Chronobiology International
Vol. 9, No. 5, pp. 380–392

Melatonin Shifts Human Circadian Rhythms According to a Phase–Response Curve

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Summary: A physiological dose of orally administered melatonin shifts circadian rhythms in humans according to a phase–response curve (PRC) that is nearly opposite in phase with the PRCs for light exposure: melatonin delays circadian rhythms when administered in the morning and advances them when administered in the afternoon or early evening. The human melatonin PRC provides critical information for using melatonin to treat circadian phase sleep and mood disorders, as well as maladaptation to shift work and transmeridional air travel. The human melatonin PRC also provides the strongest evidence to date for a function of endogenous melatonin and its suppression by light in augmenting entrainment of circadian rhythms by the light–dark cycle. **Key Words:** Circadian phase shifts—Circadian phase sleep and mood disorders—Dim light melatonin onset (DLMO)—Melatonin administration—Phase–response curve (PRC).

In most diurnal and nocturnal animals, melatonin production by the pineal gland occurs only at night. Sometime after dusk, melatonin levels increase 10- to 50-fold; levels decrease several hours later, either because of an endogenous mechanism or because of exposure to light in the morning—whichever happens first. Although the suppressant effect of light may be unique to melatonin production, the light–dark cycle entrains an endogenous pacemaker [which is thought to be located in the suprachiasmatic nuclei (SCN)] that drives most circadian rhythms, including the melatonin production rhythm (1,2). The SCN is also involved in the regulation of seasonal rhythms (3).

The most clearly delineated function of melatonin is the regulation of some seasonal rhythms, such as the breeding cycles of many photoperiodic animals, by the duration and/or phase of melatonin production that corresponds to the annual change in scotoperiod (4). A role for melatonin in the regulation of circadian rhythms is also supported by animal studies. For example, injection of melatonin at the same

Received February 25, 1992; accepted with revisions May 6, 1992.
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time every day synchronizes the free-running activity rhythms of European starlings, *Sturnus vulgaris* (5). Similarly, melatonin can entrain Long–Evans hooded rats free-running in constant darkness (6). Furthermore, melatonin injections administered to lizards (*Sceloporus occidentalis*) between circadian time (CT) 6 and CT 13 cause phase advances in activity rhythms, while injections between CT 14 and CT 5 cause delays (7). Arrhythmic, disrupted activity patterns of Long–Evans hooded rats in constant light can also be entrained by melatonin (8). Furthermore, in this species injections around CT 10.5 elicit phase advances in activity [at other times, injections caused no phase shifts except one phase delay, probably artifactual, at CT 18 (9)]. In humans, however, a function for melatonin has not yet been identified.

Following the discovery that exposure to sufficiently bright light suppresses human melatonin production (10), morning exposure has been shown to phase–advance human circadian rhythms and evening exposure to phase–delay them (11–21). Appropriately timed bright light exposure has also been shown to be therapeutic in the treatment of circadian phase sleep and mood disorders (16,22,23). Nevertheless, it is still not clear why melatonin's suppressant response to light has been retained nor what melatonin does in humans, particularly given the relative lack of seasonal reproductive rhythms (24,25).

Whereas evidence for seasonal rhythms in humans is sparse, circadian rhythms are fundamental in human physiology (26). Furthermore, melatonin receptors have recently been identified in the human SCN (27). Accordingly, a circadian function for melatonin has been postulated in humans, although evidence for phase-shifting the endogenous melatonin circadian rhythm (a widely recognized marker for SCN-driven rhythms) with exogenous melatonin has been equivocal at best in sighted people (see Discussion). However, in four out of five totally blind people with free-running melatonin production circadian rhythms (28,29), we recently found that daily administration of melatonin (5 mg orally) caused significant cumulative phase advances in the endogenous onset of melatonin production.

Blind people probably responded more than sighted people to the phase-shifting effects of exogenous melatonin primarily because there was no competing zeitgeber signal from the light–dark cycle. However, we thought that we could perhaps demonstrate phase-shifting effects in sighted people if we were to use a marker—the dim light melatonin onset (DLMO) (30)—that can reliably assess small changes in circadian phase position, particularly if melatonin levels are measured with use of an assay characterized by a high degree of sensitivity and specificity not usually obtainable with most previous radioimmunoassays. We therefore undertook the present study to determine the phase of the DLMO in sighted individuals before and after treatment with a reduced (physiological) dose of exogenous melatonin (0.5 mg orally). We found that the time of administration was critical for exogenous melatonin's phase-shifting effect. Indeed, our data provide the first evidence for a phase–response curve (PRC) for melatonin (or any nonphotic stimulus) in humans.

SUBJECTS AND METHODS

Adult volunteers were screened for medical and psychiatric disorders. Ten subjects (ages 20–48 years), nine men and one woman, participated after informed consent was obtained. One subject was eliminated from the study because of noncompliance.

Most subjects participated in multiple trials, each consisting of a 2-week protocol. Subjects took placebo (corn starch) capsules at home at assigned times for the first 6 days of week 1. On the seventh day, they were admitted between 17:00 and 18:00 h to the Oregon Health Sciences University (OHSU) Clinical Research Center (CRC) for DLMO determinations. Subjects avoided light exposure >10–50 lux for at least 1–2 h prior to their nightly onset of melatonin production and throughout the blood-drawing procedure. Blood samples were drawn every 0.5 h beginning at 18:00 h for 5–6 h. Plasma melatonin was measured with use of a modification of the highly sensitive and specific gas chromatographic–negative chemical ionization mass spectrometric (GCMS) assay of Lewy and Markey (31). The DLMO was defined as the first interpolated point above 10 pg/ml that continued to rise. During the second week of each trial, subjects continued to take capsules at the assigned times [placebo capsules for 2 days, followed by melatonin capsules (Regis Chemical, Morton Grove, IL, U.S.A.) for 4 days]. On day 14, they were admitted for another DLMO determination.

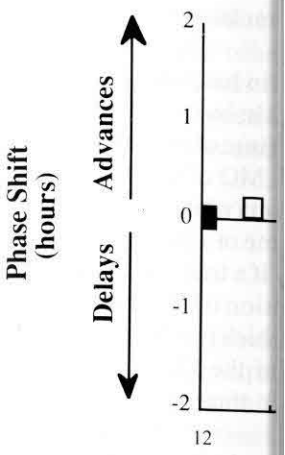
Corn starch or melatonin capsules were taken in two divided doses of 0.25 mg each, 2 h apart. The split-dose was used for the first 27 trials because it was initially thought that a few hours of melatonin stimulation would be needed to produce phase shifts. Results from the consolidated dose (0.5 mg) at the conclusion of the study indicated that the split-dose was not necessary.

For each trial, the instructions were to take capsules every day for 2 weeks, except on the last day of each week when blood was drawn for determination of the DLMO. The short $t_{1/2}$ of melatonin eliminates its exogenous administration as a confounding factor for measurement of the next day's endogenous onset (32). For melatonin administered at clock times of 01:00, 03:00, and 05:00 h, the DLMO was determined the same day as the last dose of exogenous melatonin; however, endogenous levels obtained 13–17 h later were not contaminated by exogenous melatonin, since elimination from the circulation is complete within 5 h.

The DLMO after the week of placebo administration is defined as the baseline DLMO. In order to use the baseline DLMO as a circadian reference point, the week placebo was administered always preceded the week melatonin was administered; however, subjects were not told when they were taking melatonin or placebo. An order effect in this study is not likely, given the fact that melatonin caused both phase advances and phase delays (see Results). For the same reason, feedback inhibition of the pineal gland by exogenous melatonin is also an unlikely confounding variable.

The study was conducted in three parts. In part one (conducted from July 1989 to August 1989), nine subjects were given capsules at 17:00/19:00 h (one subject failed to comply with the protocol, leaving eight subjects for data analysis). In part two, 11 trials were conducted from November 1989 to February 1990 in six of the eight subjects from part one; capsules were administered at 13:00/15:00 h, 14:00/16:00 h, 15:00/17:00 h, 16:00/18:00 h, and 19:00/21:00 h. The third part was conducted from April 1990 to October 1990 and consisted of six trials in four of the subjects from part two with capsules given between 05:30 and 14:00 h, and two trials at 20:50/22:50 h and 22:00/24:00 h. None of the above trials involved waking the subject.

Two of the subjects from part three and one additional subject also participated in three trials in which melatonin was administered at 01:00, 03:00, and 05:00 h; for these trials, subjects took melatonin (0.5 mg in one capsule) for 4 nights, but were not



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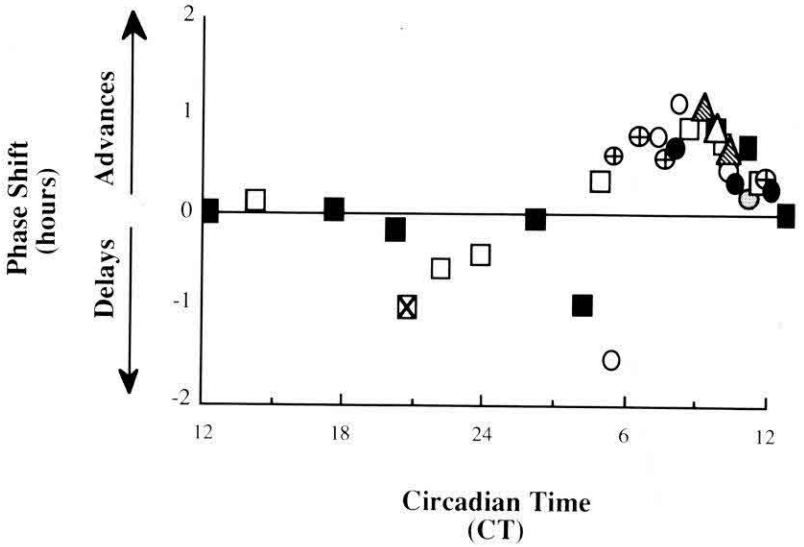

FIG. 1. Phase shifts of the dim light melatonin onset (DLMO) as a function of circadian time (CT) for all subjects' 30 trials, providing the first evidence for a human melatonin phase–response curve (PRC). Each of the nine subjects has a separate symbol. Exogenous melatonin was administered at various times with respect to the time of endogenous melatonin production (CT 14 = baseline DLMO for each trial). The time of administration appears as CT by convention and, because of interindividual variability in sleep–wake cycles, perceived light–dark cycles, and internal CT. Two subjects [J.H. (open squares) and S.E. (closed squares)] each participated in seven trials: when internal CT is referenced to the baseline DLMO, plots of the data for these two subjects nearly superimpose.

given placebo capsules so as to minimize interference with sleep. It was decided that setting alarm clocks and waking up 24 times, as opposed to four times, for taking capsules during the 2-week protocol would constitute a greater deviation from the protocol for the other trials than would consolidating the split-dose and eliminating placebo capsules (the protocol for these three trials, which were the last trials for each subject, was in all other respects identical to the protocol for the other trials). The additional subject, a co-investigator in the study (J.M.L.J.) who was not blind to the study design, took melatonin at 03:00 h. Her data point appears as the crossed square in Fig. 1. Some or all of the results from parts one and two and the first five trials in part three have been reported previously (33–36).

RESULTS

In all, 30 trials were completed. Two subjects, J.H. and S.E., completed seven trials each. To assess intraindividual variability, we determined a prebaseline DLMO for subjects J.H. and S.E. 1 week prior to the placebo week for four trials. In two cases, baseline DLMOs were slightly (13 and 21 min) delayed compared to prebaseline values, and, in two cases, they were slightly (10 and 12 min) advanced. Thus, it appears that week-to-week variability in the DLMO is small. Another measure of intraindividual variability is the standard deviation (SD) of baseline DLMOs. In the two subjects who each had seven trials, the SD of their baseline DLMOs was 17 and

22 min (in contrast, their DLMOs after exogenous melatonin administration had standard deviations of 48 and 47 min, respectively).

Although there was little intraindividual variability in baseline DLMOs, there was > a 5-hour range (18:53 to 24:00 h) between individuals; by convention and because of interindividual variability in baseline DLMOs, the time of administration appears on the abscissa as internal CT. In our studies, the DLMO of normal sighted people occurs on average at ~21:00 h, ~14 h after light onset (which occurs on average at about 07:00 h). Therefore, in order to convert clock time of administration to CT, we designate the baseline DLMO as CT 14. For example, if a trial's baseline DLMO is at a clock time of 19:00 h, then a clock time administration of melatonin at 18:00 h is converted to CT 13, 1 h before the baseline DLMO (which is defined as CT 14). Each phase shift was calculated by subtracting the time of the DLMO after melatonin administration from the time of the baseline DLMO: thus, phase advances on the ordinate are positive and phase delays are negative.

Phase shifts could not be explained by changes in sleep, which were kept to a minimum as per our instructions: during each 2-week trial, subjects were required to sleep at the same time each night of the second week as that of the corresponding night of the first week, and to record sleep times on daily logs. All data from trials in which sleep onset or offset was altered >1 h were excluded from any further analysis: this occurred in only one subject's sole trial (as mentioned before, this subject was eliminated from further participation in the study). To rule out a systematic influence of sleep in the remaining data, we plotted phase shifts in sleep onset, sleep offset, and midsleep (after averaging sleep times for the first 6 days of each week) against DLMO phase shifts for the first eight trials (one trial per subject). No significant relationship was seen. Furthermore, one trial for each subject in the entire study was chosen randomly, and the analysis was repeated: again, no correlation was seen between changes in sleep times and DLMO phase shifts. Moreover, after DLMO phase shifts from the first eight subjects' trials or the nine randomly selected trials were compared to DLMO phase shifts minus sleep shifts, results of Wilcoxon's signed rank test were not significant. Therefore, changes in sleep do not account for the DLMO shifts observed after melatonin administration.

Phase advances are apparent between CT 4 and CT 12, phase delays between CT 20 and CT 5. There appears to be a crossover point around CT 4–5, and a zone of reduced responses between CT 12 and CT 18. Although unlikely, one possible explanation of reduced responses to exogenous melatonin between CT 14 and CT 18 could be conceivably related to endogenous melatonin production that occurs between CT 14 to about CT 24.

The data for subjects J.H. and S.E. resembled the data for the group as a whole and plots of their data nearly superimpose. Given that their mean baseline DLMOs differed by almost 1.5 h [J.H.: 20:40 h ± 7 min (SEM); S.E.: 19:07 h ± 8 min], if melatonin administration were referenced to clock time, the data from these two subjects would not superimpose as well as when melatonin administration is referenced to the time of each trial's baseline DLMO (i.e., each individual's internal CT).

Because of the times when melatonin was administered, we obtained relatively fewer phase–delay shifts than phase–advance shifts. However, the phase–delay shifts are impressive because they all occur between CT 18 and CT 6, and none occurs

between CT 6 and CT 18. Half of the delay shifts were the result of melatonin administration after sleep offset, making it unlikely that the act of briefly awakening (and returning to sleep) was the cause of the delay shifts. Nevertheless, more data points will be needed between CT 18 and CT 6 before the delay portion of the human melatonin PRC and the crossover point can be clearly defined.

DISCUSSION

Previous Work

As mentioned earlier, previous attempts to demonstrate phase-shifting effects in sighted humans have been equivocal at best (we will review only the literature on the effects of exogenous melatonin on shifting the phase of the endogenous melatonin production circadian rhythm). The present data indicate that inconsistent results were probably related to internal CT variability among individuals and to the fact that melatonin was administered at suboptimal or incorrect times. Arendt and co-workers reported that exogenous melatonin (2 mg administered orally at 17:00 h for 3 weeks in Autumn, 4 weeks in spring) advanced the endogenous melatonin circadian rhythm in only five out of 11 subjects in Autumn and in only two of 12 subjects in Spring (37). Mallo and co-workers (38) did not find any phase shift after 4 days of administering melatonin (8 mg orally) at 22:00 h to six subjects, but did find a phase advance 3 days after its cessation; they also found no phase shift in the cortisol circadian rhythm. Arendt and Wever administered melatonin (5 mg orally) at bedtime to an individual in temporal isolation, which did not augment entrainment to a lengthening light–dark cycle (20,37,38); in this case, when phase–delay shifts might have promoted entrainment, melatonin was administered when it might have caused phase advances, if it were to have any phase-shifting effects at all. As a result of these studies, some of these authors concluded that exogenous melatonin's zeitgeber properties "were by no means comparable to those of bright light" [p. 274 (37)] and that exogenous melatonin "does not seem to have independent zeitgeber properties" in humans [p. 181 (20)].

Our data provide the first unequivocal demonstration of circadian phase-shifting effects of exogenous melatonin in sighted humans. Moreover, they clearly demonstrate a significant relationship between time of administration and the magnitude of the phase–shift response, particularly between CT 8 and CT 12. Our data are also consistent with a melatonin PRC that has features in common with the shapes of PRCs for other stimuli (such as light), observed in a number of species (39,40).

The phase, as well as the shape, of the human melatonin PRC resembles at least one (i.e., the lizard PRC) of the two melatonin PRCs described for other animals (7). Although both the rat and human PRCs have robust phase–advance zones between CT 9 and CT 11, the rat PRC does not have a delay zone or a crossover point (9). It is not clear why the rat and human melatonin PRCs differ. Aside from a species difference, which is probably the most likely explanation, we administered melatonin for 4 days as opposed to 1 day. Also, we used the DLMO as the reference for internal CT (defined as CT 14), whereas activity onset (defined as CT 12) was used in the rat. However, the melatonin PRC described for the lizard resembles the human melato-

nin PRC, and the methods used to derive the rat and lizard PRCs were nearly the same.

To date, three complete human light PRCs (13,19,20) have been described, including one that uses the classic single-pulse paradigm (19), and all of which approximate the human PRC for bright light that we hypothesized in 1983 (22) and 1984 (41). Light pulses for these PRCs were of varying durations. Because melatonin production occurs only during nighttime darkness, our melatonin PRC is not surprisingly nearly opposite in phase with the human PRCs for light (13,19,20). Also, the melatonin PRC predictably has the same phase as a dark-pulse PRC (42). The dark-pulse PRC in some species might represent phase shifts caused by changes in activity or rest or possibly through changes in melatonin production.

Although our methodology is not typical of that for most animal PRCs, most of the published human studies on the phase-shifting effects of light also used multiple pulses in subjects who were not free-running (11–14,16,20,21). Furthermore, PRCs for nonhuman species can be done under light–dark cycle entrainment (43). Although it will be important to obtain a melatonin PRC in humans under more classic conditions [and it appears that this work is currently being done (44)], the methodology for our PRC more closely approximates conditions under which circadian phase disorders would be treated. Apparently, administering a low daily dose of exogenous melatonin for 4 days stimulates a narrow zone of the melatonin PRC and induces measurable phase shifts, thus to some extent overriding the zeitgeber signal from a competing light–dark cycle.

The DLMO as a Marker for Circadian Phase

Although the DLMO has been important in demonstrating a melatonin PRC for humans, the question might arise as to the general use of the DLMO as a circadian phase marker. When daytime levels can be measured reliably, as with the GCMS assay (31), the endogenous melatonin onset of blind individuals and the DLMO of sighted individuals are convenient and useful circadian phase markers for both practical and theoretical reasons. Drawing blood in the evening is relatively nonstressful and minimally affects sleep. As the night progresses, melatonin levels are increasingly affected by decreasing β-adrenergic receptor sensitivity (45) and perhaps substrate availability. Therefore, the melatonin onset is probably better than any other point on the melatonin curve for assessing circadian phase and may actually be preferable to using the entire nighttime or 24-h curve that adds noise, and, therefore, artifacts to the analysis for circadian phase.

A possible criticism of the melatonin onset as a marker for circadian phase is that there may be separate circadian oscillators for the onset and the offset of melatonin production. While this may or may not be true in rats (46), if there are two oscillators in humans they appear to be tightly coupled (11,47) except under the strictest of experimental conditions (48). Even if it turns out that the DLMO provides the most accurate information about only one of many circadian oscillators, it may nonetheless be scientifically and clinically useful, in part because of the apparent tight coupling between oscillators in humans under most naturalistic conditions. A seasonal change in coupling would not significantly affect the data of the present study.

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There are many examples of the usefulness of the melatonin onset as a marker for circadian phase. In free-running blind people, it provides an assessment of the free-running period with a minimum of variability, compared to other points on the melatonin curve and compared to other circadian rhythms, such as cortisol production, although all variables measured appear to be phase-locked and to free-run at the same intrinsic period that is reproducible at a later date (30,49). Thus, the melatonin onset apparently marks the phase of the melatonin production circadian rhythm and the phase of another SCN-driven rhythm as well. When exogenous melatonin is given to free-running blind individuals, the cortisol rhythm (as marked by its nadir) shifts in tandem with the melatonin onset (29), suggesting that melatonin acts on the endogenous pacemaker that drives both of these overt circadian rhythms. In sighted people, the baseline DLMO correlates with the phase–shift response to light; that is, the later the DLMO, the greater the phase–advance response to morning light and the smaller the phase–delay response to evening light, which suggests that the DLMO marks the phase of its endogenous pacemaker's phase response curve and therefore the phase of its endogenous circadian pacemaker (30,50).

Implications of the Human Melatonin PRC

The existence of a phase–delay as well as a phase–advance zone in the human melatonin PRC provides the basis for the optimal and proper scheduling of exogenous melatonin in the treatment of both advance and delay types of circadian phase disorders currently treated with bright light: advanced (23) and delayed sleep phase syndromes (22,51), maladaptation to shift work (14) and transmeridional air travel (42), and certain types of circadian rhythm mood disorders, such as winter depression (16). At high doses (between ~2 and 80 mg), melatonin causes sedation in humans (52–57). The physiological dose (0.5 mg) used in this study causes minimal adverse effects and increases the usefulness of appropriately timed melatonin administration —alone or in conjunction with appropriately timed bright light exposure—as a specific treatment for circadian phase sleep and mood disorders, particularly when melatonin is administered during the day. It has recently been reported that melatonin administration phase-advanced the sleep–wake cycles of subjects with delayed sleep phase syndrome (58); more robust phase advances would probably have occurred if the investigators had used an earlier time of administration consistent with what we had previously reported (33,35).

Given the shape of the melatonin PRC, it is clear that the time when melatonin would be most effective in eliciting a phase delay is quite close to the time when melatonin would be most effective in eliciting a phase advance. Thus, knowledge of the time of an individual's DLMO may be important when prescribing exogenous melatonin, particularly given the marked interindividual variability in internal CT. The DLMO can, however, be predicted to some extent from sleep time. For example, the baseline DLMO in the present study correlates significantly ($p \leq 0.05$) with sleep offset, onset, and midsleep ($r = 0.71$, 0.72, and 0.73, respectively).

Nearly opposite in phase, the human melatonin and light PRCs show striking complementarities (13,19,20). The PRC's steep crossover points are not usually exposed to their respective stimuli: the crossover points of the light PRCs are in the

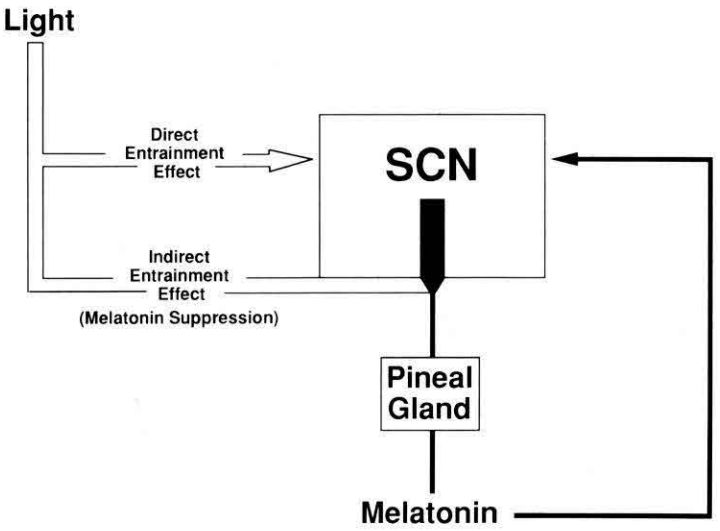

FIG. 2. Schematic diagram of some of the relationships between nighttime melatonin production by the pineal gland, the light–dark cycle, and an endogenous circadian pacemaker thought to be located in the hypothalamic suprachiasmatic nuclei (SCN). Acting on the SCN as described by the melatonin PRC (Fig. 1) at any given time of the day or night, melatonin causes phase shifts opposite to those that light would cause (indicated by the opposing arrows). However, the suppressant effect of light pares the margins of the nighttime melatonin profile (tapered arrow) and reduces endogenous melatonin's stimulation of the melatonin PRC at the day–night transitions. This second (indirect) pathway for entrainment by light is particularly significant during shifts of the light–dark cycle. Our diagram is not meant to be complete; for example, there may be a clock in the eye that may be influenced by the light–dark cycle, the SCN, and endogenous melatonin production (59).

middle of the night, when light is minimal; similarly, the crossover point of the melatonin PRC is in the middle of the day, when melatonin levels are lowest (in mammals and in most other species, darkness during the day does not induce melatonin production). The lower-amplitude zones of these PRCs occur when their respective stimuli are maximal (bright light during the day, melatonin during the night). As with the light PRCs and sufficiently bright light, the stimulus (melatonin) coincides with the higher-amplitude zones of the (melatonin) PRC primarily at the day–night transitions.

At the day–night transitions, light can apparently entrain the pacemaker in two ways (Fig. 2), directly (as described by the light PRCs) (13,19,20), and indirectly, by instantaneously altering melatonin levels that act upon the pacemaker (as described by the melatonin PRC). For example, an advance in the time of morning light exposure can advance the circadian pacemaker directly by stimulating more of the advance zone of the light PRC, and indirectly by limiting melatonin's stimulation of the delay zone of the melatonin PRC. Similar reasoning can be applied (particularly under long photoperiods) to a delay in the time of morning light exposure and can perhaps be applied to changes in the time of evening light exposure.

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Acknowledgment: We wish to t
the assistance of Gregory Clarke
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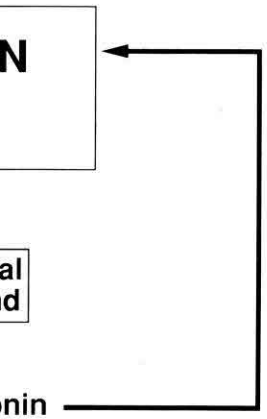

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N as described by the melatonin PRC (Fig.
se shifts opposite to those that light would
sant effect of light pares the margins of the
nous melatonin's stimulation of the mela-
athway for entrainment by light is particu-
n is not meant to be complete; for example,
ght–dark cycle, the SCN, and endogenous

larly, the crossover point of the
n melatonin levels are lowest (in
g the day does not induce melato-
se PRCs occur when their respec-
y, melatonin during the night). As
e stimulus (melatonin) coincides
) PRC primarily at the day–night

ly entrain the pacemaker in two
Cs) (13,19,20), and indirectly, by
pon the pacemaker (as described
n the time of morning light expo-
y by stimulating more of the ad-
ng melatonin's stimulation of the
ning can be applied (particularly
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ng light exposure.
n during the middle of the night
in PRC. Although the first part of

the endogenous melatonin curve appears to be acting on the dead zone, phase–delay shifts caused by exogenous melatonin coinciding with the latter part of the endogenous curve could be the result of high exogenous melatonin levels that persist into the early daylight hours. We should also mention that, at the present time, we cannot be as certain about the importance of endogenous melatonin at dusk as we can about its importance at dawn.

It is significant that the acute suppressant effect of light is always instantaneous, whereas an entrainment or phase-shifting effect is not (at least with respect to overt rhythms). The more instantaneous the change in the melatonin onset or offset, the more such a change can augment phase-shifting the pacemaker by the light–dark cycle. Once the pacemaker and its driven rhythms (including the melatonin PRC) have been phase-shifted, the steady-state relationship between the melatonin profile and the melatonin PRC resumes, a relationship that stabilizes (rather than promotes a change in) circadian phase.

CONCLUSIONS

The fact that the human melatonin and light PRCs are nearly opposite in phase suggests that endogenous melatonin may function to augment entrainment of the endogenous circadian pacemaker by the light–dark cycle. More general "circadian" hypotheses have been proposed by us and by others (27,60,61). However, the melatonin PRC allows assigning specific phase-shifting effects to the endogenous melatonin profile and permits linking phase-shifting to acute suppression of endogenous melatonin production by light exposure at the day–night transitions (33–36,62).

The suppressant effect of light via melatonin's effects on the SCN provides a second pathway for entrainment of the pacemaker, a pathway that is particularly significant during shifts of the light–dark cycle. The melatonin PRC is not redundant with a dark-pulse PRC, since melatonin production is not usually induced by daytime darkness. It is only at the day–night transitions that daytime darkness might increase melatonin levels, causing an earlier melatonin onset or a later melatonin offset.

In summary, our data describe a human melatonin PRC that appears to be nearly opposite in phase with the light PRCs. Accordingly, both phase–delay and phase–advance disorders may eventually be treated not only with appropriately timed bright light exposure, but also with appropriately timed administration of melatonin. Furthermore, our data suggest a role for melatonin and its suppression by light in providing a second pathway for augmenting entrainment of the endogenous circadian pacemaker by the light–dark cycle. The nighttime melatonin profile, as shaped by light exposure at the day–night transitions, may thus be important in the regulation of circadian as well as seasonal rhythms.

Acknowledgment: We wish to thank the nursing staff of the OHSU CRC and to acknowledge the assistance of Gregory Clarke, Mary Cardoza, Vance Bauer, Carol A. Gawryla, Neil L. Cutler, and Mary L. Blood. Supported by research grants MH 40161 (AJL), MH 00703 (AJL), and 5MO1 RR 00334 (OHSU CRC).

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Chronobiology International
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