Effects of photoperiod reduction on rat circadian rhythms of BP, heart rate, and locomotor activity

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Zhang, Bei-Li, Erika Zannou, and Frédéric Sannajust. Effects of photoperiod reduction on rat circadian rhythms of BP, heart rate, and locomotor activity. Am J Physiol Regulatory Integrative Comp Physiol 279: R169–R178, 2000.—The effects of a photoperiod reduction in the entrainment of circadian rhythms of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and spontaneous locomotor activity (SLA) were determined in conscious Wistar rats by using radiotelemetry. Two groups of seven rats were maintained in a 12:12-h light-dark (12L/12D) photoperiod for 11 wk and then placed in a reduced photoperiod of 8:16-h light-dark (8L/16D) by advancing a 4-h darkness or by advancing and delaying a 2-h darkness for 6 wk. Finally, they were resynchronized to 12L/12D. Advancing a 4-h dark phase induced a 1-h advance of acrophase for SBP, DBP, and HR, but not for SLA. The percent rhythm, amplitude, and the 12-h mean values of all parameters were significantly decreased by the photoperiod reduction. When symmetrically advancing and delaying a 2-h dark phase, a 1 h 20 min delay of acrophases and a decrease in percent rhythms and amplitudes of SBP, DBP, HR, and SLA were observed. Only the 12-h mean values of HR and SLA were decreased. Our findings show that the cardiovascular parameters differ from SLA in phase-shift response to photoperiod reduction and that the adjustment of circadian rhythms to change from 12L/12D to 8L/16D photoperiod depends on the direction of the extension of the dark period.

IN MAMMALS, THE CIRCADIAN rhythms of behavior, physiology, and metabolism are generated by an internal biological clock mainly located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (25). The endogenous timekeeping function of the SCN is complemented by its role in the entrainment of circadian rhythms to environmental stimuli. In the absence of a time-giving stimulus, the circadian clock assumes its natural frequency and free runs (2). Among the numerous environmental factors acting as synchronizers for the entrainment of endogenous circadian rhythms, the daily light/dark (L/D) cycle appears to be the most potent factor (26). The information about L/D cycle is transmitted from the retina to the SCN, which drives the daily rhythm in secretion of the pineal hormone, melatonin (26, 33), and allows organisms to synchronize the circadian rhythms of behavior and physiology. On long summer days, the nocturnal melatonin signal is short, whereas on short winter days, the signal is long (12). After transition of rats and Djungarian hamsters from long to short days, the melatonin signal extends gradually and animals adjust to changed photoperiod only after 10–12 days (14, 15). In addition, it is well established that the circadian melatonin rhythm conveys information about time of day and time of year to influence daily and seasonal physiological processes in a variety of species (2, 32).

As well as spontaneous locomotor activity (SLA), arterial blood pressure (BP) and heart rate (HR) present circadian rhythms both in animals (35) and humans (23). BP and HR are lower during resting than activity periods, which is related to the day/night rhythm (4). In addition, it is well known that life-threatening cardiovascular events, such as sudden cardiac death, stroke, or myocardial infarction occur most frequently in the early morning hours and are related to the seasonal changes (3, 24, 27). Although various mechanisms may be responsible for these disturbances, they all appear to coincide with the period of increase in the 24-h BP rhythm (28). However, how the circadian rhythms of BP and HR can be affected by changes of photoperiod has not yet been investigated. Therefore, it is likely important to clarify the influence of photoperiod reduction on the entrainment of circadian rhythms of cardiovascular parameters.

The present study was designed to characterize the circadian rhythms of systolic (SBP) and diastolic BP (DBP), HR, and SLA under standard entrained photoperiod condition of 12:12-h LD conditions (12L/12D) and to determine the effects of two processes of photoperiod reduction to 8:16-h LD conditions (8L/16D) by a 4-h extension of darkness and by symmetrically advancing and delaying a 2-h dark phase on cardiovascular and locomotor activity circadian rhythms in conscious and unrestrained Wistar rats.

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MATERIALS AND METHODS

Fourteen 9-wk-old male Wistar rats (250 ± 12 g) were obtained from Ifla-Credo (Les Oncins, Saint-Germain-sur-l’Arbresle, France). They were individually housed in Plexiglas cages under a 12L/12D cycle (light on at 0800 and light off at 2000, light intensity 150 ± 10 lx at the cage level). Ambient temperature of 21 ± 1°C and relative humidity of ~60% were maintained throughout the experiments. Animals were fed with a standard rat chow (AO3 Elevage UAR, Villemoison s’Orge, France) and had free access to tap water.

After 1-wk acclimatization to our laboratory conditions, rats were chronically instrumented by implantation of telemetry transducers. They were anesthetized with sodium methohexital (Brietal, 10 mg/kg ip, Lilly Laboratories, Saint-Cloud, France) and subjected to a surgical operation according to the procedure previously described by Brockway et al. (1). Briefly, after a midline abdominal incision, the descending aorta was exposed. The transmitter catheter (model TA11PA-C40, Data Sciences International, St. Paul, MN) was inserted into the abdominal aorta below the bifurcation of the renal arteries pointing upstream (against the flow). The catheter was secured at the point of entry to the vessel with tissue adhesive and a patch of paper fiber. The body of the transmitter was inserted in the peritoneal cavity and sutured to the abdominal musculature at the incision site. The rats received one dose (30 mg · kg⁻¹ · day⁻¹ ip) of amoxicillin (Clamoxyl, Smith-Kline and Beecham Laboratories, Nanterre, France) just before surgery and then during the 3 following postimplantation days to prevent infection. They were allowed to recover for 2 wk in the experimental room before the start of experiments.

SBP, DBP, and HR as well as SLA were continuously measured by using Dataquest IV data-acquisition system and signals were collected via RLA-1020 receivers (Data Sciences International, St. Paul, MN) located below the animal cage. BP and HR were measured via the aortic probe, whereas SLA was obtained by a system monitoring changes in the received signal strength, which occur on each animal movement. BP of each rat was continuously monitored every 2 min as a waveform curve for 5 s. Peaks and troughs in BP curve were detected, and the Dataquest IV software calculated SBP, DBP, and HR sample values. In addition to BP, SLA of each rat was measured every 2 min. Individual data from each animal were exported from the Dataquest IV program in Lotus format and then transferred to the Excel 5.0 program developed in our laboratory.

Experimental protocols. Two series of experiments were performed under three photoperiod conditions. It consisted of 5 wk discontinuous measurement and recording of SBP, DBP, HR, and SLA in 12-, 15-, 16-, 20-, and 21-wk-old rats maintained in a 12L/12D photoperiod condition. Then, these parameters were continuously monitored for 6 wk (from 22 to 27 wk of age) in a 4-h reduced photoperiod (8L/16D) process. In experimental series 1, the photoperiod reduction was carried out by advancing a 4-h dark phase (4-h darkness extension at the beginning of the night). In experimental series 2, it was performed by symmetrically advancing and delaying a 2-h dark phase (2-h darkness extension at the beginning and at the end of the night; Fig 1). Finally, rats were resynchronized to a 12L/12D standard photoperiod, and all parameters were continuously measured for 3 wk (from 28 to 30 wk of age).

Data analysis. Data are expressed as means ± SE. The parameters of circadian rhythms, such as mesor (midline-estimating statistic of rhythm, i.e., the rhythm-adjusted 24-h mean), amplitude (half of peak to trough of rhythmic change), acrophase (peak time of the rhythmic component, expressed in hours from midnight), and percent rhythm (the percentage of total variance that is accounted for by the fitted curve) were analyzed by DQ-FIT and CV-SORT programs generously given to us by Professor Björn Lemmer (37). ANOVA with repeated measures followed by the method of contrast was used to determine the difference between 7-day-period data collected under different photoperiod conditions. Kruskal-Wallis one-way ANOVA was used to determine the difference between experimental series 1 and 2. A P value <0.05 was considered to be significant.

RESULTS

12L/12D photoperiod. In two experimental series, the 12-h mean values of SBP, DBP, and SLA were identical both in light and dark phases. HR in the dark phase was slightly lower (change in HR = 17.7 ± 4.1 beats/min, P < 0.05) in series 2 than in series 1 (see Table 1). The 12-h mean values of SBP, DBP, HR, and SLA were significantly (P < 0.001) higher in dark phase than in light phase. Discontinuous measurement of these parameters from 12 to 21 wk of age showed that the diurnal and nocturnal 12-h mean values of SBP, DBP, HR, and SLA were stabilized only from 20 wk of age (data not shown). The rhythm analysis showed a dominant circadian rhythm with a 24-h period for SBP, DBP, HR, and SLA. Before photoperiod reduction, the circadian parameter analyses showed that the percent rhythm and amplitude of HR were higher than those of SBP, DBP, and SLA in both experimental series 1 and 2 (see Figs. 3 and 4). Interestingly, whether the acrophase of these parameters occurred in early morning hours, the acrophases of HR, SLA, and DBP occurred 1 h earlier than that of SBP in experimental series 1 as well as in series 2 (Fig 2).

Experimental series 1: photoperiod reduction (8L/16D) by advancing a 4-h dark phase. Similar to the 12L/12D photoperiod, a dominant circadian rhythm with a 24-h period was detected for SBP, DBP, HR, and SLA in 8L/16D. When the photoperiod was reduced by
advancing a 4-h dark phase, the acrophases of SBP, DBP, and HR were progressively advanced by 1 h 15 min, 41 min, and 42 min, respectively (Fig. 2). Advancement of the acrophase of SBP and DBP reached statistical significance 2 wk after the photoperiod reduction, whereas that of HR was obtained after 1 wk. These significant decreases were maintained until the sixth week of photoperiod reduction (see Fig. 2). The acrophase of SLA was not significantly modified after 6 wk of photoperiod reduction. Thus significant decreases in percent rhythm were found 1 wk after photoperiod reduction (Fig. 2). The decrease in the diurnal 12-h mean value of SBP was only observed after 3 wk in the 8L/16D photoperiod. The amplitudes of all parameters were also significantly decreased in 8L/16D photoperiod after 1 or 2 wk of photoperiod reduction (Fig. 4). On the other hand, since the first week of photoperiod reduction, the 12-h mean values of DBP, HR, and SLA were significantly diminished in light phase as well as in dark phase (Fig. 5). The decrease in the diurnal 12-h mean value of SBP was only observed after 3 wk in the 8L/16D photoperiod.

Experimental series 2: photoperiod reduction (8L/16D) by a 2-h dark phase advance and delay. When the photoperiod was reduced by advancing and delaying a 2-h dark phase, the acrophases were delayed from 1 h 34 min, 1 h 22 min, 1 h 13 min, and 1 h 25 min for SBP, DBP, HR, and SLA, respectively (Fig. 2). This delay of acrophase appeared at the first week of photoperiod reduction for SBP and HR, 2 wk after photoperiod reduction for DBP, and 6 wk after for SLA. The percent rhythms of HR and SLA were significantly decreased after the first week of photoperiod reduction, whereas the decreases in the percent rhythms of SBP and DBP were found after 5 wk in the 8L/16D photoperiod (see Fig. 3). Otherwise, photoperiod reduction induced a significant decrease in amplitudes of DBP, HR, and SLA, but did not alter that of SBP (Fig. 4). Finally, if the 12-h mean values of SBP and DBP were not modified by 8L/16D conditions, those of HR and SLA were predominantly decreased in dark phase (Fig. 6). These latter were more pronounced after 6 wk of photoperiod reduction.

Resynchronization of photoperiod to 12L/12D. In the group of rats maintained in 8L/16D by advancing a 4-h dark phase (experimental series 1), 1 wk after photoperiod resynchronization to 12L/12D, the acrophase of SBP tended to return to its basal value. However, this restoration was not completed even 3 wk after resynchronization (Fig. 2). The acrophases of DBP and HR were not significantly modified by the 12L/12D photoperiod. Although the percent rhythm of SBP can be restored to the initial level, those of SBP, DBP, and HR were not completely restored (Fig. 3). The amplitudes of SBP, DBP, and SLA returned to their basal levels when the photoperiod was resynchronized to 12L/12D. However, the amplitude of HR was not completely restored (Fig. 4). As shown in Fig. 5, after the first week of photoperiod resynchronization from 8L/16D to 12L/12D, the 12-h mean values of SBP and DBP, both in light and dark phases, returned to their basal values, and those of HR and SLA were not completely restored.

In the group of rats maintained on 8L/16D by advancing and delaying a 2-h dark phase (experimental series 2), the acrophases of SBP, DBP, HR, and SLA completely returned to their basal levels after the first week of photoperiod resynchronization to 12L/12D (Fig. 2). Similarly, the percent rhythms of HR and SLA were restored 1 wk after photoperiod resynchronization, whereas those of SBP and DBP were still not restored 3 wk after (Fig. 3). In addition, the amplitudes of SBP, HR, and SLA were restored by photoperiod resynchronization to 12L/12D, whereas that of DBP was not modified (Fig. 4). Finally, the 12-h mean value of HR in light phase and that of SLA in dark phase returned to their initial values during the first week of resynchronization, but the 12-h mean value of HR in dark phase was not completely restored in 12L/12D photoperiod (Fig. 6).

DISCUSSION

This is the first reported study of adaptation of circadian rhythms of BP, HR, and SLA to variations of an L/D cycle by extending a 4-h dark phase (8L/16D) in conscious and unrestrained Wistar rats. Our study shows that the adjustment of circadian rhythms of SBP, DBP, HR, and SLA to change from 12L/12D to 8L/16D photoperiod depends on the direction of the prolongation of the dark period. The entrainment of circadian rhythms differs markedly when photoperiod reduction is performed by advancing a 4-h dark phase versus when photoperiod reduction is obtained by symmetrically advancing and delaying a 2-h dark phase.
The circadian rhythm of cardiovascular parameters has been previously studied by a number of investigators in laboratory animals (17) as well as in humans (16, 29).

However, the BP monitoring methods used in the animal studies did not allow very long-lasting recording periods (up to 9 days). The radiotelemetry system used in the
present experiments allows performing continuous monitoring of BP, HR, and SLA in unrestrained and unstressed conscious animals over very long periods (3–6.5 mo) of time (1). Furthermore, the telemetry system seems to be the most accurate method for cardiovascular circadian rhythm measurement (35).

Fig. 3. Effects of photoperiod reduction from 12L/12D to 8L/16D by advancing a 4-h dark phase (experimental series 1, n = 7) or by advancing and delaying a 2-h dark phase (experimental series 2, n = 7) on percent rhythm of SBP, DBP, HR, and SLA of Wistar rats. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. 21 wk of age; †P < 0.05 and ††P < 0.01 for 28, 29, and 30 wk vs. 27 wk of age. One-way ANOVA with repeated measures followed by the method of contrast.
In the present study, the recordings were performed 2 wk after telemetry transmitter implantation to avoid the influence of anesthesia and surgical stress on cardiovascular and behavioral parameters. However, we found that in a 12L/12D photoperiod, the 12-h mean values of SBP, DBP, HR, and SLA decreased progress-
sively and tended to be stabilized only 10 wk after telemetry transmitter implantation. Therefore, in our study, the basal values obtained from the 21 wk of age in 12L/12D photoperiod were used as reference values for all parameters to assess the effects of the two different photoperiod reduction processes.

![Fig. 5. Evolution of the 12-h mean values of SBP, DBP, HR, and SLA in 7 Wistar rats exposed to an 8L/16D photoperiod by advancing a 4-h dark phase (experimental series 1). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. 21 wk of age; †P < 0.05, ††P < 0.01, and †††P < 0.001 for 28, 29, and 30 wk vs. 27 wk of age. One-way ANOVA with repeated measures followed by the method of contrast.](image-url)
As it has been previously reported in rats, BP, HR, and SLA showed dominant circadian patterns with elevated mean values during the activity period (22, 34, 35). The nocturnal behavior associated with the higher BP and HR levels seems to be due to physiological activity, such as eating and drinking, during which
sympathetic nervous system activity and plasma pressor hormones are higher (9, 21).

In this study, we have shown that a photoperiod reduction by advancing a 4-h dark phase (8L/16D) induced a 1-h advance of SBP, DBP, and HR acrophases, but it did not modify the acrophase of SLA. This dissociation in phase response to the photoperiod reduction suggests that the entrainment of cardiovascular circadian rhythms may be different from that of SLA. Similar dissociation has been previously reported by Koster-Van Hoffen et al. (19), who demonstrated a dissociation in response to an analog of melatonin (S20242) in the circadian rhythm of SLA and body temperature. This melatonin agonist exerted a beneficial influence on the amplitude and stability of body temperature but not on those of SLA in old and middle-aged Wistar rats. Although in our experimental conditions, the SLA acrophase was not modified by advancing a 4-h dark phase, the percent rhythm and amplitude of SLA were significantly decreased by photoperiod reduction as well as those of SBP, DBP, and HR. These results suggest that the photoperiod reduction by advancing a 4-h dark phase (8L/16D) may influence the central mechanism of circadian control of SLA and that the responsiveness to such a photoperiod reduction seems to be less sensitive for SLA than cardiovascular circadian system. In contrast, when the photoperiod reduction was performed by advancing and delaying a 2-h dark phase, the acrophases of all parameters were delayed from about 1 h 20 min, which was associated with pronounced decreases in circadian amplitude and percent rhythm. These findings show that the phase response to the photoperiod reduction by symmetrically advancing and delaying a 2-h dark phase differs from the photoperiod reduction by advancing a 4-h dark phase. For all parameters, the extension of darkness into evening produced an advanced acrophase, whereas symmetrical extension of darkness into morning and evening delayed their acrophase. These observations could be explained by the hypothetical existence of two circadian oscillators (the morning oscillator and the evening oscillator) that could respond in a different manner to a photoperiod reduction (8, 10, 31). In addition, we may infer that the pineal melatonin secretion rhythms are also differentially affected by the way in which photoperiodic signals are accomplished (11, 20). It has been shown that the physiological and behavioral changes induced by decreases in day length are mediated by increases in the duration of nocturnal secretion of melatonin (6), which is positively correlated with the length of daily dark phase (7, 13). Both the absolute duration of nightly melatonin secretion and the changes in the duration of nocturnal melatonin signal can influence photoperiodic responses (6, 18). Our observations agree with the previous findings in Wistar rats showing that the pineal melatonin production enzyme N-acetyltransferase rhythm is shifted toward the morning hours with symmetrical extension of the dark period into evening and morning (15). Otherwise, our findings of advancing cardiovascular circadian acrophases by a 4-h extension of the dark phase at the beginning of the darkness are also in agreement with the observations of Pitrosky et al. (30), who demonstrated that the melatonin secretion phase was advanced when extending the dark phase at the beginning of the dark period in Syrian hamsters. The fact that photoperiod reduction from 12L/12D to 8L/16D by advancing and delaying a 2-h dark phase resulted in a delayed circadian acrophase for all parameters, indicates that these parameters are predominantly entrained to a circadian rhythm by light on than by light off.

On the other hand, we have shown that the diurnal and nocturnal 12-h mean values of SBP, DBP, HR, and SLA were decreased when we extended the darkness by advancing a 4-h dark phase. By contrast, only a decrease in the nocturnal 12-h mean values of HR and SLA was found when the darkness was symmetrically extended by advancing and delaying a 2-h dark phase. These findings indicate that the entrainment of BP circadian rhythm is more efficient than that of HR and SLA when the photoperiod reduction was performed by advancing and delaying a 2-h dark phase. Similarly, during the resynchronization to the 12L/12D photoperiod, the adjustment of circadian parameters was more rapid and complete when the photoperiod reduction was done by symmetrically advancing and delaying a 2-h dark phase. All of these findings suggest that the adaptation to a photoperiod reduction is better when extending symmetrically the darkness into evening and morning. This is in agreement with the findings reported by Gorman et al. (8) in Siberian hamsters. They demonstrated that abrupt photoperiod reduction from 16L/SD to 8L/16D achieved by extending darkness into morning resulted in more rapid entrainment of locomotor activity than did extensions into the evening hours. However, the underlying circadian bases of differences in entrainment rates still remain to be elucidated.

In conclusion, the present study shows that the adjustment of the rat circadian rhythms of SBP, DBP, HR, and SLA to change from long to short photoperiod depends on the direction of the extension of the dark period. Our findings indicate that entrainment of circadian rhythms differs markedly when photoperiod reduction is performed by advancing a 4-h dark phase versus when photoperiod reduction is performed by advancing and delaying a 2-h dark phase.

**Perspectives**

Because the major finding of our study performed in chronically instrumented adult rats is that the adjustment of cardiovascular and locomotor activity circadian rhythms to two different changes of photoperiod from 12L/12D to 8L/16D depends on the direction of the extension of the dark period, we will attempt to investigate the underlying mechanism responsible for the dissociation of the circadian rhythms in the phase response to the photoperiod reduction. Particularly, as melatonin levels have been shown to be critically involved in circadian rhythm changes, we would like to simultaneously combine central (inha-
cardiovascular circadian rhythms and photoperiod

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