Melatonin or a melatonin agonist corrects age-related changes in circadian response to environmental stimulus

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Van Reeth, Olivier, Laurence Weibel, Elisabeth Olivares, Sonia Maccari, Elisabeth Mocaer, and Fred William Turek. Melatonin or a melatonin agonist corrects age-related changes in circadian response to environmental stimulus. Am J Physiol Regulatory Integrative Comp Physiol 280: R1582–R1591, 2001.—The effects of a melatonin agonist, S-20098, included in the diet were tested on a specific effect of aging in hamsters: the marked decline in the phase shifting effects of a 6-h pulse of darkness on a background of constant light. In contrast to young hamsters, old hamsters fed with the control diet showed little or no phase shifts in response to a dark pulse presented in the middle of their inactive or active period. Old hamsters fed with S-20098 showed phase shifts that were ∼70% of the ones in young animals and significantly greater than those in old controls. The phase advancing response to a dark pulse presented during the inactive period was dose dependent and reversed after S-20098 discontinuation. Melatonin included in the diet showed comparable restorative effects on the phase shifting response to a dark pulse in old hamsters. Replacement therapy with melatonin or melatonin-related compounds could prove useful in treating, preventing, or delaying disturbances of circadian rhythmicity and/or sleep in older people.

aging; circadian rhythm; sleep

AGING HAS PRONOUNCED EFFECTS on the expression of endocrine, metabolic, and behavioral circadian rhythms in a variety of mammalian species, including humans (19, 31, 38, 39). At least some of those age-related changes may be due to alteration in the functional activity of the master circadian pacemaker, the hypothalamic suprachiasmatic nuclei (SCN): aged rodents exhibit alterations in SCN glucose use (45), α1-adrenergic receptor levels (43), number, or size of vasoactive intestinal peptide and vasopressin cells (26, 32) and a decreased density of melatonin receptors associated with its blunted diurnal rhythms (44). In addition, advanced age has been associated with a decrease in the responsiveness of the circadian clock to the phase-shifting effects of a variety of pharmacological or nonpharmacological synchronizers (21, 38, 39, 48), and light-induced gene expression is also reduced in old rats and hamsters (28, 49).

Taken together, these results indicate that the aging circadian system is less sensitive to the synchronizing effects of stimuli that are normally involved in entraining the circadian clock to the 24-h changes in the external environment. Although the use of hormonal replacement therapy to reverse or attenuate the negative effects of aging on a variety of physiological and metabolic systems is an intensely active area of scientific and medical interest (16), such an approach has not yet been shown to prevent or reverse age-related changes in the circadian clock system. The pineal hormone melatonin is a particularly attractive candidate for such studies for a number of reasons, including 1) the robust 24-h rhythm in melatonin production (1) provides a reliable chemical equivalent of the temporal position of light and dark throughout the 24-h day, 2) the age-related decline in circulating melatonin levels is associated with aging of the circadian clock and other physiological systems in mammals (13, 25), 3) melatonin receptors have been localized to the mammalian SCN (42), 4) in vitro application of melatonin or melatonin agonists to SCN cells can induce changes in neuronal activity and circadian phase (47), and 5) their in vivo administration can phase shift and/or entrain circadian rhythms in rodents (9, 24, 34, 35). Furthermore, the observation that daily melatonin injections can enhance the organization of disrupted circadian rhythms (41) and that pinealectomy facilitates the loss of circadian rhythmicity in rodents kept in constant light (8) supports the hypothesis that the pineal gland and melatonin contribute to the integrity of circadian rhythmicity in mammals; this integrity is often observed to break down with advancing age.

In the present study we tested the effects of a melatonin agonist, S-20098 (3), or melatonin on a specific and easily quantifiable effect of advanced age in golden hamsters: the marked decline in the phase-shifting effects of a single 6-h pulse of darkness (36) in animals free-running in constant light (38). The results demon-
strate that chronic treatment with S-20098 or melatonin can restore responsiveness to this phase-shifting stimulus. The restorative effects of S-20098, reassessed after a wash-out of treatment, were not maintained after treatment discontinuation.

MATERIAL AND METHODS

General

Experiments were carried out on young (8 wk old) and old outbred male golden hamsters (Mesocricetus auratus) purchased from Charles River Lakeview (Newfield, NJ). Old hamsters, purchased at the age of 10 mo (retired breeders), were initially group housed and maintained under a 14:10-h light-dark cycle (LD; i.e., 14 h of light per 24 h) until they were 19–21 mo old.

Young and old hamsters were then moved to individual cages equipped with a running wheel to allow for continuous recording of locomotor activity through an electrical switch connected to a computer (Chronobiology Kit software package, Stanford Software Systems) (29). All animals were fed with regular powdered food and maintained in light-tight chambers (6 cages to a chamber) equipped with continuously operating fans.

Experimental Protocols

Effects of S-20098 on the phase advancing or phase delaying effects of a 6-h dark pulse in young and old hamsters. After 8 days, animals were weighed and transferred to constant light (LL; light intensity ~200 lx at cage floor level) at the usual time of lights-on. All young and 12 old hamsters were kept on regular powdered food, whereas the 12 remaining old hamsters were switched to powdered food containing 2,000 parts per million (ppm) S-20098. Animals were allowed to free run undisturbed until a steady-state phase of the free-running activity rhythm was established. After 16–21 days in LL, hamsters were exposed to a 6-h pulse of darkness (DP; Figs. 1 and 2) beginning around circadian time 6 (CT6; by convention CT12 refers to the onset of locomotor activity in this nocturnal species). For DP presentation, animals remained in their home cage with access to the running wheel while light was switched off for 6 h. After 13–28 days, those animals were subjected to a 6-h DP at CT 18 (Figs. 3 and 4). All young and old hamsters were then returned to the

![Fig. 1. Mean ± SE phase advances in the activity rhythm of young (left) and old (right) hamsters free running in constant light and subjected to a 6-h dark pulse (DP) beginning at circadian time (CT) 6 on 2 separate occasions (DP-1 and DP-2). Before and during exposure to both DPs, young (DP-1; DP-2) and old controls (Cont DP-1; Cont DP-2) were fed with regular powdered food. Old treated hamsters were kept on a diet containing 2,000 ppm S-20098 before and during exposure to the first DP (S-20098 DP-1) and put back to a regular powdered diet before and during the second DP (Post S-20098 DP-2). Numbers within parentheses indicate the number of animals tested for each condition.](http://apregu.physiology.org/)
original 14:10-h LD cycle for 21 days and once again fed with regular powdered food without S-20098 supplement. Those animals were then returned to LL for 17–22 days before they were exposed to a second 6-h DP beginning at CT6 (Figs. 1 and 2). Ten days later, all old hamsters were switched, in three steps, to powdered food mixed with increasing concentrations of S-20098: 500 ppm S-20098 for 30 days, 1,000 ppm for 27 days, and 2,000 ppm for 35 days. At least 2 wk (19–32 days) after the start of each of these S-20098 mixed diets, animals were subjected to a 6-h DP beginning at CT6 (Figs. 5 and 6). Variability in the numbers of days that elapsed before a DP was given applied equally to all experimental groups of hamsters.

S-20098 plasma level measurements. S-20098 plasma concentrations were measured at four different times of day in another group of 14 old hamsters (21 mo old) kept under a 14:10-h LD cycle and fed for 3 wk with 500 ppm S-20098. Blood was sampled (under pentobarbital sodium anesthesia) by cardiac puncture. Blood was centrifuged 10 min at 200 g, and plasma samples were stored at −20°C. S-20098 was assayed by liquid chromatography with fluorescence detection. Limit of quantification of the analytic method was 0.2 ng/ml.

Effects of melatonin on the phase advancing effects of a 6-h DP in young and old hamsters. After 10 days of adaptation to the cage with the running wheel, young (n = 6) and old (n = 6) hamsters were transferred to constant light (LL) and remained undisturbed in this lighting regimen until a steady-state phase of free-running activity rhythm was established. After 10 days in LL, hamsters were exposed to a 6-h DP beginning around CT6. Ten days later, animals were switched to powdered food containing melatonin (2,000 ppm). After the switch, hamsters were left undisturbed for 14 days before being exposed to a second DP at CT6. The experiment ended 10 days after this second DP.

Data Analysis

The phase-shifting effects of DPs on the activity rhythm were assessed as previously described (12, 30). Briefly, using daily onsets of wheel running activity for the last 7–9 days preceding treatment as phase reference points, a regression line fitted by eye was drawn and a time of activity onset was calculated. In addition, daily onsets of activity for the 7–9 days after treatment were used as post-treatment phase reference points, and a time of activity onset for the cycle after DP exposure was projected. The projected time of activity onset was subtracted from the time of activity onset the day after treatment, yielding the magnitude of the shift in the activity rhythm induced by treatment. Thus a negative value indicates a delay in the onset of activity, whereas a positive value indicates an advance. “Significant phase shift” refers to...
a permanent shift (advance or delay) in the onset of activity with a magnitude >20 min.

Running wheel activity in response to a DP presentation at CT6 or CT18 (first part of first experiment) was individually assessed in all studied hamsters by measuring total running wheel activity counts between the onset and the offset of each DP (Chronobiology Kit, Stanford Software Systems).

At both circadian times, the effects of S-20098 on the phase-shifting effects of DPs or their running wheel activity-inducing effect were compared between groups using an ANOVA one-way (group) factor analysis, followed by a multiple comparison test (Fisher’s protected least significant difference).

In the melatonin experiment, the phase-shifting effects of DPs were compared between groups before or during melatonin treatment with a unpaired Student’s t-test and within groups with a paired Student’s t-test.

RESULTS

Effects of S-20098 on the Phase Advancing or Delaying Effects of a 6-h DP in Young and Old Hamsters

In response to the first DP at CT6, six of seven young hamsters exhibited large phase advances in the activity rhythm (group means ± SE = 124 ± 40 min; Figs. 1 and 2). Significant phase advances were observed in only two of eight old controls (group means ± SE = 5 ± 6 min) and in nine of ten old animals fed with S-20098 (group means ± SE = 78 ± 23 min; P = 0.01; ANOVA 1-way group factor). Phase shifts in S-20098-treated old hamsters were significantly larger than in old controls (P = 0.04, multiple comparison Fisher’s test), but not different from those in young hamsters (P = 0.21, multiple comparison Fisher’s test). In response to the second DP at CT6, comparable large phase advances were seen in the activity rhythm of young hamsters (group means ± SE = 116 ± 39 min). In old hamsters fed with regular powdered food from the beginning of the study, the 6-h DP elicited a significant phase advance in only two of six tested animals (group means ± SE = 9 ± 6 min; P = 0.0009; ANOVA 1-way group factor). In old hamsters previously fed with S-20098 and returned to regular food before the second DP, phase advances were observed in only two of eight hamsters (group means ± SE = 16 ± 3 min). Phase shifts in this group were no longer significantly different from those in old control hamsters (P = 0.79, multiple comparison Fisher’s test).

In response to the 6-h DP at CT18 (Figs. 3 and 4), significant phase delays were observed in all young hamsters (group means ± SE = -56 ± 11 min). A significant phase shift was seen in only one of seven old
Fig. 4. Representative sections from the wheel running activity records of 1 young hamster (top), 1 old control hamster (middle), and 1 old hamster treated with S-20098 (bottom). Animals were housed in LL before and after they were exposed to a 6-h DP beginning at CT18. See legend of Fig. 2 for further details.
control hamsters (group means ± SE = −2 ± 5 min). In contrast, six of seven old hamsters fed with S-20098 exhibited significant phase delays in the activity rhythm (group means ± SE = −39 ± 9 min; P = 0.0008, ANOVA 1-way group factor). Phase shifts in S-20098-treated old hamsters were significantly larger than in old controls (P = 0.003, multiple comparison Fisher’s test) and not different from those in young hamsters (P = 0.19, multiple comparison Fisher’s test).

There was a great interindividual variability in the amount of running wheel activity induced by presentation of DPs, especially in old hamsters. Mean (±SE) running wheel activity counts during the first DP presented at CT6 averaged 2,306 ± 522 in young controls, 370 ± 268 in old controls, and 580 ± 216 in old hamsters fed with S-20098 (P = 0.001, ANOVA 1-way group factor). There was no significant difference in the amount of running wheel activity between old controls and those fed with S-20098 (P = 0.64, multiple comparison Fisher’s test). During the second DP at CT6 (performed after the wash-out period for the animals previously fed with S-20098) mean (±SE) running wheel activity counts averaged 320 ± 178 in old controls and 600 ± 417 in old previously fed with S-20098 (P = 0.61, ANOVA 1-way group factor). In those two groups of animals, there was no significant difference in the amount of running wheel activity in response to the two DPs at CT6 (old controls: P = 0.63, old fed with S-20098: P = 0.94; paired Student’s t-test). Running wheel activity counts during DPs presented at CT18 averaged 2,123 ± 767 in young controls, 2,523 ± 624 in old controls, and 1,240 ± 368 in old fed with S-20098 (P = 0.27, ANOVA 1-way group factor).

Dose-dependent phase shifts were observed in response to a DP at CT6 in old hamsters switched from regular diet to a diet containing increasing concentrations of S-20098 (Fig. 5). Significant phase advances were observed in 5 of 20 old hamsters when fed with regular diet (group means ± SE = 17 ± 3 min), in 12 of 15 of them under 500 ppm S-20098 (group means ±

![Fig. 5. Means ± SE phase advances in the activity rhythm in response to a 6-h DP at CT6 in old hamsters first fed with regular powdered food, then with diet mixed with increasing concentrations of S-20098 (from left to right, respectively 500, 1,000 and 2,000 ppm). Numbers within parentheses indicate the number of animals tested for each condition.](http://ajpregu.physiology.org/)
S-20098 Plasma Level Measurements

Individual S-20098 plasma levels varied between 2.4 and 12.5 ng/ml with no evidence of diurnal variation. Mean (±SE) S-20098 plasma concentrations averaged 6.3 ± 0.9 ng/ml.

Effects of Melatonin on the Phase Advancing Effects of a 6-h DP in Young and Old Hamsters

In response to the first DP at CT6, all young hamsters exhibited large phase advances in their activity rhythm (group means ± SE = 237 ± 35 min), whereas significant phase advances were observed in only two of six old hamsters (group means ± SE = 15 ± 12 min; Fig. 6). Phase shifts in young hamsters were significantly larger than in old ones before treatment (P = 0.0001, unpaired Student’s t-test).

In response to the second DP (under melatonin treatment), comparable large phase advances were seen in the activity rhythm of young hamsters (group means ± SE = 303 ± 35 min; P = 0.14, 1-tail paired Student’s t-test compared with before treatment). In old hamsters, the 6-h DP elicited a significant phase advance in six of six tested animals (group means ± SE = 97 ± 19 min). Phase shifts in response to a 6-h DP in old hamsters were significantly larger under melatonin treatment compared with before treatment (P = 0.0077, 1-tail paired Student’s t-test).

DISCUSSION

These results indicate that a pharmacological treatment can be used to restore, at least in part, responsiveness of the circadian clock of old hamsters to an environmental stimulus that is normally lost in advanced age. Chronic treatment with a melatonin agonist or with melatonin was able to render old hamsters sensitive to both the phase-advancing and phase-delaying effects of a 6-h DP on the free-running rhythm of locomotor activity. Additional experiments performed only with the melatonin agonist showed that the degree of restoration was dose dependent and that responsiveness to the phase-shifting effects of the DP was lost after termination of treatment. This indicates that such treatment did not induce a long-lasting change in circadian clock responsiveness to an environmental stimulus, but rather that the presence of the agonist at the time of stimulation was necessary for function to be restored.

The dose-response curve to S-20098 for the phase shifting effects of a 6-h DP at CT6 was built by incrementally increasing the diet concentration of this compound throughout the experiment, with no incremental decrease in its concentration afterward. However, the possibility that the observed increase in phase shift size was caused by experience rather than by S-20098 dosage is unlikely, because in the first part of this experiment old control hamsters exposed twice to the same DP did not show any increase in phase shift magnitude in response to the second DP.

Restorative effects of melatonin or a melatonin agonist on circadian clock function could be due to effects on either an input pathway to the SCN or on changes in the SCN itself. Although there is a decrease in melatonin receptor expression in the SCN of old mice, this decrease does not alter the response to the phase-shifting effects of a single injection of melatonin, indicating that a maximal response of the SCN to melatonin can be elicited by activation of a reduced number of melatonin receptors (2). These results indicate that in rodents the aged SCN is still capable of responding to the phase-shifting effects of an acute melatonin challenge, although at the present time it is not known if the chronic effects of melatonin or a melatonin agonist that restore circadian clock responsiveness to a different phase-shifting agent (i.e., DP) also involve a direct effect on SCN cells.

In view of the fact that the phase-shifting effects of a DP are thought to be due, at least in part, to the DP-induced increase in locomotor activity that is associated with the presentation of this stimulus (36), another possible site of action for the chronic effects of
Melatonin or melatonin analogs would be on the input pathway by which information about the level of activity/rest reaches the SCN. This pathway appears to involve the raphe nuclei of the midbrain as well as the intergeniculate leaflet (IGL) of the lateral geniculate nucleus of the thalamus (10, 23). It should be noted that in a previous study we demonstrated that the acute effects of another activity-inducing stimulus (i.e., the short-acting benzodiazepine, triazolam) on increased locomotor activity were similar between young and old hamsters, despite the fact that in old hamsters phase shifts in response to this stimulus were totally blocked (38). Indeed, results of the present study confirm that running wheel activity for the 6-h duration of DPs presented at CT6 or CT18 was not significantly different between old control and old hamsters treated with S-20098. Thus it appears that the restorative effects of the melatonin agonist on the response of old hamsters to activity-inducing stimuli are not due to a restorative effect on the response to the acute behavioral effects of DP (i.e., an increase in locomotor and/or total activity) but instead are due to their restorative effects on the ability of the circadian clock to respond to an increase in activity.

Pharmacological activation of the serotonergic pathways to the SCN and IGL, as well as activation of the neuropeptide Y pathway from the IGL to the SCN, all induce phase shifts in the circadian clock that are similar to those induced by a DP or other activity-inducing stimuli in young hamsters (4, 11, 18). The phase-shifting response of the circadian clock of old hamsters to both pharmacological and nonpharmacological activation of these pathways is attenuated or lost in old hamsters, and it will be of interest to determine if melatonin can restore the responsiveness of old animals to a variety of different stimuli.

In the present study, hamsters had ad libitum access to powdered food. In contrast to the clear circadian feeding rhythm of rats or mice, the feeding pattern of hamsters shows a weak diurnal variation, with only slightly higher food intake during nighttime compared with daytime (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route compared with daytime (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route compared with daytime (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route compared with daytime (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route compared with daytime (27). This suggests the possibility that comparable quantities of melatonin or the melatonin agonist were ingested through the oral route.

In view of recent data showing the influence of timing/duration of S-20098 administration on circadian entrainment in rodents (22), future protocols designed to provide or mimic high nighttime plasma levels of melatonin agonists and low daytime levels should be tested to determine whether mimicking physiological profiles of endogenous melatonin (1) will strengthen the action of chronobiologic compounds on synchronization processes.

Restoration of circadian function in old animals with melatonin or a melatonin agonist adds to the small list of successful interventions that have been found to "rejuvenate" the aging rodent circadian clock. As first reported in 1994, transplantation of fetal SCN tissue into the SCN region of old hamsters restored the feedback effects of the activity-rest cycle on the circadian clock of old hamsters (40). Later studies reported that fetal SCN grafts were able to increase the amplitude of the blunted locomotor activity rhythm in old hamsters (14) and to restore the dampened SCN circadian rhythm of Fos expression and light-induced Jun-B expression (5, 7), as well as the diurnal rhythm of hypothalamic corticotrophin-releasing hormone and anterior pituitary proopiomelanocortin mRNA in old rats (6). Whether a "young" SCN restores circadian function in old animals by its own activity on the direct expression of circadian rhythms or on an effect it may have on the host SCN is not known.

Increasing the strength of the entraining light-dark cycle has been shown to increase the amplitude of the locomotor activity rhythm of old rats (46) as well as in middle-aged, but not old, hamsters (15). In the studies on middle-aged hamsters, the overall 24-h activity patterns showed a more "youthful" profile with activity increasing particularly in the early hours of the dark period when young hamsters are normally active. So far, pharmacological approaches to improve circadian function in aging have been limited. Except for the present report using melatonin or a melatonin agonist, only one other pharmacological approach had beneficial effects: treatment with a thiamin cofactor, sulbutiamine, improved circadian function in old hamsters (33, 37). Although behavioral interventions for strengthening the entraining effects of environmental stimuli on the circadian clock in the elderly hold some promise (19, 20), this approach may be too time consuming or difficult to implement.

Perspectives

Treatment with melatonin or a melatonin agonist may be useful in enhancing how older humans respond to the natural entraining stimuli from the external environment. Elucidating the mechanisms by which pharmacological and nonpharmacological stimuli can enhance circadian function in older animals may lead to clinical approaches for treating, preventing, or delaying disturbances of circadian rhythms and sleep in older human populations.

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