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Melatonin as a chemical indicator of environmental light-dark cycle

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Mini-review

Abstract. Melatonin (N-acetyl-5-methoxytryptamine) is an evolutionary highly conserved molecule that plays an important role in conveying the clock and calendar information to all living organisms, including man. Melatonin is synthesized in the rhythmic fashion, primarily by the pineal gland, and, to a lesser degree, by extrapineal tissues - namely the retina, the Harderian gland, and the gastrointestinal tract. The rhythm of the hormone production, with maximal levels occurring at night in darkness, is generated by an endogenous circadian clock(s) and is synchronized with the photoperiodic environment to which animals are exposed. This brief outline surveys data on the regulation of rhythmic melatonin biosynthesis by a circadian pacemaker and light (full spectrum white light and monochromatic lights with wavelengths both in the visible and invisible range). Additionally, possible applications of this chronobiotic compound in agriculture and in medicine in the treatment of circadian rhythm sleep disorders are discussed.

Key words: pineal gland, retina, melatonin, light, circadian rhythm, seasonal reproduction, circadian rhythm sleep disorders

INTRODUCTION

Various organisms living under physiological conditions have evolved mechanisms that enable them to organize their responses and adapt to new or changing environments. As a result of this, animals express variety of both daily rhythms and seasonal cycles in their biochemistry, physiology, and behavior. The most obvious biological rhythms are the daily cycle of sleep and wakefulness, and the seasonal cycle of growth and reproduction. Majority of animal species use annual changes in variables, such as photoperiod, temperature, rainfall and food supply, to regulate physiological functions such as reproduction, migration, locomotor activity, feeding patterns, molt of skin, fur and plumage.

The major action of the environment is to synchronize the internal system to a period of precisely 24 h, so that this internal system and expressed rhythms it controls will be in harmony with the day-night cycle. Circadian rhythms can be entrained by only a few environmental variables, with light and temperature cycles being the dominant synchronizing agents.

In vertebrates, it is now clearly demonstrated that the pineal gland is implicated in conveying photoperiodic information *via* the daily pattern of melatonin secretion. Since the rhythmic formation of melatonin has been demonstrated in unicellular organisms, invertebrates and vertebrates, it is hypothesized that this evolutionary conserved molecule plays an important role in providing the clock and calendar information to all living organisms, including man. Although the pineal gland is widely accepted as the main source of the body melatonin in most animal species studied thus far, other extrapineal tissues, notably, the retina, the Harderian gland, and the gastrointestinal tract, are also capable of producing this compound (Binkey et al. 1979, Besharse and Iuvone 1983, Nowak et al. 1989, Reiter 1991, Zawilska and Nowak 1992, Huether 1993).

RHYTHMIC CHANGES IN MELATONIN BIOSYNTHESIS AND RELEASE

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized from the aminoacid precursor L-tryptophan by the consecutive action of four enzymes: tryptophan hydroxylase (TPH; EC 1.14.16.4), aromatic amino acid decarboxylase (AAAD; EC 4.1.1.28), serotonin N-acetyltransferase (NAT; EC 2.3.1.87) and hydroxyindole-O-methyltransferase (HIOMT; EC 2.1.1.4). The last two enzymes, i.e., NAT and HIOMT, require acetyl coenzyme A (AcCoA) and S-adenosyl-L-methionine (SAM), respectively, as their cofactors.

$$\text{L-Tryptophan} \xrightarrow{\text{TPH}} \text{5-Hydroksytryptophan}$$

$$\xrightarrow{\text{AAAD}} \text{5-Hydroxytryptamine}$$

$$\xrightarrow[\text{AcCoA}]{\text{NAT}} \text{N-acetyl-5-hydroxytryptamine}$$

$$\xrightarrow[\text{SAM}]{\text{HIOMT}} \underset{(\textit{melatonin})}{\text{N-acetyl-5-methoxytryptamine}}$$

The most striking feature of the melatonin-generating system is its diurnal (or in some species circadian) variation and enormous sensitivity to light, which suppresses its activity. Regardless of whether animals are diurnally active, nocturnally active or they exhibit a crepuscular activity pattern, melatonin levels are always highest during the dark phase of any natural or imposed light-dark illumination cycle (Reiter 1991, 1993a,b). The rate and the pattern of the nocturnal increase in melatonin production depend, among other factors, on species and tissues. Three different basic patterns of the pineal melatonin production are described in mammals (Reiter 1987). A type A, not very common among animal species and demonstrated in the Syrian hamster, Mongolian gerbil and the house mouse, is characterized by a discrete melatonin peak occurring late at night (or in the dark phase of the light-dark illumination cycle). Thus, the onset of the nocturnal melatonin production is delayed by several hours after the lights off time. After midnight, melatonin levels increase quickly to its peak values and soon thereafter, before the time of lights on, they decline to daytime values. A type B represents the most common pattern of the nocturnal pineal melatonin formation, and is characterized by a midnight melatonin peak. In animals with this pattern (e.g., domestic hen, domestic rat, guinea pig, ground squirrel, and human), pineal melatonin levels gradually rise (beginning at about the time of lights off), reaching peaks around the middle of the night, and then declining slowly during the second half of the night to reach low daytime values near the time of lights on. Animals with a type C pattern of the nocturnal melatonin synthesis are also common, and they are characterized by a prolonged peak of melatonin levels for virtually the entire night. Thus, peak melatonin production is reached soon after the onset of darkness; these high levels of the hormone are maintained for most of the night and decrease before lights onset. This pattern of the nocturnal pineal melatonin formation is present for example in the domestic sheep, domestic cat and the Djungarian hamster. Regardless of the type of the pineal melatonin pattern a given species exhibits, an extension of the night (or the dark phase) also lengthens the duration of the elevated hormone levels. Thus, the duration of elevated melatonin is positively correlated with the duration of night.

The rhythmic production of melatonin appears to be regulated primarily by distinct changes in the activity of NAT, which fluctuates in a manner similar to melatonin levels, with respect to both photoperiod and acute light exposure (Binkley et al. 1979, Reiter 1991, Zawilska and Nowak 1992). Very recently, using molecular biology techniques, diurnal variations in the amount of mRNA encoding NAT molecules, parallel to changes in the enzyme activity, have been demonstrated for the pineal gland of rat and sheep (Borjigin et al. 1995, Coon et al. 1995). An absolute amount of the synthesized melatonin is additionally dependent on serotonin availability during the whole period of high NAT activity (Thomas and Iuvone 1991). On the other hand, the activity of the last enzyme in melatonin biosynthetic pathway, i.e., HIOMT, does not seem to change as a function of photoperiod or lighting conditions (e.g., Reiter et al. 1983, Sugden et al. 1987, Nowak et al. 1989, 1993).

It has been shown that rhythms of melatonin formation and release in the pineal gland of chick, house sparrow, and pigeon, and in the retina of African clawed frog (*Xenopus laevis*), chick and quail are circadian in nature (Besharse and Iuvone 1983, Robertson and Takahashi 1988a, Zatz et al. 1988, Underwood et al. 1990, Cahill and Besharse 1991, Zawilska and Wawrocka 1993, Murakami et al. 1994). They free-run in constant darkness for several days, with levels decreasing during the subjective light phase, and increasing during the subjective dark phase (Fig. 1). The periods of these rhythms increased to about 25 h (e.g., the retina of *Xenopus*

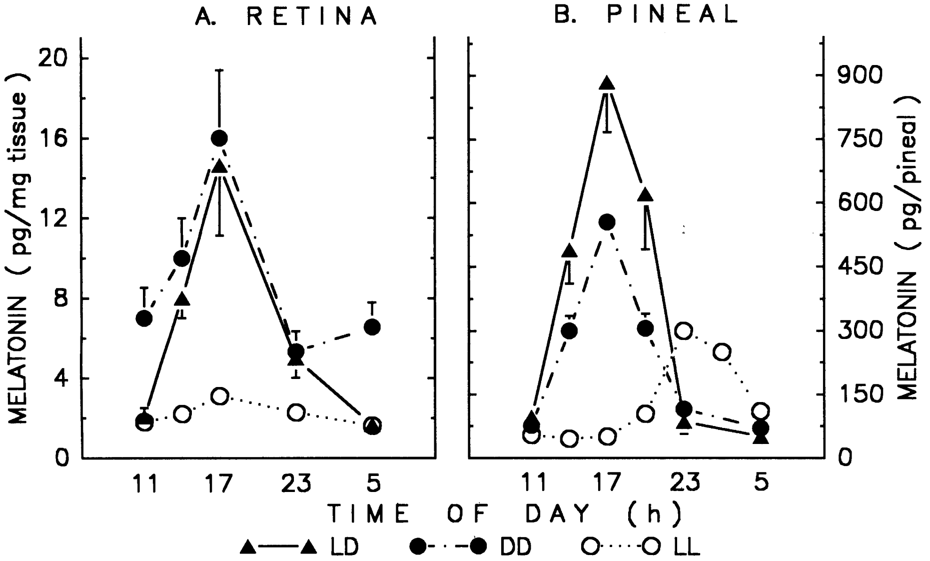

Fig. 1. Effect of constant darkness (DD) and continuous light (LL) on the rhythm of melatonin content in chick retina (A) and pineal gland (B). Chicks maintained under the 12 h light: 12 h dark (LD) illumination cycle (lights off between 11:00 and 23:00) were exposed for 4 days to the normal LD cycle, DD or LL. Data are mean ± SEM. *n* = 6-8 for each time point.

laevis and chick, the pineal gland of chick) or decreased to about 20 h (pineal cells of house sparrow and pigeon) (Robertson and Takahashi 1988a, Cahill and Besharse 1991, Zawilska and Wawrocka 1993, Murakami et al. 1994). In constant darkness the amplitude of the rhythm of melatonin biosynthesis markedly damped with time. This damping of the amplitude can be primarily due to an increase in trough levels (e.g., chick retina, pineal cells of house sparrow), or a decrease in peak levels (e.g., pineal cells of pigeon) or both (e.g., pineal cells of chick, photoreceptor cells of *Xenopus laevis* retina) (Robertson and Takahashi 1988a, Zatz et al. 1988, Cahill and Besharse 1991, Zawilska and Wawrocka 1993, Murakami et al. 1994). Interestingly, although the pineal cells of Japanase quail showed an elegant diurnal oscillation of melatonin synthesis and release under light-dark cycle, this rhythmicity was potently weakened or even abolished under conditions of constant darkness (Murakami et al. 1994). In the pineal gland and the retina of living chick cyclic changes in NAT activity and melatonin level persisted also, for up to 5 days, under conditions of continuous light (Zawilska and Wawrocka 1993). However, these two tissues differentially responded to the constant light treatment. Adaptation to light strongly suppressed melatonin production of the retina, but in spite of this, NAT activity and melatonin content of the tissue oscillated in a low-amplitude rhythm with a period close to 24 h (Fig. 1A). In the pineal gland of the same species, light exposure suppressed the level of the enzyme activity and melatonin content (the effect being substantially weaker than that observed in the retina), delayed the first peak by 3 h, and prolonged the rhythm's period to about 26 h (Fig. 1B). Thus, some tissue and species-dependent differences in the activity and/or properties of the circadian oscillators that generate the rhythm of melatonin production are likely to occur.

In mammals, the pacemaker (or driving oscillator) that controls the rhythm of the pineal melatonin biosynthesis is localized to the suprachiasmatic nuclei of the anterior hypothalamus (SCN). The retinohypothalamic fibers, whose perikarya are localized to the ganglion cell layer of the retina, synchronize the activity of the SCN to precisely 24 h and, in addition, mediate the photic information to the oscillator (reviewed by Reiter 1991). On the contrary, in birds the pineal gland is directly photosensitive and contains an endogenous pacemaker generating the circadian rhythm of melatonin production (Robertson and Takahashi 1988a, Zatz et al. 1988, Murakami et al. 1994). Whether the same holds true for lower vertebrates remains to be established. An increasing body of evidence indicates that the rhythmic biosynthesis of the retinal melatonin is under control of a biological clock(s) localized to the retina itself, most probably, to photoreceptor cells (Underwood et al. 1990, Zawilska and Iuvone 1992, Cahill and Besharse 1993).

EFFECTS OF LIGHT ON THE MELATONIN-GENERATING SYSTEM

Light is the dominant environmental factor regulating melatonin biosynthesis, and as such it has two distinct effects on the hormone production. Thus, light causes an acute suppression of NAT activity and melatonin content and release (e.g., Binkley et al. 1979, Iuvone and Besharse 1983, Robertson and Takahashi 1988b, Nowak et al. 1989, Cahill and Besharse 1991, Zawilska et al. 1994), and it resets the phase of the free-running circadian oscillator generating the rhythm of melatonin production (Robertson and Takahashi 1988b, Zatz et al. 1988, Cahill and Besharse 1991, 1993, Zawilska 1994a,b). On the other hand, numerous studies performed on various vertebrate species have shown that a short-term exposure to light has no significant effect on both the pineal and retinal HIOMT activity (e.g., Nowak et al. 1989, 1993, Reiter 1991).

The ability of light when applied at night (or the dark phase of the light-dark illumination cycle) to shut down the enzymatic machinery required for nocturnal melatonin production, with subsequent dramatic decline in melatonin levels, is common to all animal species studied thus far. Even a brief exposure of animals at night to white (or full spectrum) light of sufficient intensity will rapidly suppress the hormone formation. However, the amount of light required to do so varies considerably from species to species. In general, the melatonin-generating system of the nocturnally active animals is far more sensitive to the inhibition by light than is the system of the diurnally active species. Thus, for example in the albino rat (*Rattus norvigecus*), the most sensitive species in this respect, a light irradiance of 0.0005 $\mu W/cm^2$ (0.0017 lux) evokes marked depression of melatonin synthesis by the pineal gland, whereas in the Richardson's ground squirrel (*Spermophilus richardsoni*) light irradiance of 1,850 $\mu W/cm^2$ (6,200 lux) is needed to suppress the hormone production (Reiter 1993a). Humans require substantial amounts of light to suppress melatonin secretion at night. It has been demonstrated that in order to decrease the nighttime plasma melatonin to basal (daytime) levels, pulses of light of the intensity of 1,500 lux or higher should be applied, an observation that meets an increasing appeal in the treatment of various pathological symptoms resulting from rhythm disturbance (Brainard et al. 1993, Reiter 1993a, Arendt 1995c). It should be noted, however, that recent studies of Boivin et al. (1996) on the relationship between the resetting effect of light on the circadian rhythm of the core body temperature and the intensity of the light pulse have demonstrated that light of the intensity of only 180 lux significantly phase-shifts the human circadian pacemaker, thus indicating that humans are much more sensitive to light than it was initially thought.

The suppressive effect of white light on melatonin-generating system is mimicked, to varying degrees, by monochromatic lights (with wavelengths both in the visible and invisible range). In experiments performed on chicks, rats, and hamsters it has been demonstrated that pulses of visible monochromatic light significantly decreased the nighttime NAT activity and melatonin level of the pineal gland and retina (Figs. 2-4). Also in humans, exposure to monochromatic light at night produced a decline in plasma melatonin level (Brainard et al. 1984, e.g., Lynch et al. 1984, Takahashi et al. 1984, Podolin et al. 1987, Honma et al. 1992, Brainard et al. 1993, Zawilska et al. 1995). Of the tested wavelengths, the most potent light was that in the green-blue range (430-560 nm). Importantly, red light,

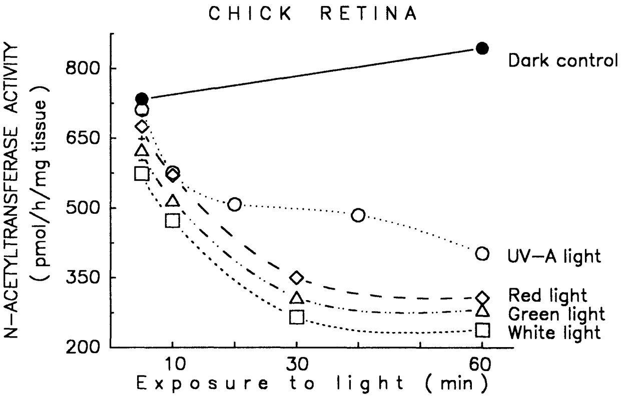

Fig. 2. Suppression of the nocturnal NAT activity of chick retina by acute exposure to white and monochromatic (*UV-A*, λ_{max} = 365 nm; *blue*, λ_{max} = 434 nm; *green*, λ_{max} = 548 nm; *red*, λ_{max} = 614 nm) lights. During the fourth hour of the dark phase of the 12 h light: 12 h dark cycle chicks were illuminated by either white or monochromatic light of an irradiance 7-11 $\mu W/cm^2$. At the end of an experiment birds were killed by decapitation. Dark control refers to the enzyme activity in tissues isolated from chicks not exposed to the tested lights and sacrificed prior to and after the experimentation. Data are mean ± SEM. *n* = 10-22/group.

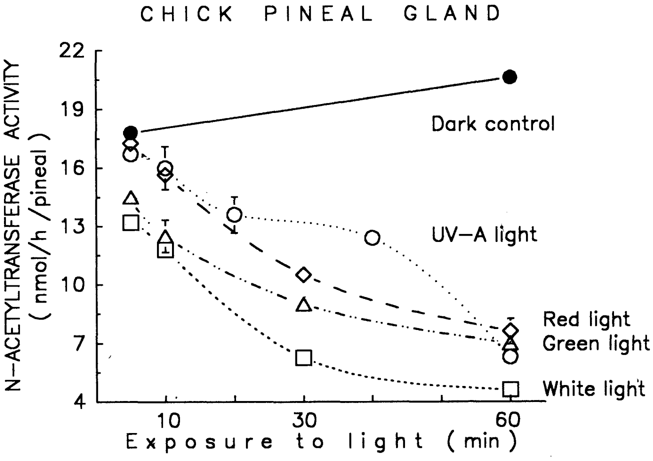

Fig. 3. Suppression of the nocturnal NAT activity of chick pineal gland by acute exposure to white and monochromatic lights. *n* = 10-24/group. For other explanations see Fig. 2.

which for a long time was assumed to have a marginal effect on the melatonin-generating system, when applied at a high intensity (Sun et al. 1993) and/or for a sufficiently long time (Zawilska et al. 1995), is capable of reducing melatonin production to the same extent as white light does (see Fig. 4). It should be noted, however, that anatomical and biochemical elements responsible for this action of red light remain to be established.

Very recently we have found out that NAT activity of the pineal gland of rat and chick and of the chick retina is also sensitive to UV-A light (λ_{max} = 365 nm; irradiance = 10 $\mu W/cm^2$). As in the case of visible monochromatic light (Zawilska et al. 1995), NAT of the rat pineal gland was far more sensitive to the inhibition by UV-A light than the enzymes of the chick tissues (Figs. 2-4). For UV-A light a time-course of NAT inactivation, as well as the enzyme reactivation (following a brief, 5 min exposure to light), was different from those observed for pulses of visible lights (Figs. 2 and 3; Zawilska et al., in preparation).

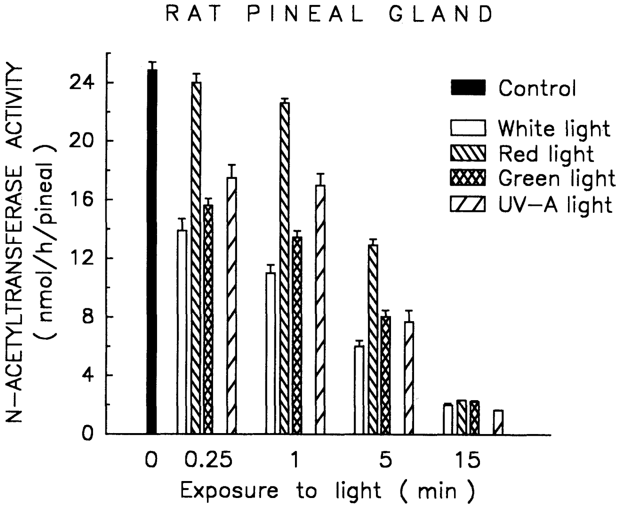

Fig. 4. Suppression of the nocturnal NAT activity of rat pineal gland by acute exposure to white and monochromatic lights. During the fifth hour of the dark phase of the 12 h light: 12 h dark cycle rats were illuminated by either white or monochromatic light, and then killed by decapitation. Control refers to the enzyme activity in pineal glands isolated from rats not exposed to the tested lights. Data are mean ± SEM. *n* = 8-14/group.

Light not only acutely suppresses melatonin biosynthesis, but it also entrains the circadian rhythm of the hormone production and release by resetting a pacemaker. It is well documented that pulses of white light caused phase-dependent phase shifts (advances or delays) visible in subsequent cycles of the circadian rhythm of melatonin formation and release, and did not significantly modify the length of the period of the rhythm. Thus, a light pulse applied early in the subjective night produced a phase delay (Fig. 5). When a light pulse (of the same intensity and duration) was applied during the late subjective night, it caused a phase advance (Fig. 6). Pulses of light given during the subjective day did not affect

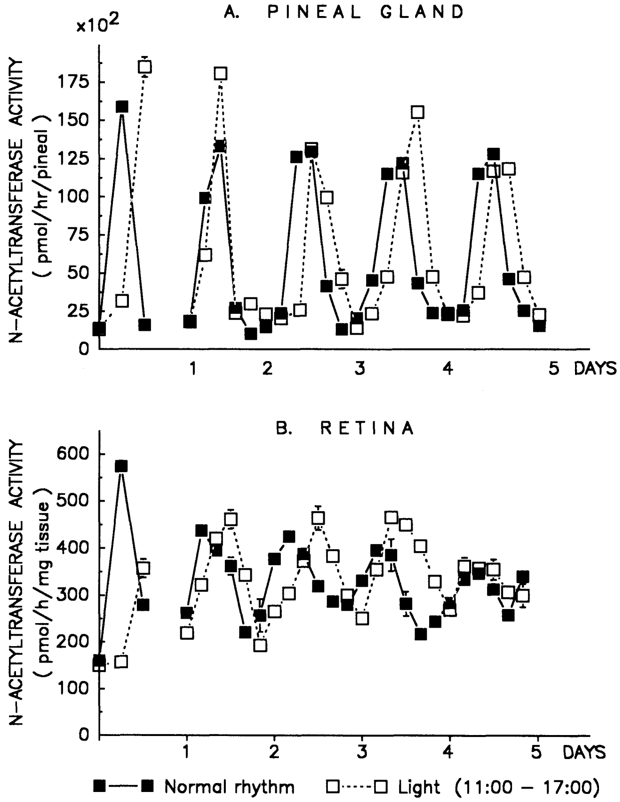

Fig. 5. Effect of light given early in the subjective night on the circadian rhythm of NAT activity in the chick pineal gland (A) and retina (B). Control chicks (normal rhythm) were maintained in constant darkness for 5 days, starting at the animals' dark onset time (i.e., 11:00). Light-treated birds were exposed to a 6-h pulse of white light (100 lux) during the first half of the subjective night (i.e., between 11:00 and 17:00), and then kept in constant darkness throughout the experiment. Data are mean ± SEM. *n* = 5-6 for each time point.

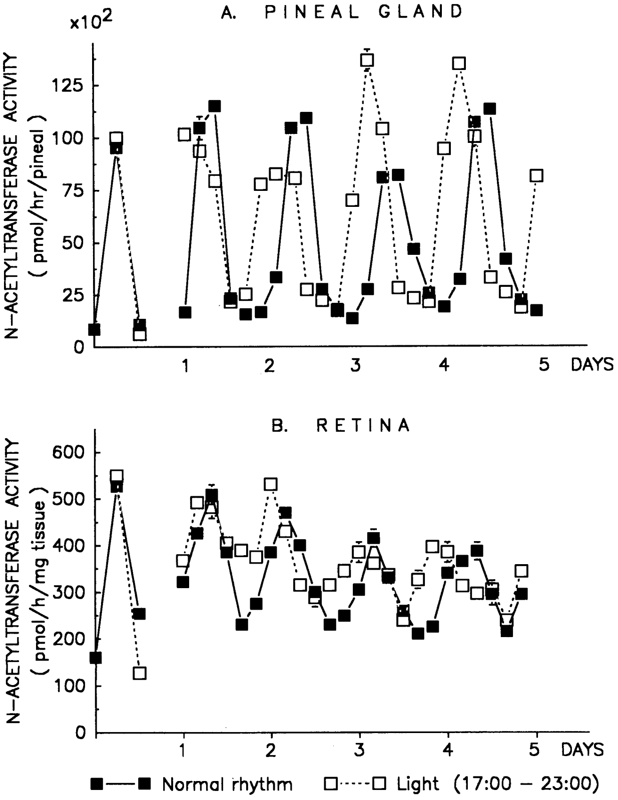

Fig. 6. Effect of light given late in the subjective night on the circadian rhythm of NAT activity in the chick pineal gland (A) and retina (B). Control chicks (normal rhythm) were maintained in constant darkness for 5 days, starting at the animals' dark onset time (i.e., 11:00). Light-treated birds were exposed to a 6-h pulse of white light (100 lux) during the second half of the subjective night (i.e. between 17:00 and 23:00)., and then kept in constant darkness throughout the experiment. Data are mean ± SEM. n = 5-6 for each time point.

the circadian rhythmicity (Robertson and Takahashi 1988b, Zatz et al. 1988, Cahill and Besharse 1991, 1993, Zawilska 1994a,b). At present, it is unknown whether in order to reset the circadian rhythm of melatonin formation a full spectrum of white light is required. Furthermore, a sensitivity of the circadian pacemaker generating the melatonin rhythm to monochromatic lights of various wavelengths should be estimated.

It has been shown that in the pineal gland of chick endogenous noradrenaline, acting through *alpha*$_2$-adrenergic receptors localized to pinealocyte membranes, is involved in mediating the suppressive effect of light on the melatonin-generating system (Voisin and Collin 1986, Pratt and Takahashi 1987, Zatz and Mullen 1988, Zawilska and Iuvone 1989). However, noradrenaline is not a factor of a major importance in the light-evoked process of entrainment of the circadian clock generating the rhythm of the hormone production (Zatz and Mullen 1988, Zawilska 1994a). In the case of retina, there is fairly good evidence that dopamine, released in response to light stimulation and acting on postsynaptic D$_4$-like (and/or D$_2$) dopamine receptors, is partially responsible for the light-induced inhibition of melatonin formation (Iuvone et al. 1987, Zawilska and Iuvone 1989, Nowak et al. 1989, Cahill and Besharse 1991, Zawilska and Nowak 1994a,b, Zawilska et al. 1994, Zawilska and Derbiszewska 1995). On the other hand, it is less clear whether the dopaminergic system is implicated in the regulation of the retinal circadian pacemaker by light (Cahill and Besharse 1991, 1993, Zawilska 1994b).

BIOLOGICAL AND THERAPEUTIC IMPLICATIONS OF THE RHYTHMIC CHANGES IN MELATONIN LEVELS

The most well established role of melatonin is to coordinate biological rhythms. Thus, this hormone acts as an internal clock for the timing of daily events and as such plays a crucial role in cueing circadian rhythms (notably the sleep-wake cycle). Melatonin also acts as an internal calendar providing photoperiodic information so that seasonal body changes will be in harmony with changing day length. Under natural photoperiodic conditions, the melatonin secretory pattern reflects the changing hours of darkness and these alterations trigger the seasonal physiological and behavioral adaptive changes (e.g., Reiter 1980, 1993b, Arendt 1995a,b). Numerous experimental data point to melatonin receptors localized to the suprachiasmatic nuclei of the anterior hypothalamus and *pars tuberalis* of the pituitary as putative target sites for chronobiological actions of melatonin on circadian rhythms and seasonal changes, respectively (reviewed by Morgan 1991, Morgan et al. 1994, Arendt 1995a,b).

An increasing body of evidence indicates that modulation of melatonin rhythm by means of administration of exogenous melatonin or inhibition of its synthesis by exposure of the individuals to bright light could be beneficial in the treatment of circadian rhythm sleep disorders, including delayed sleep phase insomnia, advance sleep phase insomnia, non-24-h sleep-wake disorder, disorders associated with re-entrainment ("intercontinental flight dysrhythmia" or "jet-lag", and shift work), irregular sleep-wake pattern, and sleep and behavioral disorders in children with multiple brain damage (Hoban et al. 1989, Amstrong 1991, Midwinter and Arendt 1991, Arendt 1995c, Brown 1995, Wirz-Justice 1995). Few clinical trials have demonstrated an effectiveness of melatonin in the treatment of insomnias in elderly patients (Haimov et al. 1994a,b). Furthermore, a seasonal affective disorder (SAD) or winter depression has been successfully treated with bright light (Rosenthal and Blehar 1989, Kjellman et al. 1993, Lingjaerde et al. 1993, Wirz-Justice 1995).

Photoperiodic species use the annual changes in daylength to time and control seasonal variations in their physiology (notably reproduction, coat growth and color, or feeding) and behavior. In all photoperiodic mammals studied to date there is fairly good evidence that the pineal gland, *via* melatonin secretion, conveys the photoperiodic message for the timing of seasonal functions. Both in long-day breeders (e.g., the Syrian hamster) and short-day breeders (e.g., sheep, goat and deer) melatonin plays an important role in adjusting seasonal reproductive capability acting as an antigonadotropic and a progonadotropic factor, respectively (reviewed by Reiter 1980, 1993b, Arendt 1995a). Noteworthy, several authors strongly suggested that the ability of melatonin to induce out-of-season breeding in sheep, goat and deer, to improve fertility in sheep, and to change the timing of seasonal changes in coat growth can be applied in agriculture (e.g., English et al. 1986, Foldes et al. 1990, Barry and Wilson 1991, Forsyth et al. 1991, Staples et al. 1991, Deveson et al. 1992). In fact, the usefulness of slow-release melatonin preparations (subcutaneous silicone-based melatonin implants and soluble glass boluses which remain in the rumen for extended period of time) in breeding of ungulates has recently been extensively studied.

GENERAL CONCLUSIONS

In vast majority of organisms which have been studied thus far melatonin is synthesized in a rhythmic fashion with high levels during the dark phase of a natural or any imposed light-dark illumination cycle. The rhythmic changes in the level of the circulating hormone provides information concerning the temporal position and duration of darkness (i.e., the clock and calendar information). By doing so, melatonin acts as a coordinator of internal physiological rhythmicity. The widely accepted role of melatonin as one of the most important endogenous mediators of photoperiodic messages points to several applications of this compound. There is an increasing interest in beneficial effects of melatonin in the therapy of circadian rhythm disorders in humans. Furthermore, an increasing body of evidence indicates that treatment with melatonin can be used as an effective and safe way to manipulate breeding of some animals such as sheep, goat and deer (possibly also domestic pig, wild pig, and horse) and to increase production of milk, wool and coat. Nevertheless, with the progress in the pineal research and melatonin pharmacology, perspectives of practical application of this chronobiotic compound (called by some authors "time in a bottle" or "circadian glue") will broaden.

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