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Evidence for a Free-Running Pituitary-Adrenal Circadian Rhythm in Constant Light-Treated Adult Rats (39606)

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In the rat, the zenith of the plasma corticosterone circadian cycle can be shifted by altering lighting conditions (1, 2), suggesting that light-dark cycles entrain the circadian rhythm of the pituitary-adrenal system. The effect of constant light on this rhythm has not been established. Group data, based on samples from different rats at each time point, demonstrated loss of rhythmicity in rats in a constant light environment (2-4). However, group data cannot distinguish between loss of synchronization of rhythms and loss of rhythmicity. To make this distinction it is necessary to examine serial samples from individual rats. Using such samples, Krieger (4) found that 80-day-old rats reared in constant light from birth did not have a circadian rhythm in plasma corticosterone.

To determine whether constant light also abolishes the circadian rhythm in individual adult rats, we studied female rats placed in constant light as adults. These rats were selected from those in an experiment designed to determine the temporal sequence and interrelation of the disappearance of estrous cycles and AM-PM variations in plasma TSH and corticosterone in constant light (5). At the time of the present study, the animals were in a state of persistent vaginal estrus and had lost the normal nyctohemeral pattern of plasma TSH and corticosterone as defined by two sample collections per day.

Materials and methods. Adult female Sprague-Dawley rats (240-260 g, Simonson, received when approximately 11 weeks old) were maintained under conditions of controlled fluorescent lighting (lights on 0600-1800 hr) and temperature (24 ± 1°). Purina lab chow and water were available at all times. After 2 weeks of acclimation to our laboratory conditions, 19 of the 29 rats were moved to an adjacent room of similar size and shape in which the lights were constantly on. Light intensity was between 10 and 20 fc. Vaginal smears, taken 5 days per week, were used as an index of estrous cycles. As reported separately (5), AM and PM blood samples were collected at various times before and after the rats were placed in constant light to determine how quickly normal AM-PM variations in TSH and corticosterone were lost. After 15 weeks in constant light, nine rats which demonstrated persistent estrus were selected for serial blood sampling. Nine of the rats which had been maintained on the usual light-dark schedule and had normal estrous and TSH cycles were used as controls.

Blood samples were taken from a tail vein at 4-hr intervals over a 44-hr period. The validity of this technique for demonstrating normal variations in concentrations of plasma corticosterone has been demonstrated (4, 6) and the method used in this experiment has been described (6). In brief, 0.3 ml of blood was obtained from a 2- to 5-mm incision over a tail vein within 3 min after removing a rat from its cage. Rats were housed in individual cages 3 days prior to and during the experiments. The bleeding order was arranged to alternate between constant light (LL) and light dark (LD) maintained rats.

Plasma corticosterone was measured with a semiautomated microfluorescence technique (7). Statistical probabilities were determined by one-way or two-way analysis of variance for repeated measures (ANOVA) (8). Statistical evaluation of corticosterone patterns in individual rats was difficult because of the small number of data points. In addition, corticosterone concentrations varied with time in the form of sharp peaks rather than as a sine wave making sinusoidal regression analysis inappropriate. The following criteria were empirically derived and

used to determine whether an individual rat had a corticosterone (B) circadian rhythm: (i) The highest plasma B value on both days occurred at the same time of day plus or minus 4 hr. (ii) There was a positive correlation coefficient correlating plasma B values on both days with time. (iii) The correlation coefficient was within 2 SD of the mean of the control LD group. (iv) The ratio of the maximum to minimum plasma B was within 2 SD of the mean of the ratio of the control group on both days. Any rat which did not meet all four criteria was considered not to have a circadian rhythm. Application of these criteria gave results which agreed with subjective judgments based on inspection of graphed data. The one control rat which was arrhythmic was not used in calculating mean and SD for the control data.

Results. Five of nine LL rats and eight of nine LD rats had fluctuations in plasma corticosterone with a periodicity of approximately 24 hr. The eight rhythmic LD rats had high plasma corticosterone concentrations at approximately the period when the lights went off on both days. Steroid peaks in LL rats were not synchronized with time of day or with those of other rats in the group (one at 0600, one at 1000, two at 1800, one at 2200). Arrhythmic fluctuations were observed in four LL and one LD rat. Corticosterone patterns of representative rats are shown in Fig. 1.

Figure 2 illustrates that the pattern of the LD group obtained by this sampling technique was consistent with normal, synchronized 24-hr periodicity. Plasma corticosterone in the LD group showed variations with time ($P < 0.01$, one-way ANOVA) and the mean peak occurred at 1800 on both days. The LL group did not demonstrate a rhythmic pattern and fluctuations were not significant during the sampling period ($P > 0.05$, one-way ANOVA).

Discussion. The presence of an asynchronous 24-hr periodicity in plasma corticosterone concentration in five of the LL rats indicates that these rats had an unentrained, "free-running," circadian rhythm in pituitary-adrenal function. The other four LL rats had aperiodic corticosterone fluctuations suggesting loss of rhythmicity. This study illustrates the importance of obtaining serial samples from individual rats to distinguish between loss of rhythmicity and loss of synchronization. The arrhythmicity in four of nine LL rats suggests that this treatment has a disruptive effect on the corticosterone circadian rhythm. The rhythm may be grad-

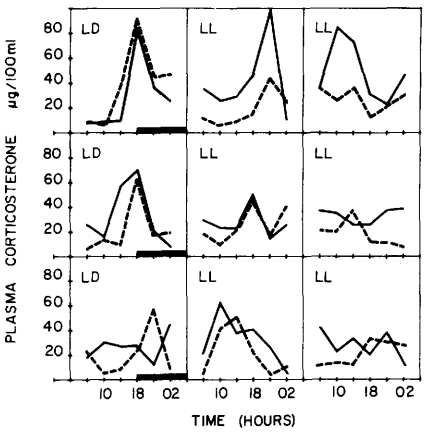

FIG. 1. Individual plasma corticosterone cycles of representative animals kept in a light-dark (LD) or constant-light (LL) environment for 15 weeks. The solid lines connect values on the first day; dashed lines connect values on the second day. The solid bars represent the dark periods. Patterns of three of the four arrhythmic LL rats are shown in the right panel, that of the arrhythmic LD rat is shown in the lower left section.

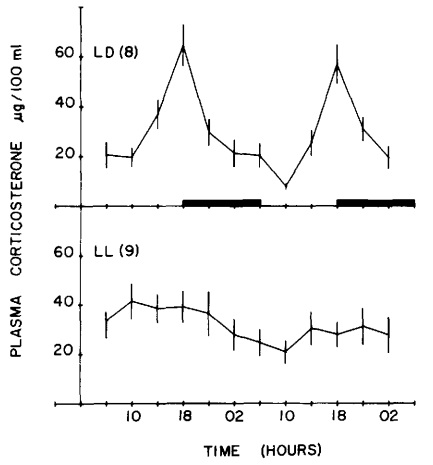

FIG. 2. Effect of constant light (LL) for 15 weeks on the group pattern of plasma corticosterone. Lines connect group means of the plasma corticosterone concentrations obtained from serial samples every 4 hr for 44 hr. Vertical lines indicate SE. The number of rats in each group is in parentheses. Dark periods for the light-dark group are indicated by the solid horizontal bars on the abscissa.

ually fading after 15 weeks without light–dark cues, or constant light may make the rhythm more vulnerable to the stressful effects of the bleeding procedure. Five of the nine LL rats demonstrated a circadian plasma B rhythm, indicating that constant light does not necessarily abolish this rhythm. All of the rats showed normal AM–PM differences in plasma B when they were returned to our standard light–dark environment (5). Similar results were obtained by Dunn *et al.* (9) in rats returned to a light–dark environment after 90 days of exposure to constant illumination.

The group data from LL rats in this study are similar to those obtained in other laboratories when different rats were sampled at each time point (2–4). There is one report using group data from constant light-treated female rats (1), indicating 24-hr corticosterone periodicity with a phase shift. The pattern in that study may have been obtained by chance or may have reflected synchronization by an environmental cue other than light.

The individual patterns of five of the LL rats in the present study differ from the arrhythmic fluctuations found by Krieger in 80-day-old rats reared from birth in constant light. The discrepancy between these findings suggests that the age at which a rat is placed in constant light is critical. A circadian periodicity in corticosterone does not appear until 20 to 30 days of age (4) and a retinohypothalamic tract appears at the same time as the onset of diurnal variations in corticosterone (10). Therefore, it is possible that light–dark cycles are necessary to initiate circadian rhythmicity in the brain–pituitary–adrenal system.

Our data indicate that light–dark cycles entrain the circadian rhythm of corticosterone but that they are not essential for rhythmicity in adult rats. This interpretation of the role of light–dark cycles in synchronizing pituitary–adrenal rhythmicity is consistent with previous data obtained from rats blinded as adults which showed a free-running corticosterone circadian rhythm 10 weeks after blinding (11).

Summary. To determine whether the circadian rhythm in pituitary–adrenal function persists in adult rats exposed to constant light, 24-hr patterns of plasma corticosterone concentrations were compared in individual constant light (LL) and light–dark (LD) (lights on 0600–18000 hr) maintained adult female rats. Nonstress plasma corticosterone concentrations were determined in serial blood samples obtained from a tail vein of each rat at 4-hr intervals for a 44-hr period 15 weeks after initiation of constant light treatment. Plasma corticosterone of eight of nine rats kept in LD had a 24-hr periodicity with peaks entrained to the onset of dark. Five of the nine LL rats demonstrated an unentrained circadian periodicity in plasma corticosterone. The other four LL rats had apparently arrhythmic fluctuations. These findings suggest that in adult rats the plasma corticosterone rhythm is not dependent on an LD environment but that constant light does have a disruptive effect on the rhythmicity of this hormone system.

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