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Differential regulation of behavioral, genomic and neuroendocrine responses by CRF infusions in rats

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Abstract

Studies suggest that behavioral, genomic, and endocrine functions mediated by central corticotropin-releasing factor (CRF)-containing circuits may be differentially regulated. However, this hypothesis has never been tested directly by simultaneous assessment of distinct CRF-mediated responses within the same animal. The present study addressed this issue by concurrently examining the effects of central CRF infusions on anxiety responses, plasma corticosterone release, and c-*fos* mRNA induction within limbic brain circuits. Bilateral intracerebroventricular (icv) infusions of CRF (0.1–10 μg total) dose-dependently reduced exploratory behavior in a novel open field, increased circulating corticosterone (CORT) levels and augmented c-*fos* mRNA expression in the central nucleus of the amygdala (CeA) and the hypothalamic paraventricular nucleus (PVN). Plasma CORT levels increased significantly after 0.1 μg CRF, whereas behavioral and genomic responses required at least 1 μg CRF, suggesting that the distinct responses mediated by CRF are differentially regulated. Further characterization of intracerebroventricular CRF at 1 μg also demonstrated a disruption of social interaction behavior. The majority of behavioral effects and the elevated c-*fos* mRNA expression were attenuated by 10 mg/kg DMP696, a CRF_1 antagonist. However, plasma CORT elevation required 30 mg/kg DMP696 for attenuation. Thus, our studies demonstrate a greater sensitivity of the hypothalamic–pituitary–adrenal axis to intracerebroventricular CRF compared with the induction of innate fear-like responses and associated genomic changes.

Keywords: Anxiety; Amygdala; Stress; Social interaction; Corticosterone; c-*fos*

1. Introduction

Corticotropin-releasing factor (CRF) is a 41-amino-acid peptide that plays a central role in coordinating the body's endocrine, immune, autonomic, and behavioral responses to stress (Vale et al., 1981). It has been hypothesized that abnormal CRF secretion or synthesis may be involved in a variety of stress-related psychiatric disorders such as anxiety, depression, eating disorders, and obsessive-compulsive disorder (Stenzel-Poore et al., 1994; Koob and Heinrichs, 1999). In support of this hypothesis, intracerebroventricular administration of CRF has been demonstrated to mimic stress responses, including elevation of circulating corticosterone (CORT) and adrenocorticotrophic hormone (ACTH) levels (Rivier and Vale, 1983), as well as the activation of limbic-associated regions within the brain (Benoit et al., 2000; Bittencourt and Sawchenko, 2000). Furthermore, central infusion of CRF is anxiogenic in a variety of behavioral models in rodents (Sutton et al., 1982; Berridge and Dunn, 1986; Baldwin et al., 1991), and transgenic overexpression results in increased anxiety responses in a battery of innate and conditioned fear paradigms (van Gaalen et al., 2002). However, several studies show that the neuroendocrine responses and behavioral anxiety responses mediated by CRF may be differentially regulated (Cador et al., 1992; Heinrichs et al., 1992; Korte et al., 1994). Consistent with this notion, CRF antagonists reverse stress-induced anxiety-like behavior at doses that do not prevent the stimulation of the HPA axis by the stressor. Additionally, Gupta and Brush (1998) report that central CRF (0.1–1 μg) infusion appears to produce nonlinear

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anxiogenic behavioral responses in a test of conditioned avoidance, but elicits a linear increase in plasma CORT levels, again, indicating a noncausative relationship between the neuroendocrine and behavioral measures. However, to date, no study has directly examined the simultaneous regulation of innate fear-like behaviors and acute neuroendocrine responses induced by central CRF infusions. Additionally, although stress and intracerebroventricular CRF have been demonstrated to induce gene expression in various stress-related circuits (Bittencourt and Sawchenko, 2000), the relationship of these genomic responses with behavioral and endocrine alterations has not been examined within the same animals. Therefore, the objective of the present study was to elucidate the relationship between CRF-induced innate fear-like behaviors, neuroendocrine effects, and genomic changes by concurrent assessment of these end-points within the same animals.

2. Methods

2.1. Animals

Male, Sprague–Dawley rats (Harlan Laboratory, Indianapolis, IN) were used for the generation of the dose-response effects of CRF. These animals weighed 245–255 g at the beginning of the experiments and were housed on a 12-hr light:dark schedule (lights on at 6 a.m.) in a temperature- and humidity-controlled environment with food and water available ad libitum. An additional set of male, Sprague–Dawley rats (Charles River Labs, Kingston, AL), in which simultaneous measures of social anxiety, open-field exploration, circulating CORT levels, and changes in gene expression were performed, weighed between 275 and 300 g at the beginning of the experiments and were housed under the same conditions.

2.2. Surgeries

Animals were allowed to acclimate to the housing facility for a minimum of 3 days prior to surgical procedures. Under ketamine anesthesia, each animal was implanted with 26GA bilateral guide cannula within the lateral ventricle (AP 0.3, ML ± 1.9, DV 3.6, angle 11°) affixed to the skull by three stainless steel screws and dental cement (Plastics One, Roanoke, VA). All coordinates were relative to Bregma (Paxinos and Watson, 1986). The animals were then allowed to recover for 5–7 days before behavioral testing.

2.3. Drugs

Synthetic rat/human CRF (Sigma, St. Louis, MO) was dissolved in artificial cerebrospinal fluid (aCSF, vehicle) consisting of (in mM): 124 NaCl, 2 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose, (pH 7.4). Drug or vehicle was applied in freely moving rats using 33GA infusion cannula (Plastics One), which projected 1 mm beyond the guides, via an automated pump connected by polyethylene (PE 50) tubing. Infusions were performed at the rate of 0.5 μl/min over 2 min. The infusion cannula was left in place for an additional 2 min to help prevent backflow into the guide cannula and facilitate diffusion. All intracerebroventricular drug doses represent total CRF infused, that is, 1 μg is equivalent to 0.5 μg per ventricle. For studies examining the role of CRF_1 receptors, DMP696 or its vehicle (1:2:17, Tween 20:Dimethylsulfoxide:H_2O) was injected intraperitoneally (1 ml/kg) 20 min prior to intracerebroventricular CRF infusion.

2.4. Behavioral testing

All behavioral testing was carried out between 10 a.m. and 2 p.m. For the dose-response effect of CRF, exploratory behavior was measured 10 min postinfusion by introducing the rats to a novel 40 × 40 × 30 cm activity chamber (Accuscan Instruments, Columbus, OH) equipped with two sets of horizontal infrared light grids for automated data collection. A lower grid set at 3 cm from the bottom of the cage was used to measure total distance and center time, while the second grid set at 15 cm from the cage bottom measured rearing behavior. Exploration data were collected for 30 min in 1-min bins using Digiscan software (Accuscan Instruments). Following behavioral testing, the animals were sacrificed to examine the neuroendocrine and genomic responses.

An additional set of animals was tested sequentially in two ethologically valid models of anxiety. First, the animals were tested in a social interaction paradigm, modified from that described by File and Hyde (1978) and Dunn and File (1987). Detailed characterization of the test paradigm used here has been described previously (Hajos-Korcsok et al., 2003). Briefly, the test animal was introduced into a novel 55 × 55 × 30 cm opaque chamber, containing a habituated resident animal, 30 min after intracerebroventricular CRF or aCSF infusion. The behavior of each test animal was assessed by a person blinded to the treatment conditions, for the amount of time spent socializing with the resident rat, as well as the proportion of time spent rearing, grooming, exploring, and freezing over a 5-min period. Evidence of social interaction required that the test animal initiate the encounter with the resident animal and included activities such as licking, sniffing, following, and crawling over/under the resident rat. Following this social interaction paradigm, test animals were immediately placed within the novel open-field activity chambers, where their behavior was monitored for an additional 5 min. These animals were then immediately sacrificed to measure changes in genomic and neuroendocrine indices.

2.5. In situ hybridization and histochemistry

Following behavioral measures, animals were decapitated and their brains rapidly removed, frozen on liquid

nitrogen, and stored at −80 °C prior to use for in situ hybridization. Each brain was cut into 20-μm thick coronal sections on a cryostat, and the cannula placement was histologically verified using a Diff-Quick stain set (Dade Behring, Newark, DE). Only animals with appropriate bilateral cannula placement were utilized. To measure changes in gene expression, the tissue sections were subjected to in situ hybridization using a ^{35}S-UTP-labeled riboprobe generated from a 1352 bp fragment of c-*fos* cDNA inserted into pBluescriptII, as described previously (Curran et al., 1987). In brief, sections were treated with 4% paraformaldehyde, rinsed in 2 × saline sodium citrate (2 × SSC), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, rinsed in 2 × SSC, and dehydrated in a graded series of ethanol. The tissue was then incubated overnight in a hybridization buffer containing 3×10^6 cpm/75 μl of ^{35}S-UTP-labeled cRNA at 55 °C. Following hybridization, the sections were washed in 2 × SSC, treated with RNAse A (20 μg/ml) at 37 °C, and then washed in 1 × SSC followed by washes in 0.2 × SSC at 60 °C. Finally, the sections were dehydrated in a graded series of ethanol and apposed to Kodak autoradiographic film. The films were developed using an automated processor and analyzed by optical densitometry using Inquiry software.

2.6. Radioimmunoassay

Radioimmunoassays for plasma CORT levels (ICN Pharmaceuticals, Orangeburg, NY) were performed according to the manufacture's protocol. In brief, plasma collected from trunk blood at the time of decapitation was diluted 1:200 in an assay buffer and incubated for 2 h in anti-CORT primary antibody with a fixed concentration of ^{125}I-CORT. The samples were then precipitated, centrifuged, and the supernatant discarded. The remaining pellets were examined using a γ-counter, and the levels of CORT were determined from a standard curve.

2.7. Statistical analyses

Statistical analysis was performed using Unistat 5.0. For dose-response studies an overall drug effect was determined by one-way ANOVA followed by Dunnett's post

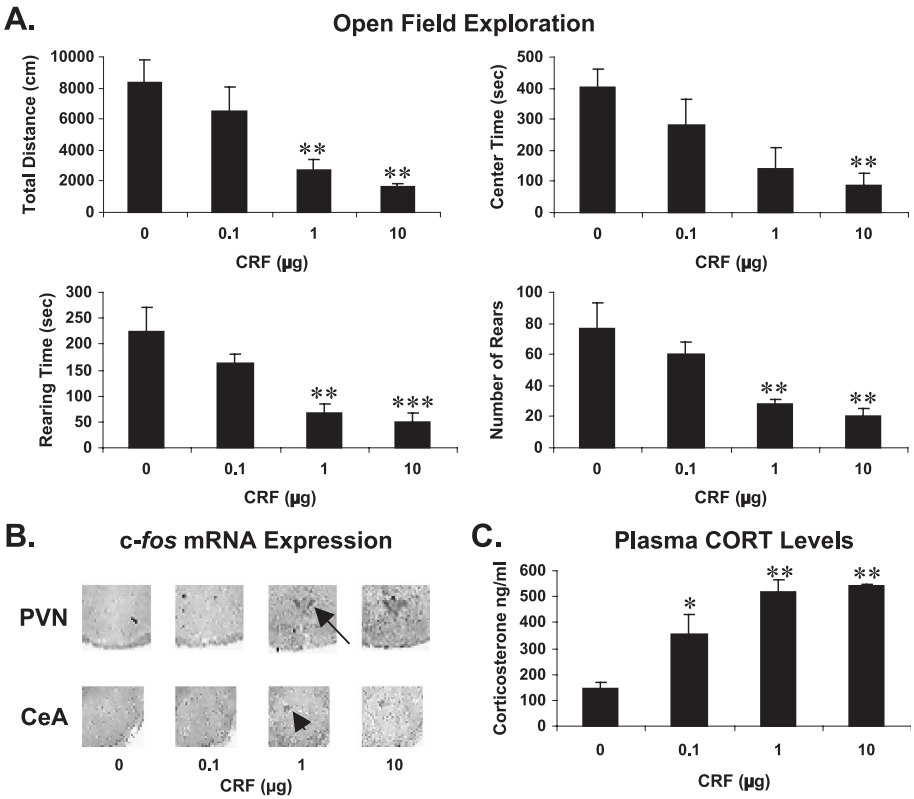

Fig. 1. Effects of intracerebroventricular CRF on open-field behaviors, plasma corticosterone secretion, and c-*fos* mRNA expression. Dose responses to intracerebroventricular-CRF-induced behavioral responses (Panel A), c-*fos* mRNA expression (Panel B), and plasma CORT levels (Panel C) are shown. Exploratory behaviors were monitored in a novel open field for 30 min, starting at 10 min after intracerebroventricular CRF infusions. Animals were sacrificed following the behavioral assessment to examine changes in c-*fos* mRNA expression in the brain and CORT levels in the plasma. Note that the minimum effective dose for CRF-induced reduction in exploratory behaviors (cumulative counts for 30 min) and c-*fos* mRNA induction in the PVN (arrow) and CeA (arrow head) was 1 μg, whereas that for plasma CORT elevation was 0.1 μg. All data represent the mean ± S.E.M. for 4–6 animals/group. * $P < .05$, ** $P < .01$, *** $P < .001$ compared with vehicle-treated animals.

hoc anaylsis to determine between-group differences. For experiments that examined interactions between intracerebroventricular CRF and DMP696, a two-way ANOVA was used, followed by Fisher's LSD post hoc analysis to identify groups differing from each other. The level of significance was set at $P < .05$.

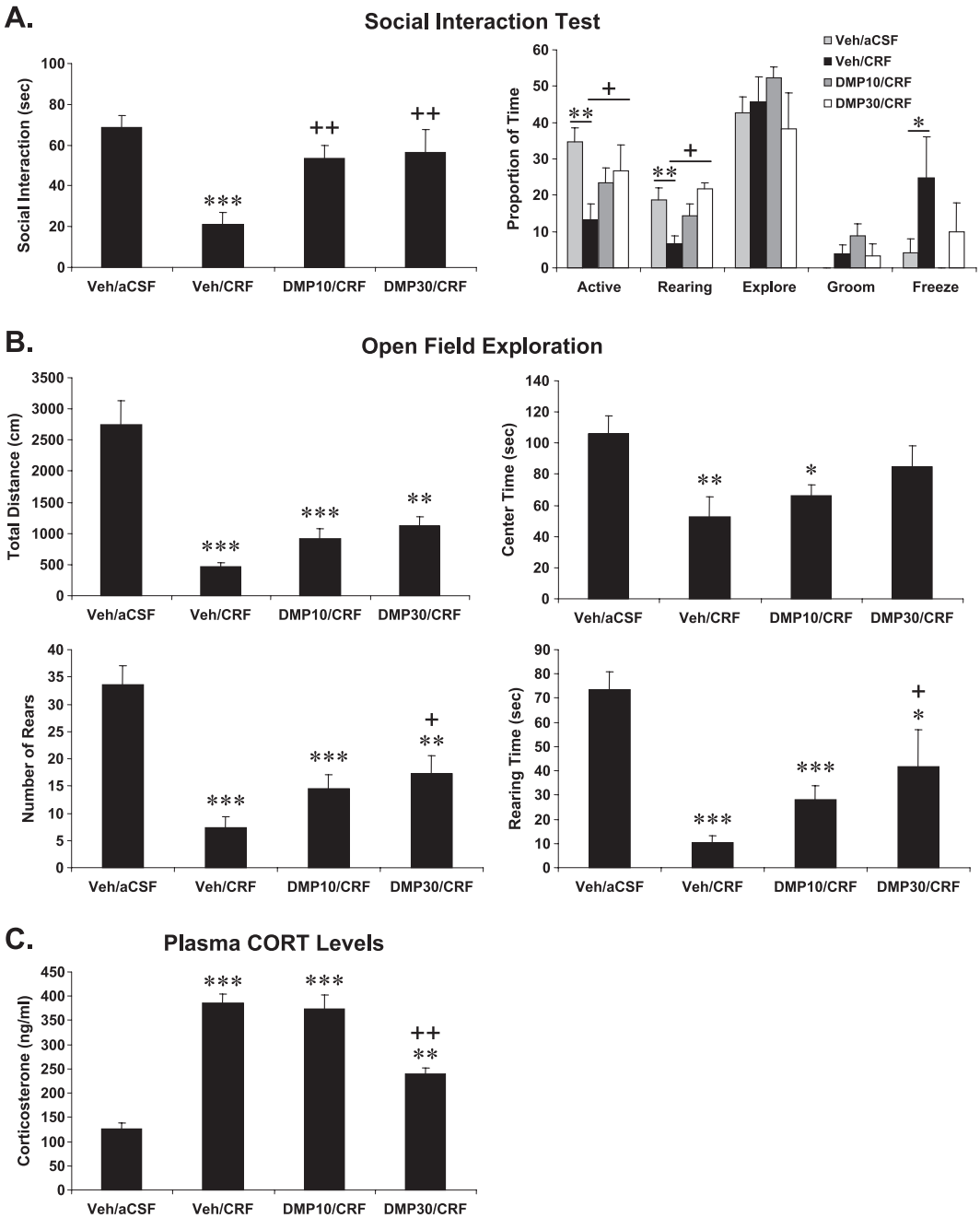

Fig. 2. Effects of DMP696 on 1 μg intracerebroventricular-CRF-induced alterations in social interactions, open-field behaviors, and plasma CORT secretion. At 30 min after intracerebroventricular CRF infusions, animals were tested for 5 min each in the social interaction paradigm, followed by novel open-field exploration, and were then sacrificed immediately to harvest the brain and plasma to evaluate changes in c-*fos* mRNA and CORT levels. Panel A shows the decreases in social interactions in intracerebroventricular CRF-treated animals and complete prevention of this effect by DMP696 at 10 and 30 mg/kg ip. Panel B shows the decreases in exploratory activity (5-min cumulative counts) in a novel open field after intracerebroventricular CRF treatment. DMP696 appeared to attenuate decreases in center time and rearing behaviors without significantly affecting the decreases in total distance traveled in the horizontal plane. Panel C shows the CRF-induced elevation in the plasma CORT level and its modulation by DMP696 and demonstrates that the minimum effective dose of DMP696 is 30 mg/kg, which only partially inhibits CORT secretion induced by CRF. All data represent the mean ± S.E.M. for 6–8 animals/group. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ compared with vehicle-treated animals, $^{+}P < .05$, $^{++}P < .01$ compared with CRF-treated animals.

3. Results

3.1. Dose-response effects of intracerebroventricular CRF on novel open-field exploration, c-fos mRNA induction, and plasma CORT levels

Infusion of CRF into the lateral ventricles resulted in a dose-dependent decrease in open-field exploration (Fig. 1). Total distance traveled, number of rears, and rearing time were all reduced significantly at 1 μg CRF, but not at 0.1 μg CRF. Further increase in the dose to 10 μg CRF did not augment the open-field behavioral responses. In addition, an increase in thigmotaxis, as measured by a decrease in the time spent in the center of the cage, trended toward significance at 1 μg CRF (P=.051), but was statistically significant only at 10 μg CRF.

Changes in c-*fos* mRNA expression were assessed qualitatively to identify brain regions showing robust changes, as well as the dose of CRF producing consistent elevation in c-*fos* mRNA.. On the basis of this study, the dose of 1 μg of CRF and two brain regions were later characterized using quantitative analysis for modulation of c-*fos* induction by a CRF_1 receptor antagonist (see below). An increase in c-*fos* gene expression was observed within the posterior region of the central nucleus of amygdala (CeA) and the hypothalamic paraventricular nucleus (PVN) following intracerebroventricular CRF infusions. These increases mirrored open-field behavioral measures in that they occurred at 1 μg CRF and did not appear to increase at 10 μg. An expression of c-*fos* mRNA was evident also within the cortex of some animals, but this effect was not different between intracerebroventricular CRF- and aCSF-treated animals.

Finally, circulating levels of CORT were measured in plasma samples collected at the end of behavioral experiments. At 40 min postinfusion, CRF significantly elevated circulating CORT levels at the lowest dose tested, 0.1 μg CRF. This response was elevated further at the 1 and 10 μg doses; however, there was no significant difference between the effects of 1 and 10 μg CRF.

3.2. Effects of intracerebroventricular CRF±DMP696 on social interaction, open-field behaviors, and gene expression within stress circuits

Since 1 μg CRF appeared to have the maximal effects on the majority of measures tested above, this dose was characterized further. At 30 min postinfusion, CRF (1 μg) decreased the amount of time that test animals engaged in social behavior in a social interaction paradigm (Fig. 2). In addition, test animals treated with CRF also displayed reduced rearing and increased freezing behavior. Furthermore, occasional bouts of grooming were observed, but the overall exploration of the testing chamber was unaltered. However, it should be noted that the assessment of percent time spent in exploration in the social interaction paradigm included such activities as walking, scratching, and sniffing the corners. In contrast, exploratory activity assessed using the Digiscan automated monitoring system in a novel open-field measures locomotion in the horizontal plane and vertical rearing behavior. Subsequent to the social interac-

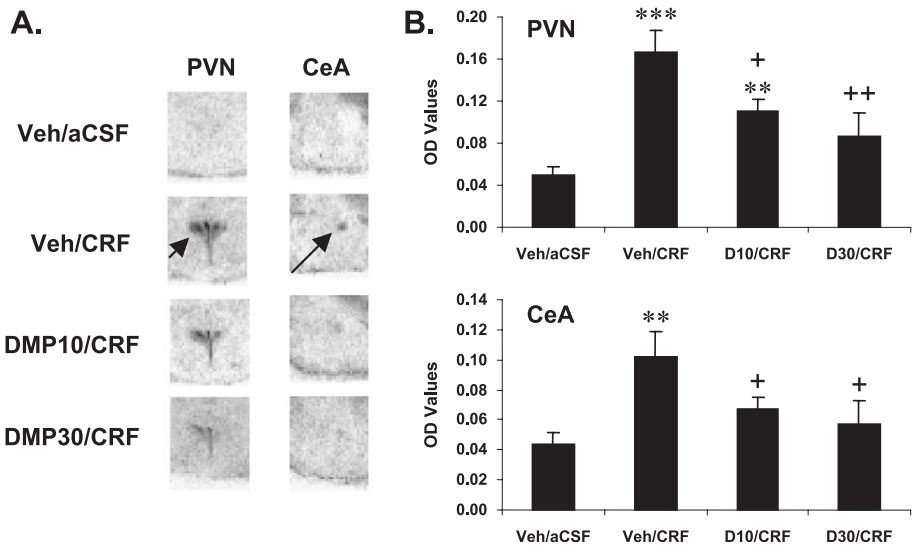

Fig. 3. Effects of DMP696 on c-*fos* mRNA induction produced by 1 μg of intracerebroventricular CRF infusion. Brains harvested from animals immediately after the behavioral tests shown in Fig. 2 were used for the analysis of c-*fos* mRNA levels by in situ hybridization histochemistry. Panel A shows representative photomicrographs depicting the expression of c-*fos* mRNA in a CRF-treated animal and its subsequent inhibition by increasing doses of DMP696 within the PVN (arrow head) and CeA (arrow). Panel B shows the quantitative densitometric analysis of autoradiograms demonstrating significant increases in c-*fos* mRNA expression within the PVN and CeA after 1 μg of intracerebroventricular CRF, and their attenuation by 10 or 30 mg/kg DMP696. All data represent the mean ± S.E.M. for 3–5 animals per group. ** $P<.01$, *** $P<.001$ compared with vehicle-treated animals; $^{+}P<.05$, $^{++}P<.01$ compared with CRF-treated animals.

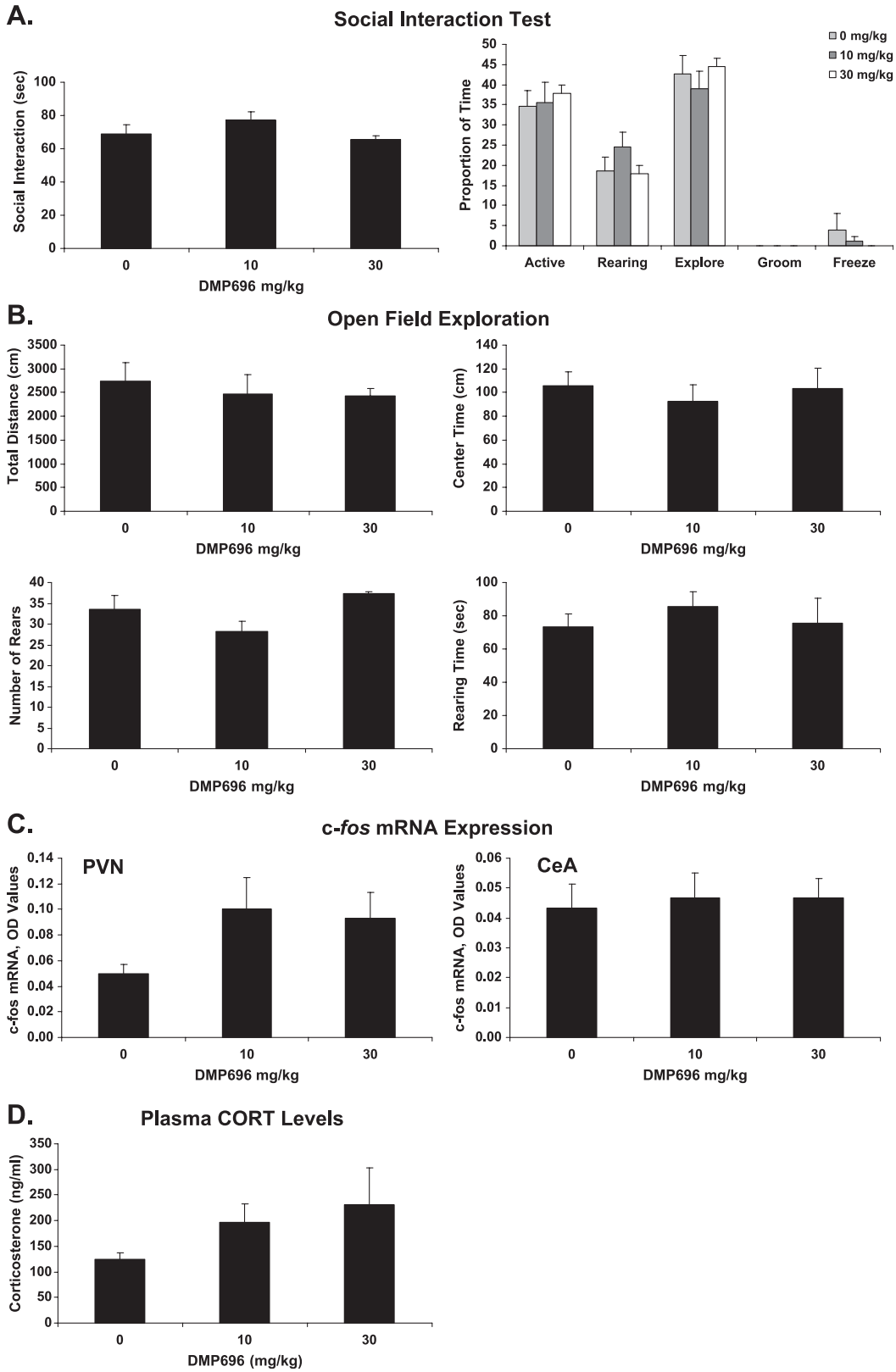

Fig. 4. Effects of DMP696 on social interaction, baseline open field behaviors and plasma CORT secretion. Effects of DMP696 were examined in the study shown above in Figs. 2 and 3, but are shown here separately for ease of data presentation. DMP696 or its vehicle was administered intraperitoneally 20 min prior to aCSF infusions. At 30 min after intracerebroventricular aCSF infusions, animals were tested for 5 min each in the social interaction paradigm, followed by novel open-field exploration and then sacrificed immediately to harvest the brain and plasma to evaluate changes in c-*fos* mRNA and CORT levels. Note that DMP696 failed to alter social interaction (Panel A), exploratory behaviors (Panel B), or c-*fos* mRNA expression (Panel C) in aCSF-treated rats. However, a nearly significant trend to elevate CORT levels was observed (Panel D). All data represent the mean ± S.E.M. for 4–6 animals per group.

tion paradigm, rats tested using the Digiscan system showed that 1 μg intracerebroventricular CRF reduced total distance traveled, rearing time, and number of rears, similar with the animals in the dose-response study (Fig. 1). In addition, a significant decrease in center time was also evident in these animals. Finally, circulating CORT levels were greatly elevated and c-*fos* mRNA induction was observed again within the PVN and CeA (Fig. 3).

The role of CRF_1 receptors in intracerebroventricular CRF-mediated responses described above was evaluated using the CRF_1 selective antagonist, DMP696 (He et al., 2000). As shown in Figs. 2 and 3, behavioral, endocrine and genomic effects induced by 1 μg of intracerebroventricular CRF were either attenuated or prevented by prior systemic application of DMP696. The doses at which this inhibition occurred, however, were test-dependent. As such, deficits in social interaction time were completely prevented by the lowest dose of DMP696 tested, 10 mg/kg. In contrast, a significant attenuation (but not blockade) of CRF-induced decreases in rearing behavior required 30 mg/kg of DMP696. Additionally, this dose did not significantly attenuate CRF-induced decreases in total distance traveled or center time, although a strong trend was noted (P=.07 to .08).

The blockade of intracerebroventricular-CRF-induced c-*fos* mRNA expression within the CeA by DMP696 followed a dose response similar with that seen with behavioral measures in the social interaction paradigm, whereas expression within the PVN was more resistant and, therefore, more consistent with HPA-axis activation and increases in plasma CORT levels. Thus,c-*fos* mRNA induction was blocked in the CeA and significantly attenuated in the PVN by 10 mg/kg DMP696, while complete blockade within the PVN required 30 mg/kg DMP696 (Fig. 3). Concurrently, plasma CORT increases were completely unaffected at 10 mg/kg of DMP696, but were significantly reduced at 30 mg/kg (Fig. 2). Finally, it is important to note that when applied without intracerebroventricular CRF, DMP696 did not alter any of the behavioral measures or c-*fos* mRNA expression (Fig. 4), although there was a trend to increase circulating CORT levels (P=.07).

4. Discussion

In the current study, we examined a range of behavioral, genomic, and neuroendocrine responses elicited by central infusion of CRF within the same animal to determine whether previously postulated differential sensitivity of CRF-induced responses could be observed when assessed simultaneously within the same test subjects. Our findings indicate that a hierarchy of effects occurs as a result of exogenous CRF application. While the induction of c-*fos* mRNA expression occurred within the limbic-associated regions in a dose range consistent with anxiogenic behavioral measures, the dose response for CRF-induced CORT release was shifted leftwards, relative to the behavior and genomic measures. Additionally, CRF-induced decreases in social interactions and c-*fos* mRNA expression were more sensitive to inhibition by antagonism of CRF_1 receptors than circulating CORT levels.

It is important to note the caveat that the differential sensitivity of plasma corticosterone release versus fear-like behaviors to intracerebroventricular CRF may result partly from the availability of CRF at the site of action for these various measures. As such, neuronal circuits containing CRF receptors closer to the ventricular system may appear more sensitive to exogenous CRF application than deeper nuclei. However, prior research using endogenous CRF release has also predicted that behavioral and neuroendocrine effects of centrally released CRF may be functionally independent and differentially regulated. Thus, studies which assessed the effects of CRF antagonists on stress-induced responses show a similar dissociation between the inhibition of behavioral and endocrine responses (Heinrichs et al., 1992; Korte et al., 1994).

In contrast to the neuroendocrine system, the genomic effects of intracerebroventricular CRF were more closely associated with behavioral fear-like responses in the current study. As such, the dose-response relationship for c-*fos* mRNA induction mirrored the behavioral responses (decreases in social interactions and novel open-field exploration), an apparent all-or-nothing response to 1 μg CRF, with no further augmentation in the effects at higher doses. Additionally, it is noteworthy that CRF-induced c-*fos* mRNA induction in the CeA, as well as fear responses assessed in the social anxiety paradigm, were completely blocked by 10 mg/kg of DMP696, whereas it required 30 mg/kg DMP696 to abolish CRF-induced c-*fos* induction in the PVN and elevation in plasma CORT levels. Although a strong correlation appears between c-*fos* gene expression in the CeA and anxiety in the social interaction paradigm on the one hand and c-*fos* mRNA induction in the PVN and plasma CORT elevation on the other hand, it remains to be established whether the regional c-*fos* gene expression and behavioral/endocrine responses are causally related. Furthermore, it should be noted that CRF-induced decreases in rearing behavior and exploratory activity measures, such as center time and total distance traveled, were only partially attenuated even at the higher dose (30 mg/kg) of DMP696. The reasons underlying the apparently greater sensitivity of the social interaction behaviors to CRF_1 antagonism or its relevance to potential clinical efficacy of CRF_1 antagonists remain to be investigated further.

Interestingly, the present study demonstrated c-*fos* mRNA induction within the same brain regions that have been reported previously following an intracerebroventricular CRF infusion in the home cages (Benoit et al., 2000; Bittencourt and Sawchenko, 2000). Since intracerebroventricular CRF administered in a familiar environment does not reduce exploratory activity, as was seen in the present study in a novel open-field environment (Koob and Thatcher-Britton, 1985; Sutton et al., 1982), it is reasonable to

conclude that the gene induction relates to direct CRF receptor activation independent of an interaction with the testing environment.

An additional point to consider with the present results is the possibility that behavioral and genomic responses may occur over a different time course than CORT release. However, ample evidence exists to support the argument that the dissociation between endocrine versus behavioral and genomic responses are not due to the time points selected for assessments. For example, Spina et al. (2002), using a defensive withdrawal paradigm, showed similar anxiogenic responses between 5 and 30 min after intracerebroventricular CRF (1μg) administration to rats using the defensive withdrawal and elevated plus maze paradigms. This finding is supported by our data in that open-field exploration is reduced whether the animals were tested 10 min following intracerebroventricular CRF infusion (Fig. 1) or after a 35 min intermission (Fig. 2). Furthermore, Kovacs and Sawchenko (1996) have shown that the time course for stress-induced changes in c-*fos* mRNA expression and CORT release occur in a similar range, with maximal effects between 30 and 60 min after CRF infusion, time points consistent with the 40-min collection time in the present report. Thus, taken together, the current available literature suggests that the diverse measures reported here should be regulated by CRF within a similar temporal range and that the divergent effects are therefore likely to be mediated by a differential sensitivity of neural systems subserving the different phenomena to CRF receptor activation.

In summary, the current experiments have examined several important aspects of the relationship between anxiety- and stress-related responses within the rodent CNS mediated by CRF receptor activation. We used innate fear behavioral paradigms following intracerebroventricular CRF administration to establish that circulating plasma CORT levels are increased at a dose lower than that required for measurable changes in either acute fear-like responses or c-*fos* mRNA expression within the CeA. These data indicate that the HPA axis is more sensitive to CRF receptor activation than either acute behavioral responses or associated genomic effects. In addition, anxiety-like behaviors and c-*fos* expression in the CeA were induced concurrently by CRF at a similar dose range, and their relationship appears to be independent of an interaction with the testing environment. Thus, by concurrent assessment of CRF- (and, specifically, CRF_1 receptor-) mediated behavior, and neuroendocrine and genomic changes, the current paper unequivocally demonstrates a differential sensitivity of neural systems subserving specific CRF_1-mediated responses. These studies indicate that it is critical to consider and utilize appropriate end-points for the discovery of novel CRF_1 antagonists for treatment of stress-related disorders such as anxiety and depression. Furthermore, they allow us to draw the conclusion that CRF_1 receptor antagonists may effectively reduce acute anxiety-related behaviors without interfering with the 'normal' HPA-axis regulation of the neuroendocrine system by stress.

References

Baldwin HA, Rassnicks SC, Britton KT, Koob GF. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology 1991;103:227–32.

Benoit SC, Thiele TE, Heinrichs SC, Rushing PA, Blake KA, Steeley RJ. Comparison of central administration of corticotropin-releasing hormone and urocortin on food intake, conditioned taste aversion, and c-*fos* expression. Peptides 2000;21:345–51.

Berridge CW, Dunn AJ. Corticotropin-releasing factor elicits naloxone sensitive stress-like alterations in exploratory behavior in mice. Regul Pept 1986;16:83–93.

Bittencourt JC, Sawchenko PE. Do centrally administered neuropeptides access cognate receptors?: An analysis in the central corticotropin-releasing factor system. J Neurosci 2000;20:1142–56.

Cador M, Ahmed SH, Koob GF, Le Moal M, Stinus L. Corticotropin-releasing factor induces a place aversion independent of its neuroendocrine role. Brain Res 1992;597:304–9.

Curran T, Gordon MB, Rubino KL, Sambucceti LC. Isolation and characterization of the c-*fos* (rat) cDNA and analysis of post-translational modification in vitro. Oncogene 1987;2:79–84.

Dunn AJ, File SE. Corticotropin-releasing factor has an anxiogenic action in the social interaction paradigm. Horm Behav 1987;21:193–202.

File SE, Hyde JR. Can social interaction be used to measure anxiety? Br J Pharmacol 1978;62:19–24.

Gupta P, Brush FR. Differential behavioral and endocrinological effects of corticotropin-releasing hormone (CRH) in the Syracuse high- and low-avoidance rats. Horm Behav 1998;34:262–7.

Hajos-Korcsok E, Robinson DD, Yu JH, Fitch CS, Walker E, Merchant KM. Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. Pharmacol Biochem Behav 2003;74:609–14.

He L, Gilligan PJ, Zaczek R, Fitzgerald LW, McElroy J, Shen HS, et al. 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2, 4-dichlorophenyl)-pyrazolo[1,5-a]-1,3,5-triazine: a potent, orally bioavailable CRF(1) receptor antagonist. J Med Chem 2000;43:449–56.

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res 1992;581:190–7.

Koob GF, Heinrichs SC. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res 1999;848:141–52.

Koob GF, Thatcher-Britton K. Stimulant and anxiogenic effects of corticotropin releasing factor. Prog Clin Biol Res 1985;192:499–506.

Korte SM, Korte-Bouws GA, Bohus B, Koob GF. Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats. Physiol Behav 1994;56:115–20.

Kovacs KJ, Sawchenko PE. Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. J Neurosci 1996;16:262–73.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.

Rivier C, Vale W. Influence of the frequency of ovine corticotropin-releasing factor administration on adrenocorticotropin and corticosterone secretion in the rat. Endocrinology 1983;113:1422–6.

Spina MG, Merlo-Pich E, Akwa Y, Balducci C, Basso AM, Zorilla EP, Britton KT, River J, Wale WW, Koob GF. Time-dependent induction of anxiogenic-like effects after central infusion of urocortin or corticotropin-releasing factor in the rat. Psychopharmacol 2002;160:113–21.

Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overpro-

duction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J Neurosci 1994;14:2579–84.
Sutton RE, Koob GF, LeMoal M, River J, Vale W. Corticotropin releasing factor produces behavioural activation in rats. Nature 1982;297:331–3.
Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 1981;213:1394–7.
van Gaalen MM, Stenzel-Poore MP, Holsboer F, Steckler T. Effects of transgenic overproduction of CRH on anxiety-like behaviour. Eur J Neurosci 2002;15:2007–15.