Thermocyclic entrainment of lizard blood plasma melatonin rhythms in constant and cyclic photic environments

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Firth, Bruce T., Ingrid Belan, David J. Kennaway, and Robert W. Moyer. Thermocyclic entrainment of lizard blood plasma melatonin rhythms in constant and cyclic photic environments. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1620–R1626, 1999.—We assessed how chronic exposure to 6-h cryophase temperatures of 15°C in an otherwise 33°C environment entrains the rhythm of blood plasma melatonin rhythms in lizards (Tiliqua rugosa) subjected to constant dark (DD), constant light (LL), and to 12:12-h light-dark cycles (12L:12D). The peak of the melatonin rhythm was entrained by the cryophase temperature of the thermocycle in DD and LL, irrespective of the time at which the cryophase temperature was applied. Comparable thermocycles of 6 h at 15°C imposed on a 12L:12D photocycle, however, affected the amplitude and phase of the melatonin rhythm, depending on the phase relationship between light and temperature. Cold pulses in the early light period and at midday resulted, respectively, either in low amplitude or nonexistent melatonin rhythms, whereas those centered in or around the dark phase elicited rhythms of high amplitude. Supplementary experiments in 12L:12D using two intermittent 6-h 15°C cryophases, one delivered in the midscotophase and another in the midphotophase, elicited melatonin rhythms comparable to those in lizards subjected to constant 33°C and 12L:12D. In contrast, lizards subjected to 12L:12D and a 33°C:15°C thermocycle, whose thermophase was aligned with the photophase, produced a threefold increase in the amplitude of the melatonin rhythm. Taken together, these results support the notion that there is an interaction between the external light and temperature cycle and a circadian clock in determining melatonin rhythms in Tiliqua rugosa.

Light and temperature are the underlying environmental parameters affecting seasonal physiological cycles in vertebrates. Numerous investigations in mammals have shown that pineal melatonin acts as a neurochemical messenger of the photoperiod in synchronizing such cycles. The situation is different for nonmammalian ectothermic vertebrates whose body temperature (Tb) under-

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The present investigation further explores the influence of thermocycles on plasma melatonin rhythms in sleepy lizards (Tiliqua rugosa), this time examining the steady-state effect of 12- to 14-day exposure to 6-h cryophase temperatures of 15°C in an otherwise 33°C environment. Our primary aim was to determine precisely how short periods of cool temperature coincident with a 12:12-h light-dark (12L:12D) photocycle affect entrainment of the blood plasma melatonin rhythm. The second objective was to find whether the melatonin rhythm can be entrained by such thermocycles in constant dark (DD) and constant light (LL). A third aim was to assess whether the blood plasma melatonin rhythm in sleepy lizards depends on the absolute ratio of thermophase-to-cryophase temperature in a 12L:12D environment or whether it is the timing or phase of such ratios that is important.

MATERIALS AND METHODS

General

Male and female T. rugosa lizards (~500-g average wt) were collected in spring and summer within a 300-km radius of Adelaide, South Australia, and housed prior to experiments in 3.5-m diameter outdoor pens. Experiments were conducted in the austral summer-autumn (December-May). Lizards held in individual containers were acclimated for 12–14 days in photothermally programmable environment chambers as described previously (12). All experiments used thermocycles of 33°C:15°C. These temperatures were chosen to respectively reflect the daytime activity temperature of T. rugosa in nature (9) and the average summer nighttime temperature in Adelaide.

Blood was sampled at 2- to 4-h intervals by cardiac puncture through the left axillary region. Nighttime samples were taken using a dim red light (maximum intensity 10 lx). Blood plasma was stored at −20°C and radioimmunoassayed for melatonin using techniques described previously (11).

Experiment 1

Effect of 6-h cold pulses (15°C) and 18 h (33°C) in DD. To determine whether the melatonin rhythm can be entrained to cold pulses in the absence of a light-dark cycle, four groups of lizards, balanced for sex and weight, were acclimated for 12–14 days in DD. Each group was subjected to an 18-h 33°C thermophase alternating with a 6-h 15°C cryophase centered at either 1200, 1800, 2400, or 0600. At the end of the acclimation time, blood samples were assayed for melatonin at 7-9 time points over a 24-h period for each of the four groups of lizards.

Experiment 2

Effect of 6-h cold pulses in LL. To ascertain whether the melatonin rhythm could be generated in LL in conjunction with a thermophase, two groups of lizards, balanced for sex and weight, were acclimated for 12–14 days in LL (average intensity in chamber of 2,800 lx). The thermophase consisted of 18 h at 33°C and 6 h at 15°C with the cryophase either at midday or midnight. At the end of the acclimation time, blood samples were assayed for melatonin at 7-9 time points over a 24-h period for each of the two groups of lizards.

Experiment 3

Effect of 6-h cold pulses in a 12L:12D photocycle. This experiment was designed to determine how thermocycles with 6-h cold pulses centered at different phases of a 12L:12D photoperiod affect the pattern of melatonin secretion. Lizards, approximately balanced for sex and weight, were subjected to a 12L:12D photoperiod for 12–14 days. Simultaneously, they were exposed to a thermophase consisting of a 33°C therophase and a 6-h cryophase of 15°C centered either at 0600, 0900, 1200, 1800, 2100, or 2400. At the end of the acclimation period, an average of eight lizards were sampled at each of the 7-9 sampling times.

Experiment 4

Effect of constant 33°C and intermittent thermocycles on melatonin rhythms. This set of experiments was designed to discern the effect on the melatonin rhythm of the phase versus the absolute amount of time that sleepy lizards were subjected to a given cryophase temperature (15°C). A control group of lizards was subjected to a constant 33°C and 12L:12D environment for 12–14 days. A second group of lizards was subjected to a thermophase of 12 h of 15°C and 33°C, concomitant with the respective scotophase and photophase of the light-dark cycle. A third group was acclimated to 12 h of 15°C, alternating with 33°C, but with the cryophase component separated such that 6 h of 15°C were delivered from 0900 to 1500 and again from 2100 to 0300. Each of these three groups of lizards was sampled for blood after 12–14 days acclimation to the appropriate regime at 7–9 sampling points over a 24-h period.

RESULTS

Experiment 1

In lizards exposed to 6-h 15°C cold pulses in an otherwise 33°C environment in DD, the peak levels of the blood plasma melatonin rhythm generally coincided with the midpoint of the cryophase (15°C) temperature of the thermocycle (Fig 1). Only when the cryophase temperature was centered at 0600 did the plasma melatonin levels peak at 0400, 2 h out of phase with the midpoint of an imposed thermocycle (Fig 1D).

In every case, there was a significant rhythm of melatonin production (P < 0.01). The time of the acrophase of melatonin secretion differed significantly (P < 0.001) among the four different application times of the 6-h cold pulse (Kruskal-Wallis one-way ANOVA). Unlike the situation in experiment 3 thermocycle in 12L:12D, the amplitude of the melatonin rhythm did not significantly differ (P > 0.05) with respect to the time of application of the cold pulse (see Figs. 1 and 3).
Experiment 2

Lizards exposed to LL, but with an imposed thermocycle, exhibited a significant rhythm of blood plasma melatonin levels (P < 0.001; Fig. 2). The rhythm peaked at 1200 or 2400, coincident with each of the times at which the 6-h 15°C cold pulse was centered. Thus the cryophase of a thermocycle entrained the blood plasma melatonin rhythm, even in bright LL.

Experiment 3

There was no significant rhythm of plasma melatonin levels when a 6-h 15°C cold pulse was centered at midday in an otherwise 33°C thermal environment and a 12L:12D photocycle (P > 0.05; Fig. 3). Cold pulses at all other times elicited a significant rhythm of melatonin secretion (P < 0.01).

The amplitude of the melatonin rhythm depended on the timing of the cryophase temperature relative to the photoperiod. For example, the rhythm of highest amplitude was when a 6-h cryophase pulse was centered at 2100 (peak > 1,000 pmol/l). The acrophase of this rhythm was significantly different (P < 0.05, two-tailed Mann-Whitney U) from that of the next highest rhythm, when the cryophase pulse was centered at 2400 (Fig. 3, E and F). Cryophase temperatures centered at all other times elicited melatonin rhythms of which the highest amplitude was generally <600 pmol/l (Fig. 3).

The results also show that the timing of the cold pulse is accompanied by a phase shift in the peak of the rhythm.
melatonin rhythm. Apart from when a cryophase pulse was centered at 1200 (which abolished the melatonin rhythm), there was a melatonin peak that coincided with some phase aspect of the cryophase temperature, although the peak was always contained within the scotophase of the 12L:12D photocycle (Fig. 3). Thus a cryophase pulse at 0600 elicited a peak at 0200, whereas at 0900, the peak was at 0400. When the pulse was centered at 1800, the melatonin peak was at 2000, whereas at 2100, it was at 0300.

Figure 4 compares the amount of melatonin produced when 6-h cold pulses were delivered in 12L:12D with those delivered in DD. In 12L:12D, the amount of melatonin varied, depending on the time of application of the cold pulse. In contrast, in DD, the time at which the cold pulse was applied had little effect on the amount of melatonin produced.

Experiment 4

This experiment further demonstrates that the timing of the cryophase pulse, rather than its absolute duration, is important in determining the blood plasma melatonin rhythm in sleepy lizards. When a 12-h cryophase temperature of 15°C coincided with the dark phase of a 12L:12D photocycle, the resultant melatonin rhythm was one of high amplitude (~1,400 pmol/l; Fig. 5C) and amount (1,421 arbitrary units) compared with that at constant 33°C. When a comparable 12-h cryophase temperature was delivered in two components, each of 6 h, and centered either at midscotophase or midphotophase, the melatonin rhythm was of low amplitude (~450 pmol/l; Fig. 5B) and amount (295 arbitrary units). This rhythm was similar in amplitude (300 pmol/l; Fig. 5A) and absolute amount of melatonin (387 arbitrary units) to that exhibited in constant 33°C and a light cycle of 12L:12D.

DISCUSSION

Our results support the notion that temperature cycles in ectotherms such as lizards are important synchronizers of the rhythm of melatonin production. Previous studies in T. rugosa have shown that the zenith of a rhythm of blood plasma melatonin production coincides with the nadir (cryophase) of the thermocycle in a 12L:12D cycle (10–14). The current study in T. rugosa suggests that a 6-h cryophase temperature of 15°C in an otherwise 33°C environment, imposed at any stage of the subjective day or night in DD, can entrain the plasma melatonin rhythm. In the North American iguanid lizard Anolis carolinensis, Underwood and Calaban (35) also found that in DD, thermocycles of 32°C:20°C entrained the melatonin rhythm, the peak of which coincided with the cryophase, as shown also by in vitro studies of pineal organs in the fish Catostomus commersoni (40) and geckos (26). Paradoxically, in another fish species Esox lucius, the melatonin peak coincided with the thermophase of a 20°C: 10°C thermocycle in DD (8). The reasons for these contrasting outcomes are not yet obvious, but nevertheless, demonstrate the apparent evolutionary plasticity of melatonin synthesis and the underlying circadian system controlling melatonin secretion in vertebrates (23).

Our demonstration that temperature cycles can entrain melatonin rhythms in LL is supported by two other studies, both in reptiles. In Anolis carolinensis, a cryophase temperature of 20°C imposed at an unspecified time of the subjective day or night in conjunction with a thermophase of 32°C could entrain the melatonin rhythm in dim LL of ≤80 lx, levels of light intensity...
well below those of the present study (32, 35). In the gecko Christinus marmoratus, Moyer et al. (26) also demonstrated in vitro a melatonin rhythm in LL (500 lx) in the presence of a 30°C:20°C temperature cycle, with the peak of the rhythm coinciding with the cryophase. The observation that thermocycles can entrain a rhythm of melatonin secretion in LL suggests a difference in the regulation of melatonin from that seen in mammals in which light acutely suppresses nighttime melatonin secretion (20). For example, in humans, 2,500 lx (compared with 2,800 lx in the present study) of light delivered at night can suppress melatonin secretion to daytime levels, and nocturnal rodents are sensitive to much lower intensities and durations of light (20). Among nonmammalian species, acute light exposure at night lowers the nighttime melatonin levels in fishes (22, 39), but not in reptiles (33, 38).

When a thermocycle is superimposed on a photocycle, the blood plasma melatonin rhythm of T. rugosa shows an interesting pattern. Cold pulses of 6-h duration can phase shift the melatonin rhythm in a manner that is different from that in constant photic environments, indicating that light and temperature interact in determining the amplitude and phase of the melatonin rhythm. Both of these parameters are affected by the phase at which the 6-h cold pulse is applied relative to the 12L:12D photoperiod (Fig. 3). Cold pulses in the early light period and at midday resulted, respectively, either in low amplitude or nonexistent melatonin rhythms, whereas those centered in the dark phase (especially at 2100) elicited high-amplitude rhythms. These results are in accordance with those of earlier observations (12) in which the converse situation of 6-h thermophase temperatures of 33°C were applied at four different phases relative to the 12L:12D photoperiod. In that study, the blood plasma melatonin rhythm was eliminated when the thermophase was centered at midnight, possibly because the melatonin peak was dampened when shifted into the subsequent photophase. In vitro studies using thermocycles and photocycles in fishes (40) and gekkonid lizards (26) provide further evidence that the amplitude of the melatonin rhythm is dampened when the warm pulse of the thermocycle coincides with the mid-dark phase of the photocycle. The situation, however, seems to be different in the pike, a teleost fish. Supersaturated pike pineal organs in a 12L:12D light cycle and 10°C day:20°C night thermocycle augmented the amplitude of scotophase melatonin production compared with those pines with the nighttime temperature at 10°C. In neither case, however, was the melatonin rhythm abolished (8). Similarly, in Anolis lizards, reversed thermocycles (cold light, warm dark) of 20°C:33°C did not abolish the melatonin rhythm, unlike the comparable situation in T. rugosa. Rather, in Anolis the melatonin levels in the light phase were similar in amplitude to those when the cryophase coincided with the scotophase (32). Recent studies on melatonin output from cultured eye cups of the frog Rana perezi using various thermoderoids in a 12L:12D photoperiod represent a further variation in that the nighttime temperature differentially influences the amplitude of the melatonin rhythm (37). For example, in a thermoperiod of 25°C:15°C, the nighttime melatonin output is higher when the cryophase coincides with the scotophase, whereas, in a thermoperiod of 25°C:5°C, the output is higher when the cryophase coincides with the photophase. The biochemical, ecological, and phylogenetic bases of these interspecific differences in the photic and thermoderoid responsiveness of melatonin are yet to be resolved.

Our results in experiment 4 confirm the previous observation (11) that a thermocycle in conjunction with a photocycle elicits a more robust rhythm of melatonin production than a photocycle in constant high temperature. Implicit in these present results also is that light and temperature interact with a circadian clock in shaping the rhythm of melatonin production in sleepy lizards. When only 6 h of a 12-h 15°C cryophase temperature were delivered in the scotophase, the subsequent melatonin rhythm was less than one-third of the amplitude and amount that resulted from a 12-h cryophase temperature coincident with the scotophase. The circadian system may, therefore, express the cryophase signal only when it coincides either wholly or partly with the scotophase, as also indicated by our results in experiment 3. In keeping with this notion, phase-response curves for locomotor activity, derived from photoperiodic studies in mammalian circadian rhythms, indicate that phase delays and advances occur mainly in the subjective night (see Ref. 27).

Surprisingly, the phase-shifting effect of temperature cycles on melatonin rhythms also extends to obligate endotherms. In dispersed cell cultures of chick pineal (1), warm 6-h temperature cycles of 42°C in an otherwise 37°C environment resulted in phase-response curves (rhythmic shifts in response to light/temperature perturbations) that were identical to those elicited by 6-h light cycles. The authors concluded that both light and temperature influence the same circadian oscillator(s). It is interesting, therefore, that, like ectotherms, some endothermic homeotherms are capable of interpreting thermocycles as well as light cycles with respect to the rhythm of melatonin production. Whether this capability is limited to birds, the immediate phylogenetic relatives of reptiles (6), and not to mammals remains to be determined.

Perspectives

The ecophysiological implications of photothermoperiodic interaction in ectothermic vertebrates are poorly understood. Terrestrial ectotherms, such as lizards, undergo wide daily and seasonal variations of Tb. Many diurnal reptiles in nature raise their Tb to species-typical constant levels during the day and submit to ambient temperatures at night. This voluntary hypothermia can be replicated in laboratory thermal gradients (9, 31), and it has been shown to vary seasonally in its pattern in Tiliqua rugosa (9) and in other lizard species (29). Because the cryophase temperature of a lizard differentially coincides with the prevailing photoperiod at different seasons, pineal melatonin production would change accordingly. Such a photothermope-
EFFECT OF THERMOCYCLES ON LIZARD MELATONIN RHYTHMS

R1625

periodic signal could provide a relatively stable, noise-free seasonal timing signal for functions such as reproductive and thermal adaptation. Although photoperiod/thermoperiod interactions have been well demonstrated in the control of diapause in insects (2), their functions have been explored only cursorily in lizards and other vertebrates (21). For example, seasonal reproductive cycles in the lizard Anolis carolinensis depend on photoperiodic time measurement, which functions only when temperatures exceed a threshold level (36). Experiments in this species using night-break, T-cycle, resonance lighting schedules, and melatonin replacement studies further indicate that photoperiodic time measurement of the reproductive cycle is correlated with the phase of its melatonin rhythm rather than the rhythm’s amplitude or duration (17, 18). In this context, the present study in T. rugosa shows that the phase of the melatonin rhythm can be dramatically altered by thermocycles. These data, together with those demonstrating the involvement of the lizard pineal gland in circadian organization (see review, Ref. 34), strongly suggest that aspects of the daily and seasonal physiology of lizards are directed by a circadian clock dependent on thermoperiodically as well as photoperiodically mediated melatonin.

We thank Gail Hermanis, Shawn Rowe, and Rehema White for technical assistance.

This study was supported by funds from the Australian Research Council and the Faculty of Medicine at the Univ. of Adelaide. The research was conducted with the permission of the South Australian National Parks and Wildlife Service (C21465) and the Univ. of Adelaide animal ethics committee (7/65/89).

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Received 18 February 1999; accepted in final form 19 July 1999.

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