BEHAVIORAL AND PHYSIOLOGICAL CONSEQUENCES OF REPEATED DAILY INTRACEREBROVENTRICULAR INJECTION OF CORTICOTROPIN-RELEASING FACTOR IN THE RAT

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SUMMARY

The present study was conducted to investigate the long-term consequences of repeated daily bolus injections of corticotropin-releasing factor (CRF) intracerebroventricularly (ICV) on ongoing locomotor activity and physiology in the home cage of individually housed rats. For this purpose ovine CRF (1 μg/3 μl) was injected once daily during the early resting phase into the lateral ventricle for a period of 10 days. Changes in daily rhythms in heart rate, body temperature and motor activity were recorded telemetrically before and during the treatment period. Daily central CRF injection delayed the body weight gain, increased adrenal weight, and decreased the weight of the thymus at the end of the experiment. The acute behavioral and physiological responses to CRF did not habituate with repetition of treatment. CRF treatment also failed to affect the long-term regulation of baseline heart rate, body temperature and motor activity during the light phase, as measured during the hour preceding the daily CRF injection. Mean heart rate during the dark phase was, however, significantly decreased in CRF-treated rats during the whole experimental 10-day period, without any sign of habituation. The failure of episodic CRF to affect long-term regulation of baseline body temperature during the light as well as the dark phase was noteworthy because an increased daytime body temperature lasting for several days is a characteristic marker of various behavioral stressors. Since a previous study showed that the temperature response during chronic CRF infusion was similar to the long-term effects of behavioral stress it is hypothesized that chronic but not episodic increases in central CRF levels are related to the induction and persistence of part of the stress-related behavioral and physiological disorders. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

The majority of studies describing behavioral and physiological responses to various stressors focus on acute stress responses that are short-lasting and return to baseline values within minutes or hours. An increasing number of reports indicate that, depending on the nature of the stressor and the parameter that is analyzed, behavioral changes and
adaptations in autonomic nervous, neuroendocrine and neurochemical functioning can persist for days or even weeks. These long-term changes have been described in animals exposed to chronic stress situations (Fuchs and Flügge, 1995; Kant et al., 1991), and repeated stressors (Meerlo et al., 1996; Tornatzky and Miczek, 1993). Even a single stressor (Koolhaas et al., 1990, 1997; Meerlo et al., 1996; Van Dijken et al., 1993) was shown to induce persistent alterations in the physiological and behavioral state of the animal.

One of the neuropeptides that appears to be crucially involved in the regulation and coordination of the stress response is corticotropin-releasing factor (CRF) (Dunn and Berridge, 1990). This neuropeptide is intimately involved in the expression of autonomic, endocrine and behavioral responses to stress. CRF functions both as a neurohormone in the hypothalamic–pituitary–adrenal axis to elicit ACTH secretion (Vale et al., 1981) and as a neurotransmitter in various extrahypothalamic regions to initiate physiological and behavioral components of the stress response (Sawchenko et al., 1993). In several areas of the brain CRF acts as a neurotransmitter enhancing sympathetic nervous and adrenomedullary activity (Fisher, 1989). When injected centrally into the cerebrospinal fluid of experimental animals, CRF activates brain regions related to stress responses (Imaki et al., 1993), produces stress-like activation of brain catecholaminergic systems (Dunn and Berridge, 1987) and stress-related behavioral changes which depend on the state of arousal of the animal (Koob et al., 1993; Korte et al., 1992; Takahashi et al., 1989). Conversely, it has been shown that acute and chronic behavioral stressors increase the biosynthesis and release of CRF in various brain regions (Chappell et al., 1986; Imaki et al., 1991) and affect the number and sensitivity (Fuchs and Flügge, 1995; Sapolsky, 1989) of CRF binding sites in the brain and pituitary. The hypothesis that the central nervous CRF system is basically involved in the adaptation of the organism’s behavioral, autonomic and immune responses to stress is especially strengthened by the blockade of behavioral and physiological stress responses by central application of CRF antagonists (Heinrichs et al., 1992; Menzaghi et al., 1994; Swerdlow et al., 1989).

Based on the similarities between many of the short-lasting effects of centrally administered CRF and the behavioral signs and physiological symptoms that occur in human affective disorders, an important role for CRF in the pathophysiology of affective disorders was suggested (Nemeroff et al., 1984). Clinical studies have found direct and indirect evidence supporting the hypothesis that CRF is hypersecreted from one or more populations of neurons in the brain of patients diagnosed for major depressive disorder, as indicated by the increased concentrations of CRF in the cerebrospinal fluid of these patients (Nemeroff et al., 1984).

Several animal studies focused on the behavioral, physiological and anatomical consequences of chronic elevation of centrally circulating CRF by infusing CRF into the brain in order to mimic the hypothesized CRF hypersecretion in depressed patients. Hauger et al. (1993) showed that chronic intracerebroventricular (ICV) CRF infusion induced a downregulation of CRF receptors in the amygdala but not in the anterior pituitary. Body weight and food intake were clearly reduced (Arase et al., 1988); thermogenesis in brown adipose tissue was increased (Lefèuvre et al., 1987) as was hypothalamic-pituitary-adrenocortical axis activity (Cunningham et al., 1988; Labeur et al., 1995; Miyanaga et al. 1990). Behavior in a variety of tests was affected (Song et al., 1995) and some immune responses were suppressed (Hauger et al., 1993; Labeur et al., 1995). Many of these changes induced by chronic increases of intracerebral CRF concentration are similar to features of major depressive disorder (DSM-III-R, 1987).
In view of the crucial role of CRF in the stress response and the reported alterations in central CRF systems in depression it is important to realize that stressful life events are considered to play an important role in the etiology of human depressive disorders (Anisman and Zacharko, 1982). Therefore, we recently studied to what extent the long-term consequences of a serious behavioral stressor could be mimicked by the central application of CRF (Buwalda et al., 1997). For this purpose, we compared continuous long-term recordings of behavior and physiology during chronic CRF administration in the brain with the response to social defeat, which is regarded as an animal model for the study of mechanisms involved in the pathogenesis of affective disorders (Kant et al., 1991; Koolhaas et al., 1990; Meerlo et al., 1996; Tornatzky and Miczek, 1993). The effects of chronic ICV CRF administration on daily rhythms of body temperature and motor activity in the home cage of individually-housed rats were assessed with the aid of biotelemetry. Baseline body temperature was increased for several days, in particular during the resting phase. The effects on temperature during the night was less prominent. Motor activity in the home cage was initially slightly increased during the resting phase. The CRF treatment furthermore increased adrenal weights after 10 days and elicited an increased anxiety on an elevated plus-maze 1 week after start of CRF infusion (Buwalda et al., 1997). These effects of a chronic CRF treatment showed a number of striking similarities with the long-term consequences of stress of social defeat.

Many chronic stress paradigms consist of single or repeated exposure to various stressors eliciting acute episodic—and not necessary chronic—release of central CRF. Therefore, the present study was conducted to investigate whether repeated daily bolus injections with a relatively high dose of CRF into the lateral ventricle of the rat brain can evoke similar long-term changes in daily rhythms of body temperature, heart rate and motor activity as observed following stress of social defeat and during chronic CRF administration.

**METHODS**

**Animals**

Male Wistar rats ($n = 11$; six vehicle and five CRF treated), weighing $394 \pm 5$ g at the start of the experiments were housed individually in clear Plexiglas cages (25 $\times$ 25 $\times$ 30 cm) on a layer of wood shavings in a room with constant temperature (21 $\pm$ 2°C) and fixed, 12 h light–dark regime (light on at 0800h). The animals were daily weighed at 0900h and had free access to standard rat chow and tap water.

**Biotelemetric surgery**

Heart rate, body temperature and gross locomotor activity were recorded prior to and throughout the entire 10 day treatment period by means of radiotelemetry. For this purpose a telemetry transmitter (model CTA10TA-F40, Data Sciences, St. Paul, MN) was implanted intraperitoneally under halothane anesthesia. To obtain optimal electrocardiographic (ECG) recordings the wire electrodes were placed as previously described by Sgoifo et al. (1996) avoiding loss of signal during increased physical activity.
Biotelemetric data acquisition

The transmitters produced temperature—and heart rate dependent frequency—modulated signals, which were received with an antenna board (model RA1010, Data Sciences) underneath the cage. Locomotor activity was obtained by monitoring changes in the received signal strength that resulted from movement of the animal. Changes in signal strength beyond a predetermined threshold generated a pulse that was counted by the acquisition system. For detection of movement the transmitter had to move. Therefore, with the transmitter implanted in the peritoneal cavity, movements of the upper part of the body during grooming or eating were not registered as activity. Data were collected and processed by a computer with a specialized recording and analysis system (Dataquest IV, Data Sciences). Heart rate and body temperature were sampled for 10 s every 5 min. Locomotor activity was recorded continuously and stored at 10-min intervals. Averages (1 h) were calculated and presented in this paper.

ICV cannulation and peptide injections

Together with the implantation of the transmitter a 22G stainless steel guide cannula (C313; Plastics one; Roanoke, VA) was stereotaxically placed 1 mm above the right lateral ventricle with the toothbar at –3.3 mm according to the Paxinos brain atlas (AP –1.0 mm and L 1.6 mm from bregma and DV 3.6 mm). The insertion cannula protruded 1 mm below the tip of the guide cannula. After 3 weeks of recovery the rats were injected by gravity once daily between 1000 and 1200 h with either 3 μl of the vehicle solution (saline with 0.1% bovine serum albumin (BSA) and 0.01% ascorbic acid) or with ovine CRF (American Peptide, Sunnyvale, CA.; 1 μg/3 μl vehicle solution).

Ten days after the first injection the rats were sacrificed between 1000 and 1200 h. On this day adrenals and thymus were removed and weighed. Location of the cannula was checked and only animals with correctly located cannula’s were included in the study.

Statistical analysis

To assess the overall acute and long-term effects of vehicle and CRF injections on heart rate, body temperature and activity, mean group values were compared using analysis of variance (ANOVA) repeated measures over the 10-day period following start of CRF administration. Mean values are presented with SEM. Telemetric sampling and subsequent analysis of one vehicle treated rat was problematic and therefore the continuous recordings of this animal were discarded. For post hoc analysis the Dunnett’s test was used to determine significance between pairs of means. Within-group changes were analyzed with a paired t-test. Effects of CRF administration on body and organ weight and on behavior in the elevated plus-maze were analyzed with a one-way ANOVA.

RESULTS

Body weight

On the first day of treatment the mean body weight of the group of animals receiving vehicle solution was 402 ± 6 g which was similar to that of CRF treated animals 412 ± 10 g. The vehicle administered controls gradually gained weight whereas the growth of CRF treated rats lagged significantly behind that of controls resulting in a treatment effect
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*(F(1,9) = 10.89; p < 0.01)* which was based on lower body weight gain in CRF treated rats on day 2, 3, 4, 7, 8, 9 and 10 (*p* < 0.05). (Fig. 1)

Heart rate, body temperature and activity in the home cage

The hourly recordings of heart rate, temperature and activity (Fig. 2) show that daily injection of ICV CRF elicited acute and rapid increases in all three parameters. In vehicle administered rats these activity and physiological responses also occurred to the injection procedure, however, the effects were only minor compared with the CRF induced rises. The sharp increase in motor activity following CRF injection can even be regarded as a demarcation of the daily injections. The acute response to the first injection is shown in more detail in Fig. 3. Since the detailed figures of the next nine daily injections were not essentially different from the first (Fig. 4) they are not individually presented. Pre-injection baseline resting levels of heart rate, temperature and activity were similar in both groups on this first treatment day. Also the immediate increase in the three parameters to handling, insertion of the internal cannula into the guide cannula and fluid injection was similar in vehicle and CRF-treated rats. Following vehicle injection the heart rate returned within 10 min to baseline levels, whereas it remained elevated in CRF-treated rats for nearly 4 h resulting in a significant treatment effect *(F(1,8) = 22.5; p < 0.001)* and an interaction between treatment and time *(F(54,432) = 2.22; p < 0.001)*. The effects of the injection procedure on body temperature in vehicle-treated controls was more prolonged than the increase in heart rate. Baseline values were regained not before 100 min after injection. This prolonged vehicle effect is probably responsible for the lack of a significant treatment effect during the 4 h post-injection period. There was, however, a highly significant interaction between treatment and time *(F(54, 432) = 4.42; p < 0.001)*. This was caused by the biphasic increase in body temperature that was seen following ICV CRF.

![Fig. 1. Changes in mean body weight (± SEM) following ICV infusion of CRF or vehicle. * p < 0.05; ** p < 0.01 Significant difference between CRF and vehicle treated groups, one-way ANOVA.](image)
Fig. 2. The effect of ten daily ICV injections of 1 μg CRF on daily rhythms of heart rate, body temperature and home cage activity compared with vehicle administration. The data represent hourly averaged values. The first injection is indicated by the arrow at the x-axis.

Injection while vehicle administration produced a monophasic increase. The first rise in body temperature of vehicle- and CRF-treated rats was similar. Compared with vehicle-treated animals, CRF injected rats more rapidly regained baseline temperatures. CRF induced a second rise in body temperature starting 80 min after the start of injection. The increase in motor activity following vehicle injection rapidly returned to baseline similar to the heart rate response. After CRF administration the animals stayed hyperactive for more than 3 h (treatment and treatment interaction with time: $F(1,8) = 13.74; p < 0.01$ and $F(54,432) = 1.55; p < 0.01$, respectively).

From Fig. 2 it already becomes apparent that there was little habituation of the acute heart rate, body temperature and activity responses to CRF throughout the treatment period. The lack of habituation can clearly be seen from the presentation of the daily acute responses in Fig. 4 where the average response was calculated over the first 2 h (reflecting the first temperature rise) and 2–4 h (second temperature rise) following ICV injection. Except for the body temperature response immediately (0–2 h) following vehicle or CRF injection, there was always a significant CRF treatment effect on the mean daily response values ($p < 0.01$ as indicated in the legend of Fig. 4). The absence of a significant time effect confirms the lack of habituation in the behavioral and physiological response to CRF administration.
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Fig. 3. Acute response in heart rate, body temperature and motor activity in the home cage following 1 μg CRF/3 μl vehicle solution or vehicle solution alone (arrow on x-axis at t = 0 min). Time is presented in minutes before and after the injection. Motor activity data are stacked for vehicle and CRF treated rats. Figures show mean values + SEM.
Fig. 4. On the left the mean values of heart rate, temperature and activity during the 2 h immediately following CRF or vehicle ICV injection (0–2 h) from day 1 to 10 are presented. The figures on the right side show the mean values of the next 2 h (2–4 h). Statistical analysis showed during 0–2 h after injection the following significant differences: a significant treatment effect for heart rate ($F(1,8) = 19.46; p = 0.002$); for temperature a significant interaction between treatment and time ($F(9,72) = 2.18; p = 0.03$); for activity a treatment effect ($F(1,8) = 16.55; p = 0.004$). For the next 2 h (figures on the right side): for heart rate a treatment effect: ($F(1,8) = 34.87; p = 0.000$); a treatment effect for temperature ($F(1,8) = 17.38; p = 0.003$) and for activity ($F(1,8) = 11.11; p = 0.01$). Figures show means $\pm$ SEM.
The upper panel in Fig. 5 shows the effects of repeated daily CRF injection on the baseline values of resting (light phase) heart rate, temperature and activity as measured during 60 min prior to CRF or vehicle injection (daily between 1000 and 1100h). An ANOVA for repeated measures did not reveal any significant treatment effect or an interaction with treatment and time on heart rate, body temperature, and motor activity, indicating that there was no effect of repeated CRF injection on baseline resting heart rate, body temperature or activity. The lower panel of Fig. 5, however, shows that active (dark phase) heart rate values were affected by repeated CRF injections. Twelve hour means of

Fig. 5. Upper panel shows mean values (+ SEM) of heart rate (left), temperature (middle) and activity (right) during the light phase as measured during 60 min between 1000 and 1100h representing baseline resting values prior to the injection procedure. Lower panel shows 12 h means of heart rate, body temperature and motor activity during the dark phase. During the dark phase the heart rate was a significant decreased in CRF treated rats as reflected in a treatment effect ($F(1,8) = 27.18; p < 0.01$).
Table I. Adrenal and thymus weight of vehicle- and CRF-administered rats

<table>
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<th>Adrenal (mg/100 g body weight)</th>
<th>Thymus (mg/100 g body weight)</th>
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<tbody>
<tr>
<td>Control</td>
<td>8.9 ± 0.5</td>
<td>121.4 ± 3.9</td>
</tr>
<tr>
<td>CRF</td>
<td>11.2 ± 0.5*</td>
<td>93.9 ± 11.3*</td>
</tr>
</tbody>
</table>

Mean ± SEM
*p < 0.05, Indicates significant difference from control treated rats, one-way ANOVA.

The night recordings following the injections in the light phase show that heart rate in CRF-treated rats was significantly lower ($F(1,8) = 27.18; p < 0.01$) every night as compared with vehicles. There was no interaction of treatment with time indicating again the lack of habituation. Night time body temperature was not affected by repeated CRF injection. Motor activity was not significantly decreased. Although there was no significant treatment effect, within-group statistics indicated a decreased activity during every night except for the fifth as compared with pre-injection night activity in the CRF treated animals ($p < 0.05$).

**Organ weights**

The animals were sacrificed on day 11 at the end of the experiment and adrenals and thymus were removed and weighed. Table I shows that daily CRF injection resulted in significant increase in the adrenal weight and a decrease in weight of the thymus.

**DISCUSSION**

The present report shows the acute and long-term effects of ten daily ICV CRF injections on diurnal rhythms of heart rate, body temperature and activity of individually housed rats tested in their home cage. Daily central CRF injection delayed the body weight gain, increased the weight of the adrenals and suppressed the thymus weight at the end of the experiment. The continuous biotelemetric recordings showed that throughout the treatment period CRF did not affect baseline resting heart rate, body temperature and motor activity as measured during the light phase. Mean heart rate and motor activity during the active (dark) phase were, however, significantly decreased in CRF-treated rats during the whole experimental 10-day period. Although the effects of CRF are likely to be mediated through the peptide’s central nervous actions, a peripheral contribution to the effects can not be excluded completely since Martins et al. (1996) showed a rapid CRF transport out of the brain.

The reduced gain in body weight after episodic CRF administration is in line with other studies applying CRF chronically (Arase et al., 1988; Buwalda et al., 1997; Labeur et al., 1995). It resembles the delay in body weight gain observed in rats subjected to behavioral stress (Meerlo et al., 1996). The effects of CRF on body weight can be attributed in part, but not completely, to the inhibition of food intake. This last effect appears to be mediated by CRF$_2$ receptors (Spina et al., 1996). A change in metabolism is likely to contribute to the reduced growth as well (Arase et al., 1988; Meerlo et al., 1996). In the case of CRF administration the sympathetic activation (Fisher, 1989) together with glucocorticoid-evoked catabolic effects, as indicated by the increased adrenal weight at the end of the infusion period, plays an important role in the metabolic change. Increased levels of
circulating glucocorticoids (Arase et al., 1988; Labeur et al., 1995) may also largely be responsible for the reduced thymus weight in the CRF-treated animals.

The acute effect of CRF injections in a familiar environment, in the form of a long-lasting monophasic increase in heart rate and motor activity immediately following administration are well known and described earlier in literature (Korte et al., 1992; Sutton et al., 1982), whereas publications showing acute effects of ICV CRF injection on body temperature longer than the 1 h observations made by Morimoto et al. (1993) are not available. The present biphasic temperature response is, however, identical in time course and magnitude with other findings (Linthorst et al., personal communication). The temperature increase is similar in time dynamics to the biphasic temperature changes observed following ICV injection of interleukins (LeMay et al., 1990; Lundkvist et al., 1996). There is evidence that the thermogenic response to IL-1β is mediated by CRF since it is abrogated by prior central injection of the α-helical CRF antagonist or antibodies to CRF (Rothwell, 1989) and peripheral injection of a non peptidic CRF antagonist (Lundkvist et al., 1996).

The daily heart rate, body temperature and motor activity responses failed to habituate to the repeated administration of CRF. When administered repeatedly, studies have shown that, depending on the dose and the behavioral and physiological parameter under study, the effects of CRF are either unchanged (Sutton et al., 1982), increased (Glowa and Gold, 1991) or decreased (Ahlers and Salander, 1993). Tolerance appears to be related to high doses (5–10 μg) of daily episodic CRF injections. Chronic infusion of CRF into the brain also caused a tolerance to the effects of the peptide on temperature increase and motor activation (Buwalda et al., 1997). Furthermore, chronic CRF was shown to induce CRF receptor downregulation (Hauger et al., 1993) and an attenuated pituitary-adrenocortical activation (Cunningham et al., 1988). Apparently the dose of 1 μg of CRF and the 24 h delay between subsequent injections used in the present study did not alter CRF signalling in the regions involved in the regulation of acute heart rate, temperature and motor activity responses. On a longer term the episodic CRF injections impaired the regulation of heart rate and activity during the night. Mean heart rate was lower in CRF treated animals for the whole 10-day period whereas locomotor activity was decreased when pre- and post-treatment days were compared. This long-term regulation of heart rate and activity during night time also failed to habituate during the injection period. The decrease in heart rate and activity might primarily be the consequence of a compensatory behavioral response to the drastic acute effects of CRF injection during the preceding day. An adjustment of cardiac output to somatic activity has been described previously (Obrist, 1981) and therefore the decreased activity probably initiates an attenuated sympathetic drive to the heart. The lack of an effect of CRF administration on night time temperature might be due to an overruling effect of the circadian increase.

A striking difference with intermittent chronic behavioral stressors is the absence of an effect on baseline resting temperature regulation during the day as measured during the hour preceding the daily injection. A number of reports show that one of the most characteristic long-term effects of various stressors is the increased body temperature during the resting phase (Kant et al., 1991; Meerlo et al., 1996; Tornatzky and Miezek, 1993). Since episodic behavioral stressors like these involve a high activation of central CRF systems (Chappell et al., 1986; Fuchs and Flügge, 1995; Imaki et al., 1991) and, conversely, central CRF administration mimics many of the behavioral, physiological and anatomical responses to behavioral stress (Dunn and Berridge, 1987; Imaki et al., 1993; Korte et al., 1992; Song et al., 1995), the failure of repeated CRF injection to affect
day time body temperature was remarkable. In contrast with this absent shift in long-term temperature regulation following episodic CRF challenge, chronic CRF infusion into the brain did induce an increased day time body temperature similar in time dynamics as observed after social stress (Buwalda et al., 1997). Morimoto et al. (1993) showed that a CRF antagonist severely reduced the stress-induced temperature increase. The differences in long-term consequences of chronic and episodic administration of CRF suggest that the long-term alterations in the behavioral and physiological state of the animals following intermittent behavioral stressors like the social defeat may induce a more chronic elevation of centrally circulating CRF or a release of CRF achieving concentrations far beyond that reached with the presently administered doses of 1 μg.

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