlier findings that melatonin's main function is to impose synchronicity on the multitudes of daily rhythms occurring in all cells of our body (38, 46, 55, 61, 62). Melatonin being highly lipophilic in nature permeates through all cells of our body with great ease (55). Interaction of melatonin and the SCN in the regulation of circadian rhythms is shown in Fig. 3 and it may be mentioned here that the author had also presented this concept in 1991 (3).

Biological rhythm disorders

Clinical studies

All our internal physiological rhythms are synchronized with environmental light dark cycles and for this adaptation both



ROLE OF PINEALGLAND IN THE CONTROL OF CIRCADIAN RHYTHMS

Fig. 3 : Melatonin influences circadian rythums by acting on ML 1, ML 2, receptors present in SCN and also directly by permieating through cells and tissues of the body.

suprachiasmatic nucleus and the pineal gland are very much essential (12).

Disorganization of the circadian time keeping system can give rise to various biological rhythm disorders (2, 3, 4, 6). The study of these disorders will not only help us to understand the possible involvement of melatonin in these disorders but also will give us directions for effective control and management of these conditions in future by using only simple harmless substances such as melatonin or its analogues. Biological rhythm disorder also form the major thrust areas for research since they involve not only the health of the individual concerned but also the safety of the society in which he lives (31, 80). Major industrial, air, train, or road accidents are mainly due to the inefficient handling of the situations due to the decreased alertness exhibited by the individuals suffering from major biological rhythm disorders (31, 74, 79, 80).

Circadian rhythm sleep disorders

In these disorders, the duration of sleep is not very much affected but the timing of sleep onset and wakefulness are very much altered and is precipitated either due to the intrinsic malfunctioning of the circadian oscillator or due to the temporary or chronic mismatch between endogenous bodily rhythms and environmental light-dark cycles. Alteration of melatonin rhythms have been documented in some of these conditions implicating melatonin in these disorders (33, 64).

Delayed sleep phase syndrome (DSPS) :

Delayed sleep phase syndrome (DSPS) was first identified by weitzman and his co-workers in 1981 (66). The onset time and wakefulness time are very much delayed and this condition is encountered only in young adults. People may fall asleep as late as 1.00 PM and may wake up at 11.00 AM. Repetition of this unusual procedure day to day will result in progressive sleep debt as these individuals will be forced to wake up early in the morning by the demands of the society (55). Conventional methods of treatment have failed to induce complete recovery from this illness. Following successful application of phototherapy in winter depression in 1984, Rosenthal and

his co-workers (67) used bright light for treating DSPS. Exposure of the individuals suffering from DSPS to bright light of 2500 lux for 2 hrs in the morning between 6 AM to 9 AM and then shielding them off from light by making them to wear dark goggles from 4 PM to dusk for a period of one week, advanced not only their sleep onset time but improved very much their day time alertness also even at the second week of treatment (67).

Since melatonin has been implicated in the mechanism of action of phototherapy this pineal hormone also has been tried directly in treating DSPS. Melatonin in the dose of 5 mgm/day was administered orally at 22.00 hrs to a group of 8 subjects suffering from DSPS. The duration of treatment was 4 weeks. Melatonin not only improved the sleep quality but also significantly advanced the mean sleep onset time by 82 min with a range from 19–124 min. The mean wakefulness time also was advanced by 117 minutes, but the total duration of sleep remained more or less 8 hrs 12 min (68).

Advanced sleep phase syndrome (ASPS) : As age advances changes in sleep patterns are noted and these are attributed to changes in the functioning of the circadian oscillator (56). A phase advance of sleep-wakefulness rhythm with sleep onset occurring around 8.00 PM and wake onset occurring around 3.00 AM was noted in ASPS. The quality of sleep was also very much affected as seen by increased nocturnal awakenings noted in these individuals (4). Ability to maintain phase relationship of different biological rhythms including the sleep wakefulness rhythm deteriorates with aging (19) and alteration or attenuation of melatonin rhythm has been found as one of the causes (22, 69). Recent studies have shown that melatonin improves the sleep quality (51, 70) and its usefulness in correcting the sleep wake rhythm disorders of the elderly has been very much indicated (46).

Jet lag : Rapid intercontinental jet flight across several time zones results in sudden exposure of the traveller to a new environmental light-dark cycle causing a temporary mismatch between his endogenous bodily rhythms and the imposed new environmental cycle (71). All endogenous circadian rhythms are shifted in the direction of flight; an east bound flight is followed by phase-advance of rhythms while west bound flight results in the phase delay or rhythms (72). The different bodily rhythms take different times to establish their normal phase relationships and time lag for resynchronization of internal rhythms is referred to as 'Jet Lag' (6, 55, 64). Palmer (1976) defined Jet lag cited in (57) as a "malange of symptoms dominated by disrupted sleep pattern occurring when one's physiological rhythms are out of phase with ambient light dark cycle after transmeridional flight". Features of endogenous depression like weight loss, anorexia, irritability, fatigue are very common in individuals affected by Jet lag (71), but susceptibility to Jet lag is highly individual and may vary from person to person (72). Melatonin treatment 5 mg/day reduced the symptoms of jet lag by 50% (73), and is found equally effective irrespective of the direction either towards east or west (20). Apart from field investigation studies, simulated conditions also revealed that melatonin treatment or bright light therapy

has been found effective in reducing the symptoms of jet lag. Sudden exposure of individuals after a rapid 9 hr phase shift of the light-dark cycle induced in these studies did not produce symptoms of jet lag if the subjects have already undergone prior exposure to either bright-light treatment or melatonin administration (72).

Shift-work disorder : In our modern industrialized society, large number of people are engaged in work schedules that involve both day time and night time work. These individuals are forced to forego their nocturnal sleep but are forced to sleep during the day. This inversion of activity and sleep-wake rhythms give rise to severe disruptions not only in their sleep but also affects very much the individual's alertness and performance while they are engaged in work (74, 75, 76), and they suffer from a constellation of signs and symptoms in various systems of the body giving rise to a new medical problem known as shift-work disorders (77). These individuals suffer from insomnia, delayed sleep onset, persisting fatigue that does not disappear after sleep, digestive troubles like epigastric pain dyspepsia, indigestion, peptic ulcer, cardiovascular complaints and changes in behaviour that give rise to frequent irritability etc. (74, 77).

The continuous exposure of shift workers to low intensities of light during their night shift work and their exposure to bright natural sunlight during "off duty hours" in the morning prevents the circadian adaptation of their physiological rhythms to their desired sleep-wake cycles (75). Hence phototherapy using bright artificial light has been tried for treating this disorders.

Shift workers were exposed to various intensities of bright light during their nocturnal working hours and were asked to remain in completely darkened rooms during their off-duty hours during the day. This method adapted by Czeisler and his co-workers (1990) significantly improved the physiological adaptation of the circadian rhythms of night shift workers to their inverted sleep-wake schedules. The endogenous circadian rhythms of body temperature, subjective alertness, cognition, performance got completely adjusted to their new work schedules immediately on exposure to four consecutive cycles of bright light therapy (14). Recently it has been documented that light intensity as low as 180–500 lux that usually equals ordinary indoor room light also can effectively alter the phase positions of endogenous circadian rhythms in normal healthy individuals (32). This method can be employed with night shift workers to improve the physiological adaptation of their endogenous rhythms to the imposed light-dark cycle caused by the alteration of rest activity cycle.

Recently it has been noted that melatonin treatment not only improved night time alertness and performance significantly during working hours but also induced quiet restful sleep in the day during their "off-duty" hours in shift workers of the study (15, 20, 79). This proves the efficacy of this hormone in improving tolerance to night shift work. In this context it is noteworthy that permanent night shift workers have the period of melatonin elevation shifted to day time (33). Thus in order to explore the night shift workers adaptation to the inverted rest-activity/ sleep-wake cycle, it is absolutely essential

to measure the plasma melatonin rhythm and adequate daytime plasma melatonin levels should be maintained for inducing restful sleep by melatonin administration.

Melatonin and mood disorders

Biological rhythm disturbances occur in certain (i) endogenous depressives, (ii) in manic-depressive psychosis and (iii) in seasonal disorder (SAD) or winter depression.

Endogenous depression: In endogenous depression REM sleep occurs during early phase of sleep episode (81, 85). In addition to this phase advance of a number of other circadian rhythms also occurs. The nocturnal rise of prolactin secretion, major nocturnal pulse of GH secretion, the nadir of cortisol secretion, core body temperature are all phase advanced by 2 to 3 hrs in endogenous depressives (31, 80). Healy in 1987, cited in (12), claimed that "all mood disorders in some way or other involve rhythm disturbances based on internal or external desynchronization". The finding of low nocturnal levels of melatonin in depressives (82, 83) led to the concept of "low melatonin syndrome" and depressions linked biochemically to a disturbance of melatonin production, secretion or function (84). However, recent studies of melatonin measurements in 22 subjects of age 8-17 years suffering primarily from major depression, showed elevated nocturnal serum melatonin levels and the authors state that dysregulation of the pineal gland in the form of increased serum melatonin exists in youth with major depression (5, 96). The fact that depressed adults with psychosis had low serum melatonin in this study points to the possibility that higher levels of melatonin may help to protect a depressed patient from psychotic symptoms (96). Because "the pineal gland and its hormone melatonin contribute to the regulation and entrainment of circadian rhythms to 24 hr cycle", the increased or decreased nocturnal serum melatonin in depressives only reflect the disregulation of pineal gland function in depressive disorder (96). Supportive evidences for this has been obtained from animal and human studies in which administration of psychoactive drugs produces elevation of plasma melatonin or pineal melatonin (78).

Manic-depressive illness (MDP) : MDP is recurrent illness in which episodes of mania and depression occur and remit spontaneously. Several clinical features of manic-depressive illness point out that disturbances in the timing of phase position of circadian rhythms play a crucial role in its pathophysiology (85). Melatonin secretion itself has been found to be abnormal in some bipolar patients (MDP) and study of this rhythm will be useful in investigating the biochemical basis for this disorder (65). Patients with MDP exhibit circa-bi-dian sleep wake rhythm in which patients spent one complete sleepless night (85) in between two nights of normal sleep.

Seasonal affective disorder (SAD) : SAD or winter depression is characterized by recurrent episodes of depression during winter months and euthymia or hypomania in spring or summer seasons and was first documented by Rosenthal and his co-workers in 1984 (67). The major symptoms of this disorder include hypersomnia, hyperphagia, carbohydrate craving, weight gain, etc. Endogenous circadian rhythms are all phase delayed in this condition. Most of

these patients have delayed onset of melatonin secretion and shifting of melatonin rhythm has been suggested as one of the predisposing factor for triggering winter depression (65). Moreover, SAD patients are more sensitive to dawn and one is inclined to believe that winter depression is related to circadian rhythm disturbance rather than to seasonal rhythms (86).

Phototherapy of SAD: Application of bright light in the morning (2500 lux) phase advanced all the endogenous rhythms and caused clinical remission in patients with SAD within a week (98). Phototherapy has been suggested to act through retinal melatonin, whose main function is to regulate dopamine release in the eye where it acts as the main neurotransmitter. The retina-SCN-pineal gland forms a link, which is disturbed in SAD. By suppressing retinal melatonin, phototherapy has been suggested to correct the underlying biochemical abnormality seen in SAD and thereby inducing clinical remission (87). Bright light has been shown to suppress melatonin secretion even in blind subjects (97) which substantiates the fact that non-visual photoreceptors in the eye mediate the circadian rhythm regulating effects of light (31). Recently it has been noted that exposure of SAD patients to natural sunlight in the morning hours for a period of one week resulted in complete remission of depressive symptoms (86). This seems to be a significant advancement in the etiology of SAD, that links light with mood disorders. However, the exact role of melatonin in this condition is yet to be fully understood.

Melatonin and seasonal reproduction

Melatonin acts as a photoperiod messenger molecule that transmits information about the length of the photoperiod in seasonal breeders (88). Gonadal atrophy and regression occur in syrian hamsters during winter when nights are of longer duration and days are short. Melatonin secretions are higher during long winter nights of these species (89). In ewes and sheeps also melatonin secretions are higher during winter months, but gonadal activity is initiated during winter nights (90). Administered melatonin also induces the same effects of photoperiodic changes on seasonal reproduction. It is possible to mimick winter season during summer by judicious melatonin implants and sheep production can be achieved to a significant degree (90, 91). Animal studies indicate that melatonin can also act as a seasonal cue regulating the physiological status of the reproductive system of these animals on a seasonal basis. An inverse seasonal relationship between pineal and ovarian secretion has also been demonstrated by Kauppila and his co-workers in human population living at high altitudes (cited in 19).

Melatonin and temperature regulation

The phase-shifting of the endogenous circadian rhythms by melatonin is usually preceded by its effect on core body temperature and a direct relationship seems to exist between these two phenomena (20).

Melatonin administration induces an acute transient suppression of core body temperature and is dose dependent (80). Deacon and Arendt (1995) have suggested that melatonin influences body temperature by acting centrally on the preoptic area and

anterior hypothalamus which are the main thermo regulatory centres. Indeed high affinity melatonin binding receptors have been found in many areas of the hypothalamus (38, 46, 47). The acute changes in body temperature produced by melatonin has been suggested as the primary event that is responsible for the phase-shifting effects of melatonin on bodily rhythms and thus the two mechanisms seems to be closely inter linked to each other (80).

Melatonin as an antioxidant

Recent studies carried out from several laboratories particularly from Reiter's laboratory (USA) have shown that melatonin has anti oxidant properties (100, 101, 102, 103, 104). Melatonin acts as a highly efficient scavenger for hydroxyl (–OH) and peroxyl (ROO–) radicals. The hydroxyl radical which is highly toxic can damage macro–molecules like DNA, proteins, carbohydrates and lipids and DNA damage by –OH radical can even lead to cancer. Melatonin protects these macromolecules like the nuclear DNA from oxidative stress by acting as a intracellular free radical scavanger and it does not require any membrane receptor (100). In cultured cells, organs as well as in direct *in vivo* test, melatonin has demonstrated its antioxidant potency (103). The genomic damage inflicted on the cultured human lymphocytes by ionizing radiation has been shown to be reduced by 60% in the presence of either 2 mM melatonin or 2 M dimethyl sulfoxide (DMSO) a known free radical scavenger showing thereby, the potency of melatonin as antioxidant (101). Melatonin also seems to be a more effective scavenger of peroxyl radicals than vitamin E. It is likely that the antioxidant actions of melatonin are also due to its regulation of antioxidant enzymes in the cell like glutathione peroxidase (GSH-PX) (103) since this enzyme in the brain is stimulated both by pharmacological as well as physiological doses of melatonin as shown by Pablos and his co-workers (cited in 104). These observations lend support to the concept that melatonin also contributes to the total antioxidative defence system of the organism (104).

Melatonin : other functions

Melatonin has been implicated as one of the factors that determines longevity (19). It also exerts inhibitory effect on neoplastic growth (92). Low melatonin levels are found in patients either with breast cancer (93) or prostatic cancer (94) suggesting the possible involvement of melatonin in cancer. The nocturnal serum melatonin concentrations are also low in patients with coronary heart diseases (95). This suggest the role of melatonin is normally to prevent the nocturnal rise of catecholamines which is the main predisposing factor for coronary heart diseases. As for the role of melatonin as an anti-ageing compound, much more research and large scale clinical trials are needed to demonstrate the exciting therapeutic uses of melatonin (43).

Melatonin analogues

Following the identification and characterization of melatonin receptors, many melatonin analogues with increased affinity to receptors have been developed. 2-iodo–melatonin, and 2-phenylmelatonin and their agonists show 10-fold improvement over melatonin (99). 5-methoxy-caronyl-

amino-N-acetyltryptamine (5-MCA-NAT) a recently developed melatonin analogue also binds with increased affinity to ML1 receptors (38). Substitution of the indole nucleus by naphthaline ring produces the compound namely N-2-7-methoxy-1-naphthqlenyl ethylacetamide has been found effective in regulating behavioural circadian rhythms. 2-amido tetralins with a methoxy group in position at 8 acts at ML1 receptors. Luzindole (0877) is a competitive ML1 receptor antagonist. These melatonin analogues that have been listed above will be of use not only for understanding the cellular and molecular mechanisms of actions of melatonin and also for uncovering the other physiological functions of melatonin (38). In addition to this, they may be found beneficial for treating symptoms of jet lag, shift-work disorder, circadian based sleep disorders and non–circadian sleep disorders etc., (47, 99).

CONCLUSION

Melatonin, the main pineal hormone is secreted in a circadian fashion and this rhythm is regulated by SCN, the biological clock that controls all our circadian rhythm light-dark cycle by acting through the retino-hypothalamic pathway entrains our circadian rhythms to 24 hour cycle. The recent discovery of non-visual photoreceptors that mediate circadian rhythms regulating effects of light and high affinity melatonin receptors in SCN suggest that melatonin plays a crucial role in the regulation of circadian rhythms. Disorders like DSPS, jet lag, shift work disorder and SAD, respond well either to phototherapy or melatonin suggesting that altered melatonin rhythm and dysfunctions of pineal gland may be the main factors that give rise to these disorders. The finding of either low or increased melatonin levels in depressives points to the possibility of dysfunction of the pineal gland as the main factor underlying mood disorders. Melatonin plays an important role in temperature regulation, control of sleep and seasonal reproduction. Recent studies have shown that melatonin is a powerful antioxidant which can neutralize hydroxyl radical and peroxyl radical. The recent discovery of melatonin ML1 and ML2 receptors in SCN, and the development of melatonin analogues which interacts with these receptors suggests that the analogues will be of value in treating biological rhythm disorders. The exciting genomic actions of melatonin points to the possibility that melatonin as a hormone, and as drug has a much larger role to play than the current ones that have been discussed here. The finding of low levels of melatonin in certain categories of cancer patients, low nocturnal melatonin in patient suffering from coronary heart disease indicate that melatonin may be involved in the pathogenesis of these disorders.

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