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Effects of Corticotropin-Releasing Factor on Circadian Locomotor Rhythm in the Golden Hamster

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SEIFRITZ, E., H. KLEMFUSS, J. M. MONTES, K. T. BRITTON AND C. L. EHLERS. *Effects of corticotropin-releasing factor on circadian locomotor rhythm in the golden hamster.* PHARMACOL BIOCHEM BEHAV **60**(4) 855–862 1998.—Stress produces a reduction in the amplitude of some circadian rhythms. The neurochemical mechanisms underlying stress-induced changes in circadian rhythms are not known. To investigate a possible role of corticotropin-releasing factor (CRF) in this phenomenon, three related experiments were carried out: activity rhythms of male golden hamsters (10/14 hours light/dark entrained, lights on at 0800 h) were measured 1) following the intracerebroventricular administration of CRF (0.5, 1.0, 2.0, or 4.0 μg) at two different times of day, 2) following social stress (30-min resident–intruder confrontation), 3) and following the administration of the CRF-antagonist α-helical $CRF_{9–41}$ (2.0 μg) prior to a 15-min resident–intruder confrontation. CRF produced a significant, dose-related decrease in circadian rhythm amplitude following administration in the morning hours, but not in the afternoon. CRF also induced transient increases in activity post injection concomitant with an activation of the hypothalamic–pituitary–adrenocortical (HPA) system. Stress similarly reduced the amplitude of activity patterns and stimulated the HPA system. The stress-induced depression of circadian rhythm amplitude was significantly attenuated following α-helical $CRF_{9–41}$. These data suggest a role for CRF in the stress-related modulation of circadian locomotor rhythm amplitude. © 1998 Elsevier Science Inc.

Corticotropin-releasing factor (CRF) CRF-antagonist (α-helical $CRF_{9–41}$) Hypothalamic–pituitary–adrenocortical (HPA) system Social stress Circadian rhythm Amplitude Golden hamster Depression Aging

CIRCADIAN rhythm entrainment is a result of an interaction between the circadian pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN) and environmental synchronizers (zeitgebers). Under experimental conditions, environmental stressors can perturb circadian rhythms, primarily by reducing or "damping" amplitudes of locomotor, autonomic, and core body temperature rhythms (7,11,19,24-27,37,41). Reduced circadian rhythm amplitudes, which are also seen in aging and depression (6,45), can result in rhythms that are less stable overall (10,17, 36,45). The neurochemical mechanisms underlying stress-induced disruptions of the circadian system remain unclear.

The 41-amino-acid residue single-chain polypeptide corticotropin-releasing factor (CRF) (46) is a central coordinator and modulator of the endocrine, autonomic, and behavioral responses to stress (9,33). Acute stress, in addition to activating the hypothalamic–pituitary–adrenocortical (HPA) system, also produces increases in CRF in the limbic system, the locus coeruleus, and discrete hypothalamic nuclei (5). The intracerebroventricular (ICV) administration of CRF can produce a variety of behavioral effects that are similar to those associated with stress (2). However, the role of CRF in stress-induced modulations of circadian rhythms has been little explored.

The aim of the present study was to investigate the effects of CRF on circadian locomotor rhythms in hamsters. We addressed this question in three separate but related experiments and investigated circadian wheel running rhythms in

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male golden hamsters 1) following ICV injections of CRF, 2) following social stress elicited by a resident–intruder confrontation, and 3) following the combination of stress and the ICV injection of α-helical CRF_{9-41}, a competitive CRF antagonist (39). Because of its involvement in stress response, we hypothesized that CRF, similar to stress, reduces circadian wheel running amplitude and that anti-CRF inhibits the circadian effects of stress.

METHOD

Animals and Procedures

Adult male golden hamsters (Charles River; weighing 110–120 g) were used as experimental subjects. Six weeks prior to the experiments, under pentobarbital anesthesia (50 mg/kg IP), a 23-gauge stainless steel guide cannula was implanted stereotaxically approximately 1.0 mm above the lateral ventricle [AP: −1.0 mm, ML: ±2.0 mm (relative to bregma), DV: −1.5 mm (relative to skull); incisor bar: 2 mm below interaural line]. Prior to experiments, cannula placement was controlled in five animals to verify the stereotaxic coordinates utilized in both studies. Furthermore, at the end of Study II, Evans blue was injected through the ICV cannula of 20 animals to confirm that the injected drugs were actually reaching cerebrospinal fluid in the lateral ventricle. Two days after the surgical procedures, animals were housed individually in cages equipped with running wheels. Cages were located in cabinets (12 cages each) in a room with an ambient temperature of 21–23°C and a photoperiod of 10/14 hours light/dark (lights on: 0800 h, lights off: 1800 h; light intensity: 30 ± 5, and <0.1 lax, respectively). Food (Teklad 4% rodent diet) and water were provided ad lib. The animal holding room in which the behavioral measurements were made was separated from the room in which the experimental manipulations were carried out.

Study I

Thirty hamsters were entered into this study investigating the effects of different doses of CRF and social stress on locomotor activity. All animals were tested on five separate occasions (see below). For each experiment, animals were randomly assigned to treatment groups after stratification based on rhythm stability [which was estimated by the multiple regression coefficient *R* derived from the modified periodogram (20) over the 3 days preceding each experimental session]. Treatment groups were compared in a cross-sectional fashion.

ICV injections were carried out over a 60-s period using a 30-gauge infusion needle, which extended 1 mm beyond the tip of the guide cannula, and which was connected through plastic tubing to a 10 μl Hamilton syringe. CRF (Dr. Jean Rivier, Salk Institute, La Jolla, CA) was dissolved in 1 μl vehicle (VEH; 0.9% saline). Doses of CRF were 0.5, 1.0, 2.0, and 4.0 μg. Injections of CRF were made at two times of day, 0945–1045 h (morning injections), and 1345–1445 h (afternoon injections). These times were chosen to increase CRF when CRF activity is normally lowest (morning) and just prior to the nocturnal rise (afternoon). Due to limited sample size, not all doses of CRF were compared directly in the same experimental session. Direct comparisons included the 0.5 μg dose of CRF vs. the 1.0 μg dose vs. VEH, and the 2 μg dose of CRF vs. the 4 μg dose vs. VEH. Thus, each hamster received injections of VEH or one of two CRF doses (i.e., 0.5 or 1.0 μg, and 2.0 or 4.0 μg) in both morning and afternoon experimental sessions.

Social conflict stress was produced using a modified resident–intruder confrontation (30) carried out at 0945–1045 h. A hamster (termed intruder) was placed into the home cage of a conspecific (termed resident) for 30 min (wheel access blocked). Intruders and residents were selected randomly after stratification according to rhythm stability. Physical attacks occurred after an initial period of mutual threat behavior, including erect posture and aggressive vocalizations (34). Social defeat was defined by the occurrence of submissive or supine posture after an attack has taken place. Following defeat, however, threats, pursuits and fights continued until the end of exposure. When necessary, animals were separated to avoid physical harm.

Trunk blood for determination of HPA response [corticotropin (ACTH), cortisol, corticosterone] was collected between 0945–1045 h by rapid decapitation 30 min following the ICV administration of VEH or CRF 4.0 μg, or following a 30-min resident–intruder confrontation. The CRF dose for endocrine testing and comparison with social stress effects was chosen based on the results of the wheel running analysis.

Wheel-Running Rhythm Analysis

Wheel revolutions of each animal were recorded in 5-min epochs (bins). Data were submitted offline to a set of analyses using the Ratman software (21). All algorithms have been described and discussed in detail elsewhere (20) and are explained here only briefly. Amplitude of the circadian wheel-running rhythm was calculated using the "cosinor" method. This procedure fits a sine wave at a base frequency (here: 24 h were given by the photoperiod), and calculates the difference between mesor (mean activity) and acrophase of the fitted curve. Because the assumption of sinusoidality (of the cosinor method) reflects the real waveform of locomotor behavior in hamsters only in part, amplitude was also estimated using the "nocturnality" method (24). This alternative approach fits a square wave to the real data and calculates the difference between the mesor/min during the dark and the mesor/min during the light interval. Phase was estimated by the onset periodogram technique. Each 5-min epoch was classified as a hit if running activity exceeded the mesor. The percentage of hits during the preceding 6 h was then subtracted from the percentage of hits during the following hour. Difference scores below mesor were set to 0. The resulting data series were then subjected to the modified periodogram with a fixed period of 24 h. Acute effects on wheel-running activity were calculated by comparing the activity during 120 min before treatment vs. 120 min after treatment. Except for the onset periodogram, 5-min activity bins were transformed with natural logarithm prior to analyses.

Hormone Assay

Following anticoagulation of blood samples with 50 mg/ml EDTA and centrifugation for 10 min at 4000 × g, supernatant was decanted and stored frozen at −70°C until assay. Plasma concentrations of ACTH were measured with highly sensitive IRMA (Nichols Institute, San Juan Capistrano, CA), plasma concentrations of cortisol and corticosterone were measured with RIA (cortisol: Diagnostic Products Corporation, Los Angeles, CA; corticosterone: ICN Biochemicals, Costa Mesa, CA). Intra- and interassay coefficients of variation were <4% and <6% for cortisol and corticosterone, and <3% and <7% for ACTH.

Statistical Analyses

Repeated measures analysis of variance (ANOVA) using the multivariate approach with Wilks λ was applied to evaluate statistical differences. The between-subjects factor was "treatment" (control vs. active treatment), the within-subjects factor was "time" [baseline day prior to treatment (day −1), day of treatment (day 0), and the 2 days following treatment (day 1, day 2)]. To determine acute changes in amplitude and phase, orthonormalized within-subjects contrasts were calculated for planned comparisons of day −1 vs. day 0. In experiments with three different treatments in one experimental session, orthonormalized between-groups contrasts were calculated for the comparison of active vs. inactive treatment. Amplitude changes determined with the cosinor method were double checked using the nocturnality method. The results were compared using paired *t*-tests. This analysis was done to address the possibility that decreases in cosinor amplitude were due only to acute increases of postinjection wheel-running activity, rather than concomittant decreases in nocturnal activity. Endocrine data were analyzed using one-way ANOVA. In the case of a significant *F*-ratio, Duncan tests were used for posthoc analyses. The level of α was set at 0.05 (two tailed).

Study II

A second set of 30 male golden hamsters was chronically implanted with ICV cannulae and maintained as described in Study I. Of the 30 animals, those 20 animals with the strongest rhythm stability (greatest *R*) were stratified on circadian locomotor amplitude and then randomly assigned to two groups. These experimental groups were enrolled in a counterbalanced cross-over design comparing two treatments, i.e., VEH + stress vs. CRF antagonist + stress. On each occasion, animals were administered either 2 μg α-helical $CRF_{9–41}$ (courtesy Dr. J. Rivier) diluted in 1 μl VEH, or 1 μl VEH by slow ICV injection. The 2 μg dose of α-helical $CRF_{9–41}$ was chosen based on previous experience with this CRF antagonist on stress-induced behavior. A recent study found that pretreatment with α-helical $CRF_{9–41}$ was effective to antagonize behavioral stress effects at a dose of 1 μg, but not at a dose of 5 or 25 μg (13). Furthermore, higher doses of this CRF antagonist may have agonistic properties (28). Fifteen minutes after injection, animals were exposed to social stress by placing them into the home cage of a conspecific for 15 min. The duration of social stress was chosen to avoid possible floor effects of using a 30-min stress combined with an ICV injection. Thereafter, animals were put back into the home cages. Circadian amplitude was computed using the cosinor procedure as described above. Acute effects on wheel running were calculated comparing activity during 120 min before they were removed from wheel cages vs. 120 min after they were put back to the wheel cages. Inferential statistics were done with a repeated measures ANOVA with the within-subjects factors "time" (prior vs. after treatment) and "drug" (CRF antagonist vs. VEH).

RESULTS

Study I

Amplitude. CRF: under light-entrained conditions, undisturbed hamsters maintain a stable rhythm of consolidated locomotor activity in the dark, and of relative inactivity in the light interval. For illustration purposes, activity rhythms of one representative animal are displayed in Fig. 1. The first few days of data show typical running activity in undisturbed animals (this figure will be described in more detail below). Injection of CRF in the morning hours produced a depression of locomotor rhythm amplitude, as determined by the cosinor method (Fig. 2). This was substantiated by interactions at both dose levels of CRF, which approached statistical significance at the lower doses and met statistical significance at the higher doses, i.e., a) group contrast [VEH vs. (CRF 0.5 and 1.0 μg)] × time, $F(3, 23) = 2.4, \lambda = 0.76, p = 0.09$ (Fig. 2A); b) group contrast [VEH vs. (CRF 2.0 and 4.0 μg)] × time, $F(3, 21) = 7.3, \lambda = 0.49, p < 0.01$ (Fig. 2B). The interactions group × time contrasts (day −1 vs. day 0) were significant for both dose levels [VEH vs. (CRF 0.5 and 1.0 μg): $F(1, 25) = 6.2, p < 0.05$; VEH vs. (CRF 2.0 and 4.0 μg): $F(1, 23) = 15.5, p < 0.001$)]. The effects of CRF were transient and returned to normal the first day following injection. On a descriptive level, it appeared that the effects of CRF were linearly related to dose. In contrast to the above effects produced by the injections in the morning hours, no changes in circadian amplitude were found following injections of any dose (0.5 through 4.0 μg) of CRF administered in the afternoon hours (all *F*-values for group × time contrasts were <1).

Stress: the resident–intruder interaction-induced threat behavior and active fighting rapidly following exposure in the same cage with a conspecific. Serious physical harm was prevented by the experimenters. As an example, the effects of social stress on activity rhythms in one representative animal are shown in Fig. 1. Following exposure to stress, nocturnal wheel running activity is strongly decreased and recovers throughout the following few days. The quantitation by cosinor analysis is shown in Fig. 2C. Depression of amplitude was statistically significant, as reflected by an interaction group contrast [undisturbed control vs. (resident and intruder)] × time (day −1 through day 2), $F(3, 19) = 5.5, \lambda = -0.53, p < 0.01$), as well as by a group × time contrast interaction (day −1 vs. day 0), $F(1, 21) = 15.7, p < 0.001$. Amplitude returned to normal the first or second day after treatment. No statistically significant

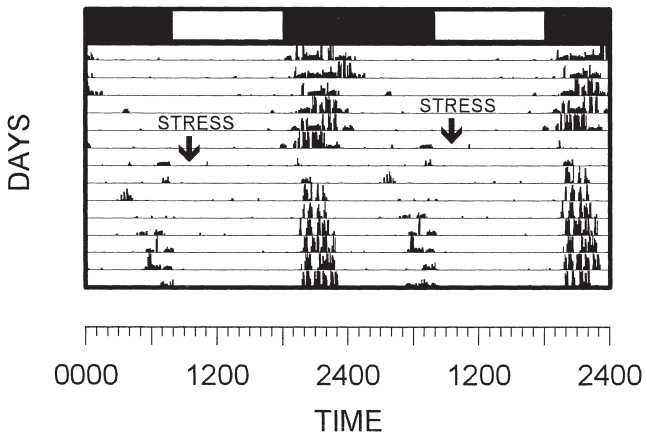

FIG. 1. Regular circadian wheel running activity of a representative hamster during 5 days. Thereafter, disturbed rhythms after a single 30-min resident–intruder confrontation stress (arrows) are shown. White and black bars on top of figure indicate entrainment to 10/14 hours light/dark cycle. Locomotor activity (wheel revolutions) is double plotted for two consecutive 24-h cycles on each line of the actigram, i.e., the data of the second day are replotted on the line below the data of the first day. Each bin represents the locomotor activity cumulated during 5-min epochs.

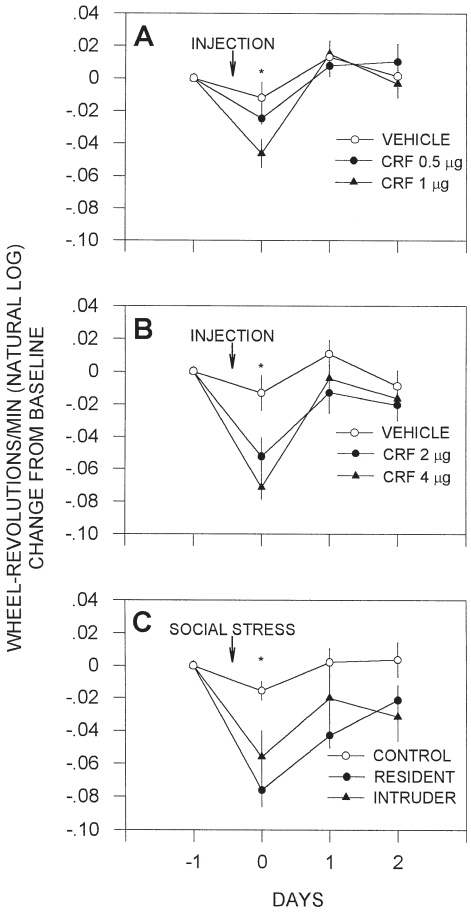

FIG. 2. The amplitude of the circadian wheel-running rhythm (as calculated by the cosinor method) decreased in animals injected ICV with CRF 0.5 and 1.0 μg (A), with CRF 2.0 and 4.0 μg (B), and following 30-min exposure to a resident–intruder confrontation (C). Statistically, this was substantiated by a significant group × time interaction (*) between day −1 and day 0 in vehicle-treated vs. CRF-treated animals, and in control vs. fighter animals. All treatments were administered at ∼1000 h. Data are mean ± SEM, N = 8–10/group.

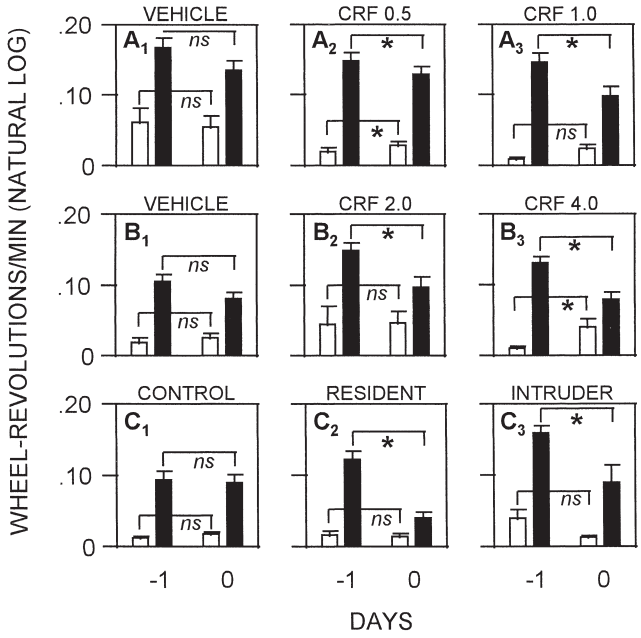

FIG. 3. To exclude that cosinor amplitude changes were due only to increases in acute poststimulation locomotor activity and not to decreases in activity during the dark period, the mesors of day- and nighttime activities on day −1 vs. day 0 were compared by paired t-tests (* denotes $p < 0.05$; ns denotes not significant). White bars indicate activity during lights-on periods, black bars indicate activity during lights-off periods. Data are mean ± SEM, n = 8–10/group.

group × time contrast interactions were found for the comparison of residents vs. intruders, nor of defeated vs. undefeated animals ($F < 1$).

To exclude systematic artifacts of amplitude measurements due to the CRF- or stress-induced acute wheel running during the day, amplitude was recalculated using a time window that excluded the daytime activity before and after injections, but no divergent results were obtained. Furthermore, analysis of the nocturnality data (i.e., change of day vs. night mesor difference between day −1 and day 0) supported the results of the cosinor analysis and yielded following results (illustrated in Fig. 3: A1 (VEH): $t(8) = 0.65$, $p = 0.54$; A2 (CRF 0.5 μg): $t(8) = 6.6$, $p < .001$; A3 (CRF 1.0 μg): $t(9) = 5.3$, $p < 0.001$; B1 (VEH): $t(8) = 0.31$, $p = 0.77$; B2 (CRF 2.0 μg): $t(9) = 1.5$, $p = 0.18$ [note: the comparison of activity during the dark period yielded a significant decrease after the injection of CRF 2.0 μg: $t(9) = 3.7$, $p < 0.05$]; B3 (CRF 4.0 μg): $t(7) = 4.2$, $p < 0.01$; C1 (controls): $t(9) = 1.3$, $p = 2.2$; C2 (residents): $t(9) = 4.9$, $p < 0.001$; C3 (intruders): $t(9) = 2.6$, $p < 0.05$. A repeated measures ANOVA did not yield differential effects in residents vs. intruders: while there was a significant effect for time, [$F(1, 18) = 28.3$, $p < 0.001$], there was neither an effect for group, [$F(1, 18) = 2.3$, $p = 1.5$] nor an interaction group × time, [$F(1, 18) = 2.7$, $p = 0.12$]. The results of separate pre vs. poststimulation comparisons of activity during the light and the dark periods are given in Fig. 3.

Phase

Compared to control animals, activity onset time was not significantly affected by CRF injections. All group × time contrast interactions, as well as group contrast × time interactions, were not statistically significant ($F < 1$). Stress also did not produce a statistically significant effect on phase (data not shown).

Acute Locomotor Effects

Compared to VEH, CRF injections administered at ∼1000 h produced an acute increase in wheel running during the 2 h postinjection. Repeated measures ANOVA with the 2 h preinjection vs. the 2 h postinjection yielded significant group contrast × time interactions for both comparisons involving CRF [i.e., VEH vs. CRF 0.5 μg and 1.0 μg, $F(1, 25) = 5.6$, $p < 0.05$; VEH vs. CRF 2 μg and 4 μg, $F(1, 23) = 6.5$, $p < 0.05$]. In the afternoon injections, no group × time interactions were found. However, there was a significant main effect for time. The resident–intruder confrontation also resulted in transient but significant wheel running during the 2 h post-exposure, [$F(1, 22) = 31.7$, $p < 0.0001$]. The second orthogonal contrast yielded a significantly higher activation of resident than intruder animals, [$F(1, 22) = 5.3$, $p < 0.05$]. It is noteworthy that wheel-running activation following the social interaction was approximately half as great as the activation following CRF.

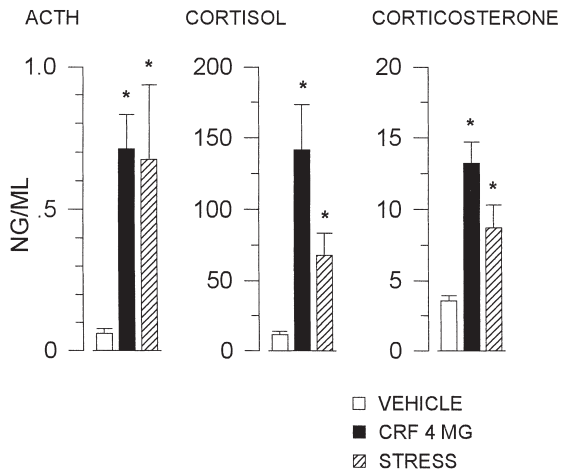

FIG. 4. Activation of the hypothalamic–pituitary–adrenocortical system by ICV CRF (4.0 μg) and social stress (30-min resident–intruder confrontation). Experiment was carried out at ~1000 h. As determined by one-way ANOVA and post hoc Duncan test, both corticotropin (ACTH) and corticosteroid concentrations significantly (*) increased following CRF or social stress, compared to vehicle. Because there were no differences between residents and intruders, data were pooled. The cortisol concentrations following CRF were also significantly higher than those following stress. Note that cortisol levels were 10 times higher than corticosterone levels. Data are mean ± SEM, n = 7–9/group.

Endocrine Effects of CRF and Social Stress

Plasma hormone concentrations are shown in Fig. 4. Data of residents (n = 4) and intruders (n = 4) were pooled to increase statistical power and are used to determine stress effects. Nine animals received VEH, and nine received CRF 4 μg. One-way ANOVA yielded significant effects for ACTH, [$F(2, 20) = 6.3$, $p < 0.01$], cortisol, [$F(2, 23) = 9.8$, $p < 0.001$], and corticosterone, [$F(2, 23) = 14.0$, $p < 0.0001$]. Duncan tests indicated that all CRF- and stress-stimulated hormone levels were larger than those following VEH injections. The CRF-stimulated cortisol levels were also significantly larger than those following stress. ACTH and corticosterone levels, however, did not statistically differ between CRF- and stress-induced stimulation. Due to technical problems, hormone levels could not be determined in all animals. Possibly related to the sample size (type II error), but consistent with the circadian wheel running behavior, we were unable to discern differences in hormone response between residents and intruders, or between dominant and defeated animals, as suggested previously (15).

Study II

Reduction of amplitude in response to social stress was replicated in this second study [main effect for time: $F(1, 19) = 207.4$, $p < 0.001$]. As shown in Fig. 5, the comparison between the effect of pretreatment with VEH vs. α-helical $CRF_{9–41}$ showed that the amplitude depression elicited by stress was significantly attenuated following the CRF antagonist. This was substantiated by an interaction between "time" × "drug," [$F(1, 19) = 4.6$, $p < 0.05$]. It is noteworthy that the stress effects were only reduced by anti-CRF, but not completely blocked. Both treatments also induced a transient increase in wheel running, peaking at about 60 min after the animals were put back to the wheel cages [significant main effect for time (pre- vs. posttreatment): $F(1, 19) = 9.2$, $p < 0.01$]. This acute effect, however, was not inhibited by α-helical $CRF_{9–41}$ [interaction time × drug not significant, $F(1, 19) = 1.3$].

DISCUSSION

Consistent with reports on other species, social stress, as evoked by a modified resident–intruder paradigm, was found to reduce the amplitude of circadian wheel-running rhythm in

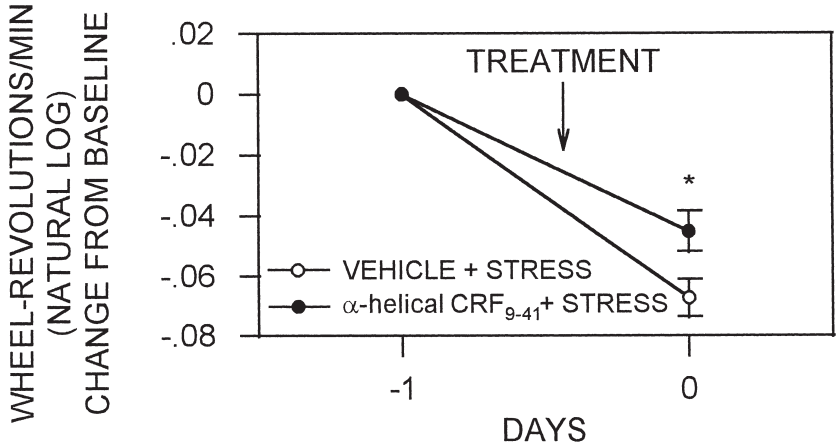

FIG. 5. In a crossover design, the effects of pretreatment with vehicle vs. 2 μg α-helical $CRF_{9–41}$ on stress-induced decrease in circadian amplitude were compared. Social stress was elicited by a modified 15-min resident–intruder interaction. Vehicle or CRF antagonist were administred ICV 15 min prior to stress. The amplitude of the circadian wheel-running rhythm was significantly decreased following stress [main effect for "time" (day −1 vs. day 0); $p < 0.001$]), but this decrease was significantly inhibited following pretreatment with CRF antagonist [interaction "time" (day −1 vs. day 0) × "drug" (vehicle vs. α-helical $CRF_{9–41}$); *$p < 0.05$]. Experiments were carried out at ~1000 h. Data are mean ± SEM, n = 20.

hamsters. Similarly, the ICV injection of CRF was also found to produce a significant reduction in circadian locomotor rhythm amplitude, a finding not previously described. The alteration in circadian amplitude following these treatments was accompanied by a stimulation of the HPA system, but not by changes in circadian phase. The amplitude-depressing effect of social stress was also found to be attenuated by the pretreatment with α-helical $CRF_{9–41}$. Taken together, these data might suggest that CRF plays a role in stress-induced circadian rhythm changes. However, because there is no direct evidence for an involvement of CRF in stress in hamsters on one hand, and because stress involves also pathways different from the HPA system, this conclusion needs to be evaluated in further studies.

The ability of CRF to reduce circadian amplitude was dose related, and was restricted to injections carried out during the morning hours. Rhythm amplitude was found to return to normal the second day following injection. Consistent with behavioral activation observed in previous studies in rats (43), CRF also produced an acute increase in wheel running in hamsters. It is possible that the acute increase in CRF-induced wheel running, rather than a direct neurotropic action of CRF on this behavior, may have produced a feedback effect on circadian rhythms. In fact, there are data suggesting that stimulated locomotion in rodents may induce phase shifts (47,49). Although we did not observe phase shifts following CRF, this possibility cannot be excluded in the present study because the fixed light–dark cycle has powerful resetting effects on phase. The phase–response curve of locomotor-induced phase shifts would suggest a maximal effect approximately 3 h, rather than 10 h, prior to circadian activity onset. Interestingly, the acute effects of CRF on locomotion, like the effects on amplitude, were present only following the injections carried out in the morning, but not in the afternoon. The concentrations of central CRF in rats (22,32) and of plasma corticosteroids in hamsters (1) are lowest during the light and greatest during the dark periods. The apparent circadian difference in responsivity to CRF is consistent with the previously reported reduction in magnitude of autonomic and behavioral effects of CRF in the afternoon (8), suggesting altered feedback mechanisms.

As expected, CRF produced an acute activation of the HPA system. This has been established in various other species (38), but has not yet been demonstrated in hamsters. In contrast to rats, hamster corticosteroids include both cortisol and corticosterone (31). It is noteworthy that in our study cortisol levels were approximately ten times higher than corticosterone levels. This is in contrast to previous reports in hamsters in which these corticosteroids were found to be in the same order of magnitude (1,15,31), but in agreement with others (42).

The threat behavior and active fighting that occurred rapidly after the confrontation between a resident and an intruder supports the observation that the natural aggressiveness of individually housed hamsters may make them especially suited for studies of social stress (34,35). As anticipated, based on previous investigations using repeated (11,12,19,44), or single stress (i.e., 60 min resident–intruder interaction) (24–27) in rats, short-term (15 or 30 min) social stress elicited by agonistic interaction in our experiments produced acute decreases in amplitude of the circadian rhythm. Also consistent with earlier studies in hamsters exposed to environmental stress (15), and in rats exposed to a resident–intruder stress (14,29,40), we found elevated plasma levels of ACTH, cortisol, and corticosterone following this manipulation. It would be interesting to evaluate whether time of day may modify the effect of stress on locomotor activity. Unfortunately, we were only able to examine the effects of stress applied during the morning hours.

The findings that depression of rhythm amplitude following social stress was mimicked by CRF injections, and that this stress effect was attenuated by pretreatment with a CRF antagonist, are consistent with the large body of evidence showing that CRF has an influence on virtually all (patho)-physiological responses to stress, including behavioral, endocrine, and autonomic events (9,33). Furthermore, these findings are in line with previous data showing that the anxiogenic effects of social stress can be prevented by the central administration of α-helical $CRF_{9–41}$ (13,14) or by pretreatment with antisense oligodeoxynucleotide to CRF receptor mRNA (23,40). Peripheral immunoneutralization of CRF (29) or the systemic administration of dexamethasone (2), however, do not have these properties. On a molecular level, ICV administration of CRF and exposure to acute social or physical stress exert an almost identical pattern of c-*fos* mRNA expression in the rat brain (16), including activation in the limbic system, hypothalamic nuclei, and the locus coeruleus (4,16).

Although there is no direct evidence, it can be assumed based on data from other species that CRF is involved in social stress effects also in hamsters. The mechanisms by which CRF affects circadian rhythms remain to be elucidated. It cannot be discerned, at this point, whether the observed changes following CRF and stress are a result of direct or indirect modifications of the circadian oscillator in the SCN, or whether they are a result of non-SCN mechanisms. For instance, recent evidence suggests that the effects of social stress on rhythms is possibly not mediated through a direct interaction with the SCN (27). It is unlikely, however, that the observed effects were mediated at the adrenocortical level, because peripheral administration of cortisol and corticosterone in adrenalectomized hamsters does not have significant effects on circadian rhythms (1). Although there are neuronal projections from the SCN to CRF-producing neurons in the paraventricular nucleus (3,48), no CRF-ergic neuronal inputs to the SCN have been described. Recent data suggest that the SCN drives the circadian rhythm of corticosterone, at least in part, via inhibitory vasopressinergic hypothalamic projections (18). It has also recently been reported that social stress stimulates vasopressin release within the hypothalamic paraventricular nucleus (50). These interactions might tentatively suggest the possibility that vasopressin participates in circadian amplitude changes elicited by CRF or stress. Furthermore, the finding that anti-CRF only attenuated, but not prevented, stress effects on circadian amplitude may point to the involvement of systems apart from CRF. In any event, the present data suggest that decreases in circadian rhythm amplitudes following stress are mediated, at least in part, by CRF.

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