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THE CENTRAL ROLE OF CORTICOTROPHIN-RELEASING FACTOR (CRF-41) IN PSYCHOLOGICAL STRESS IN RATS

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SUMMARY

1. We investigated the central role of corticotrophin-releasing factor (CRF-41) in psychological stress-induced responses, including cardiovascular, thermoregulatory and locomotive activity in free-moving rats.

2. Psychological stress was induced by cage-switch stress. After rats were placed in the novel environment, blood pressure, heart rate, body temperature and locomotive activity significantly increased. The intracerebroventricular (I.C.V.) injection of α-helical CRF(9–41), a CRF-41 receptor antagonist, significantly attenuated the stress-induced hypertension, tachycardia, hyperthermia and increase in locomotive activity. However, in unstressed rats, the I.C.V. injection of α-helical CRF(9–41) had no effect on physiological parameters measured in this study.

3. In unstressed rats, the I.C.V. injection of CRF-41 (1 μg and 10 μg) increased blood pressure, heart rate, body temperature and locomotive activity in a dose-dependent manner. The changes in these responses were quite similar to those observed during cage-switch stress.

4. The results suggest that central CRF-41 plays an important role in psychological stress-induced hypertension, hyperthermia, tachycardia and increase in locomotive activity. However, it is likely that central CRF-41 does not contribute to normal cardiovascular and body temperature regulation when rats are free from stress.

INTRODUCTION

Stressful conditions cause many physiological responses such as stimulations of the cardiovascular system, changes in body temperature and releases of several kinds of hormones. As a characteristic response to stressful stimuli, it is generally recognized that the coincident activation of the sympathetic nervous system and the hypothalamo-pituitary adrenocortical axis (HPA axis) occurs during stress. The activation of the HPA axis is triggered by neurosecretion of hypothalamic corticotrophin-releasing factor (CRF-41) into the hypophyseal portal vein.

On the other hand, increased synthesis of CRF-41 has been observed in many

regions of the brain, including the hypothalamus during many kinds of stress (Suemaru, Hashimoto, Hattori, Inoue, Kageyama & Ota, 1986; Haas & George, 1988; Murakami, Akana, Dallman & Ganong, 1989). In addition, central administration of CRF-41 has been shown to cause several responses accompanying activation of the sympathetic nervous system, which are usually observed during stress (Brown & Fisher, 1985; Fisher, 1989). Furthermore, recent studies have shown that I.C.V. administration of α-helical CRF(9–41), a CRF-41 receptor antagonist, attenuates the elevation of blood catecholamine levels caused by stresses such as ether exposure, insulin-induced hypoglycaemia and haemorrhage (Brown, Fisher, Webb, Vale & Rivier, 1985; Brown, Gray & Fisher, 1986). Kregel, Overton, Seals, Tipton & Fisher (1990) have also reported that I.C.V. administration of α-helical CRF-(9–41) significantly attenuates running exercise-induced cardiovascular responses. Therefore, CRF-41 has been believed to act in the central nervous system as a neuropeptide which induces several responses during stress.

Because of experimental difficulties involved with the observation of physiological variables in conscious animals without stress, it has been impossible to investigate whether central CRF-41 is practically involved in responses induced by a mild or psychological stress. We have acquired a recently developed telemetric system (Lange, Brockway & Azar, 1991), which enables us to measure many physiological parameters simultaneously in conscious animals with minimal stress. Using this telemetric system, we investigated stress-induced responses in free-moving rats during cage-switch stress which is thought to be a mild or psychological stress (Morimoto, Watanabe, Morimoto, Nakamori & Murakami, 1991). In the present study, to clarify the possible involvement of central CRF-41 in the development of psychological stress-induced responses, we examined the effects of centrally administered α-helical CRF(9–41), a CRF-41 receptor antagonist, on changes in blood pressure, heart rate, body temperature and locomotive activity after exposure to psychological stress in free-moving rats. Furthermore, to confirm that the central CRF-41 plays a role in psychological stress-induced responses, we compared the responses induced by psychological stress with those by I.C.V. injection of CRF-41.

METHODS

Male albino (Wistar strain) rats weighing 300–420 g were used in this study. The rats were housed in individual cages (40 × 25 × 25 cm; length × width × depth) lined with wood-chip bedding. They were kept in a room maintained at 26 ± 1 °C, a temperature within the thermoneutral zone for rats, with a 12–12 h light–dark cycle, and lights on at 7.00 h. Tap water and rodent chow were available *ad libitum*.

Blood pressure, heart rate, body temperature and locomotive activity were simultaneously measured by a biotelemetric system (DATAQUEST IV, Data Sciences Inc., USA). A battery-operated transmitter (TL10M2-C50-PT) was implanted intraperitoneally (I.P.) into each rat at least 10 days before the start of experiments under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.). During surgery, the tip of the cannula which measures the blood pressure and heart rate was inserted into the distal descending aorta. Output (frequency in Hz) from the transmitter was monitored by two antennae mounted in a receiver board that was placed under each animal's cage. The data were fed into a peripheral processor connected to a microcomputer (Kyocera, Japan). The methods of this telemetric system were described in detail in the recent paper (Lange *et al.* 1991).

Psychological stress was induced by a protocol called cage-switch. Throughout the experiment, each physiological variable was continuously measured by the telemetric system described above.

The cage-switch stress was evoked by removing the rat from its home cage and placing it into another identical plastic cage which contained 1 cm deep water at 36 °C. The rat remained in this cage for 60 min. To minimize the confounding physiological effects of the circadian rhythm, all experiments were performed between 10.00–13.00 h.

For I.C.V. injection, the rats had been implanted previously with a stainless-steel cannula (0·8 mm o.d.) at co-ordinates AP 0·2, L 0, V 9 mm according to the rat brain atlas of Pellegrino, Pellegrino & Cushman (1979) by standard stereotaxic techniques. This implantation was done 10 days before surgery for implantation of a transmitter, under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.).

CRF(1–41) (Sigma, USA) dissolved in sterile saline at a concentration of 0·5 μg/μl and 5 μg/μl and α-helical CRF(9–41) (Sigma, USA) dissolved in sterile saline at a concentration of 5 μg/μl were used for I.C.V. injection. The volume infused was always 2 μl.

The study consisted of three experiments. In experiment 1, the effects of I.C.V. injection of saline (2 μl) or α-helical CRF(9–41) (10 μg) on changes in blood pressure, heart rate, body temperature, and locomotive activity during cage-switch stress were investigated. Saline or α-helical CRF(9–41) was injected just before the start of cage-switch stress. Each rat was injected I.C.V. with both saline and α-helical CRF(9–41) on different days. To minimize the effect of habituation to the cage-switch stress, the order of the injections of saline or α-helical CRF(9–41) was randomized. In experiment 2, the effects of I.C.V. injection of saline (2 μl) or α-helical CRF(9–41) (10 μg) on blood pressure, heart rate, body temperature, and locomotive activity in unstressed rats were investigated. After injection, animals were returned to their home cage. In experiment 3, the effects of I.C.V. injection of CRF-41 (1 μg or 10 μg) or saline (2 μl) on blood pressure, heart rate, body temperature, and locomotive activity were investigated. After injection, animals were returned to their home cage.

After the completion of experiments, the animals were killed by a large overdose of sodium pentobarbitone. Carbon solution (2 μl; Rotering, FRG) was then injected I.C.V. to mark the ventricular space. The brain sections were visually examined to verify that the tip of the stainless-steel cannula was located in the third cerebral ventricle.

The data were analysed for statistical significance by Student's t test or ANOVA.

RESULTS

Figure 1 shows the effect of I.C.V. injection of saline (2 μl) or α-helical CRF(9–41) (10 μg) on changes in blood pressure (Fig. 1*A*) and heart rate (Fig. 1*B*) of rats during cage-switch stress. The cage-switch stress started at time zero. Both saline and α-helical CRF(9–41) were injected I.C.V. just before the start of cage-switch stress. As shown in Fig. 1*A*, the animals injected with saline showed significant and immediate elevations of blood pressure. These changes in elevations of the systolic, mean and diastolic pressures were almost in parallel and lasted for 60 min. The average elevations of the systolic, mean and diastolic pressures during cage-switch stress were $25 \pm 2{\cdot}4$, $24 \pm 2{\cdot}2$ and $22 \pm 2{\cdot}7$ mmHg. In contrast, the I.C.V. injection of α-helical CRF(9–41) significantly ($P < 0{\cdot}01$) suppressed the elevation of blood pressure. The average elevations of the systolic, mean and diastolic pressure in rats injected with α-helical CRF(9–41) during cage-switch stress were $13 \pm 2{\cdot}0$, $13 \pm 1{\cdot}8$ and $10 \pm 1{\cdot}8$ mmHg. In Fig. 1*B*, the animals injected with saline showed a significant and immediate increase in heart rate, and this increase lasted for 60 min. The average increase in heart rate during cage-switch stress was $115 \pm 8{\cdot}3$ beats/min. In contrast, the I.C.V. injection of α-helical CRF(9–41) significantly ($P < 0{\cdot}01$) suppressed the increase in heart rate after cage-switch stress. The average increase in heart rate during cage-switch stress in rats injected with α-helical CRF(9–41) was $59 \pm 7{\cdot}2$ beats/min.

Figure 2 shows the effect of I.C.V. injection of saline (2 μl) and α-helical CRF(9–41) (10 μg) on changes in body temperature (Fig. 2*A*) and locomotive activity (Fig. 2*B*) of rats during cage-switch stress. Changes in the body temperature are expressed as

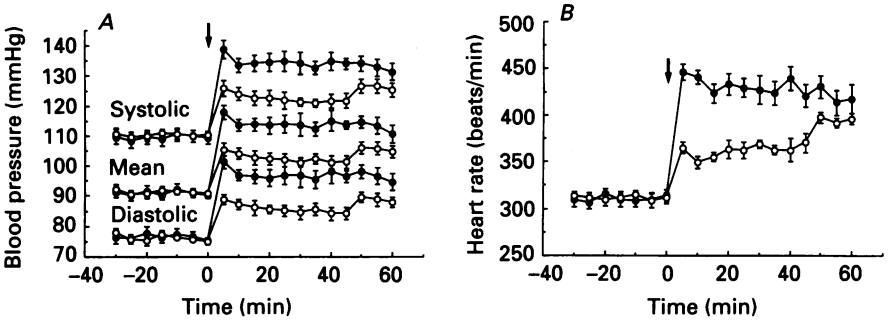

Fig. 1. Mean changes (±S.E.M.) in the blood pressure (*A*) and heart rate (*B*) of six rats during cage-switch stress. Saline (●) or α-helical CRF(9–41) (○) was injected I.C.V. just before exposure to stress. Arrows indicate the start time of the 60 min cage-switch stress.

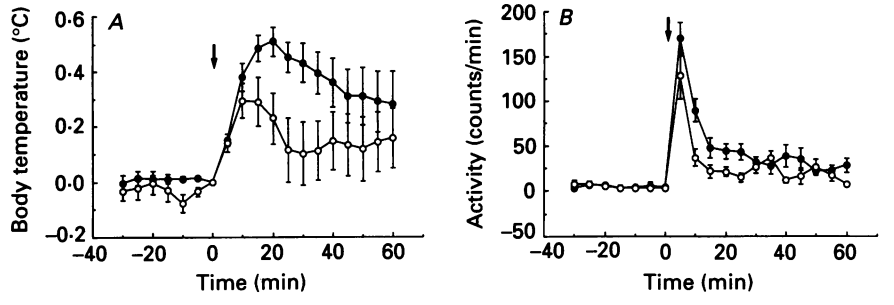

Fig. 2. Mean changes (±S.E.M.) in the body temperature (*A*) and locomotive activity (*B*) of six rats during cage-switch stress. Saline (●) or α-helical CRF(9–41) (○) was injected I.C.V. just before exposure to stress. Arrows indicate the start time of the 60 min cage-switch stress.

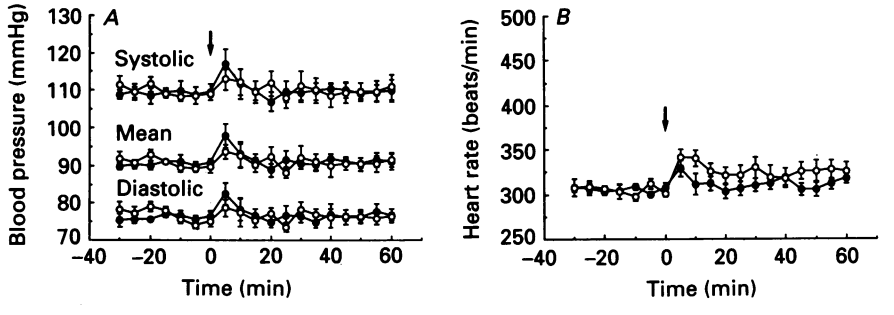

Fig. 3. Mean changes (±S.E.M.) in the blood pressure (*A*) and heart rate (*B*) of six rats. Arrows indicate the time of I.C.V. injection of saline (●) or α-helical CRF(9–41) (○).

a deviation from the baseline recorded at the start time of the cage-switch stress. Both saline and α-helical CRF(9–41) were injected just before the start of cage-switch stress. As shown in Fig. 2*A*, the animals injected with saline showed a significant elevation of body temperature. The body temperature reached a peak at

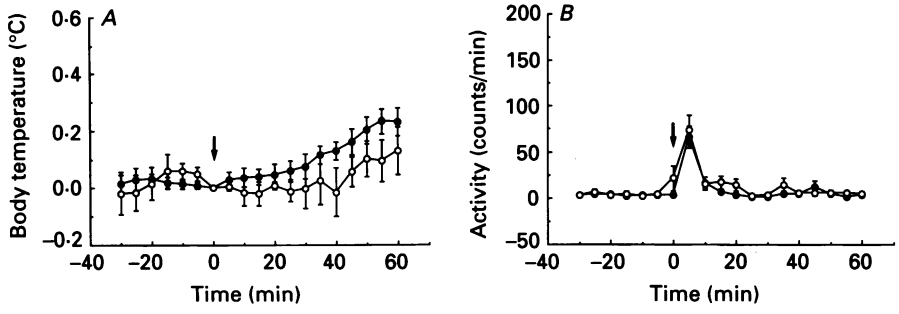

Fig. 4. Mean changes (±S.E.M.) in the body temperature (*A*) and locomotive activity (*B*) of six rats. Arrows indicate the time of I.C.V. injection of saline (●) or α-helical CRF-(9–41) (○).

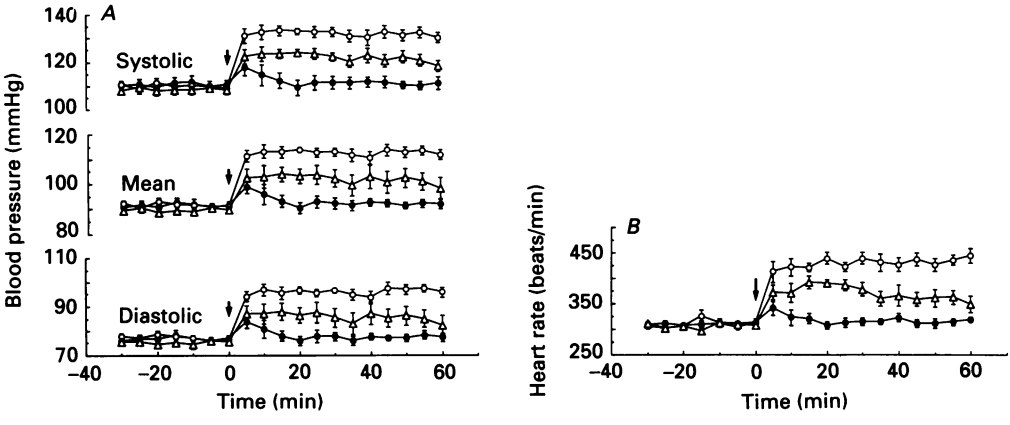

Fig. 5. Mean changes (±S.E.M.) in the blood pressure (*A*) and heart rate (*B*) of six rats. Arrows indicate the time of I.C.V. injection of saline (●) or CRF-41, 1 μg (△) or 10 μg (○).

20 min after the start of cage-switch stress, then gradually declined. The average maximum elevation of body temperature was 0·5±0·05 °C. In contrast, α-helical CRF(9–41) significantly ($P < 0·05$) suppressed this elevation of body temperature. In Fig. 2*B*, the animals injected with saline showed a significant increase in locomotive activity. The increase in this activity reached a peak at 5 min after the start of cage-switch stress, then gradually declined. In contrast, α-helical CRF(9–41) significantly ($P < 0·05$) suppressed the increase in locomotive activity for the initial 20 min. The time to the peak activity was almost the same as that for saline-injected animals but then the increased activity quickly declined to initial level.

Figure 3 shows the effect of I.C.V. injection of saline and α-helical CRF(9–41) on blood pressure (Fig. 3*A*) and heart rate (Fig. 3*B*) in unstressed rats. The I.C.V. injection of either saline or α-helical CRF(9–41) did not cause significant changes in blood pressure or heart rate. Figure 4 shows the effect of I.C.V. injection of saline or α-helical CRF(9–41) on body temperature (Fig. 4*A*), and locomotive activity (Fig.

4*B*) in unstressed rats. As shown in Fig. 4*A*, after injections of either saline or α-helical CRF(9–41), the body temperature gradually rose but there were no significant differences between those two groups. In Fig. 4*B*, after injection of either saline or α-helical CRF(9–41), the locomotive activity also transiently increased but

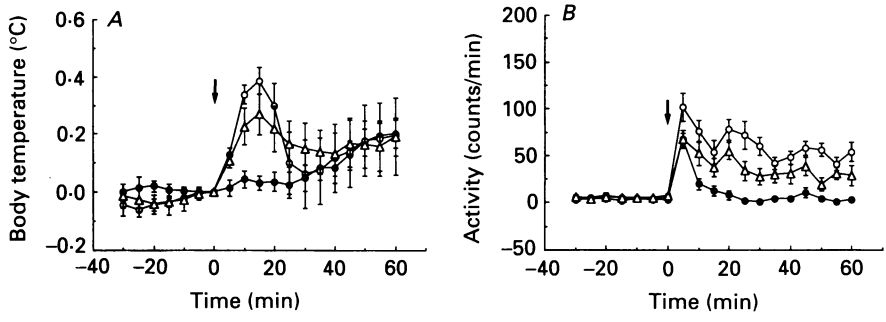

Fig. 6. Mean changes (±S.E.M.) in the body temperature (*A*) and locomotive activity (*B*) of six rats. Arrows indicate the time of I.C.V. injection of saline (●) or CRF-41, 1 μg (△) or 10 μg (○).

immediately returned to the baseline level, and there were no significant differences between those two groups.

Figure 5 shows the effect of I.C.V. injection of saline (2 μl) or CRF-41 (1 μg or 10 μg) on changes in blood pressure (Fig. 5*A*) and heart rate (Fig. 5*B*) in unstressed rats. As shown in Fig. 5*A*, the I.C.V. injection of CRF-41 increased the blood pressure in a dose-dependent manner. The changes in systolic and diastolic pressures occurred almost in parallel and lasted for more than 60 min. In Fig. 5*B*, the I.C.V. injection of CRF-41 also increased the heart rate in a dose-dependent manner. The increase in heart rate lasted for 60 min. In contrast, the animals injected with saline showed only transient increases in blood pressure and heart rate just after injection, but these variables quickly returned to their initial levels.

Figure 6 shows the effects of I.C.V. injection of saline (2 μl) or CRF-41 (1 μg or 10 μg) on changes in body temperature (Fig. 6*A*) and locomotive activity (Fig. 6*B*). As shown in Fig. 6*A*, the animals injected with CRF-41 showed a significant elevation of body temperature ($P < 0{\cdot}05$) in a dose-dependent manner in the first 20 min after injection. The body temperature reached a peak at 15 min after injection, then gradually declined. In contrast, the animals injected with saline did not show significant changes in body temperature for the initial 30 min, but thereafter it gradually rose. For the latter 20 min, there were no significant differences between CRF-41-injected and saline-injected groups. In Fig. 6*B*, the animals injected with CRF-41 showed a significant increase in locomotive activity in a dose-dependent manner ($P < 0{\cdot}01$). The increase of this activity reached a peak at 5 min after injection then gradually declined but still remained at a higher level than baseline. In animals injected with saline, locomotive activity also significantly increased and reached a peak at 5 min. However, this increase quickly declined and soon returned to the initial level.

DISCUSSION

Although severe stress is rarely encountered in life, mild stressors are frequently experienced. However, it has been difficult to observe physiological responses during mild stress because previous measurement methods often caused much larger stress than the mild stress under study. By using a new telemetric system, we were able to investigate responses evoked by a mild stress, cage-switch, with little or no additional stress caused by the measurement procedures.

In this study, the blood pressure, heart rate, body temperature and locomotive activity were significantly increased by cage-switch stress. These changes were mimicked by an I.C.V. injection of CRF-41. Furthermore, responses observed during cage-switch stress were significantly attenuated by pretreatment with I.C.V. injection of α-helical CRF(9–41). Therefore, these results strongly suggest that, during a mild or psychological stress, central CRF-41 is involved in the cardiovascular responses, and increases in body temperature and locomotive activity. In addition, since I.C.V. injection of α-helical CRF(9–41) did not induce significant responses in unstressed rats, it is likely that the central CRF-41 does not contribute to normal cardiovascular and body temperature regulation during the resting state.

Animals generally respond to stress in a stereotyped manner that includes activation of the sympathetic nervous system. As in the present results, many researchers have previously reported that the I.C.V. injection of CRF-41 induces several responses mediated by activation of the sympathetic nervous system, responses which are similar to those observed during stressful conditions (Brown & Fisher, 1985; Fisher, 1989). In the present study, it is likely that activation of the sympathetic nervous system lasted throughout the cage-switch procedure, because blood pressure and heart rate remained elevated during the stress. After pretreatment with an I.C.V. injection of α-helical CRF(9–41), cardiovascular responses during stress were significantly suppressed. Therefore, this result suggests that central CRF-41 is involved in the cardiovascular responses mediated by sympathetic nervous activation during cage-switch stress. However, we must take into account the possibility that ACTH or vasopressin released from the pituitary gland, when stimulated by I.C.V. injection of CRF-41 or by stress, is also involved in stress-induced hypertension (Gruber, Klein, Hutchins, Buckalew & Lymangrover, 1984; Lenz, 1987; Lebrun, Rohmeiss, Demmert, Rettig & Unger, 1987). During sympathetic nervous activation, it is inferred that non-shivering thermogenesis, including thermogenesis by brown adipose tissue, increases through β-adrenergic stimulation (Schönbaum, Johnson, Sellers & Gill, 1966). Furthermore, several researchers have reported that I.C.V. injection of CRF-41 causes non-shivering thermogenesis in rats (LeFeuvre, Rothwell & Stock, 1987; Rothwell, 1990). However, the peak elevation of body temperature occurred within 20 min of the start of cage-switch stress, then gradually returned to the initial level, although increases in blood pressure and heart rate continued. In the present and previous studies (Diamant & De Wied, 1991), a similar phenomenon was observed after I.C.V. injection of CRF-41. One possible explanation is that the threshold concentration of CRF-41 to induce thermogenesis may be higher than that to induce cardiovascular responses, because it is speculated that CRF-41 concentration is higher at the beginning of stress or at

the bolus injection of CRF-41. Another explanation for the temporal dissociation of the differences in the duration of the cardiovascular and thermogenic responses to CRF-41 may be that the injected peptide is cleared more rapidly from areas adjacent to the receptors effecting the thermogenic responses. However, the details still remain to be elucidated.

In summary, the present results clearly demonstrate that during a mild stress, central CRF-41 is involved as an important neuropeptide in the development of stress-induced hypertension, tachycardia, hyperthermia, and increase in locomotive activity. However, it is likely that central CRF-41 does not contribute to normal cardiovascular and body temperature regulation when rats are free from stress.

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