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Buwalda, Bauke; de Boer, Sietse; VanKalkeren, AA; Koolhaas, JM; Kalkeren, A.A. van

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PHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF CHRONIC INTRACEREBROVENTRICULAR INFUSION OF CORTICOTROPIN-RELEASING FACTOR IN THE RAT

B. Buwalda, S. F. de Boer, A. A. Van Kalkeren and J. M. Koolhaas

Department of Physiology and Behavior, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

SUMMARY

The present study was conducted to investigate the long-term effects of chronic elevation of centrally circulating levels of corticotropin-releasing factor (CRF) on behavior and physiology. For this purpose ovine CRF was infused continuously for a period of 10 days into the lateral ventricle of rats with the aid of osmotic pumps (calculated CRF delivery was 4.9 μg/day). Changes in daily rhythms in body temperature and home cage motor activity were recorded telemetrically during the infusion period. The most prominent physiological findings were a delayed body weight gain and a long-lasting hyperthermia following CRF infusion. The peptide treatment furthermore increased adrenal weight and suppressed the weight of the thymus at the end of the experiment. Behaviorally, CRF administration elicited a short-lasting increase in activity during the light phase and an increased anxiety in an elevated plus-maze 1 week after the start of infusion. The similarities between the present results and the long-term changes previously described in behaviorally stressed rats indicate that chronically elevated levels of CRF in the brain might play an important role in the induction and persistence of stress-related behavioral and physiological disorders. © 1997 Elsevier Science Ltd

INTRODUCTION

Corticotropin-releasing factor (CRF) is intimately involved in the expression of autonomic, endocrine and behavioral responses to stress. The peptide functions both as a neurohormone in the hypothalamic-pituitary axis to elicit adrenocorticotropin (ACTH) secretion (Vale et al., 1981) and as a neurotransmitter in various extrahypothalamic regions to initiate autonomic and behavioral components of the stress response (Sawchenko et al., 1993). When injected centrally into the cerebrospinal fluid of experimental animals, CRF produces 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). CRF further acts in several areas of the brain as a transmitter enhancing sympathetic nervous and adrenomedullary activity

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Address correspondence and reprint requests to: Bauke Buwalda, University of Groningen, Department of Animal Physiology, P.O. Box 14, 9750 AA Haren, The Netherlands (Tel: +31 50 3632345; Fax +31 50 3635205; E-mail: buwaldab@biol.rug.nl).

(Fisher, 1989). By a concurrent inhibition of vagal nerve activity (Fisher, 1989; Wiersma et al., 1993) CRF causes a shift in balance towards the sympathetic division of the autonomic nervous system.

The majority of the studies mentioned above describe acute responses to intracerebroventricular (ICV) CRF administration. Based on the similarities between many of these 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). Several 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. Nemeroff et al. (1984) demonstrated increased concentrations of CRF in the cerebrospinal fluid of these patients. In addition, there are reports of a blunted ACTH response to intravenously administered CRF in depressed patients (Holsboer et al., 1984). It is hypothesized that this hyporesponsivity is caused by a downregulation in CRF binding sites following CRF hypersecretion in major depression (Owens and Nemeroff, 1993). Several animal studies focussed on the behavioral, physiological and anatomical consequences of the chronic elevation of centrally circulating CRF by infusing CRF into the brain, mimicking the hypothesized CRF hypersecretion in depressed patients. Hauger et al. (1993) showed that chronic 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 (LeFeuvre 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 parts of the immune system were suppressed (Hauger et al., 1993; Labeur et al., 1995). Many of these CRF-induced changes are similar to features of major depressive disorder (DSM-III-R, 1987). There are no experimental animal or clinical studies that present continuous long-term recordings of behavioral and physiological data during chronic CRF administration into the brain.

Considering both the crucial role of CRF in the stress response as well as the 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). This causal relationship between stress and depresssion has led to the development of animal models for psychological disorders. The long-term behavioral effects of psychosocial stressors such as social defeat in rats suggest the development of a long-lasting state of depression in defeated animals (Koolhaas et al., 1990). The fact that the physiological and behavioral alterations following psychosocial stress can be counteracted by the application of antidepressants and sleep deprivation further strengthens the idea that these animal models might be valuable tools in the study of the mechanisms involved in the pathogenesis of depressive disorders (Fuchs et al., 1996; Koolhaas et al., 1990; Meerlo et al., 1996b). Telemetric studies showed that daily rhythms of body temperature, heart rate and motor activity are affected for several days in defeated rats (Kant et al., 1991; Meerlo et al., 1996a, 1996b; Tornatzky and Miczek, 1993). Because acute and chronic behavioral stressors are reported to 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, high concentrations of central CRF might be hypothesized to play an important role in the

induction and persistence of the altered physiology and behavior of the defeated animals. This hypothesis is strengthened by the finding that a CRF antagonist administered ICV reduces emotionality in socially defeated rats (Heinrichs et al., 1992).

To test further the hypothesis that chronically enhanced levels of CRF may explain the behavioral and physiological changes observed in behaviorally stressed rats, the present study was conducted to investigate the long-term effects of chronic elevation of centrally circulating levels of CRF on behavior and physiology. For this purpose CRF was infused into the lateral ventricle of rats with the aid of osmotic minipumps, in order to obtain a sustained increased concentration of CRF in the central nervous system. Subsequent changes in daily rhythms in body temperature and motor activity were recorded telemetrically for a period of 10 days, in order to compare the effects of CRF administration with the previously described long-term behavioral and physiological effects of social defeat. Effects of CRF infusion on emotionality was studied after 1 week of infusion by exposing the animals to an elevated plus-maze.

METHODS

Animals

Male Wistar rats, weighing 320–390 g at the start of the experiments, were housed individually in clear Plexiglas cages (25 × 25 × 30 cm) on a layer of wood shavings in a room with constant temperature (21 ± 2°C) and fixed, reversed 12 h light-dark regime (light on at 2000h). The animals had free access to standard rat chow and tap water.

Surgery and Data Acquisition

Body temperature and gross locomotor activity were recorded prior to and throughout the entire 10-day infusion period by means of radiotelemetry. For this purpose a transmitter (model TA10TA-F40, Data Sciences, St Paul, MN) was implanted intraperitoneally under ether anesthesia. The transmitters produced a temperature-dependent frequency-modulated signal, which was 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. It is important to note that for detection of activity the transmitter had to move. Therefore, with the transmitter implanted in the peritoneal cavity, slight head movements 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). Body temperature was sampled for 10 s every 10 min. Locomotor activity was recorded continuously and stored at 10-min intervals. One hour averages were calculated and are presented in this paper.

Together with the implantation of the transmitter, a stainless steel cannula (Alzet brain infusion cannula, Alza Corp., Palo Alto, CA) was stereotaxically placed into the right lateral ventricle. The cannula was connected with a subcutaneous polyvinylchloride catheter tube which was sealed at one end. Tubing and cannula were filled with a standard amount of sterile saline (calculated for ± 18 h pumping) in order to avoid CRF delivery immediately following the implantation of osmotic minipumps.

After 3 weeks of recovery the catheters were attached to osmotic minipumps (Alzet, model 2002, having a constant pumping rate of 0.5 μl/h for 14 days) which were inserted
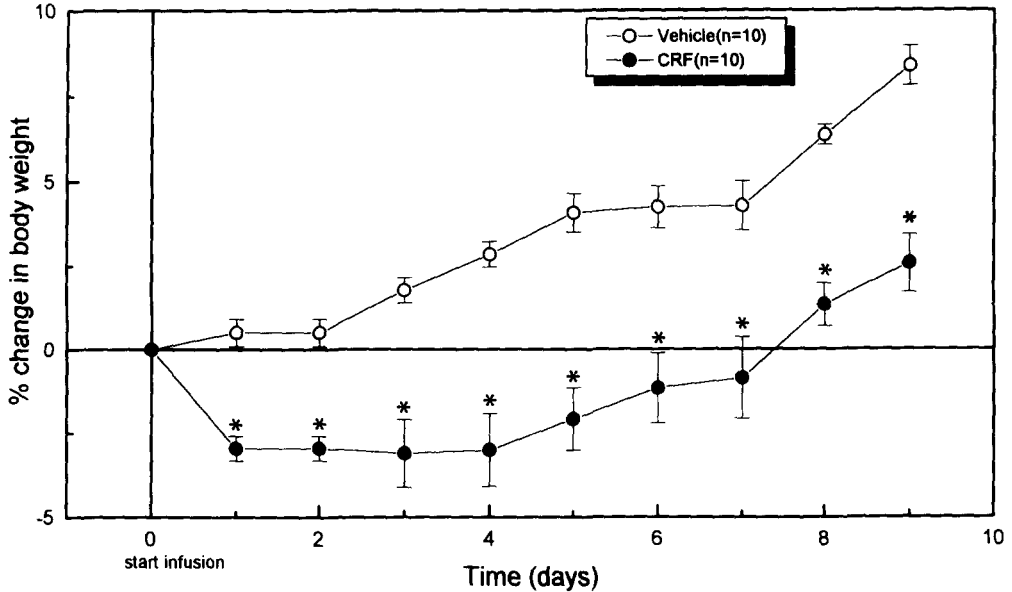
Fig. 1. Changes in body weight following ICV infusion of CRF or vehicle. *p < .05; significant
difference between CRF- and vehicle-treated groups, one-way ANOVA.

subcutaneously at the level of the scapulae. The placement of these pumps was performed
under brief (less than 5 min) ether anesthesia. The pumps contained either vehicle (saline
with 0.1% bovine serum albumin (BSA) and 0.01% ascorbic acid) or ovine CRF dissolved in
the vehicle solution. The experiments were performed in two cohorts of animals, each cohort
receiving CRF from a different distributor (Sigma, St Louis, MO and American Peptide Co.,
Sunnyvale, CA). The behavioral and physiological findings were similar in both CRF-treated
groups. The calculated CRF delivery was 4.9 μg/day.

Ten days after the start of infusion the rats were killed. On this day adrenals and thymus
were removed and weighed.

Behavior in the Elevated Plus-maze

On the seventh day after the start of either CRF or vehicle infusion the rats were exposed
to the elevated plus-maze in order to study the effect of chronic CRF infusion on levels of
anxiety. The procedure of the test has been described in detail by Pellow et al. (1985). In
short, the apparatus was a black wooden, plus-shaped maze, elevated to a height of 50 cm.
Two opposite arms were open (50 × 10 cm), whereas the other two were enclosed with
walls (50 × 10 × 40 cm). Rats were placed individually in the center of the maze with their
head inside one of the closed arms. The number of entries into open and closed arms and the
time spent on open and closed arms was scored during 5 min exposure to the maze. In
addition, the percentage time spent on the open arms (open time/(open time + closed time)
× 100) and percentage entries into open arms (entries open/total entries × 100) were
calculated. The light intensity on the open arms varied, ranging from 10 lux close to the
center of the maze to 80 lux on the end of the open arms. In the closed arms it was less than
1 lux.

Statistical Analysis

Body temperature and home cage activity were averaged over 12 h light and dark phases. The amplitude of the rhythm was defined as the difference between an average dark value and the subsequent average light value. Statistical analysis was performed on mean light and dark values and amplitude values. To assess the overall effects of vehicle and CRF infusion on body temperature and activity, groups were compared with analyses of variance (ANOVAs) for repeated measures over the 10-day period following the start of CRF infusion. For *post hoc* analysis the Dunnett's test was used to determine significance between pairs of means. Within-group changes were analysed with a paired *t*-test. Effects of CRF administration on body and organ weight and on behavior in the elevated plus-maze were analysed with a one-way ANOVA. *p* values less than .05 were considered to be statistically significant.

RESULTS

Body Weight

At the start of infusion the mean body weight of the group of animals receiving vehicle solution was higher than that of CRF-treated animals (403.9 ± 9.8 vs. 357.1 ± 6.8). For this reason changes in body weight following infusion were expressed as percentage change relative to the weight at the start of the treatment. The vehicle-administered controls gradually gained weight, whereas CRF caused an initial loss of weight during the first 2 days after start of the infusion (see Fig. 1) ($p < .05$), after which the rats regained weight but failed to catch up with the controls. There was a significant treatment effect $[F(1,8) = 189,70, \quad p < .001]$ and an interaction between treatment and time $[F(8,64) = 17.16, p < .001]$.

Body Temperature and Activity

The hourly registrations of temperature and activity (Fig. 2) and the calculated amplitudes between light and dark periods (Fig. 4) show that implantation and connection of the osmotic minipump (indicated by arrows in Fig. 2 and Fig. 4) reduced temperature amplitude on the day following implantation ($p < .05$). In vehicle-infused rats this reduction was transient and lasted only 24 h, whereas for the rest of the infusion period temperature amplitudes were similar to baseline values, indicating that ICV infusion of the saline vehicle did not affect temperature regulation. The implantation procedure and infusion of saline did not disturb the stable course of home cage motor activity during light and dark periods, as indicated by the results in controls. CRF infusion, however, caused a long-lasting increase in body temperature during both the light and the dark phase. Effects on motor activity were less pronounced, although an initial decrease in the circadian amplitude (Fig. 4) occurred due to increased activity during the light phase. Comparison of the mean values of body temperature and activity during the light and the dark phase (Fig. 3) revealed a significant effect of CRF treatment on body temperature following implantation of the osmotic pump $[F(1,18) = 74.57, p < .001]$. There was also a significant interaction between treatment and time $[F(23,414) = 5.99, p < .001]$. *Post hoc* analysis showed that the body temperature was significantly higher in CRF-treated animals compared to controls during the whole period following the start of infusion except for the first night (dark phase of day 0) and at the end the infusion period (dark phase of day 7 and light phase of day 8). Five days after start of infusion the night temperature was not significantly increased compared to baseline levels 2
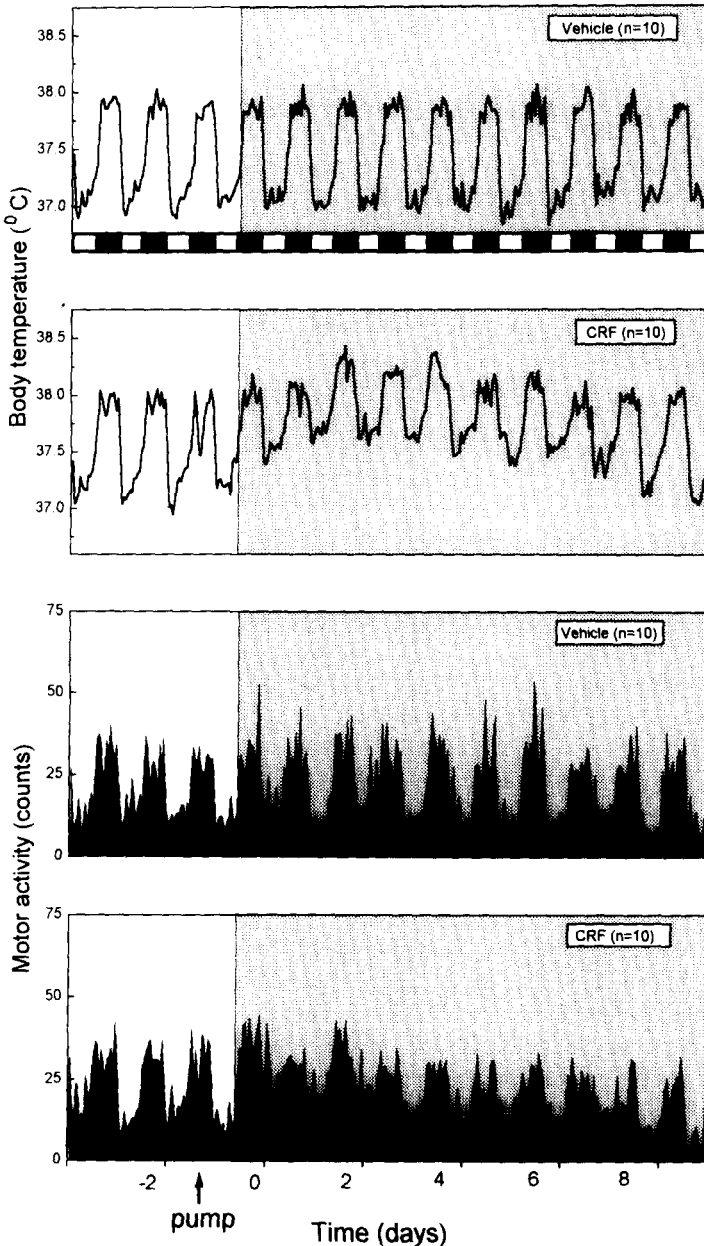
Fig. 2. The effect of chronic ICV infusion of CRF by means of SC osmotic minipumps on daily rhythms of body temperature and home cage activity compared to vehicle infusion. Pumps were implanted and connected to ICV cannulas ±18 h (indicated by arrow on X-axis) before the start of actual CRF infusion into the brain. This effective peptide infusion period is shaded in the figure. The temperature and activity data represent hourly averaged values.

days before start of infusion. Light phase temperature was back to baseline on the last day. These findings indicate that the effect of CRF administration on body temperature habituates during the infusion period. No overall effect of CRF was measured on motor activity,
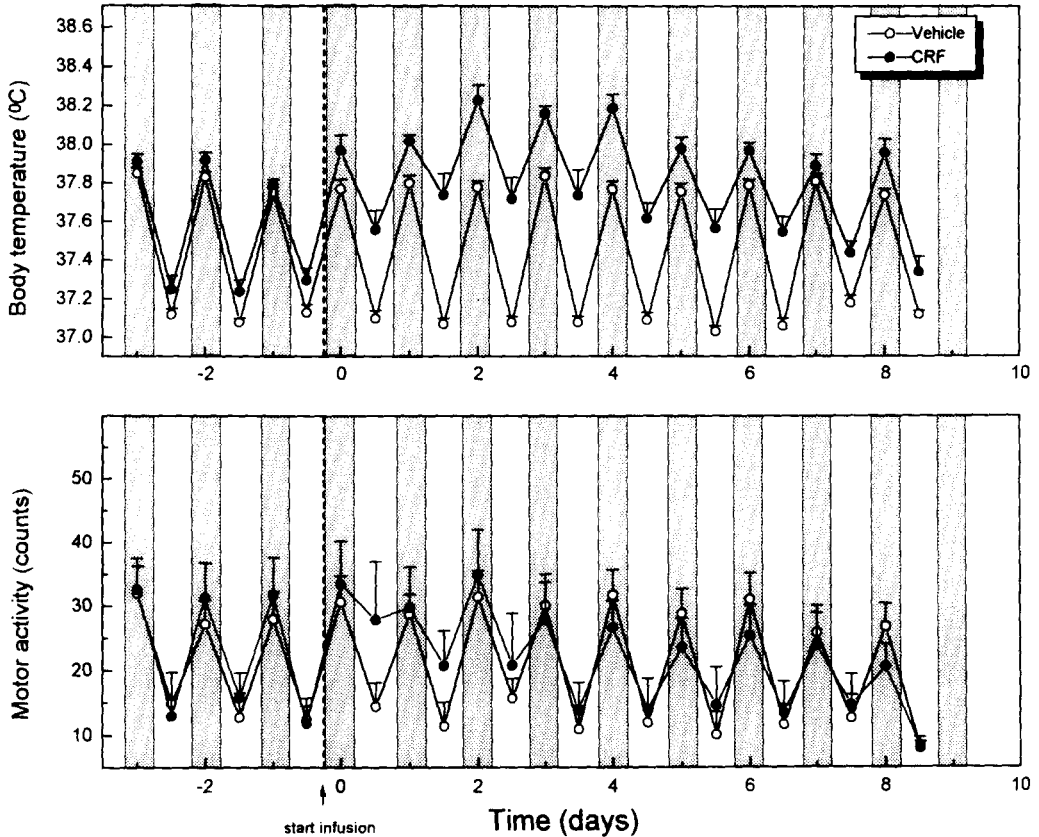
Fig. 3. Mean 12 h light and dark phase values of body temperature and home cage activity before and during ICV infusion of CRF or vehicle. Dark phase is indicated by shaded columns.

indicated by a non-significant treatment effect. Measurement of the daily amplitudes between night and day values (Fig. 4) showed a significant interaction between treatment and time for both the body temperature and motor activity [$F(12,216) = 2.54$, $p = .004$ and $F(12,216) = 2.01$, $p = .02$, respectively]. *Post hoc* analysis indicated a decreased amplitude of daily temperature ($p < .05$) in CRF-treated rats on days 0, 1, 3, 5, 6 and 7. The amplitude of activity was significantly lower than in vehicle-treated rats on day 0 only.

Behavior in the Elevated Plus-maze

When tested 7 days after the infusions started, CRF-treated rats spent significantly less time on the open arms of the plus-maze ([$F(1,19) = 7.36$, $p = .01$] and also made fewer entries into open arms [$F(1,19) = 7.11$, $p = .02$] (Fig. 5). The total number of entries was similar in controls and CRF-treated rats (13 ± 1 and 14 ± 1, respectively).

Organ Weights

Table I shows that ICV CRF infusion caused a significant increase in adrenal organ weight ($p < .01$) and decreased the weight of the thymus ($p < .05$).
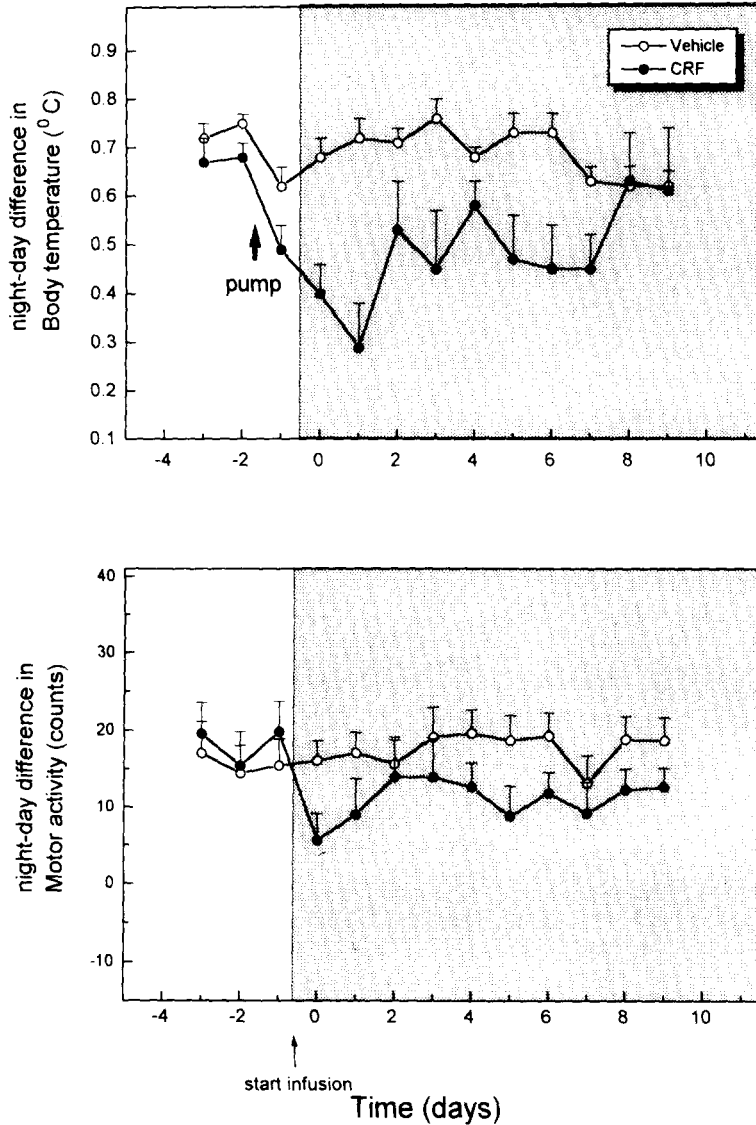
Fig. 4. Circadian amplitudes of daily rhythms of temperature and activity. Amplitudes were calculated as the difference between an average dark value and the subsequent light value. The arrow indicates the moment of pump implantation under brief ether anesthesia.

DISCUSSION

The present report is the first that shows the effects of chronic ICV CRF infusion on the daily rhythms of body temperature and home cage activity by means of telemetry. The most prominent physiological findings were a delayed body weight gain and a long-lasting hyperthermia following CRF infusion. The chronic peptide treatment increased adrenal weight and suppressed the weight of the thymus. Behaviorally, CRF administration elicited a
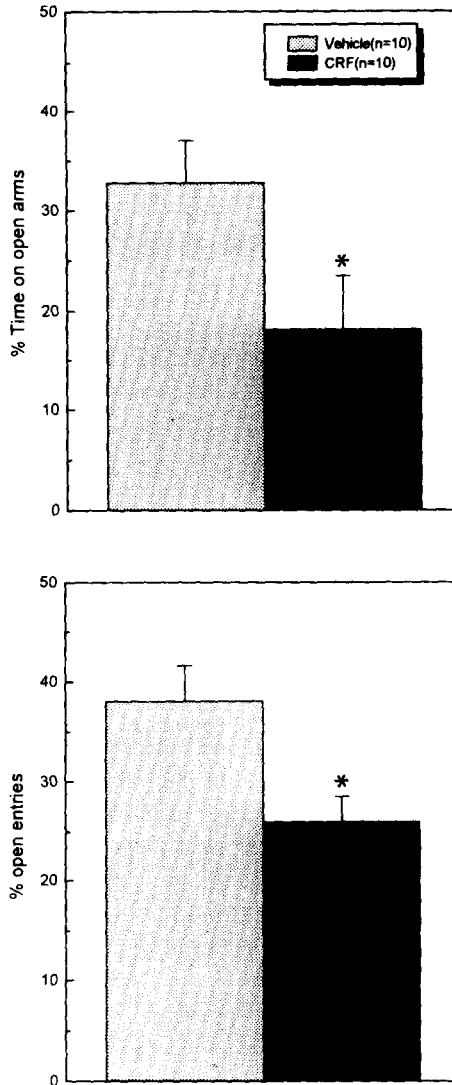
Fig. 5. Behavior on an elevated plus-maze 7 days after the start of ICV CRF or vehicle infusion. Percentage time spent on open arms and percentage of open entries is presented. $*p < .05$; significant difference between CRF- and vehicle-treated groups, one-way ANOVA.

short-lasting increase in activity during the light phase and an increased anxiety in an elevated plus-maze 1 week after the start of infusion. Although we expect the effects of this relatively low concentration of CRF to be mediated through the central nervous actions of CRF, 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 chronic CRF administration is in line with other studies (Arase et al., 1988; Labeur et al., 1995), and closely resembles the delay in body weight gain observed in rats subjected to social defeat (Meerlo et al., 1996c). It is also

Table I. Adrenal and thymus weight of vehicle- and CRF-administered rats

	Adrenal (mg/100 g bw)	Thymus (mg/100 g bw)
Control	6.95 ± 0.41	112.16 ± 9.92
CRF	9.75 ± 0.57**	74.49 ± 8.3*

*$p < .05$, indicates significant difference from control-treated rats, one-way ANOVA.
**$p < .01$.

similar to observations in depressed patients (DSM-III-R, 1987). Both the effects of CRF and of psychosocial stress on body weight seem to be attributed in part, but not completely, to the inhibition of food intake. The recently described potent reduction of appetite by CRF_2 receptors might be an indication of how CRF can be responsible for the effects on food intake (Spina et al., 1996) and body weight. A change in metabolism is also likely to contribute to the reduced growth (Arase et al., 1988; Meerlo et al., 1996c). 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, play an important role in the metabolic change. Increased levels of circulating glucocorticoids following chronic ICV CRF infusion (Arase et al., 1988; Labeur et al., 1995) may also be largely responsible for the reduced thymus weight in the peptide-treated animals.

The long-lasting hyperthermia during both day and night-time following chronic CRF infusion has not been described before. This increase in body temperature gradually habituates during the infusion period, indicating a decreasing efficacy in time of the infused peptide to influence this physiological parameter. This habituation is possibly due to a downregulation of CRF receptors and not to a decreased physiological potency of the peptide in the osmotic pumps after 10 days, as indicated by the study of Hauger et al. (1993). A number of studies show the involvement of central CRF in temperature regulation. An acute short-lasting hyperthermia follows ICV injection of CRF and a stress-induced hyperthermic response is attenuated by administration of the CRF receptor antagonist α-helical CRF (Morimoto et al., 1993). Given the well-known stimulating effect of CRF within the brain on sympathetic outflow, it was hypothesized that sympathetically induced non-shivering thermogenesis by the activation of brown adipose tissue (Hardwick et al., 1989; Holt and York, 1989; LeFeuvre et al., 1987) might play an important role in the hyperthermia following CRF injection. Whether this is also the case for the long-term hyperthermia in the present study is not known. The central actions of CRF on acute thermogenesis might be mediated in part by the pro-opiomelanocortin products α_1-MSH and β-endorphin (Rothwell et al., 1991). Finally the blockade of the pyrogenic actions of interleukin-1β by ICV administration of α-helical CRF (Rothwell, 1989) further indicates the important role of central CRF systems in temperature regulation.

Comparison of the temperature data with clinical studies indicate a similarity with drug-free patients with endogenous depression who had significantly higher temperature minima than did normol controls, also resulting in a reduction of the amplitude of daily temperature rhythm (Beersma et al., 1983; Daimon et al., 1992). The time course of the temperature response during chronic CRF infusion, as observed in the present study, furthermore at first sight shows a striking similarity to that of the temperature response following social defeat

(Meerlo et al., 1996a). The reduction in temperature amplitude after social stress, however, was caused by an increased temperature restricted mainly to the light phase, whereas the CRF infusion also increased body temperature during the dark phase. A likely explanation is that the high concentration of administered CRF by osmotic minipumps in the present experiments is able to increase the already elevated night body temperature further, whereas social stress is less severe in this respect. Another interesting hypothesis might be that social stress increases CRF secretion mainly during daytime, and in this way interferes with the existing diurnal variation in CRF concentration that exists in various brain regions (Owens et al., 1990). The constantly elevated levels of centrally circulating CRF in our study possibly interfere with these diurnal fluctuations in regional CRF concentration.

The short-lasting increase of ICV CRF infusion on motor activity during the light phase in the home cage is in line with reports on the acute effects of CRF on behavioral activity in a familiar environment (Koob and Bloom, 1985; Korte et al., 1992). Compared with the long-lasting increase of body temperature, the short-lasting effect of chronic CRF infusion on motor activity is somewhat surprising. A possible methodological explanation is that the biotelemetric way of measuring locomotor activity is not sensitive enough to produce striking differences in relatively small individual home cages. Because only the amount of horizontal movements is measured, changes in other types of motor activity such as grooming or rearing are poorly monitored. However, it is also possible that tolerance to chronic CRF infusion is actually differential in time for motor activity in a familiar environment and physiology due to different central mechanisms involved.

An increased anxiety after 1 week of central CRF infusion is demonstrated by the decrease in time spent on and fewer entries into the open arms of an elevated plus-maze. A similar acute effect of CRF on behavior in this model of anxiety has been published before (Adamec et al., 1991). The stress of social defeat also results in a long-term increase in emotionality in various behavioral test situations (Koolhaas et al., 1990; Meerlo et al., 1996a, 1996b).

The results in this study show that there are many similarities between the long-term behavioral and physiological consequences of chronic ICV CRF infusion and a single behavioral stressor such as social defeat, giving support to the hypothesis that chronically elevated levels of centrally circulating CRF might play an important role in the induction and persistence of the depressed behavioral and physiological state following psychosocial stress.

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