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Role of corticotropin-releasing factor in forced swimming test

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Abstract

Several aspects of the role of corticotropin-releasing factor (CRF) in the forced swimming test were investigated in this study by using two different administration schedules. I.c.v. microinjection of CRF produced a dose-dependent increase in swimming activity when the administration schedule originally reported for this test to screen antidepressant drugs was followed. The most effective doses were 1 and 3 μg of CRF. A lower dose of CRF (0.5 μg) was also effective when repetitive experimental stress was present. CRF receptor antagonist, *alpha-helical* CRF-(9-41) (*α-helical* CRF-(9-41)), was able to block CRF-induced increases in swimming in all sessions of the forced swimming test. However, the effects of CRF and CRF receptor antagonist depended on the administration schedule. A decrease in swimming in the forced swimming test was observed when CRF and CRF receptor antagonist were given together, using a different administration schedule. I.c.v. CRF was ineffective and CRF receptor antagonist alone produced an increase in swimming when administered according to this schedule. These behavioural responses were maintained after twelve days without any treatment. The results of the current study suggest that endogenous CRF seems to play a determinant role in behavioural responses in the forced swimming test. The involvement of the level of activation and memory processes in these behavioural responses is discussed. © 1998 Elsevier Science B.V.

Keywords: CRF (Corticotropin-releasing factor); Alpha-helical CRF-(9-41); Forced swimming test; Stress; Learning and memory; Behavioural adaptation

1. Introduction

The rat forced swimming test is a non-escapable stressful situation (Porsolt et al., 1977) and as such is widely used for screening substances with a potential antidepressant effect. Briefly, when rats or mice are forced to swim in an inescapable situation, they tend to become immobile ('floating') after vigorous activity. This immobility was qualified as a symptom of 'behavioural despair' and the forced swimming test has been suggested as an animal model of human depression. Substances that decrease immobility could potentially have antidepressant-like properties (Porsolt, 1981). However, other authors suggest that immobility represents an adaptative response to inescapable stress rather than behavioural despair. Such an adaptative process would allow animals to save energy and may confer a survival advantage when other strategies are not successful (West, 1990). Thus, learning and memory processes may be involved in the forced swimming test and the progressive immobility can represent a learned adaptative response to an inescapable stressful situation (Hawkins et al., 1978; Jefferys et al., 1984, Borsini et al., 1986; De Pablo et al., 1989).

Corticotropin-releasing factor (CRF), a 41-amino-acid polypeptide, has been shown to be a major mediator of the activation of the pituitary–adrenocortical (PA) system, which results in ACTH and β-endorphin secretion (Vale et al., 1981). The anatomical localization of CRF-immunoreactive neurons is not restricted to the neurosecretory system, suggesting that the role of CRF is not limited to its neuroendocrine effects and that it could act as a neuromodulator in extrahypothalamic regions (Swanson et al., 1983). Intracerebral infusion of CRF produces physiological and behavioural effects similar to those produced by stress, suggesting that this peptide plays an important role in the responses to stressful situations (Dunn and Berridge, 1990). CRF has also been demonstrated to influ-

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ence learning and memory processes in several experimental paradigms. Thus, CRF can facilitate or impair acquisition and/or retention of some learning tasks. Overall, higher doses of CRF seem to impair these processes while lower doses induce the opposite effect, although the threshold of this biphasic effect depends on the test (Veldhuis and De Wied, 1984; Koob and Bloom, 1985; Lee and Sung, 1989).

A specific competitive antagonist of CRF, alpha-helical CRF-(9-41) (α-helical CRF-(9-41)), a CRF analogue, has been synthesized (Rivier et al., 1984). This compound represents an important tool for the study of the physiological and behavioural function of endogenous CRF. The administration of α-helical CRF-(9-41) has been demonstrated to block CRF-induced effects on some behavioural parameters such as place aversion (Cador et al., 1992), conflict test (Britton et al., 1986), schedule-controlled responding in pigeons (Barrett et al., 1989), acoustic startle response (Swerdlow et al., 1989), locomotor activity (Britton et al., 1986; Winslow et al., 1989) and food intake (Krahn et al., 1986). Centrally administered α-helical CRF-(9-41) has been demonstrated to possess stress-protective properties in several behavioural paradigms like exploratory behaviour (Berridge and Dunn, 1987), 'fighting' (Tazi et al., 1987), 'freezing' (Kalin et al., 1988), acoustic startle response (Swerdlow et al., 1989), and to block anxiogenic responses produced by ethanol withdrawal (Baldwin et al., 1991), conditioned emotional response (Cole et al., 1987) and defensive withdrawal in response to novel situations or odors (Takahashi et al., 1989, 1990).

In the present paper, we address several aspects of the role of CRF in the forced swimming test, which has not been previously investigated, (a) to determine the dose–response relationship for CRF in the forced swimming test when CRF was administered according to the schedule previously proposed for screening antidepressant drugs (experiment 1), (b) to test if CRF receptor antagonist is able to block the CRF effect in the forced swimming test (experiment 2), (c) to evaluate the possible role of endogenous CRF in the forced swimming test by administration of CRF receptor antagonist alone (experiment 3) and (d) to study the role of CRF and CRF receptor antagonist in the adaptation of the swimming response to the forced swimming test by changing the administration schedule (experiment 4).

2. Materials and methods

2.1. Subjects

Male Wistar rats (*Rattus norvegicus albinus*, Criffa, France), weighing 240–260 g at the start of the experiment, were housed with food and water continuously available in a room maintained at 20 ± 2°C, with a controlled light–dark cycle (light: 08.00 to 20.00 h). Subjects were handled to minimize the effects of nonspecific stress and acclimatized to the animal quarters for 1 week prior to any experimental procedure.

2.2. Surgery and verification of cannula placement

Rats were anesthetized i.p. with a mixture of atropine (1 mg/ml, Palex), ketamine (40 mg/ml, Parke-Davis) and diazepam (5 mg/ml, Roche) and mounted in a Narishige stereotaxic instrument. Subjects were unilaterally implanted with a 23 gauge stainless steel guide cannula 1 mm above the right lateral ventricle, which was fixed to the skull with screws and dental cement. Implantation coordinates were: A/P, −0.8 mm from bregma; M/L, 1.5 mm and D/V, 3.5 mm from the surface of the skull (Paxinos and Watson, 1986). Following surgery, a 30-gauge stylet was placed into the guide cannula and rats were allowed to recover for at least 1 week.

In a preliminary experiment, we verified that i.c.v. cannula implantation and chronic vehicle administration did not produce any effect on swimming in the forced swimming test. A similar result has been previously reported after a single session and i.c.v. administration (Weiss et al., 1986). Immediately after the experiments, rats received i.c.v. dye microinfusions and were killed by decapitation. Cannula placement was verified by visual examination of slices made with a cryostat (Reichert-Jung, France), using a transmitted-light stand (bright/dark field). Only data from those rats with correct dye localization in the ventricular system were included in the data analysis. This verification was performed without knowledge of the behavioural response of each animal.

2.3. Forced swimming test

Individuals rats were forced to swim inside a Plexiglas cylinder (height: 60 cm, diameter: 19 cm) containing 19 cm of water at 25°C. The subjects were removed after 15 min in the cylinder (SESSION1) and allowed to dry. 24 h later, animals were returned to the cylinder and forced to swim for 5 min (SESSION2). This last procedure was repeated every 24 h for three days (SESSION3, SESSION4 and SESSION5) and twelve days after SESSION5 (SESSION6) depending on the experimental design. An automatic recording system (Panlab Animal Activity System, Panlab, Barcelona) to measure swimming was used. Briefly, frequency variations in electromagnetic field of the sensory unit produced by swimming are transformed into voltage changes which, in turn, are converted into impulses (De Pablo et al., 1989). Swimming was represented primarily by struggling behaviour because minimal changes due to swimming or floating could not be detected with the procedure used. It has been proposed that struggling is a more reliable measure of antidepressant action than other behaviours seen in the forced swimming test (Armario et al., 1988). Several sessions were used in the present study since a longer test procedure has been re-

ported to increase the forced swimming test sensitivity to a chronic treatment (Rusakov and Valdman, 1982; Yamada et al., 1989).

2.4. I.c.v. microinjection procedure and drugs

Microinfusions were administered using a 30-gauge injector connected to a Hamilton automatic microsyringe (CR-700-20) by PE-20 tubing in a volume of 2 μl. The injector was left in place for 60 s to prevent backflow leakage and the stylet was then replaced.

Rat/human CRF and α-helical CRF-(9-41) were provided by Sigma Chemical Co. CRF was disolved in artificial cerebrospinal fluid (CSF) and pH was adjusted to 7.4 by bubbling with CO_2. Alpha-helical CRF-(9-41) was dissolved in distilled water and the pH was adjusted to 6.7 with 1 N NaOH. All solutions were divided into aliquots and stored frozen.

Peptides and control solutions were infused following two different administration schedules. In experiments 1, 2 and 3, microinjections were administered 23.45, 5 and 1 h immediately before SESSION2, as previously reported for screening antidepressant drugs (Porsolt et al., 1977). Thus, in SESSION1 the rats were not treated. Microinjections were given 1 h before the other sessions (SESSION3, SESSION4, SESSION5). In experiment 4, the administration schedule was changed and microinjections were given 1 h before SESSION1 and immediately after the other sessions (SESSION2, SESSION3 and SESSION4) in order to evaluate the influence of treatments on the adaptation of swimming response in the forced swimming test. In experiments where both peptides were used, CRF receptor antagonist or vehicle was always administered 40 min after CRF or CSF administration.

2.5. Experimental procedures

2.5.1. Experiment 1

Fifty-six male rats were randomly assigned to six groups. Subjects were infused i.c.v. with 0, 0.1, 0.5, 1 and 3 μg of CRF following the administration schedule proposed by Porsolt (Porsolt et al., 1977). As we have previously indicated: three microinjections of CRF were administered 23.45, 5 and 1 h before SESSION2. In the other sessions, CRF microinjections were given 1 h before. An additional sixth group of animals received the highest dose of CRF (3 μg) only before SESSION2. In this group, CRF was replaced with artificial CSF in the other sessions (SESSION3, SESSION4 and SESSION5) to test a possible long-lasting effect of CRF.

2.5.2. Experiment 2

Fifty male rats were randomly assigned to five groups. Three groups of subjects were infused i.c.v. with 0, 0.5 or 1 μg of CRF. These intermediate doses of CRF were chosen on the basis of experiment 1. The other two groups received 0.5 or 1 μg of CRF and 25 μg of CRF receptor antagonist, α-helical CRF-(9-41). The administration schedule was that used in experiment 1.

2.5.3. Experiment 3

Twenty-six male rats were randomly assigned to three groups. Subjects were infused i.c.v. with 0, 25 or 50 μg of CRF receptor antagonist, α-helical CRF-(9-41). The administration schedule followed in this experiment was that used in the two previous experiments.

2.5.4. Experiment 4

Forty male rats were randomly assigned to four groups. Two groups of subjects were infused i.c.v. with 0 or 1 μg of CRF. The other two groups received i.c.v. 25 μg of α-helical CRF-(9-41) alone or with 1 μg of CRF. Doses of both peptides were chosen on the basis of experiments 1 and 2. The i.c.v. administration schedule was changed with respect to that used in the previous experiments. CRF was administered 1 h before SESSION1 and immediately after the other sessions. In order to better evaluate the effect of CRF, swimming in SESSION1 was measured in two ways: in the first 5 min and during the entire session (15 min). An additional session (SESSION6) was held twelve days after SESSION5 without any treatment between the two sessions to test long-term retention of the behavioural adaptation to the forced swimming test, as previously reported (Mitchell and Meaney, 1991).

2.6. Statistical analysis

In experiments 1, 2 and 3, swimming activity (number of impulses) per session was compared by using a repeated measures analysis of covariance (ANCOVA), with 'treatment' as between-subjects factor, and 'session' as within-subjects factor. Analysis of covariance was used in order to eliminate the source of variation due to individual reactivity to the test. This kind of analysis separates the effect of the covariate from the relationship between the treatments and the dependent variable (Huitema, 1980). Thus, swimming in SESSION1 was used as constant covariate since it was not affected by treatment. Swimming in the first 5 min of this session was a more appropriate covariate than was the swimming in the entire 15 min of the session. Fisher's protected LSD procedure was used for the comparison of treatment means, as previously proposed (Huitema, 1980).

In experiment 4, swimming (number of impulses) was analyzed by a repeated measure analysis of variance (ANOVA), with 'treatment' as between-subjects factor and 'session' as within-subjects factor. In this case, analysis of

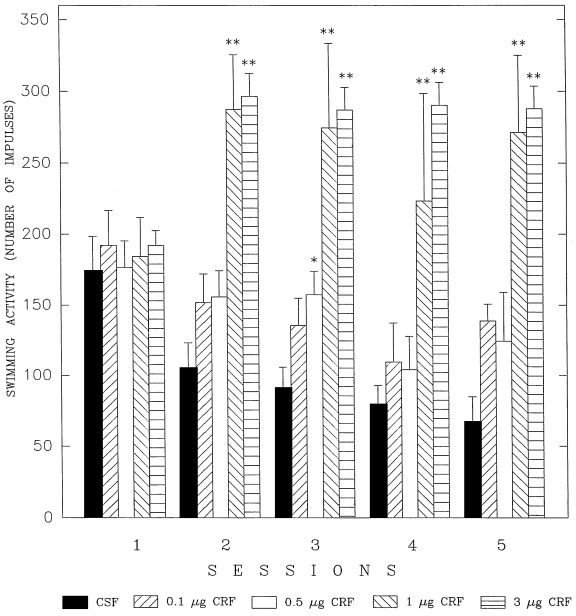

Fig. 1. Effect of different doses of i.c.v. CRF on swimming in the forced swimming test. Data represent the mean swimming (±S.E.M.) in each session, as measured by the number of impulses. The treatment groups were as follows: 0 ($n=6$), 0.1 μg ($n=7$), 0.5 μg ($n=6$), 1 μg ($n=6$) and 3 μg ($n=7$). Fisher's LSD: $^{*}P<0.05$, $^{**}P<0.01$ compared to CSF group.

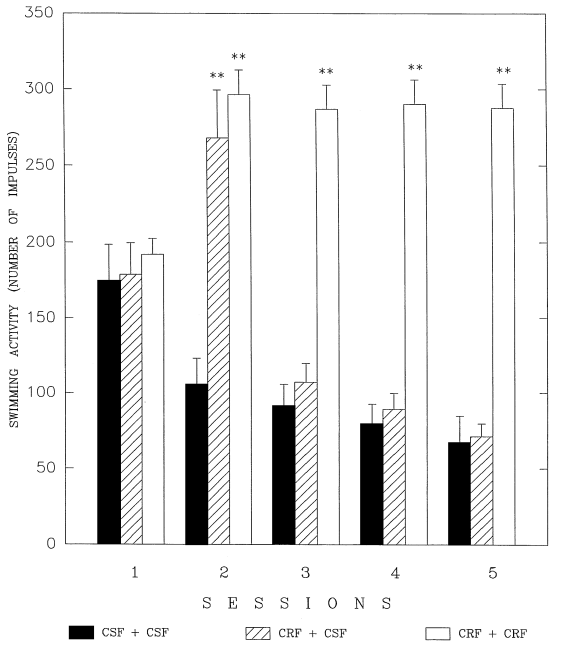

Fig. 2. The effect of i.c.v. administration of 3 μg of CRF on swimming in the forced swimming test is dependent on its daily administration. Data represent the mean swimming (±S.E.M.) in each session, as measured by the number of impulses. The treatment groups were as follows: CSF+CSF group received CSF before each session ($n=6$), CRF+CSF group received 3 μg of CRF before SESSION2 and CSF before the other sessions ($n=7$) and CRF+CRF group received 3 μg of CRF before each session ($n=7$). Fisher's LSD: $^{**}P<0.01$ compared to CSF+CSF group.

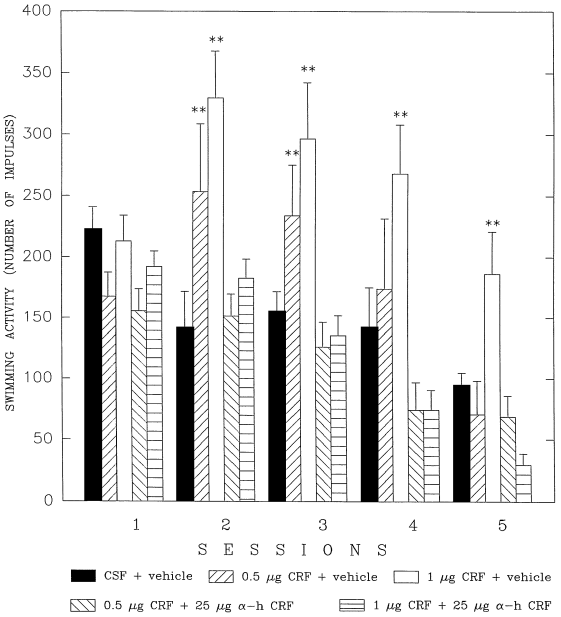

Fig. 3. Reversal of CRF-induced increase in swimming in the forced swimming test by i.c.v. administration of α-helical CRF-(9-41). Data represent the mean swimming (±S.E.M.) in each session, as measured by the number of impulses. The treatment groups were as follows: CSF+vehicle ($n=6$), 0.5 μg CRF+vehicle ($n=5$), 1 μg CRF+vehicle ($n=7$), 0.5 μg CRF+25 μg α-helical CRF-(9-41) ($n=6$) and 1 μg CRF+25 μg α-helical CRF-(9-41) ($n=8$). Fisher's LSD: $^{**}P<0.01$ compared to CSF+vehicle group.

covariance was not used since the covariate was affected by treatment. Post-hoc comparisons of individual means were made by a Student–Newman–Keuls test (SNK).

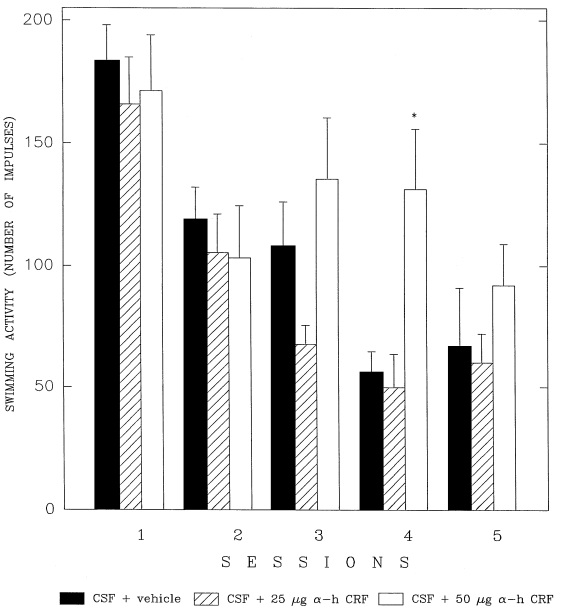

Fig. 4. Effect of i.c.v. administration of α-helical CRF-(9-41) (25 and 50 μg) on swimming in the forced swimming test. Data represent the mean swimming (±S.E.M.), as measured by the number of impulses. The treatment groups were as follows: CSF+vehicle ($n=5$), CSF+25 μg α-helical CRF-(9-41) ($n=6$), CSF+50 μg α-helical CRF-(9-41) ($n=7$). Fisher's LSD: $^{*}P<0.025$ compared to CSF+vehicle group.

3. Results

3.1. Experiment 1

Fig. 1 shows the effect of different doses of i.c.v. CRF on swimming during all sessions of the forced swimming test. Statistical analysis revealed a significant effect of 'treatment' ($F_{5,32} = 14.49$, $P < 0.001$), 'session' ($F_{3,99} = 22.52$, $P < 0.001$) and an interaction between 'treatment' and 'session' ($F_{15,99} = 6.6$, $P < 0.001$). As expected, the control group displayed a lasting decrease in swimming across all sessions in the forced swimming test (Yamada et al., 1989). I.c.v. CRF clearly increased swimming in the

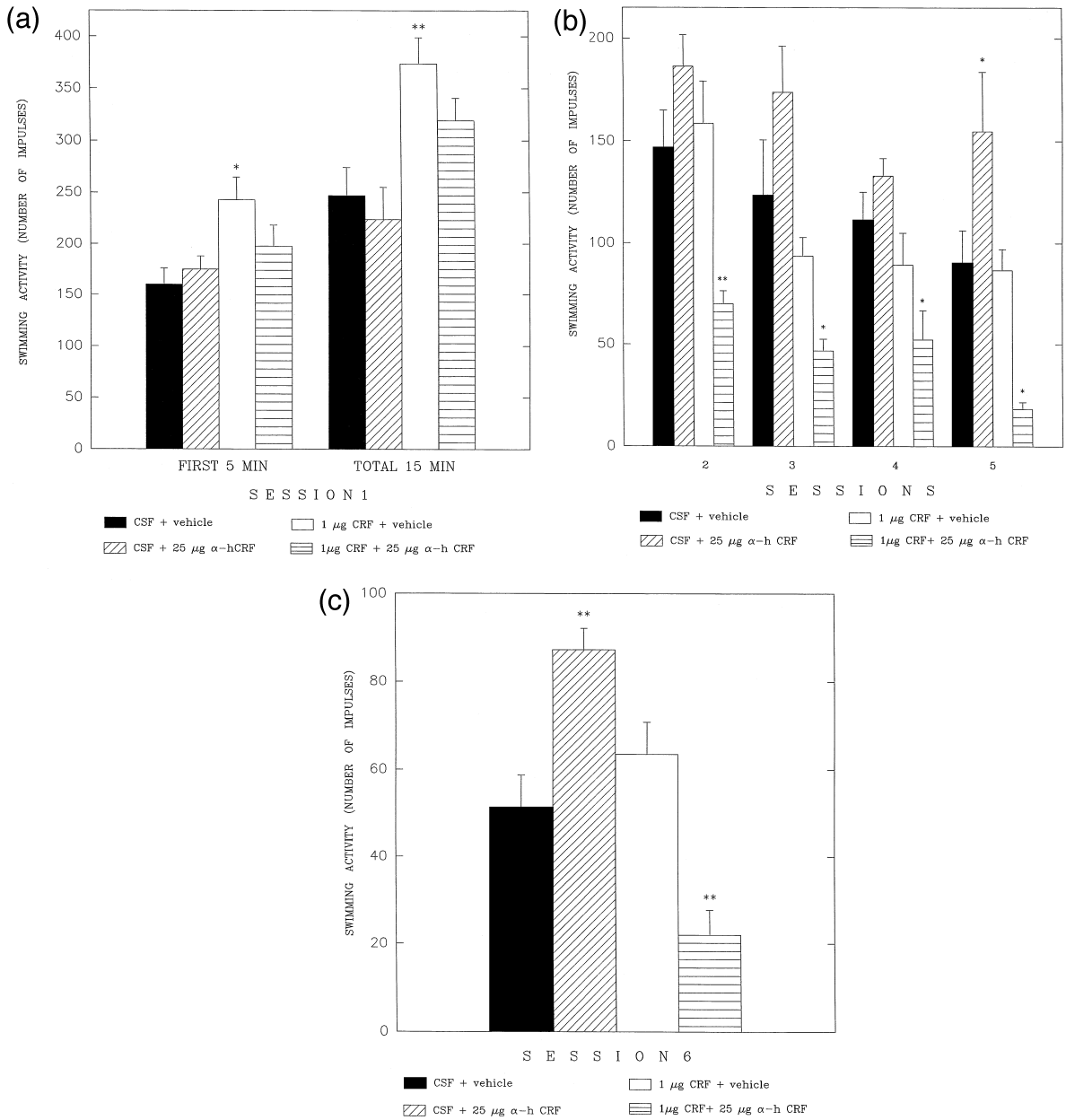

Fig. 5. (a) Effect of i.c.v. CRF and α-helical CRF-(9-41) administered according to a different schedule, on swimming in the first 5 min and during the entire 15 min of SESSION1 in the forced swimming test. Data represent the mean swimming ($\pm$S.E.M.), as measured by the number of impulses. The treatment groups were as follows: CSF + vehicle ($n = 6$), CSF + 25 μg α-helical CRF-(9-41) ($n = 6$), 1 μg CRF + vehicle ($n = 6$) and 1 μg CRF + 25 μg α-helical CRF-(9-41) ($n = 6$). SNK's test: $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to CSF + vehicle group. (b) Effect of i.c.v. CRF and α-helical CRF-(9-41) administered according to a new schedule, on swimming in 5 min of SESSION2 to SESSION5 in the forced swimming test. Data represent the mean swimming ($\pm$S.E.M.), as measured by the number of impulses. The treatment groups were as follows: CSF + vehicle ($n = 6$), CSF + 25 μg α-helical CRF-(9-41) ($n = 6$), 1 μg CRF + vehicle ($n = 6$) and 1 μg CRF + 25 μg α-helical CRF-(9-41) ($n = 6$). SNK's test: $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to CSF + vehicle group. (c) Effect of i.c.v. CRF and α-helical CRF-(9-41) administered according to a new schedule, on swimming in an additional session (SESSION6) in the forced swimming test after twelve days without any treatment. Data represent the mean swimming ($\pm$S.E.M.), as measured by the number of impulses. The treatment groups were as follows: CSF + vehicle ($n = 6$), CSF + 25 μg α-helical CRF-(9-41) ($n = 6$), 1 μg CRF + vehicle ($n = 6$) and 1 μg CRF + 25 μg α-helical CRF-(9-41) ($n = 6$). SNK's test: $^{**}P < 0.01$ compared to CSF + vehicle group.

forced swimming test, but significant differences were only attained with the highest doses of CRF, 1 and 3 μg doses ($P < 0.01$). Significant differences were not found when the additional sixth group (to test a possible long-lasting effect of CRF) was compared to the control group in sessions in which CRF was replaced with CSF (CRF + CSF group) (Fig. 2).

3.2. Experiment 2

Fig. 3 shows the effect of i.c.v. α-helical CRF-(9-41) on the CRF-induced increase in swimming in the forced swimming test. Statistical analysis revealed a significant effect of 'treatment' ($F_{4,26} = 11.49$, $P < 0.001$) and 'session' ($F_{3,81} = 32.9$, $P < 0.001$). The 'treatment' × 'session' interaction was not significant. I.c.v. CRF (1 μg) increased swimming in all sessions of the forced swimming test ($P < 0.01$). The dose of 0.5 μg of CRF was only effective in SESSION2 and SESSION3 ($P < 0.01$). Alpha-helical CRF-(9-41) (25 μg) was able to block the CRF-induced increase in swimming in all sessions of the forced swimming test. In a previous study, we found that a higher dose of CRF receptor antagonist (50 μg) was also able to block the increases in swimming produced by 1 μg of CRF (data not shown).

3.3. Experiment 3

Fig. 4 shows the effect of i.c.v. α-helical CRF-(9-41) alone on swimming in the forced swimming test. Statistical analysis revealed a significant effect of 'treatment' ($F_{2,14} = 4.09$, $P < 0.05$), 'session' ($F_{3,45} = 4.87$, $P < 0.01$) and an interaction between 'treatment' and 'session' ($F_{6,45} = 2.66$, $P < 0.01$). Alpha-helical CRF-(9-41), 25 and 50 μg had no effect on swimming in SESSION2. However, the highest dose of the CRF receptor antagonist (50 μg) significantly increased swimming in SESSION4 ($P < 0.025$). The lowest dose of the CRF receptor antagonist (25 μg) slightly decreased swimming, but the differences were not significant.

3.4. Experiment 4

Fig. 5a shows the effect of i.c.v. administration of CRF and α-helical CRF-(9-41) on swimming in the first 5 min and over the entire 15 min of SESSION1 of the forced swimming test and Fig. 5b shows the effects in the other sessions of forced swimming test. Statistical analysis revealed a significant effect of 'treatment' ($F_{3,20} = 11.89$, $P < 0.001$), 'session' ($F_{5,100} = 54.19$, $P < 0.001$) and a 'treatment' × 'session' interaction ($F_{15,100} = 5.22$, $P < 0.001$). 'Treatment' was also significant for the entire 15 min of SESSION1 ($F_{3,20} = 6.64$, $P < 0.01$). I.c.v. CRF (1 μg) produced an increase in swimming, but only when it was administered before SESSION1. This increase was significant for both the first 5 min ($P < 0.05$) and the entire 15 min of total session ($P < 0.01$) (Fig. 5A). In the other sessions, CRF failed to produce any effect on swimming (Fig. 5b).

Alpha-helical CRF-(9-41) blocked the CRF-induced increase in swimming in SESSION1 in the first 5 min and during the 15 min of the total session (Fig. 5A). However, administration of both peptides produced a significant decrease in swimming in the other sessions ($P < 0.01$ in SESSION2 and $P < 0.05$ in SESSION3, 4 and 5) (Fig. 5B). Alpha-helical CRF-(9-41) alone failed to produce any effect on swimming in SESSION1, but increased swimming across all sessions, reaching a significant effect in SESSION5 ($P < 0.05$) (Fig. 5b).

Data obtained for the additional session (SESSION6), to test the long-term retention of the behavioural adaptation to the forced swimming test, showed an increase in swimming in the group treated with CRF receptor antagonist alone ($P < 0.01$) (Fig. 5c). Subjects treated with CRF and CRF receptor antagonist together showed a significant decrease in swimming ($P < 0.01$) (Fig. 5c). A significant effect of 'session' was found between SESSION5 and SESSION6 ($F_{1,20} = 13$, $P < 0.01$). Thus, all groups showed a decrease in swimming in the last additional session after twelve days without any treatment.

4. Discussion

The present results support a role for i.c.v. CRF in promoting behavioural activation in the forced swimming test. This activating effect produces a dose-dependent increase in swimming depending on the daily presence of the peptide (experiment 1). However, this effect was only shown when CRF was administered before the test, whatever the session (experiment 1 and 4). The most effective doses were 1 and 3 μg, but a low dose (0.5 μg) was also effective (experiment 1 and 2), probably due to a synergistic action between endogenous CRF, released as a result of experimental stress (i.e. handling during a second i.c.v. microinjection) and exogenous CRF, i.c.v. administered. CRF has been shown to be more effective when its i.c.v. administration involved handling of the animals in a social isolation test and this effectiveness was paralleled by increased plasma cortisol levels (Hennessy et al., 1992) and in acoustic startle response (Swerdlow et al., 1986; Liang et al., 1992). In addition, stressful situations, such as single handling of animals, have been reported to increase the delivery of CRF from hypothalamic PVN (Kitayama et al., 1989) and to increase ir-CRF in the median eminence (Murakami et al., 1989) and hypothalamus (Haas and George, 1988). These data also suggest that the level of activation previous to the forced swimming test can be a critical factor that could determine a drug's effectiveness in this test.

The results found in our study are in agreement with those of a previous work reporting an i.c.v. CRF-induced

reduction of immobility in the forced swimming test over a similar range of doses (Butler et al., 1990). Swimming was used as a behavioural parameter in the forced swimming test rather than immobility (Weiss et al., 1982; Plaznik et al., 1985; De Pablo et al., 1989). In this respect, it has been previously demonstrated that there is a highly negative correlation between immobility and swimming (De Pablo et al., 1989). Given the administration schedule used in experiment 1, the results could be interpreted as evidence for an antidepressant-like property of CRF (Porsolt et al., 1977; Porsolt, 1981). However, clinical studies have found elevated levels of CRF in the cerebrospinal-fluid of depressive patients and, in consequence, an increased secretion of CRF has been proposed to be implicated in depressive states (Nemeroff et al., 1984). Therefore, CRF seems to test 'false positive' in the forced swimming test, as do other drugs (Browne, 1979; Wallach and Hedley, 1979; Betin et al., 1982; De Pablo et al., 1989). Alpha-helical CRF-(9-41) has been proposed as an antidepressant drug (Nemeroff, 1988), but data from experiment 3 do not support this suggestion. Thus, doses of 25 and 50 μg of CRF receptor antagonist given according to the administration schedule used to screen substances with antidepressant properties (drug effect on second session is analyzed) failed to produce any change in swimming in the forced swimming test (Porsolt et al., 1977; Borsini and Meli, 1988).

The highest dose of CRF receptor antagonist alone (50 μg) increased swimming (experiment 3). In agreement, previous studies have reported agonist-like activity when α-helical CRF-(9-41) is administered at high doses. Thus, CRF receptor antagonist (50 μg) produces an anxiogenic effect in the elevated plus maze (Baldwin et al., 1991) and possess activating properties, increasing arousal, vigilance and aggressive behaviours, as does CRF (Winslow et al., 1989). In addition, higher doses of α-helical CRF-(9-41) are reported to be less effective in blocking the effect produced by CRF or stress than are lower doses (Berridge and Dunn, 1987; Kalin et al., 1988; Barrett et al., 1989).

CRF has a dose-dependent biphasic effect on rat locomotor activity. Higher doses of CRF (≥ 1 μg) decrease locomotor activity in a novel environment, while lower doses increase it (Sutton et al., 1982; Veldhuis and De Wied, 1984). Thus, the activating properties of CRF found in the present work could be attributed to its effect on locomotor activity. However, in our study high doses of CRF produced an increase and not a decrease in swimming in SESSION1, as would be predicted for animals exposed to a novel environment. The range of CRF doses effective in the forced swimming test is similar to that of doses that increase arousal (Ehlers et al., 1983) and anxiogenic effects (Cole et al., 1987; Dunn and File, 1987). Behavioural activation in the forced swimming test has been reported following infusion of CRF into the Locus Coeruleus without there being an effect on locomotor activity (Butler et al., 1990), suggesting that arousal-related effects could be involved in the CRF-induced behavioural activation in the forced swimming test mediated through the LC, rather than increased locomotor activity. In addition, chronic administration of CRF receptor antagonist (25 and 50 μg) has no effect (Heinrichs et al., 1992) or decreases (Yang et al., 1990) locomotor activity. In our study, both doses of CRF receptor antagonist produced a significant increase in swimming (experiment 3 and 4). These data suggest that, in the forced swimming test, CRF and α-helical CRF-(9-41) could affect other parameters rather than locomotor activity.

Alpha-helical CRF-(9-41) was able to block the CRF-induced increase in swimming when administered i.c.v. before the sessions (experiment 2 and 4). However, a change in administration schedule revealed a different effect: when CRF and CRF receptor antagonist were administered together there was a decrease in swimming (experiment 4). Other authors have found a similar result when both peptides were microinjected together into the central nucleus of the amygdala (Wiersma et al., 1993). This decreased swimming only appeared in those sessions in which the i.c.v. microinjection was administered to the subjects before the test. Therefore, the results of this experiment suggest that both peptides could exert this effect by influencing memory processes. The decrease in swimming produced by both peptides was also found in the last additional session (sixth session). In addition, all groups showed decreased swimming in this session compared to fifth session. These data together suggest an involvement of memory processes in the long-term retention of this behaviour in the forced swimming test.

The progressive increase in immobility in the forced swimming test can be viewed as an adaptative response to the stressful situation (Hawkins et al., 1978; Jefferys et al., 1984; Borsini et al., 1986; De Pablo et al., 1989) which allows the animals to save energy (West, 1990). Thus, the progressive decrease in swimming could be interpreted in a similar way. Indeed, the normal adaptation process to this experimental situation results in a decrease in swimming throughout the consecutive sessions, as could be seen in our control groups. The dramatic decrease in swimming produced by CRF and CRF receptor antagonist could suggest that administration of both peptides together facilitated not only behavioural adaptation to the forced swimming test, but also long-term retention of this behaviour. This effect was not only due to CRF receptor antagonist, because the antagonist produced an opposite effect when it was administered alone (experiment 4). These data could be explained by a differential blockade by the CRF receptor antagonist of some central behavioural effects of CRF. Thus, CRF receptor antagonist could block the anxiogenic effects of CRF in the forced swimming test while at the same time allowing other effects of CRF (i.e. facilitating learning and/or memory processes) which could improve behavioural adaptation to the forced swimming test. Other authors have found that α-helical CRF-(9-41) can block

CRF-induced decreases in food and water intake, but not increases in grooming (Krahn et al., 1986). In the same manner, α-helical CRF-(9-41) blocks CRF-induced increases in locomotor activity, but not increases in vigilance (Winslow et al., 1989). In this respect, it has been shown that marked differences exist in the ability of i.c.v. α-helical CRF-(9-41) to inhibit several biological effects of i.c.v. CRF. Thus, an antagonist:agonist ratio between 6:1 and 12:1 is required to abolish CRF-induced increases in plasma catecholamine levels while a ratio of 3000:1 is required for a total blockade of CRF-induced increases in plasma ACTH and β-endorphin levels (Fisher et al., 1991). These data suggest that these different effects of α-helical CRF-(9-41) could be exerted through different CRF receptor subtypes (Chalmers et al., 1996).

The facilitating role of CRF and α-helical CRF-(9-41) in the behavioural adaptation to the forced swimming test was only seen with the second administration schedule. In this respect, it is known that the effect of a substance on acquisition and/or retention processes (consolidation) depends on the time of administration in relation to the training session (Martínez et al., 1983). Thus, there are substances that have no effect in the forced swimming test when they are administered according to the schedule proposed by Porsolt, but which are effective when the time of administration is changed, i.e. pentobarbital (Kitada et al., 1981; De Pablo et al., 1991), diazepam (De Pablo et al., 1991) and naloxone (Jefferys et al., 1984). In addition, the impaired behavioural adaptation found with anisomycine again supports the involvement of memory processes in behaviour in the forced swimming test (De Pablo et al., 1989).

Data obtained with CRF receptor antagonist alone (experiment 4) suggest that endogenous CRF may participate in the behavioural adaptation to the forced swimming test. Blockade of endogenous CRF, due to i.c.v. CRF receptor antagonist administration, seemed to produce an impairment of the adaptative behaviour to the forced swimming test because the subjects behaved the same in the fifth and first session. An impairment of the retention of this behaviour was also observed in the last additional session (sixth session), suggesting that CRF is not only necessary for a normal behavioural adaptation to forced swimming test, but also for a long-term retention of this behaviour. These data are in agreement with other results showing that the same dose of α-helical CRF-(9-41) impairs acquisition of some learning paradigms like conditioned emotional response (Cole et al., 1987) and schedule-induced-polydipsia (Cole and Koob, 1991).

In summary, the results of our study indicate that the effects of CRF in the forced swimming test are complex (depending on the administration schedule and interaction with experimental stress, at least) and that swimming could be modulated by the level of activation and memory processes. In these processes, endogenous CRF may play a determinant role. Different administration schedules should be considered in pharmacological studies with the forced swimming test to clarify the neurobiological bases underlying the behaviour displayed in this test.

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