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ORIGINAL INVESTIGATION

Ana M. Basso · Mariarosa Spina · Jean Rivier
Wylie Vale · George F. Koob

Corticotropin-releasing factor antagonist attenuates the "anxiogenic-like" effect in the defensive burying paradigm but not in the elevated plus-maze following chronic cocaine in rats

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Abstract *Rationale*: Chronic cocaine abuse is associated with the development of anxiogenic states in humans. Corticotropin-releasing factor (CRF) is an endogenous neurotropic factor well known to modulate stress responses. It has been postulated that CRF is involved in the neurobiological mechanisms underlying the anxiety and/or stress responses associated with removal of cocaine after chronic administration. *Objective*: The present study investigated the role of endogenous CRF in mediating the "anxiety-like" effect 48 h after the cessation of saline or chronic cocaine treatment in rats, using the defensive burying paradigm and the elevated plus-maze. *Methods*: Rats received daily injections of cocaine (20 mg/kg IP, for 14 consecutive days) or vehicle. Forty-eight hours after the last injection, animals were tested in the plus-maze and then in the defensive burying paradigm. In a second experiment, intracerebroventricular (ICV) cannulae were implanted at the lateral ventricle. Animals were allowed a 1-week period for recovery before starting the chronic drug treatment. The defensive burying testing took place 48 h after cessation of the treatment. The CRF antagonist [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$Me Leu^{37}] r/h CRF$_{(12–41)}$, (also known as D-phe CRF$_{(12–41)}$) (0.04, 0.2 and 1.0 μg/5 μl) was injected 5 min before the 15-min testing. *Results*: An "anxiogenic-like" effect following chronic cocaine treatment was demonstrated with the defensive burying paradigm, but not with the elevated plus-maze. This "anxiety-like" response was attenuated by ICV pretreatment with the CRF antagonist D-Phe CRF$_{(12–41)}$, with the highest dose of the CRF antagonist reversing the observed "anxiogenic-like" response. *Conclusions*: These data suggest that brain CRF may be substantially involved in the development of "anxiety-like" responses related to cocaine withdrawal and could be important for future drug dependence treatments.

Key words Cocaine · Chronic treatment · Withdrawal · Anxiety · Defensive burying paradigm · Elevated plus-maze · Corticotropin-releasing factor · Corticotropin-releasing factor antagonist · D-Phe CRF$_{(12–41)}$

A.M. Basso · M. Spina · G.F. Koob
Department of Neuropharmacology CVN-7,
10550 North Torrey Pines Road,
La Jolla, CA 92037, USA

J. Rivier · W. Vale
Clayton Foundation Laboratories for Peptide Biology,
The Salk Institute, 10010 North Torrey Pines Road,
La Jolla, CA 92037, USA

A.M. Basso (✉)
Universidad Nacional de Cordoba, Departamento de
Farmacologia, Facultad de Ciencias Quimicas, Agencia Postal 4,
C.C. 61, 5000 Cordoba, Argentina
e-mail: ambasso@fcq.unc.edu.ar
Fax: +54-351-4334434

Introduction

Cocaine abuse and dependence continue to constitute major societal problems. Clinical studies show that subjects often manifest major changes in mood and behavior associated with chronic cocaine abuse and dependence (Gawin and Kleber 1986; Weddington et al. 1990). Anxiety, panic attacks, depression and anhedonia are the major symptoms experienced by chronic cocaine abusers following cessation of drug intake (Resnick and Schuyten-Resnick 1977; Aronson and Craig 1986; Gawin and Kleber 1986; Gawin 1991). Severe anxiety and depression may provide part of the negative reinforcement associated with cocaine dependence and may be considered important motivational factors for relapse and maintenance of repetitive cycles of cocaine abuse (Gawin and Kleber 1986; Markou et al. 1992). Associated with behavioral changes, neurochemical and neuroendocrine alterations have

been described in humans as well as in experimental animals (Gawin 1991). However, the neurochemical mechanisms involved in behavioral changes, and the stress and anxiety associated with cocaine withdrawal, are not completely understood.

Several reports have demonstrated that cocaine stimulates the hypothalamic release of corticotropin-releasing factor (CRF) (Rivier and Vale 1987; Calogero et al. 1989), and induces alterations in CRF brain systems (Goeders et al. 1990; Sarnyai et al. 1993; Richter et al. 1995). CRF is a 41-amino acid neuropeptide considered to be an important neurotropic factor for the initiation of behavioral responses to stress (Koob and Bloom 1985; Baldwin et al. 1990; Dunn and Berridge 1990; Koob et al. 1994). CRF is known to have a neuroendocrine role through stress-induced activation of the hypothalamic-pituitary-adrenal axis (HPA) (Vale et al. 1981, 1983; Rivier and Plotsky 1986), and also a neurotransmitter role in the central nervous system (CNS) (Valentino and Wehby 1988; Dunn and Berridge 1990; Owens and Nemeroff 1991; Valentino et al. 1993; Koob et al. 1994). In fact, CRF immunoreactivity and CRF receptors have been reported to be widely distributed within the rat CNS (Swanson et al. 1983; De Souza et al. 1985; De Souza 1987; Perrin et al. 1993). Central administration of CRF induces behavioral and physiological changes that resemble "stress-like" responses (Koob et al. 1994). Thus, administration of CRF increases the spontaneous firing of the locus coeruleus (Valentino et al. 1983; Valentino and Foote 1988), mimics sympathetic activation (Brown et al. 1982), and produces a wide variety of behavioral effects characterized by an increase in arousal and/or "anxiogenic-like" effects (Britton et al. 1982; Sutton et al. 1982; Takahashi et al. 1989; Dunn and Berridge 1990; Koob et al. 1994). Consistent with these actions, central injection of CRF antagonists such as α-helical $CRF_{(9–41)}$ or D-Phe $CRF_{(12–41)}$, antagonize autonomic and behavioral effects induced by CRF or stress exposure (Britton et al. 1986c; Tazi et al. 1987; Kalin et al. 1988; Heinrichs et al. 1992; Korte et al. 1994; Menzaghi et al. 1994). These behavioral effects are centrally mediated and are independent of the HPA axis (Eaves et al. 1985; Britton et al. 1986a,b; Heinrichs et al. 1992).

Chronic cocaine administration has been associated with "anxiety-like" states; however, its effects have not been extensively studied in experimental models. One paradigm used to evaluate anxiety associated with cessation of chronic drug administration has been drug discrimination (Wood and Lal 1987; Emmett-Oglesby et al. 1990). Other studies attempting to evaluate anxiety-like behaviors following cessation of chronic cocaine have used the conflict paradigm (Fontana and Commissaris 1989), the light-dark test box (Costall et al. 1990), the elevated plus-maze (Sarnyai et al. 1995), and the defensive burying paradigm (Harris and Aston-Jones 1993). The burying response is a fundamental, rodent-specific defense reaction to aversive stimulation (Pinel and Treit 1978; Wilkie et al. 1979; Treit et al. 1980). Burying behavior is an active strategy of a rodent to cope with a stressor. In this paradigm, rats shocked once from an electrified probe subsequently push bedding material of the chamber toward or over the stationary probe (Treit et al. 1981). Anxiolytic drugs suppress this behavior in a dose-related fashion, with a potency comparable to those in clinical settings (Treit et al. 1981; Treit 1985a,b), whereas anxiogenic treatments increase this behavior (Treit et al. 1980; Diamant et al. 1992). Another extensively validated model for experimental study of anxiety is the elevated plus-maze. In this paradigm, animals are allowed to explore a novel environment defined by the two elevated open arms and the two elevated closed arms of the maze. The normal exploratory behavior of rodents is in favor of the closed arms with a lower level of open-arm activity. Anxiogenic-like treatments will increase this tendency to stay in the closed arms, while anxiolytic-like treatments, such as drugs that reduce anxiety in humans, will decrease the natural aversion toward the open arms and increase the exploration and activity in the open arms (Pellow et al. 1985; Pellow and File 1986). These paradigms constitute pharmacologically validated models for the study of the neuronal mechanisms associated with anxiety and the behavioral responses to stress.

The hypothesis that activation of extrahypothalamic CRF systems are participating in the behavioral response to stress associated with chronic drug administration also is supported by numerous independent observations, involving CRF systems in the mediation of an "anxiogenic-like" response during drug-withdrawal (File et al. 1989; Baldwin et al. 1991; Merlo-Pich et al. 1995; Rodriguez de Fonseca et al. 1997).

Since CRF may play a critical role in the development of stress and depression, and because anxiety is recognized as an important result of cessation of chronic drug administration, it is possible to postulate that brain CRF modulates the neurobiological mechanisms that underlie the anxiety and/or stress associated with chronic drug administration. Because the anxiety experienced following cessation of chronic cocaine administration is considered one of the major factors involved in drug relapse, the study of the mechanisms mediating this process could provide basic new knowledge critical for treatment of cocaine abuse. The hypothesis that cessation of cocaine administration following a chronic drug treatment produces an anxiogenic-like effect mediated in part by brain CRF was tested in the present study using the defensive burying paradigm and the elevated plus-maze, animal models predictive of drugs and treatments that modulate anxiety in humans.

Materials and methods

Animals

Adult male Wistar rats (Charles River Laboratories, Kingston, NY, USA) weighing 220–250 g at the beginning of the experiments were used in this study. Rats were group-housed (three per cage) in a temperature controlled environment and maintained on a 12-h light/dark cycle (lights on at 6:00 a.m.). Animals had access to standard rat food and water ad libitum.

All animals were allowed at least 1 week of acclimation to the animal facility and they were handled once before the start of the experiments. Experiments were carried out between 8:00 a.m. and 4:00 p.m.

Surgery

Unilateral cannulae aimed at the lateral ventricle were implanted in rats at the beginning of the experiments. Animals were anesthetized with halothane (1–2% v/v in oxygen) and placed in a Kopf stereotaxic frame (Kopf Instruments, Tujunga, Calif., USA) with the tooth bar set at +5.0 mm above the interaural line. A cannula made of 23-gauge stainless steel tubing, 7 mm long, was secured to the surface of the skull by using four stainless steel screws and dental cement and sealed with a wire stylet. The cannula was positioned 1.0 mm above the lateral ventricle at the following coordinates: 0.6 mm posterior to bregma, ±2.0 mm lateral and −3.2 mm below the surface of the skull at the point of entry (Pellegrino et al. 1979). Animals were allowed 1 week for postsurgery recovery; cannula patency then was checked by gravity flow of isotonic saline solution. This procedure also allowed the animals to become familiar with this experimental manipulation.

Drugs

Cocaine hydrochloride (provided by the National Institute on Drug Abuse, Bethesda, Md., USA) was dissolved in an isotonic saline solution (0.9% NaCl) and injected at a dose of 20 mg/kg. Control animals received saline injections. All animals were injected intraperitoneally (IP) with 0.1 ml/100 g body weight. [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$Me Leu^{37}] r/h CRF$_{(12–41)}$ (D-Phe CRF$_{(12–41)}$) (molecular weight 4604) was synthesized and provided by The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, Calif., USA. The peptide was dissolved in distilled water pH = 6.7 and injected at doses ranging from 0 to 1.0 μg/5 μl. Once prepared, the solution was kept in ice and used within 2–3 h.

Intracerebroventricular (ICV) injections

For injections, the stylet was removed from the guide cannula, and an 8.5-mm long, 30-gauge injector was connected to a calibrated polyethylene tube inserted through the guide and placed into the lateral ventricle. After the tube was raised, 5 μl of the solution flowed by gravity over a period of 30–60 s. The injector then was left in the cannula for an additional 30 s to avoid backflow leakage. After completion of the injection, the stylet was replaced. The location of the cannulae was confirmed at the end of the experiment by the injection of methylene blue solution. Rats were deeply anesthetized with pentobarbital and then injected with blue dye by gravity, following the same procedure used for ICV injections. Methylene blue dyes the lateral ventricle where the cannula was placed, and the stain can be observed after removing and slicing the brain. Only those animals with accurate placements were considered for statistical analysis.

Apparatus

Elevated plus-maze

The elevated plus-maze apparatus consisted of four arms made of black Plexiglas (50cm long × 10 cm wide), elevated 50cm above the floor, making the shape of a plus sign. Two arms were open (0.5-cm high edges) while the other two arms were closed (40-cm high walls). Each arm was positioned at a 90 degree angle to the adjacent arm. Behavioral testing was conducted in a quiet and dimmed room providing a constant illumination along the two open arms (1.5–2.0 lux). In order to habituate the animals to the testing room, they were moved to the pre-room (with dim illumination) and kept there for 2 h before starting the experiment. For testing, each animal was placed onto the center of the apparatus facing a closed arm and evaluated for 5-min testing (Pellow et al. 1985). The maze was wiped with a damp sponge and carefully dried between each trial. The behavior during the 5-min test period was monitored and automatically recorded with a computer program through photobeam sensors placed at the entrance of each arm of the maze. The automated recording system used in the laboratory is sensitive to the position of the four paws of the rat, thus avoiding spurious movements of the animal in the center of the maze being registered as an arm entry. This system provided information about the time spent exploring each section of the maze and overall activity, expressed as number of entries onto each arm. Data are presented as the percentage of time spent in the open arms (percentage of time spent in the open arms compared to the total time spent in open and closed arms), and as total number of entries in the open and closed arms. Each animal was tested only once in this paradigm.

Defensive burying paradigm

Testing was performed in a transparent, Plexiglas test chamber (43 × 20 × 20 cm), the floor of which was covered with wood shavings (height 3.5 cm). A removable, electrified Teflon probe (6.5 cm long x 1.0 cm diameter), wrapped with two exposed wires, was placed through a small hole, 2 cm above the bedding material, in the center of one of the longest walls of the cage. The aversive stimulus was generated by a shock generator-scrambler (Staco Energy Products Dayton, Ohio, USA) and delivered through the wires of the shock probe whenever the animal touched with a forepaw or the snout to both wires simultaneously.

Defensive burying paradigm

Habituation sessions

Subjects were placed in the test chamber in groups of two or three (depending on the number of animals in the home cage), for 45–60 min habituation sessions on 2 consecutive days before the testing day. The probe was not present during these habituation sessions. The bedding material was changed and the chamber cleaned between each habituation and testing trial. The first habituation session was done 4 h after the last injection of the chronic treatment.

Test session

During the testing day (48 h after the last injection), animals were placed in the pre-room for habituation, with dim illumination and red lights on for 1 h before starting the experiment. For testing, rats were placed individually in the chamber with the shock probe present. Upon contact with the wire-wrapped probe, usually with

the mouth or forepaws, the rat received a 1.5 mA shock for the duration of contact with the probe (less than 1 s). Only animals that contacted the probe and received the shock were included in the data analysis. Animals received only one shock; after that the probe was de-energized. After shock, animals were removed from the chamber and received the ICV injection either with vehicle or different doses of D-Phe $CRF_{(12–41)}$ (0.04, 0.2 and 1.0 μg/5 μl). Rats were placed in a holding cage for 5 min after the injection and placed back in the testing chamber with the de-energized probe, and the behavior recorded with a videocamera for 15 min. All animals were treated and tested only once.

Behavioral measurements

To avoid disturbance of the animals' behavior, rats were observed with a television monitor by a trained observer in an adjacent room.

The following parameters were evaluated: latency to start burying, duration of burying (time spent pushing and spraying bedding material toward the probe with forepaws and nose) and height of the bedding material at the junction between the probe and the wall at the end of the 15-min test.

Chronic drug treatment

Rats received IP cocaine injections once daily, 20 mg/kg for 14 consecutive days. Control animals received IP injections with an isotonic saline solution. Forty-eight hours after the last saline or cocaine injection, animals were subjected to the plus-maze and defensive burying test in order to evaluate the "anxiogenic-like" effect following chronic cocaine treatment. The cocaine dose used in these experiments, and the time periods of testing following completion of the treatments, was based on previous reports (Harris and Aston-Jones 1993; Sarnyai et al. 1995) and on preliminary studies in this laboratory.

Procedure

Experiment 1

Nineteen rats not submitted to cannula implantation surgery received daily injections of cocaine (20 mg/kg, IP, for 14 consecutive days) or vehicle (saline 1 ml/kg). Forty-eight hours after the last injection, animals were tested in the plus-maze and then in the defensive burying paradigm.

To ensure that the anxiety-like behavior observed with the defensive burying paradigm was an actual consequence of chronic cocaine treatment and not due to an acute cocaine effect from the last cocaine injection, an additional experiment was done. In this way, 14 animals received only one injection of either saline or cocaine (20 mg/kg, IP) and were then tested in the defensive burying paradigm after 48 h. The experimental protocol was the same as described above.

Experiment 2

Ninety-one animals were used in this experiment. ICV cannulae were previously implanted at the lateral ventricle. Animals were allowed a 1-week period for recovery before starting the chronic drug treatment. The defensive burying testing took place 48 h after the treatment. In order to avoid any interference of the CRF antagonist on the aversiveness and/or perception of the stressful stimulus, D-Phe $CRF_{(12–41)}$ was injected after the animal touched the probe and received the electric shock. After the completed ICV injection, animals were placed in a different cage for 5 min and then placed back in the defensive-burying testing cage for 15-min testing.

Data analysis

Data are presented as mean ± SEM. All the experiments were designed according to a between-subject experimental design. Behavioral data for experiment 1 were analyzed by Student's *t*-test. Data for experiment 2 were analyzed using a two-way analysis of variance (ANOVA), 2 × 4 factorial for each parameter, where the factors under consideration were (1) chronic treatment (saline or cocaine) and (2) CRF antagonist acute treatment (0, 0.04, 0.2 and 1.0 μg/5 μl). Significant interactions were followed by comparisons of individual means by the Duncan test post-hoc analysis with a level of significance set at $P < 0.05$.

Results

Experiment 1

No differences were observed between rats with a chronic cocaine history and controls on the elevated plus-maze. Table 1 shows the behavioral effects of the chronic cocaine history in rats tested in the elevated plus-maze 48 h after the chronic cocaine treatment. Comparisons between cocaine- and saline-treated rats indicated no significant differences either in the percentage of time spent in the open arms or in the total number of entries or in the percentage of entries to open arms. Extensive subsequent experiments using the elevated plus-maze failed to reveal any differences in the cocaine- exposed animals at any time point. Thus, this paradigm was not pursued.

Figure 1 shows the anxiogenic-like effect following chronic cocaine treatment as measured by the defensive burying paradigm. Animals submitted to the chronic cocaine treatment (20 mg/kg, IP, daily for 14 days) showed a significant reduction in the latency to begin burying [$t(17) = 6.93$, $P < 0.05$], a significant increase in the total time spent burying [$t\ (17) = 4.53$, $P < 0.05$], and a significantly higher pile of bedding material covering the probe [$t(17) = 4.78$, $P < 0.05$], compared to animals chronically injected with saline (Figs 2A, B and C, respectively). Together, these results suggest an anxiogenic-like response and confirm a previous report demonstrating anxiety-like behavior during cocaine-withdrawal in cocaine-dependent rats (Harris and Aston-Jones 1993).

Table 1 Effects of cessation of chronic cocaine administration on the plus-maze in rats. Animals were submitted to chronic cocaine (20 mg/kg daily, IP) or saline (1 ml/kg, IP) treatment for 14 consecutive days and tested in the elevated plus-maze 48 h after the last injection. Data are presented as the mean ± SEM. Each group contained nine to ten rats. No significant difference was found between the cocaine and saline group

Treatment	% Time spent on open arms	Total number of entries	% Entries to open arms
Saline	52.14 ± 5.16	23.00 ± 2.47	33.99 ± 3.52
Cocaine	47.5 ± 4.07	27.44 ± 2.29	36.77 ± 4.9

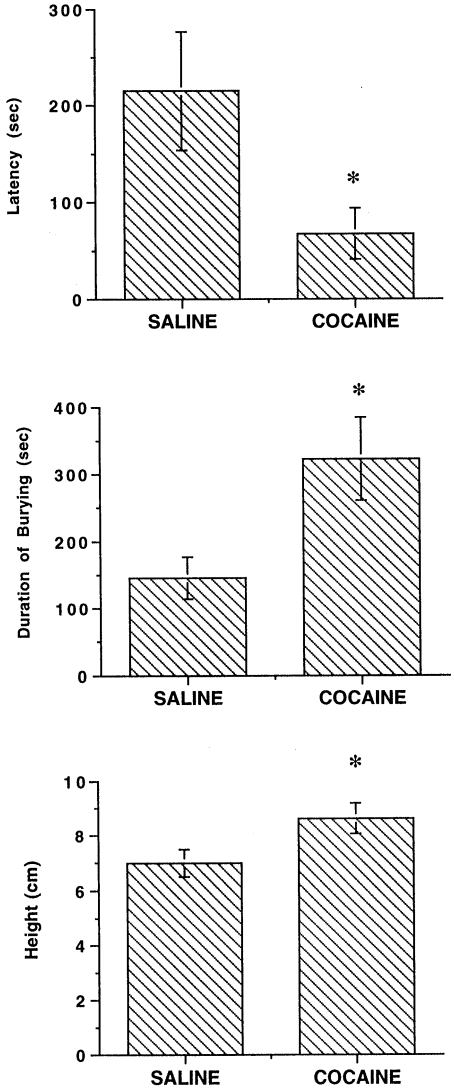

Fig. 1 Behavioral effects of cessation of chronic cocaine administration in rats on the defensive burying paradigm. Rats were submitted to chronic cocaine (20 mg/kg, IP) or saline (1 ml/kg, IP) treatment for 14 consecutive days. Animals then were tested in the defensive burying paradigm 48 h after the last injection. All data are presented as the mean ± SEM. Each group contained nine or ten animals. The *top panel* shows the latency to begin burying expressed in seconds for both experimental groups (*$P < 0.05$ compared to saline group). The *middle panel* represents the total duration of burying, expressed in seconds, during the 15-min testing period (*$P < 0.05$ compared to saline group). The *bottom panel* shows the height of bedding material (cm) at the junction between the probe and the wall of the testing cage at the end of the 15-min session (*$P < 0.05$ compared to saline group)

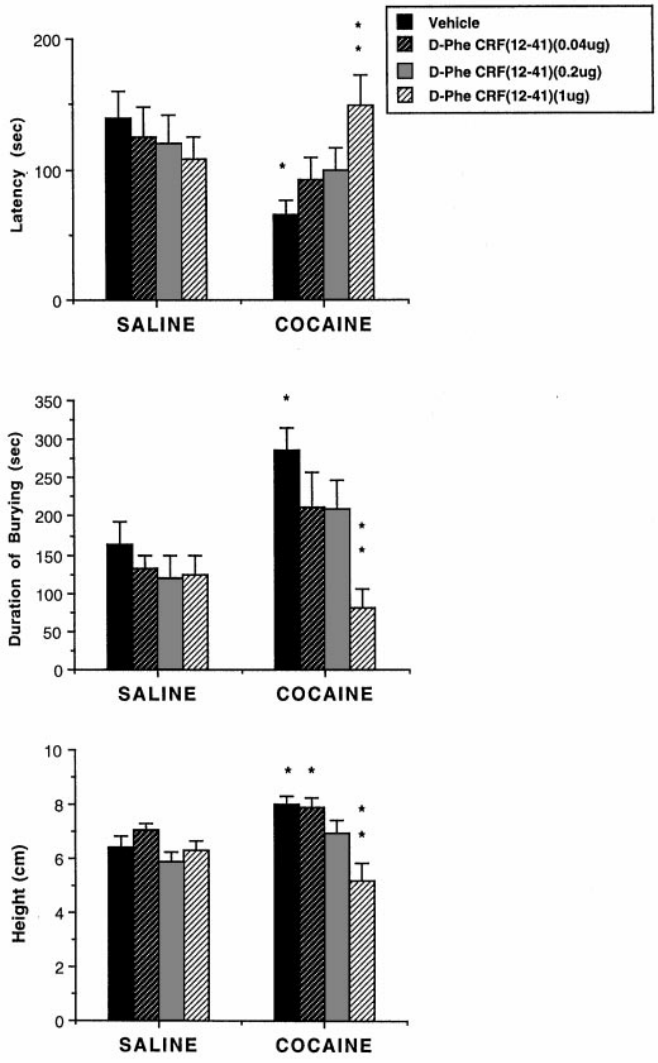

Fig. 2 Effect of ICV administration of the CRF antagonist D-Phe $CRF_{(12–41)}$ on the anxiogenic-like effect following chronic cocaine administration. Rats received chronic cocaine (20 mg/kg, IP, for 14 days) or saline (1 ml/kg, IP) treatment. Animals then were tested in the defensive burying paradigm 48 h after the last injection. The CRF antagonist D-Phe $CRF_{(12–41)}$ (0, 0.04, 0.2 and 1.0 μg/5 μl), was administered ICV immediately after the animal touched the electrified probe and received the shock and 5 min before the testing session. Data are presented as the mean ± SEM. Each group contained 10–14 animals. The *top panel* shows the latency to start burying (in seconds) for all the experimental groups [*$P < 0.05$ compared to saline/vehicle group; **$P < 0.01$ compared to cocaine/vehicle group (Duncan post-hoc analysis)]. The *middle panel* represents the total duration of burying behavior expressed in seconds for all the experimental groups [*$P < 0.05$ compared to chronically saline-treated groups; **$P < 0.01$ compared to cocaine/veh group (Duncan post-hoc analysis)]. The *bottom panel* represents the height of bedding material, expressed in cm, at the junction between the probe and the wall of the testing cage [*$P < 0.05$ compared to saline/veh group; **$P < 0.01$ compared to other chronically cocaine-treated groups (Duncan post-hoc analysis)]

No significant differences were observed in any of the behavioral parameters evaluated in the defensive burying test 48 h after acute administration of either a single injection of cocaine (20 mg/kg, IP) or saline (data not shown). These results indicate that the anxiogenic-like behavior observed in the previous experiment is a consequence of the chronic cocaine treatment (20 mg/kg, IP, daily for 14 days).

Experiment 2

Figure 2 shows the effect of the CRF antagonist D-Phe $CRF_{(12–41)}$ injected ICV in the anxiety-like

behavior induced by a history of chronic cocaine administration in rats. Animals submitted to chronic cocaine treatment and injected ICV with vehicle showed significantly shorter latencies to initiate burying, a significant increase in the total duration of burying, and a significant difference in the height of bedding material compared to animals treated with saline and then injected ICV with vehicle before testing. The CRF-antagonist pretreatment dose-dependently antagonized this anxiogenic-like effect in the chronic cocaine rats. ICV administration of D-Phe $CRF_{(12–41)}$ before testing attenuated the decrease in the latency to begin the burying behavior, the increase in total duration of burying, and the higher amount of bedding material piled over the probe. Although there was a tendency to block these effects with lower doses of the antagonist, only the highest dose of D-Phe $CRF_{(12–41)}$ (1.0 μg/5 μl) was statistically significant compared to chronic cocaine/vehicle (ICV)-treated animals for all the parameters analyzed.

ANOVA for the latency to initiate burying indicated no significant effects for either the chronic treatment factor or the CRF-antagonist treatment factor, but it did indicate a significant chronic treatment × CRF-antagonist treatment interaction [$F(3,83) = 3.00$, $P < 0.05$]. Individual post-hoc comparisons using the Duncan post-hoc analysis indicated a significant decrease in the latency to begin burying in the cocaine/vehicle group compared to saline/vehicle ($P < 0.05$) and cocaine/D-Phe $CRF_{(12–41)}$ 1.0 μg groups ($P < 0.01$).

Figure 2 also shows the effect of chronic cocaine treatment and CRF-antagonist administration on the total duration of burying. A two-way ANOVA revealed a significant effect of the chronic treatment factor [$F(1,83) = 8.01$, $P < 0.01$], a significant CRF-antagonist treatment effect [$F(3,83) = 5.42$, $P < 0.01$] and a significant chronic treatment × CRF-antagonist treatment interaction [$F(3,83) = 2.77$, $P < 0.05$]. The Duncan test for comparison of individual means showed a significant increase in the time spent burying in the cocaine/vehicle group compared to the chronic saline-treated groups ($P < 0.05$) and the cocaine/D-Phe $CRF_{(12–41)}$ 1.0 μg group ($P < 0.01$).

A two-way ANOVA for the height of sawdust covering the probe revealed a statistically significant chronic treatment factor [$F(1,83) = 4.78$, $P < 0.05$], a significant CRF-antagonist treatment effect [$F(3,83) = 8.13$, $P < 0.001$] and a statistically significant interaction between the chronic treatment factor × CRF-antagonist treatment factor [$F(3,83) = 4.39$, $P < 0.01$]. A posteriori comparisons for individual means with the Duncan test analysis indicated a significant increase in the pile of sawdust in the cocaine/vehicle group related to the cocaine/D-Phe $CRF_{(12–41)}$ 1.0 μg group ($P < 0.01$) and the saline/vehicle group ($P < 0.05$). Moreover, the cocaine/D-Phe $CRF_{(12–41)}$ 1.0 μg group was statistically different from all the other chronic cocaine-treated groups ($P < 0.01$).

No statistically significant difference was found between the groups chronically treated with saline and those injected with different doses of CRF antagonist (0, 0.04, 0.2 and 1.0 μg/5 μl) for any of the behavioral parameters analyzed in this study using the defensive burying paradigm.

Discussion

This study provides additional evidence that cessation of repeated administration of cocaine is associated with "anxiogenic-like" behavior, as measured by the defensive burying paradigm, and supports a role for brain CRF systems in the mediation of this particular behavioral stress response associated with chronic cocaine treatment. Rats undergoing cessation of cocaine administration following a chronic cocaine treatment showed a decreased latency to begin burying, and an increased burying behavior compared to saline-treated animals as demonstrated by the defensive burying paradigm, a pharmacologically validated animal model for evaluating drugs that modulate anxiety in humans. Although no evidence of other signs of cocaine withdrawal were observed in these chronically cocaine-treated rats, these findings are indicative of a higher reactivity to an aversive stimulus. Pretreatment with the CRF antagonist D-Phe $CRF_{(12–41)}$ administered ICV antagonized this "anxiogenic-like" behavior, with the highest dose of the CRF antagonist (1.0 μg/5 μl) significantly reversing the increased burying behavior observed in chronic cocaine-treated animals. Lower doses (0.04 and 0.2 μg/5 μl), however, showed a tendency to antagonize this effect. D-Phe $CRF_{(12–41)}$ had no significant effect at any of the doses used in the defensive burying paradigm in animals subjected to chronic saline injections. The present study is consistent with previous reports which found anxiety-like behavior following cessation of chronic cocaine in animals using different paradigms to evaluate fear and anxiety (Wood and Lal 1987; Fontana and Commissaris 1989; Costall et al. 1990; Emmett-Oglesby et al. 1990; Harris and Aston-Jones 1993; Sarnyai et al. 1995). Moreover, this study also provides evidence for the involvement of extrahypothalamic CRF systems in fear- and stress-related processes associated with repeated cocaine administration.

Because α-hel $CRF_{(9–41)}$ has been found to attenuate burying behavior compared to vehicle-treated rats, it has been suggested that endogenous CRF is involved in the expression of defensive burying behavior (Korte et al. 1994). Also, it has been postulated that CRF receptors that modulate defensive burying behavior could be different from CRF receptors that regulate the endocrine response, since the CRF antagonist was able to antagonize behavioral expression but not the enhanced release of plasma ACTH and corticosterone

in stressed animals (Korte et al. 1994). No significant difference was found in the defensive burying paradigm in saline-treated animals administered ICV with any dose of D-Phe $CRF_{(12–41)}$, indicating that the CRF antagonist had no obvious intrinsic "anxiolytic-like" properties in the present study. However, 1.0 μg D-Phe $CRF_{(12–41)}$ did completely antagonize the increased burying behavior observed following chronic cocaine administration. A possible explanation for the difference between our results and those of Korte et al. (1994) may be that the baseline burying behavior was significantly different between the two studies. In the present study, a different experimental procedure was carried out, including daily handling and chronic saline injections, which decreased baseline burying behavior. Consistent with the present findings, other studies also have demonstrated that CRF antagonists α-hel $CRF_{(9–41)}$ and D-Phe $CRF_{(12–41)}$ do not have an intrinsic anxiolytic-like profile when tested in several anxiety tests without a pre-stress shift in baseline (Britton et al. 1986b; Dunn and File 1987; Heinrichs et al. 1992; Menzaghi et al. 1994), but they are effective in reversing the anxiogenic-like effects associated with an increased activity of endogenous CRF brain systems produced by exposure to stressors (Baldwin et al. 1991; Heinrichs et al. 1992; Menzaghi et al. 1994). This latter observation also supports the hypothesis that animals chronically exposed to cocaine present dysregulation or hyperactivity of endogenous brain CRF systems.

Although, an anxiogenic-like effect using the defensive burying paradigm was shown following the cessation of chronic cocaine, an anxiogenic-like response using another widely known test of anxiety, the elevated plus-maze, was not observed. Sarnyai et al. (1995) showed that cessation of chronic cocaine induced anxiogenic-like behavior as measured with the elevated plus-maze. Although these authors used the same protocol for chronic cocaine treatment, discrepancies in the results could be due to different methodologies or different strains of the rats used in both experiments. We have not been able to demonstrate an anxiogenic-like response in the plus-maze in animals undergoing cocaine withdrawal. Negative results were obtained following an extensive study using different cocaine treatments and time courses following the cessation of the drug treatment. The elevated plus-maze is a particularly sensitive test, markedly affected by numerous variables such as animal strain, age, previous handling, environmental conditions, housing of the animals, and aversiveness of the test conditions (Pellow et al. 1985; Rodgers and Cole 1993; Dawson and Tricklebank 1995; Hogg 1996). In addition, complex emotional states such as anxiety cannot be simplistically considered as the expression of only one established behavior. Since the behavior evaluated in both animal models of anxiety involve different behavioral patterns (inhibition of the exploration in the elevated plus-maze and an active behavioral response against the aversive stimulus in the defensive burying), it is possible that the neurobiological substrates that underlie these behavioral responses to stress could be differentially expressed in different animal models of anxiety. Other studies also have shown differences in anxiolytic-like effects or fear-enhanced behavior in the elevated plus-maze compared to the defensive burying paradigm or the fear-potentiated startle paradigm (Korte et al. 1995, 1996). Identical stressor conditions can induce different behavioral patterns on the plus-maze and the social interaction test (Zangrossi and File 1992; Grahn et al. 1995). Moreover, negative or inconsistent results can be elicited by several anxiolytic drugs using only one animal model of anxiety (File 1985). Thus, anxiolytic drugs such as 5-HT_{1A} agonists and 5-HT_3 antagonists showed inconsistencies in anxiolytic-like behavior in experimental models such as the plus-maze and social-interaction test (Chopin and Briley 1987; Griebel 1995). The neurobiological basis that underlies the expression of behavioral responses in these two validated models of anxiety is still unclear. Dissociation of the anti-fear effects with two pharmacologically validated models of rat anxiety – the elevated plus-maze and the defensive burying paradigm – have been described using septum and amygdaloid lesions (Treit et al. 1993). Lesions of the posterior septum, but not amygdaloid lesions, induced an anxiolytic-like effect in the plus-maze and defensive burying paradigm. Amygdaloid lesions also suppressed fear-potentiated startle (Davis 1992), and increased the number of contacts with the electrified probe, both anxiolytic-like effects not observed with septal lesions (Treit et al. 1993). In addition, a recent study indicated that the anxiolytic properties of 5-HT_{1A} agonists into limbic structures also can be also test-specific and differentially mediated by septum and hippocampus (Menard and Treit 1997). Thus, 8 OH-DPAT when injected in the hippocampus induced an anxiolytic-like behavior as evaluated in the plus-maze but not in the defensive burying paradigms. On the other hand, intraseptal injection of the 5-HT_{1A} agonist produced anxiolytic-like behavior in the defensive burying paradigm but not in the plus-maze (Menard and Treit 1997), indicating that anxiolytic properties of drugs also can be test-specific and differentially mediated by brain structures. Other evidence that lends-support to this hypothesis is the dissociation between distinct amygdaloid nuclei in the mediation of different types of fear-conditioned behavior (Killcross et al. 1997), suggesting the role of different neural subsystems in the integration of emotional responses. These results provide impetus for further neurobiological studies that would focus on the role of different brain areas in the expression of anxiety- and/or fear-related behaviors. Together, these results suggest that activation of CRF brain systems after chronic cocaine treatment could involve specific brain areas that specifically regulate different behavioral responses associated with stress.

It could be argued that the increased burying behavior induced by chronic cocaine administration may be due to the increased aversiveness of a single shock (i.e., an altered threshold for a nociceptive stimulus). This interpretation is unlikely, since identical threshold analgesia responses were observed in animals submitted to the tail flick test (water temperature 52°C), regardless of the animals' exposure to chronic cocaine or chronic saline treatment when tested 48 h after the last injection [saline-treated rats (s) = 3.00 ± 0.258; cocaine-treated rats (s) = 3.06 ± 0.274]. Moreover, the possibility that conditioned-aversion to the test chamber during habituation might contribute to the response observed during the test session does not account, since animals undergoing cocaine withdrawal but which are not shocked do not show the burying behavior against the probe or similar behavior without it.

It is well established that cocaine stimulates the activity of the sympathetic nervous system, which is also activated during the stress response. The relationship between cocaine and anxiety is suggested partially because of the cocaine-induced secretion of adrenocorticotropin hormone (ACTH) through a CRF-mediated mechanism in vivo (Rivier and Vale 1987). Cocaine also stimulates the hypothalamic release of CRF in vitro (Calogero et al. 1989). Moreover, chronic cocaine administration results in decreased CRF receptor labeling in mesolimbic/mesocortical dopaminergic system (Goeders et al. 1990) and sensitizes the cocaine-stimulated increases in extracellular levels of CRF from the amygdala (Richter et al. 1995), a brain region well known to play a significant role in mediating behavioral and emotional consequences of stress. Thus, it is possible to hypothesize that extrahypothalamic CRF systems may mediate the anxiogenic-like response observed in rats with a history of chronic cocaine treatment. However, further experiments will be necessary in order to determine the specific brain areas involved in the increased activity of CRF systems associated with an anxiogenic-like behavior caused by a history of chronic drug administration.

In summary, this study has demonstrated that the stress response associated with chronic drug administration could be mediated by hyperactivity of endogenous CRF brain systems. Antagonism of CRF receptors with the CRF antagonist D-Phe $CRF_{(12–41)}$ was able to block the anxiety-like response following the cessation of chronic cocaine administration. However, the involvement of other neurotransmitter systems in this process cannot be ruled out. These results are consistent with clinical evidence indicating that cessation of chronic cocaine abuse is associated with behavioral disturbances such as severe anxiety in cocaine addicts (Gawin and Kleber 1986; Gawin 1991) and suggests that brain CRF systems may have an important role in the development and expression of drug dependence.

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