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REVIEW

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The chronobiotic properties of melatonin

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Abstract In mammals, the exact role of melatonin (Mel) in the circadian timing system remains to be determined. However, exogenously administered Mel, as reported in the present mini-review, has been shown to affect the circadian clock. The sites and mechanisms of action involved in this "chronobiotic" effect of Mel have begun to be characterized. The suprachiasmatic nuclei (SCN) appear to be an important site for the entrainment effect of Mel and the presence of Mel receptors appears to be a prerequisite. However, the pharmacological dose of Mel needed to entrain circadian rhythms means that very probably other sites and mechanisms also play a role.

Keywords Melatonin · Circadian clock · SCN

Introduction

The day/night organization of physiological processes relies on endogenous circadian clock(s) that generate rhythms and are capable of being entrained to cyclic environmental factors (e.g., light/dark cycle). Such clocks convey circadian information to the rest of the organism via nervous and/or endocrine pathways.

In most non-mammalian vertebrates the rhythmic synthesis and secretion of melatonin (Mel) is the direct output of these clocks located within some or all the photoreceptive pinealocytes of the pineal and within some or all the retinal photoreceptors. The pineal may not be universally important in the circadian system per se but rhythmic changes in circulating Mel (different proportions coming from different sources) are fundamental to circadian rhythmicity. Under constant environmental conditions pinealectomy in several fishes, amphibians, lizards and birds alters or abolishes the circadian rhythms in locomotor activity. However, in some bird and lizard species that do not become arrhythmic after pinealectomy alone, arrhythmicity results from the combination of pinealectomy and removal of the eyes, the second important source of Mel in these species (review in Cassone 1998).

Mel synthesis in the mammalian pineal gland also occurs within the pinealocytes. Although in mammals, pinealocytes do not retain clock and photoreceptive properties (cf. Korf et al. 1998), the rhythmic synthesis of Mel is still a direct output of the circadian clock. Pineal Mel production is driven through multisynaptic neural pathways by the circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore 1996; Teclemariam-Mesbah et al. 1999; Ganguly et al. 2002: this issue; Moller 2002: this issue). It is generally assumed that in mammals the pineal gland is not involved in the generation and maintenance of circadian rhythmicity. Pinealectomy indeed appears to have little effect on the circadian rhythm of locomotor activity (Underwood and Goldman 1987). Therefore, it has been concluded that, contrary to non-mammalian species, circulating Mel has a very limited role in circadian organization. The Mel rhythm, however, is only one of the efferent signals of the clock. It is probable that for the circadian organization of complex functions such as locomotor activity, circadian information is distributed via a number of different efferent clock signals. If pinealectomy has little effect on circadian organization it is perhaps because, even without Mel, the circadian signal can be integrated through other clock outputs (Kramer et al. 2001). This will not preclude an important role for Mel in circadian organization (see also Stehle et al. 2002: this issue). After pinealectomy subtle desynchrony of several physiological functions has been described (Lima et al. 1998) and the reentrainment of rat locomotor activity rhythm is modified after a phase-shift of the light/dark (LD) cycle (Armstrong 1989). One week after pinealectomy the firing rate rhythm of SCN neurons in vitro is altered, as

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well as the daily rhythm of responsiveness to Mel (Rusak and Yu 1993). Mel is also known to interfere with the metabolic activity (glucose utilization and protein synthesis) of the SCN (Cassone et al. 1988).

In addition to this role of Mel on clock function, as Mel synthesis is under SCN control, the SCN may also use the daily Mel signal to distribute the circadian message to any system than can "read" it, i.e., to any structure/organ possessing Mel receptors (Cardinali and Pévet 1998; Pévet 1998). For example, in the rat pars tuberalis, the circadian rhythm of melatonin receptor density is suppressed after pinealectomy and Mel drives this rhythm independently of the SCN or the LD cycle (Gauer et al. 1994). Mel is also known to directly control the circadian expression of the clock gene *Per1* in the pars tuberalis (Messager et al. 1999, 2000; von Gall et al. 2002a; Stehle et al. 2002: this issue). Thus, although clear experimental evidence is still scarce, it appears that in mammals, like in non-mammalian vertebrates, Mel is involved in some aspects of the control of circadian rhythmicity.

All the data described above concern the role of endogenous Mel. The effect of exogenous Mel has to be considered also and, in this mini-review, we will concentrate on that issue.

Chronobiotic properties of melatonin

A chronobiotic effect means that exogenous melatonin is able to influence, directly or indirectly, the phase and/or the period of the circadian clock. For a long time, it has been known that administration of Mel can entrain free-running activity rhythms in rodents (for review, see Weaver 1999; Cassone and Natesan 1997). For example, Redman et al. (1983) demonstrated that daily subcutaneous injections of Mel to rats strongly affect the locomotor activity rhythm. Mel entrains the free-running locomotor activity rhythm of animals and entrainment only occurs when the Mel injection time coincided with the onset of activity. If the injection is given at any other time, the rhythm continues to free-run until this coincidence occurs. However, all these experiments (Redman et al. 1983; Cassone and Natesan 1997) were based on bolus administration of Mel. Behavioral arousal (1–4 h before activity onset; Hastings et al. 1992; Cutrera et al. 1996) is known to induce a phase advance of the locomotor activity rhythm in Syrian hamster. Consequently, the arousal associated with the injection-induced daily handling of the rats may also interfere with the results. In support of this idea is the fact that a small percentage of the control animals became entrained to vehicle administration in the early experiments (Redman et al. 1983).

In order to analyze the direct action of exogenous Mel on circadian organization, we consider it necessary to administer Mel in a way that does not by itself induce entrainment and to eliminate the non-specific disturbance of the animals. Mel administration via timed access to drinking water has been shown to be an efficient way to entrain free-running activity rhythms in the rat; entrainment occurs at the same circadian phase and with the same phase angle to Mel onset (Slotten et al. 1999). However, this technique of administering Mel via drinking water, like the bolus administration experiments, does not permit precise control of the duration of the Mel peak signal. The duration of Mel is known to provide essential information, at least in photoperiodic terms. To address these points a chronic infusion device allowing the animal to move freely in its cage and providing continuous drug infusion (over several months) of controlled duration and dose without handling the animals has been developed (Kirsch et al. 1993; Pitrosky et al. 1999).

Daily infusions of Mel for 1, 8 or 16 h or 2×1 h entrained the circadian rhythms of core body temperature, running wheel and general activity to a 24-h period. Nevertheless regardless of the dose, the efficiency of Mel infusion decreased if it lasted a long time (16 h). During entrainment, when the intrinsic period of the circadian pacemaker is equal to the period of the zeitgeber, it is assumed that the pacemaker maintains a constant phase relation with the zeitgeber. With daily injection or oral administration of Mel, the onset of activity is linked to the time of administration and the phase angle is close to zero. When Mel is administered by daily infusion, the phase angle difference between the entrained rhythm and the zeitgeber (Mel) depends upon the duration of the infusion period. A negative phase angle is observed and its value is increased with the duration of the infusion period (Fig. 1). In addition to the effects on phase angle, another response has been observed. With an 8-h infusion and more evidently with a 16-h infusion, Mel administration induced a change in the free-running period in the first days (Fig. 1). The period was lengthened compared with the saline infusion, suggesting that melatonin delays the pacemaker each day until entrainment occurs. In other words, with a long duration of infusion, entrainment occurs earlier than predicted by the model based on the Mel injection experiments. Moreover, the magnitude of the change in period increased significantly with the duration of that infusion. These observations cannot be explained on the basis of a sensitivity window but rather suggest that the chronobiotic properties of melatonin imply an active mechanism on the circadian clock. This conclusion is supported by the results obtained after a "skeleton" infusion: 2×1-h infusions with an interval of 15 h, corresponding to the extremities of the 16-h infusion. Under these conditions Mel induced entrainment after a time during which circadian periods were either lengthened in a fraction of the animals or shortened in the others (Fig. 2). However, all animals responded in such a way that, once entrained, their active phase occurred in the shorter time interval between the Mel signals. This finding suggests that to achieve entrainment, Mel has to induce either a phase delay (when the period was shortened) or a phase advance (when the period was lengthened). Such a dual effect of Mel has also been reported in other studies. For example, when sub-

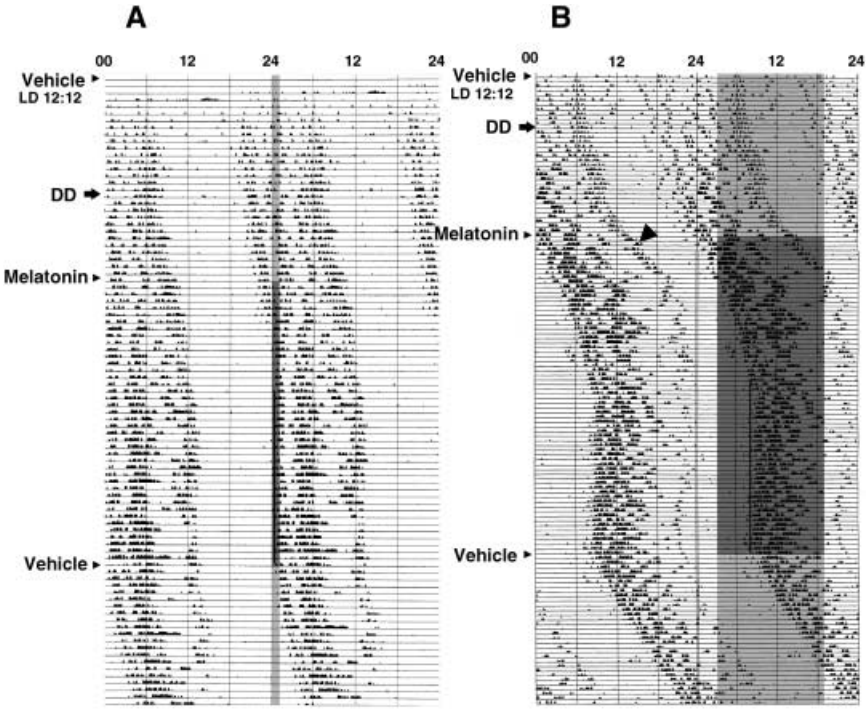

Fig. 1 Double-plotted graphs of running-wheel activity of rats infused for either 1 h **(A)** or 16 h/day **(B)** with melatonin (100 µg/h). *Vertical white lines* represent estimated eye-fitted onset of activity and illustrate the phase angle difference after infusion onset. Note that the 16-h infusion of melatonin induced in the first days (*arrowhead*) a marked lengthening of the period, which was not observed with 1 h infusion. Modified from Pitrosky et al. (1999)

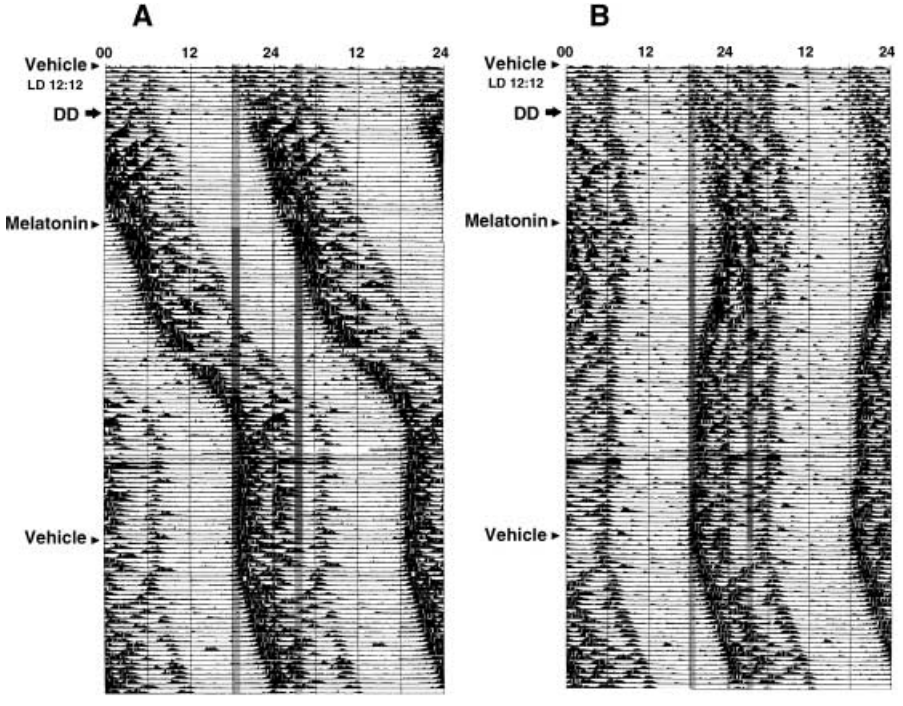

Fig. 2A, B Effects of 2×1-h melatonin infusions (100 µg/h) on body temperature of two rats. Melatonin was able to induce a true entrainment of the rhythm to a period of 24 h. The animals synchronized their rhythm with the shortest time interval between the infusions (e.g., between 1800 and 0400 hours). The free-running rhythm observed in DD could be entrained in two different ways either by melatonin-induced phase advances **(A)** or melatonin-induced phase delays **(B)**. The body temperatures are represented on a linear scale ranging from 36.5°C to 39.5°C. Modified from Pitrosky et al. (1999)

mitted to a 5-h phase advance of the dark onset in LD conditions, rats injected daily at the new dark onset reentrained with a decreased latency, some of the animals doing it by phase delays, whereas others did it by phase advances (Redman and Armstrong 1988). Infusion of Mel has been reported to entrain hamsters or *Arvicanthis ansorgei*, a diurnal rodent, by inducing phase advances when the free-running period was longer than 24 h and phase delays when the period was shorter than 24 h (Kirsch et al. 1993; Slotten et al. 2002a). All these observations strongly suggest that the effects of exogenous Mel are dependent on the period before entrainment.

Does melatonin cause "true" entrainment?

To entrain circadian rhythms a zeitgeber must induce a daily phase shift by either delaying or advancing the rhythm. Thus, the period of the observed rhythm must adjust to and equal the zeitgeber cycle (*T*), and a stable phase relation must be established between the rhythm and zeitgeber cycle (Moore-Ede et al. 1982). According to the non-parametric model of entrainment, this synchronization process occurs through daily phase shifts ($\Delta\varphi$), with the size and direction of shifts defined in the phase response curve (PRC). However, entrainment does not occur at any given value of *T*, because the phase relation depends on factors such as duration, intensity, and timing of the zeitgeber as well as the circadian clock's sensitivity to the stimulus considered (Pittendrigh 1981). In principle, the maximum phase advance and phase delay values on the PRC indicate the *range* of *T* values to which a circadian clock can entrain (Saunders 1977). This has been demonstrated by experiments using light as the zeitgeber and little is known about the zeitgeber properties of Mel or other non-photic stimuli (e.g., scheduled feeding, forced activity). Moreover, if, as reported above, daily administration of Mel has the capacity to entrain free-running circadian rhythms in mammals, the limits of entrainment to Mel are not yet well defined. In a recent study (Slotten et al. 2002b), we have investigated these entrainment limits by administering Mel for a series of *T* values. The period of the Mel cycle (*T*) was initially kept at 24 h until stable entrainment was established. The *T* cycle was then changed in a stepwise manner. Our results indicate that the limiting phase advance value, to which the rat activity rhythm entrains to Mel infusion, is approximately 35 min (Fig. 3). Entrainment occurred at about CT 12; thus, at this phase of the activity cycle, Mel infusion induced the phase advance necessary to entrain the rhythm. The maximum $\Delta\varphi$ values defined by the Mel PRC (Armstrong et al. 1989) and the magnitude of phase shift responses to a single Mel injection (Warren et al. 1993) range from 15 to 52 min. The entrainment limits found in our study correspond quite well to these maximum phase advance values. This relation is in accordance with the non-parametric model of entrainment. The negative phase angle difference between activity onset and Mel onset increased as *T* values approached the entrainment limit, whereas no change in the duration of the daily activity bouts was found. No difference was observed between pre- and post-treatment values in the free-running period, hence revealing no aftereffects of any *T* cycle. With the present study, in which Mel was administered at *T* values different from 24 h for the first time, we can conclude that, in the rat, daily acute Mel administration causes "true" entrainment as defined by Enright (1981).

Although light and exogenous Mel represent two qualitatively different kinds of zeitgebers, the functional properties of entrainment to these stimuli resemble each other closely. Especially, the relation between features of the PRC and entrainment for the respective stimuli appears similar. Thus, concerning integration of the zeitgeber signal in the circadian pacemaker, these results suggest that entrainment to MEL or light involves at one level or another a common mechanism even if their input pathways to the pacemaker differ.

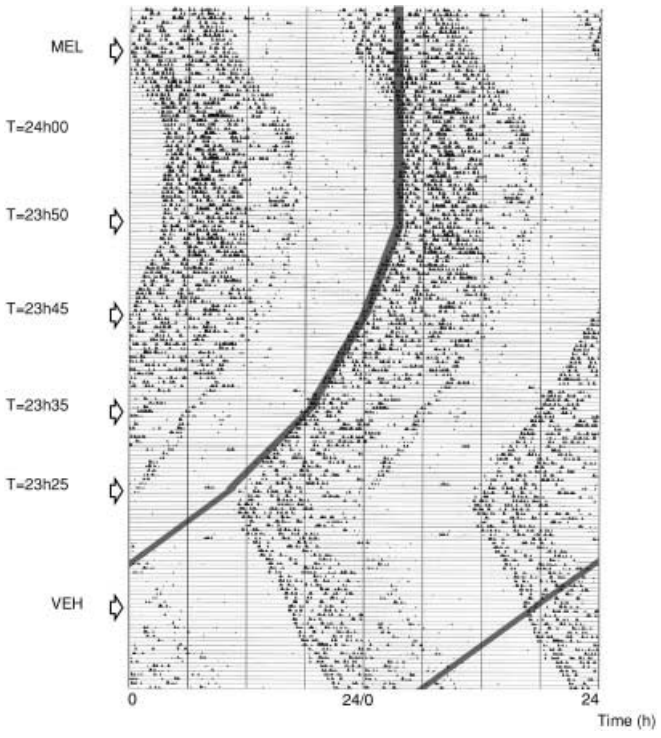

Fig. 3 Double-plotted graph of running wheel activity of a rat infused 1 h daily (*vertical grey bar*) with melatonin (100 µg/h). *T* cycle value is indicated in the margin. Following entrainment to LD (not shown), the animal was transferred to DD and displayed a free-running rhythm under vehicle administration. Then, melatonin was administered at *T*=24 h, and when activity onset and melatonin coincided, entrainment was obtained. Then the value of *T* was changed. The animal presented in this picture was entrained until *T*=23.45. When *T* was further decreased, the rat starts to free run again. Modified from Slotten et al. (2002)

Sites and mechanisms of action of melatonin

Considering the observed chronobiotic properties of Mel, the sites and mechanisms of action remain an open question.

In all the experiments reported above, responsiveness to Mel is restricted to a narrow window of sensitivity which is generally late in the subjective afternoon but depends upon the duration of the Mel signal as well as the previous free-running period. The finding that pinealectomized rats entrain to daily Mel administration (Warren et al. 1993; Pitrosky et al. 1999) indicates that endogenous melatonin is not necessary for the entrainment effect of exogenous Mel, for example, by entraining a window of sensitivity to Mel (Pitrosky et al. 1995).

Nocturnal Mel production is a direct output of the SCN circadian clock. Exogenous Mel is effective at a time when endogenous Mel is not produced or present in the general circulation. Consequently the effects of Mel administration in vivo, as important as they are in terms of potential clinical applications, are apparently not related to the role of endogenous melatonin on circadian function. This conclusion is reinforced by the observation that to obtain entrainment of the circadian activity rhythm of rodents kept under constant darkness (DD), high doses of Mel have to be used independent of the mode of administration (Cassone et al. 1986; Pitrosky et al. 1999; Slotten et al. 1999). These doses of Mel produce peak serum levels 100- to 1000-fold higher than the endogenous Mel nighttime levels. The necessity of such a high dose of Mel is unlikely to be a consequence of its rapid metabolism. Appropriate photoperiodic response is, indeed, obtained when Mel is administered via a similar subcutaneous infusion system with a dose which mimics the endogenous secretion profile (Pitrosky et al. 1991, 1995). The suggestion that endogenous Mel makes receptors less sensitive to exogenous Mel (Warren et al. 1993) cannot be an explanation since entrainment is also obtained in pinealectomized rats. Most likely, this high concentration of Mel is needed because it is an integral part of the response observed. However, even if the chronobiotic effect of Mel appears to be pharmacological rather than physiological, knowledge of the sites involved will provide information on the entrainment mechanisms of the clock.

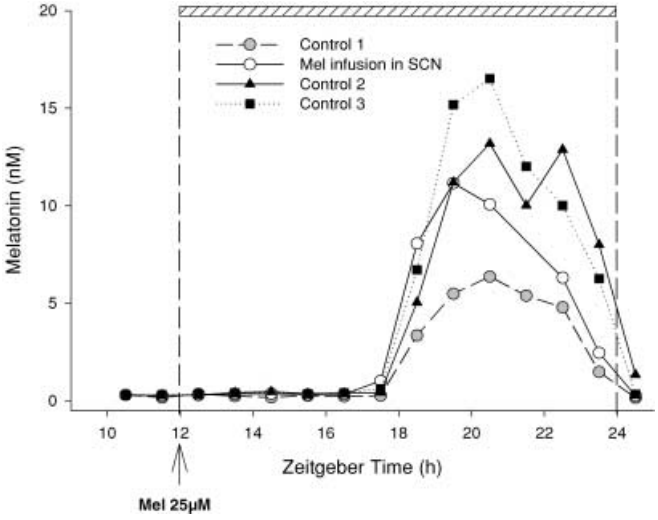

Fig. 4 Effect of exogenous melatonin infused locally within the SCN of a rat by reverse microdialysis on the endogenous nocturnal melatonin peak determined by intrapineal microdialysis. In the animal presented in this picture, melatonin (25 µM) (*Mel infusion in SCN*) was applied at ZT12. Pineal dialysates were collected the day before (*Control 1*), the day of administration and the 2 subsequent days (*Control 2 and 3*). The *hatched bar on top* represents the dark period of the L/D cycle. Note the large increase in amplitude of melatonin profiles which is observed after melatonin administration within the SCN and lasts for at least 2 days

Suprachiasmatic nuclei and melatonin receptors

Several lines of evidence support the view that the SCN are the primary sites of the Mel entrainment effect. The SCN, indeed, are affected by Mel in vivo as well as in vitro. For example, in vivo, subcutaneous Mel injections in rats inhibit the uptake of the metabolic marker 2-deoxy[^{14}C]glucose within the SCN during the late subjective day but have no effect during the early subjective day or night (Cassone et al. 1988). Superfusion or iontophoresis of Mel on rat hypothalamic slice preparation in vitro inhibits SCN electrical activity (Shibata et al. 1989; Stehle et al. 1989; van den Top et al. 2001) and, when administered late in the subjective day, phase shift the firing rate of SCN neurons (rat and mouse) (Gillette and McArthur 1996). It has also been observed that SCN-lesioned hamsters whose rhythmicity had been restored with fetal hypothalamic grafts are entrained by daily Mel injections, and Mel is known to accelerate the reentrainment of circadian rhythms (locomotor activity as well as *N*-acetyltransferase, AANAT, activity) in rats subjected to a shift in the LD cycle (Redman and Armstrong 1988; Humlova and Illnerova 1989). It is thus probable that the chronobiotic effect of Mel may result from a direct action on the SCN clock. If this were true, as the rhythmic synthesis of Mel by the pineal is a direct output of the clock, an effect of exogenous Mel on endogenous Mel rhythm is to be expected. In a recent study we have followed the effect of exogenous Mel (acute administration) on pineal Mel synthesis in vivo. Daily Mel profiles were measured by transpineal microdialysis over four consecutive days and Mel was administered (day 2) by reverse microdialysis directly into the SCN. As expected, we observed a phase advance of the nocturnal Mel peak in some animals (Fig. 4) (Bothorel et al. 2002). This finding confirms the data on melatonin and AANAT rhythms obtained after subcutaneous administration of Mel (Bothorel et al. 2002; Illnerova et al. 1989). Interestingly, following Mel administration, either subcutaneously or directly into the SCN, a significant increase in the amplitude of the melatonin peak (which persisted for at least 3 days) was observed (Fig. 4), suggesting a direct action on the amplitude of the clock oscillations, in addition to the phase-shifting effect. This effect was only obtained when exogenous Mel was administered at CT12 or ZT12 (Bothorel et al. 2002). This time window of clock sensitivity is similar to that observed for Mel's phase-shifting effect.

It is generally believed that Mel mediates these effects through the high-affinity Mel receptors located within the SCN (Gauer et al. 1993; Vanecek et al. 1987; von Gall et al. 2002b). This view is supported by the high correlation between the density of Mel receptors within the SCN and the ability of daily Mel administration to entrain the free-running activity rhythm in mammals. Contrary to the rat, mouse and Djungarian hamster, which are able to be entrained by daily Mel administrations and in which a high density of Mel receptors is ob-

served within the SCN, the mink (*Mustela vison*) does not appear to have specific Mel receptors (at least 2-iodo-melatonin-binding sites) within the SCN. This animal does not entrain to Mel (Bonnefond et al. 1993). Newborn Syrian hamsters express Mel receptors in the SCN but shortly after birth the receptor number decreases (Gauer et al. 1998; Maywood et al. 1995). Young hamsters are entrainable by daily acute Mel administration while, in the adult, Mel is not able to entrain (Davis and Manion 1988; Grosse et al. 1996; Hastings et al. 1992) or can entrain only under particular experimental conditions (e.g., long-term infusions) (Kirsch et al. 1993; Schulher et al. 2002). Moreover, a Mel receptor antagonist blocks the phase-advancing effect of Mel (Weibel et al. 1999; Hunt et al. 2001) and after administration of various Mel receptor agonists (Drijfhout et al. 1999) an increase in the amplitude of the nocturnal Mel peak is also observed.

Although the presence of high-affinity melatonin receptors (MT1/MT2) appears to be a necessary condition for the chronobiotic effect of Mel, it seems evident that other mechanisms could also be involved. If the high-affinity MT1 and MT2 Mel receptors were the only receptors involved, it would be difficult to explain why such a high dose of Mel is needed in vivo to obtain a chronobiotic effect. Even in the in vitro experiments, where effects are obtained with physiological doses of Mel, the mechanisms involved appear more complex. For example, two distinct effects of Mel have been described: an acute inhibitory effect on neuronal firing and a phase-shifting effect in the rhythm in electrical activity (Liu et al. 1997). Until recently it was assumed that the inhibition of electrical activity was part of the cellular mechanism underlying the phase-shifting effect of Mel. However, in mice with a targeted deletion of the MT1 receptor, the inhibitory effect of Mel was abolished, while the phase-shifting effect remained intact (Liu et al. 1997; von Gall et al. 2002b). In contrast to previous studies, van den Top et al. (2001) have recently demonstrated the absence of a particular window of sensitivity for Mel to inhibit SCN neuronal activity. Such a lack is in contrast to Mel's phase-shifting effect, and indicates that the cellular mechanism involved in the acute inhibitory effect is distinct from that in the phase-shifting effect of Mel. This may be related to the two types of effects observed in vivo after the daily 8- or 16-h Mel perfusions (Pitrosky et al. 1999) described above.

Serotonergic fibers and other potential sites

The high dose of Mel used in all the in vivo studies produces supraphysiological concentrations. The possibility that such high levels can pharmacologically influence other types of receptors or neurotransmitters within the SCN clock has to be considered because it would provide a mechanism by which Mel can have a parallel effect on SCN activity. Supraphysiological levels of Mel are known to inhibit 5-hydroxytryptamine (5-HT) reuptake in rat nerve endings (Cardinali et al. 1975; Miguez et al. 1995). Administration of serotonin receptor agonists in vivo also phase-shift rodent circadian clock. Involvement of 5-HT fibers in the entraining properties of Mel may thus be critical. To test this idea, bilateral neurotoxic (5, 7-dihydroxytryptamine) lesions of the serotonergic fibers were made in the SCN itself (Slotten et al. 2001). Both lesioned and intact animals entrained to Mel daily administrations. No differences were observed in parameters such as the phase-angle difference between Mel onset and activity onset, and core body temperature acrophase. This experiment establishes that the 5-HT terminals in the SCN are not necessary for the expression of the chronobiotic effect of Mel or, more correctly, that the inhibiting effect of MEL on 5-HT reuptake is not crucial for the Mel effect on the circadian rhythms.

The present data, however, only exclude an entraining action of Mel through induced changes in 5-HT release from the nerve endings. They do not exclude a possible interaction between Mel and the 5-HT system. For example, Mel might act at the level of the postsynaptic 5-HT receptors. This idea is supported by the work of Dugovic et al. (1989), who have shown that, in vivo, exogenous Mel counteracts the sleep induced by 5-HT_{2A} agonists [DOM (1-2, 5-dimethoxy-4-methylphenyl-2-aminopropane)]. In addition, Eison et al. (1995) demonstrated a modulation of 5-HT_{2A} receptor-mediated behavioral responses by high doses of exogenous Mel, and Ying et al. (1993) found that high doses of the hormone exerted inhibitory effects on firing rates in intergeniculate leaflet (IGL) cells by mimicking the effects of 5-HT agonists.

In humans, administration of Mel has been shown to be able to entrain some but not all the circadian rhythms recorded. For example, Folkard et al. (1990) observed in a blind subject that Mel entrained the free-running sleep-wake rhythm but not the free-running rhythm of rectal temperature or urinary cortisol excretion. Even if the same group (Lockley et al. 2000) observed that under some conditions Mel can also entrain cortisol rhythms, the observation of Folkard et al. (1990) cannot be explained by a direct action of Mel on the SCN and suggests an action at a different level of the circadian timing system, at least to synchronize the sleep-wake cycle. Mel may act on structures efferent to the SCN, such as the paraventricular nuclei (PVT) of the thalamus, which express 2-iodo-melatonin-binding sites (Weaver et al. 1989; Williams et al. 1989) and which are elements in the mesolimbic structures involved in the generation of motor behavior (Berendse and Groenewegen 1990). Mel may also act on structures or fibers afferent to the SCN. The IGL is one of them and could be a target for Mel since the hormone suppresses both spontaneous and light-activated firing of IGL cells in Syrian hamsters (Ying et al. 1993). The involvement of 5-HT afferents to the SCN in the circadian organization of locomotor activity is well known (Schuhler et al. 1998; Cutrera et al. 1994; Challet et al. 1997). In the midbrain 5-HT is present in cell bodies of neurons located in the raphe nuclei

(Dahlström and Fuxe 1964). Mel injections induce an increase in the 5-HT concentration in the midbrain (Anton-Tay et al. 1968). Thus, it cannot be excluded that this increase in 5-HT represents a signal which reaches directly, or indirectly via the IGL, the circadian clock (Meyer-Bernstein and Morin 1996; Schuhler et al. 1999).

Conclusions

Exogenously administered Mel has clear effects on circadian functions. The hormone possesses all the criteria necessary to be classified as a "chronobiotic agent" and, consequently, is an attractive candidate for manipulating circadian rhythms in humans. Although this chronobiotic effect seems to be pharmacological rather than physiological, the functional properties of entrainment to Mel resemble that described for light and other non-photic stimuli. Even if the input pathways to the pacemaker differ, the present data suggest involvement of common timing mechanisms. Definition of the sites and mechanisms of the Mel action involved in this well-characterized chronobiotic effect will allow us to clarify the role of endogenous Mel in circadian organization.

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