Psychopharmacology (1998) 137 : 184–190

ORIGINAL INVESTIGATION

Yavin Shaham · Suzanne Erb · Shirley Leung
Yvona Buczek · Jane Stewart

CP-154,526, a selective, non-peptide antagonist of the corticotropin-releasing factor$_1$ receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats

Received: 12 July 1997/Final version: 20 October 1997

Abstract We have found that peptide antagonists of corticotropin-releasing factor (CRF) receptors attenuate reinstatement of heroin and cocaine seeking induced by footshock. Here we examined the effect of a non-peptide, selective CRF_1 receptor antagonist, CP-154,526, on reinstatement of heroin and cocaine seeking induced by footshock. Rats were trained to self-administer heroin or cocaine (0.1 and 1.0 mg/kg per infusion, IV, respectively) for 9–12 days. Extinction sessions were given for up to 14 days, during which saline was substituted for the drugs. Tests for reinstatement were then conducted after exposure to intermittent footshock (10 or 15 min, 0.5 mA). The footshock stressor reliably reinstated extinguished cocaine- and heroin-taking behavior. Pretreatment with CP-154,526 (15 and 30 mg/kg, SC) significantly attenuated the reinstatement effect of the stressor in both heroin- and cocaine-trained rats. CP-154,526, administered in the absence of the footshock stressor, did not affect extinguished drug seeking. In addition, in a separate experiment, CP-154,526 was shown not to alter high rates of lever pressing for a 10% sucrose solution, suggesting that the suppression of lever pressing in stress-induced reinstatement is not caused by a performance deficit. These results extend previous reports on the role of CRF in reinstatement of drug seeking induced by stressors. The present data also suggest that, to the extent that exposure to environmental stressors provoke relapse to drug use in humans, systemically effective CRF receptor antagonists may be of use in the treatment of relapse to drug use.

Key words Cocaine · Corticotropin-releasing factor · CRF receptor · Drug self-administration · Heroin · Reinstatement · Relapse · Stress

Y. Shaham (✉) · S. Leung · Y. Buczek
Biobehavioral Research Department,
Addiction Research Foundation, 33 Russell Street,
Toronto, Ontario, Canada M5S 2S1

Y. Shaham
Department of Psychology, University of Toronto,
Toronto, Ontario, Canada
Fax: +1-416-595-6922, e-mail: yshaham@arf.org

S. Erb · J. Stewart
Center for Studies in Behavioral Neurobiology,
Department of Psychology, Concordia University,
Montreal, Quebec, Canada

Introduction

Relapse to drug use after prolonged periods of abstinence remains a threat to individuals with a history of cocaine and heroin use (Jaffe 1990). Previous studies indicate that a "taste" of the drug itself increases craving for cocaine and heroin and provokes relapse to drug use in drug-free individuals (Meyer and Mirin 1979; Jaffe et al. 1989). Similarly, in laboratory animals trained to press a lever for heroin or cocaine, reexposure to the self-administered drug reliably reinstates drug seeking after periods of extinction (e.g., Gerber and Stretch 1975; de Wit and Stewart 1981, 1983; see Carroll and Comer 1996 and de Wit 1996 for reviews).

It is obvious, however, that conditions other than reexposure to a drug contribute to relapse; drug seeking in humans is antecedent to drug exposure. One factor thought to contribute to relapse in humans is exposure to stress (see Whitehead 1974; Shiffman and Wills 1985; Kosten et al. 1986). Recently, we and others have shown using a reinstatement procedure that in rats, brief exposure to a footshock stressor is a powerful stimulus for reinstatement of cocaine or heroin seeking after extended drug-free periods (Shaham and Stewart 1995; Erb et al. 1996; Ahmed and Koob 1997). The reinstatement procedure is regarded as a valid preclinical model of relapse (Stewart and de Wit 1987; de Wit 1996).

In subsequent studies, we have examined the role of corticotropin-releasing factor (CRF) in stress-induced relapse. CRF is one of the primary neuropeptides involved in coordination of the stress response (Vale et al. 1981). A large body of evidence indicates that CRF has effects on behaviors that are independent of its effects on the hypothalamic-pituitary-adrenal (HPA) axis (see Dunn and Berridge 1990; Menzhagi et al. 1993). CRF-containing neurons and receptors are found throughout the brain (Joseph and Knigge 1983; Swanson et al. 1983; de Souza 1985; see De Souza 1995 and Behan et al. 1996 for reviews). The evidence for widespread anatomical distribution of the CRF system, together with observations of abnormal functioning of the CRF system in depression, anxiety and anorexia nervosa, has led to the suggestion that CRF plays a role in psychiatric disorders (De Souza 1995; Nemeroff 1996).

We have found that in animals trained to self-administer heroin, footshock stress-induced, but not heroin-induced, reinstatement is attenuated by intracerebroventricular (ICV) injections of a peptide corticotropin-releasing factor (CRF) receptor antagonist, alpha-helical CRF. We have also found that the effect of the stressor on reinstatement is partially mimicked by ICV injections of CRF, itself. In contrast, inhibition of corticosterone release and synthesis does not prevent the reinstatement induced by the footshock stressor (Shaham et al. 1997). Similarly, in cocaine-trained animals, we have found recently that footshock-induced, but not by cocaine-induced, reinstatement is blocked by ICV injections of the newer peptide CRF receptor antagonist, *d*-Phe CRF (Erb et al. 1997). These findings made it appear that centrally acting CRF plays a unique role in stress-induced relapse to both cocaine and heroin seeking after an extended period of extinction and when animals have been drug-free for up to 2 weeks.

Though of theoretical importance, these findings are of limited applicability because of the fact that the CRF receptor antagonists available until now are large peptide molecules and had to be administered intraventricularly. Recently, however, there have been reports of a potent, selective, and behaviorally effective non-peptide antagonist of the CRF_1 receptor subtype, CP-154,526 (Chen et al. 1997), that can be administered systemically. This compound has been shown to inhibit the CRF-induced cell firing of noradrenergic neurons in the locus coeruleus, to block CRF-induced ACTH release, to block anxiogenic responses as measured by the acoustic startle and the elevated plus-maze procedures, and to exert antidepressant-like effects in the learned helplessness procedure (Lundkvist et al. 1996; Schulz et al. 1996; Mansbach et al. 1997).

We now report that CP-154,526 given systemically attenuates stress-induced reinstatement of drug seeking in both cocaine- and heroin-trained rats. Rats trained to self-administer either cocaine or heroin IV were given up to 14 days of extinction during which the drug was unavailable. Tests for footshock-induced reinstatement of drug seeking were given over the next several days in which animals were tested after subcutaneous (SC) injections of either CP-154,526 or vehicle. In addition, we tested the effect of CP-154,526 on high rates of lever pressing for a sucrose solution, in order to rule out the possibility that the drug attenuates stress-induced reinstatement by causing performance deficits.

Materials and methods

Experiment 1: reinstatement

Subjects

Ten male Long-Evans rats (Charles River, Quebec; 375–450 g) served as subjects for the tests for reinstatement of cocaine seeking and 11 male Long-Evans rats (Charles River, Quebec; 300–400 g) served as subjects for the tests for reinstatement of heroin seeking. The animals were housed in the colony room for 1–3 weeks before surgery and were allowed to recover for 1–2 weeks after surgery. The rats were then transferred to the operant chambers, where they were housed permanently for the duration of the experiment on a reverse light-dark cycle (lights on 9:00 p.m.–9:00 a.m. or 10:00 p.m.–10:00 a.m. for cocaine-trained and heroin-trained rats, respectively) with free access to food and water. For animals tested for reinstatement of heroin seeking, food was removed from the self-administration chambers during the 3-h test sessions. The experiment with heroin-trained rats was carried out at the ARF and the experiment with cocaine-trained rats was carried out at Concordia University.

Surgery

The animals were surgically implanted with IV silastic catheters (Dow Corning, Midland, Mich., USA) in the right jugular vein under anesthesia: a mixture of xylazine (Haver, Etobicoke, Ontario, Canada; 10 mg/kg, IP) and ketamine HCl (Vetrepharm, London, Ontario, Canada; 100 mg/kg, IP) for heroin-trained rats or sodium pentobarbital (MTC Pharmaceutical, Cambridge, Ontario, Canada; 65 mg/kg, IP) for cocaine-trained rats. Atropine sulfate (MTC Pharmaceutical; 0.6 mg/ml; 0.3 ml/animal) and Penicillin B (Wyeth-Ayerst, Montreal, Canada; 300000 IU; 0.2 ml/animal) were given at the time of surgery. Buprenorphine (0.01 mg/kg, SC) was given after the surgery to the rats in the experiment with heroin (the ACC regulations at the ARF require post-operative analgesia with buprenorphine). The catheter was secured to the vein with a silk suture and passed subcutaneously to the top of the skull, where it exited into a connector (a modified 22 gauge cannula; Plastics One, Roanoke, Va., USA) mounted to the skull with jewellers' screws and dental cement. The catheters were flushed every 24–48 h with about 0.05 ml of a saline-heparin solution (5 or 15 (IU)/ml; heparin obtained from ICN Biochemical, Cleveland, Ohio, USA).

Apparatus

The operant chambers had two levers located 9 cm above the floor, but only one lever (an active, retractable lever; Med Associates) activated the infusion pump (Razel Sci., Stamford, Conn., USA). Presses on the other lever (an inactive, stationary lever) were recorded, but did not activate the infusion pump. A given drug dose

was infused at a volume of 0.13 ml during a 20-s period. During the infusion, a light above the active lever was lit for 20 s. Lever presses during those 20-s timeout periods were counted, but did not lead to further infusions. Throughout the experiment, each session began with the introduction of the retractable lever into the cage and the illumination of the white light above the lever for 30 s. A red house light was turned on for the entire session. The grid floors of the chambers were connected to electric shock generators (Grason-Stadler, West Concord, Mass. or Med Associates, Georgia, Vt., USA).

Drugs

Cocaine HCl was obtained from BDH Chemicals (Toronto, Ontario, Canada) and diacetylmorphine HCl (heroin) was obtained from National Institute for Drug Abuse, USA; both drugs were dissolved in physiological saline. CP-154,526, butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo [2,3-d] pyrimidin-4-yl]-ethyl-amine (generously provided by Pfizer, Groton, Conn., USA), was dissolved in distilled water containing 0.1% methylcellulose (pH adjusted to 5.5–6.5). CP-154,526 (15 or 30 mg/kg) or the vehicle was injected SC at a volume of 2 ml/kg. These doses are based on previous reports (Schulz et al. 1996; Mansbach et al. 1997).

Procedure

The experiments were run in three phases: self-administration training, extinction, and testing for reinstatement.

Training

Cocaine self-administration. Rats were trained to self-administer cocaine (1.0 mg/kg per infusion, IV) on a fixed-ratio-1 schedule of reinforcement during single daily sessions, 3–4 h after lights off. Animals were given between ten and 14 training sessions; training conditions continued until stable responding (20% or less of variation from the mean) was maintained over at least eight consecutive sessions. No priming injections of cocaine were given at the start of the training sessions.

Heroin self-administration. Rats were trained to self-administer heroin (0.1 mg/kg per infusion, IV) for 9 days. Each day was divided into three 3-h sessions separated by 3 h. The first session of each day started at the beginning of the dark period. Under these conditions, stable drug-taking behavior (20 % or less of variation from the mean) is obtained after 4–5 days. No priming injections of heroin were given at the start of the training sessions. The training conditions for heroin and cocaine were different because our attempts to apply the training procedure used for heroin-trained rats to cocaine-trained rats were not successful. That is, cocaine-trained rats did not maintain good health with multiple sessions per day and did not initiate drug taking without priming injections of cocaine when the sessions started at the beginning of the dark cycle.

Extinction

Cocaine-trained rats. During extinction the conditions remained the same as in training, except that saline was substituted for cocaine and animals were allowed to self-administer during one to two 3-h sessions each day (when more than one extinction session was given per day, the two sessions were separated by 2 h). These extinction conditions remained in place until the rats made fewer than 15 responses (saline infusions + timeout responses) on the active lever in a 3-h session. In subsequent sessions, SC injections of the vehicle were given before each session to habituate the animals to the injections until a baseline criterion level of responding of ten or fewer responses (saline infusions + timeout responses) in a 3-h session was reached. The total number of extinction sessions ranged from 6 to 26 over 4–14 days. When an animal reached the criterion level of responding, testing for reinstatement began for that animal.

Heroin-trained rats. Extinction conditions were similar to those described for cocaine-trained animals except that three 3-h self-administration sessions were given each day for 5 days. Subsequently, the extinction sessions were given once per day for 1–2 additional days. During these days, the animals were given a vehicle injection before the start of the sessions until a baseline criterion of less than 15 responses (saline infusions + timeout responses) on the active lever was reached.

Testing for reinstatement

Both cocaine- and heroin-trained rats received six tests for reinstatement on 6 consecutive days during a single 3-h session. The vehicle alone condition was given first and the other five conditions (vehicle pretreatment + footshock, 15 mg/kg CP-154,526 + footshock, 30 mg/kg CP + footshock, 15 mg/kg CP alone, 30 mg/kg CP alone) were given in a counterbalanced order (i.e., the rats were exposed to different sequences of the test conditions). For footshock tests, cocaine- and heroin-trained rats received 10 or 15 min of footshock (0.5 mA, 0.5 s on, with a mean off period of 40 s), respectively, immediately before the start of the test session. The footshock parameters are based on our previous studies. Within-subject design was employed because we have found in our previous work little or no habituation to the effect of footshock on reinstatement when it is given repeatedly over several daily test sessions (Shaham and Stewart 1996; Shaham et al. 1997). Vehicle or CP was injected 30–40 min before exposure to the footshock or no footshock test conditions. During the test sessions, lever pressing continued to result in IV infusions of saline.

Experiment 2: sucrose self-administration

Subjects

Nine male Long-Evans rats (Charles River, Quebec, Canada, 300–350 g) served as the subjects. The animals were housed in the colony room under a reverse light-dark cycle (lights on 7:00 p.m.–7:00 a.m.). The rats were maintained on 20 g food per day with free access to water.

Apparatus

The self-administration system consists of operant chambers that were constructed by the technical staff of the ARF. Each chamber is equipped with two levers, symmetrically centered on the side panel. Responding on one lever (an active lever) activates the infusion pump (Razel Sci. Stanford, Conn., USA) which results in delivery of 0.18 ml of a 10% sucrose solution to a liquid drop receptacle located between the two levers over a period of 5 s. During the infusion, a stimulus light above the active lever is turned on for 5 s. Lever presses during this timeout period are recorded but do not lead to further infusions. Presses on the other lever (an inactive lever) are recorded but do not activate the pump.

Procedure

The training sessions were conducted during the dark cycle and rats were given their daily food ration after the daily sessions. Training

started after a 24-h period of food deprivation for 60 min per day (FR-1 schedule; 5-s timeout) for 6 days and then for 3 days for 30 min per day. Subsequently, the session duration was decreased to 20 min and the timeout period was increased to 20 s in order to prevent the rats from emptying the 20 ml sucrose syringes. Tests for the effect of CP-154,526 (15 and 30 mg/kg, SC; 45 min before the session) started after 3 days of a stable baseline (20% or less of variation from the mean) following vehicle injections. During the next 2 days, the rats were injected, in a counterbalanced order, with the two doses of CP-154,526, and lever pressing for sucrose was determined.

Statistical analyses

The data for cocaine- and heroin-trained rats were analyzed separately. The main dependent measures for the tests for reinstatement were the number of responses on the active lever (saline infusions + timeout responses) and the number of responses on the inactive lever at each hour of testing. Separate analyses were conducted for responding on each lever. CP-154,526 (0, 15, 30 mg/kg), Test condition (footshock, no footshock) and Hour (hour of testing) were the within-subject factors. When appropriate, post hoc differences between the various experimental conditions were analyzed by contrasts (SAS, General Linear Model) comparing the vehicle condition to the drug conditions. Data from the sucrose study were analyzed with a repeated measures ANOVA. Significant differences are reported for P values of less than 0.05.

Results

Experiment 1: reinstatement

Reinstatement of cocaine seeking

During training the rats self-administered approximately six or seven infusions of 1.0 mg/kg cocaine per hour. The mean (±SEM) number of infusions made in the 3-h session on the last day of training was 19.7±1.9. On day 1 of extinction, the total number of responses in 3 h on the active lever (saline infusions + timeout responses) was 41.7 ± 8.8 (20.6 ± 2.1 infusions); by the end of extinction in the test with vehicle + no shock, responding was extinguished to 6.6 ± 1.3 responses (4.6 ± 1.1 infusions). The number of responses on the inactive lever on day 1 of extinction and in the vehicle + no shock test condition were 4.7 ± 1.5 and 1.5 ± 0.9, respectively.

The mean number of responses made on the active lever during the 3-h tests for reinstatement are shown in Fig. 1a under vehicle and CP-154,526 pretreatment in the footshock and the no footshock tests. Fig. 2a shows the mean number of responses made on the active lever in each hour in tests for footshock-induced reinstatement. Exposure to intermittent footshock reinstated cocaine seeking, an effect that was due to increased responding in the first hour of testing. More importantly, this effect of footshock was attenuated by the CRF receptor antagonist. The statistical analysis revealed significant effects of CP dose [$F(2,18) = 4.7$, $P < 0.03$], Test condition [$F(1,9) = 11.2$, $P < 0.01$],

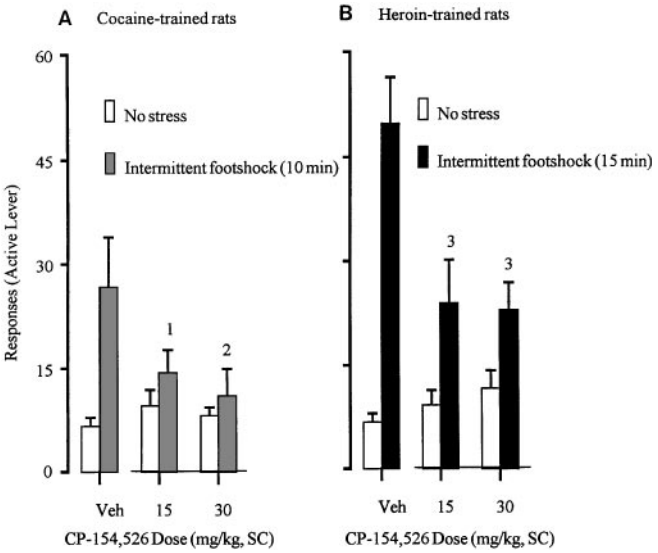

Fig. 1 **A** Mean (± SEM) number of responses (infusions + timeout responses) on the previously active lever in the 3 h after exposure to 10 min of intermittent footshock stress or no stress in rats previously trained to self-administered cocaine ($n = 10$). **B** Mean number of responses on the previously active lever in the 3 h after exposure to 15 min of intermittent footshock stress or no stress in rats previously trained to self-administer heroin ($n = 11$). Rats were pretreated with vehicle or CP-154,526 (15 and 30 mg/kg, SC) 30–40 min prior to the exposure to the footshock stress. Lever presses resulted in saline infusions during the tests. [1]Marginally significantly different from the vehicle condition, $P < 0.06$. [2]Significantly different from the vehicle condition, $P < 0.05$. [3]Significantly different from the vehicle condition, $P < 0.01$

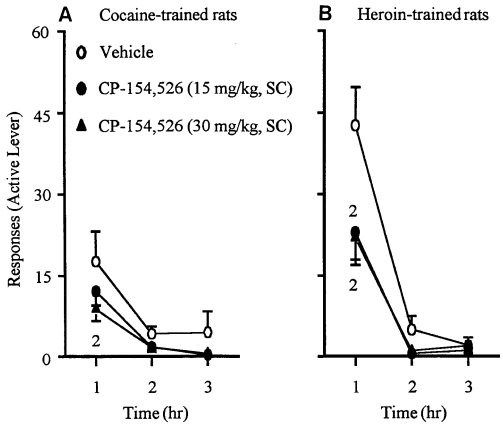

Fig. 2 **A** Mean (± SEM) number of responses (infusion + timeout responses) on the previously active lever in each hour after exposure to 10 min of footshock stress in rats previously trained to self-administer cocaine ($n = 10$). **B** Mean number of responses on the previously active lever in each hr after exposure to 15 min of footshock stress in rats previously trained to self-administer heroin ($n = 11$). Rats were pretreated with vehicle or CP-154,526 (15 and 30 mg/kg, SC) 30–40 min prior to the exposure to the footshock stress. Lever presses resulted in saline infusions during the tests. [2]Significantly different from the vehicle condition, $P < 0.05$

CP dose by Test condition [$F(2,18) = 19.3$, $P < 0.01$], and CP dose by Time [$F(2,18) = 11.9$, $P < 0.01$]. Post hoc analyses, comparing each dose CP-154,426 with the vehicle condition, on total responses on the active

lever after exposure to footshock revealed a significant effect at the high dose ($P < 0.05$) and a marginally significant effect at the low dose ($P < 0.06$, see Fig. 1). The CRF receptor antagonist did not alter responding on the inactive lever [$F(2,18) = 1.1$, NS] and, regardless of the test condition, responses on the inactive lever did not exceed six responses per 3 h. In addition, the number of responses on the active lever in the no stress condition after injections of the CRF receptor antagonist did not differ from that after injections of the vehicle (see Fig. 1a). Differences between conditions as determined by post hoc tests are indicated in Figs. 1a and 2a.

Reinstatement of heroin seeking

During training, the rats self-administered approximately three or four infusions per hour of 0.1 mg/kg per infusion of heroin. On the last day of training, the mean number of infusions in 3 h was 9.4 ± 0.7. On day 1 of extinction, the total number of responses in 3 h on the active lever (saline infusions + timeout responses) was 172.7 ± 41.4 (32.9 ± 4.8 infusions); by the end of extinction, in the test with vehicle + no shock, responding was extinguished to 6.7 ± 1.3 responses (4.2 ± 0.7 infusions). The number of responses on the inactive lever in day 1 of extinction and in the vehicle + no shock test condition were 1.3 ± 0.4 and 0.6 ± 0.4, respectively.

Figure 1b shows the mean number of responses made on the active lever during the 3-h tests for reinstatement under vehicle and CP-154,526 pretreatment conditions and in the footshock and no footshock tests. Figure 2b shows the mean number of responses made on the active lever in each hour of the tests for reinstatement after exposure to footshock. Exposure to intermittent footshock reinstated heroin seeking. It can be seen that most of the responding occurred in the first hr of testing. This effect of footshock was attenuated by the CRF receptor antagonist at both doses. The statistical analysis revealed significant effects of CP dose [$F(2,20) = 7.5$, $P < 0.01$], Test condition [$F(1, 10) = 19.7$, $P < 0.01$], CP dose by Test condition [$F(2, 20) = 19.3$, $P < 0.01$] Test condition by Hour [$F(2, 20) = 37.1$, $P < 0.01$], and Antagonist dose by test condition by Hour [$F(4,40) = 4.5$, $P < 0.01$]. The CRF receptor antagonist did not alter responding on the inactive lever [$F(2,20) = 2.4$, NS] and, regardless of the test condition, the number of responses on the inactive lever did not exceed four per 3 h. In addition, injections of the CRF receptor antagonist did not alter the number of responses on the active lever in the no stress condition compared with the vehicle condition (see Fig. 1b). Differences between conditions as determined by post hoc tests are indicated in Figs. 1b and 2b.

Experiment 2: sucrose self-administration

Rats maintained high rates of responding for the 10% sucrose solution. The mean number of reinforcers earned and total responses (reinforcers earned + timeout responses) during the last three 20-min sessions in which the vehicle injections were given were 36.9 ± 2.9 and 136.6 ± 21.4, respectively. Pretreatment with 15 and 30 mg/kg CP-154,526, however, did not alter the response rates for sucrose. For the 15 mg/kg dose the mean number of reinforcers earned and total number of responses (reinforcers earned + timeout responses) were 37.1 ± 2.7 and 112.4 ± 19.7, respectively; for the 30 mg/kg dose they were 39.6 ± 2.9 and 129.8 ± 18.6, respectively. No significant effects of drug pretreatment were observed for either the number of reinforcers earned or the total responses [$F(2,16) = 1.2$, NS, and $F(2,16) = 1.1$, NS, respectively].

Discussion

The main finding of the present report is that CP-154,526, a systemically active, non-peptide CRF_1 receptor antagonist, attenuated footshock-induced reinstatement in both heroin- and cocaine-experienced rats. The CRF receptor antagonist attenuated stress-induced reinstatement at doses that did not affect baseline responding (no stress condition) and at doses that in a separate experiment did not alter high rates of operant responding for a sucrose solution. Together, these findings suggest that CP-154,526 had a specific pharmacological effect on the behavioral response to the footshock stressor in drug-trained animals. These data are in agreement with our recent reports of attenuation of footshock-induced reinstatement of heroin and cocaine seeking by peptide CRF receptor antagonists injected ICV (Erb et al. 1997; Shaham et al. 1997).

The peptide CRF receptor antagonists used in our previous studies, *d*-Phe-CRF and alpha-helical-CRF, have similar affinities (in the nanomolar range) for the two cloned CRF receptors, CRF_1 and CRF_2 (Behan et al. 1996). In contrast, CP-154,526 binds selectively to the CRF_1 receptor subtype at nanomolar concentrations (Schulz et al. 1996; Chen et al. 1997). A comparison between the data in the present study and those in our previous studies shows that the peptide CRF receptor antagonists and CP-154,526 have similar behavioral effects on reinstatement of cocaine and heroin seeking induced by footshock. CP-154,526 was as effective as the peptide antagonists in attenuating drug seeking induced by footshock stress, despite the fact that it is pharmacologically selective for the CRF_1 receptor subtype. That is, in heroin-trained rats we found that both alpha-helical CRF, ICV (Shaham et al. 1997) and CP-154,526 (present report) caused about a 50% reduction in the number of responses

induced by footshock in tests for reinstatement. In both cases, footshock-induced responding after pretreatment with the CRF receptor antagonists remained significantly higher than in the no shock condition, suggesting the involvement of other neurotransmitters in stress-induced relapse. In cocaine-trained rats, we found that both *d*-Phe CRF (Erb et al. 1997) and CP-154,526 (present report) attenuated footshock-induced responding to levels that were not significantly different than in the no shock condition. It should be pointed out that although these observations suggest that CRF_1, but not CRF_2, receptors are involved in stress-induced reinstatement, a study with selective CRF_2 receptor antagonists (that are not yet available) is needed to confirm this possibility.

It should be pointed out that our data on the role of CRF in footshock-induced resinstatement and stress-induced reinstatement in general were obtained with high training doses of heroin and cocaine that engender relatively low rates of responding under the fixed-ratio 1 schedule used in our studies. It is likely, however, that the results obtained generalize to different training conditions with lower unit doses and different schedules. Studies using the reinstatement procedure indicate that the effect of priming injections of drugs on reinstatement is not dependent on the training dose (see Comer et al. 1995) and is obtained under different schedules of reinforcement with different drug classes (see Stewart and de Wit 1987; de Wit 1996). It is likely that a similar conclusion will be eventually reached concerning the effect of footshock stress on reinstatement. Ahmed and Koob (1997) found that footshock, at parameters similar to the ones used in our studies, reinstate cocaine-taking behavior previously maintained on a training dose of 0.25 mg/kg which is 4 times lower than the one used in our studies. Lê et al. (1998) found that footshock potently reinstates alcohol-taking behavior that previously engendered high rate of responding under an FR-3 schedule.

Recent reports indicate that CRF is involved in anxiogenic and aversive symptoms of withdrawal from several drugs of abuse, including alcohol (Menzaghi et al. 1994), opioid drugs (Heinrichs et al. 1995) and cocaine (Sarnyai 1995). These effects of CRF are probably related to the extra-hypothalamic effects of CRF in limbic structures such as the amygdala (see Heinrichs et al. 1995; Merlo Pich et al. 1995; and Koob 1996 for a review). These data on the role of CRF in withdrawal from drugs, together with our data on the involvement of CRF in reinstatement of drug seeking induced by stressors, suggest that brain CRF may be involved in processes underlying excessive drug use. Furthermore, it would appear that CRF does not affect mechanisms underlying the reinforcing effects of drugs directly. The CRF receptor antagonists, alpha-helical CRF and *d*-Phe-CRF, do not attenuate the priming effect of heroin and cocaine (Erb et al. 1997; Shaham et al. 1997). In addition, a recent study found that *d*-Phe-CRF (0.04–1.5 μg, ICV) did not alter stable IV cocaine self-administration maintained on a unit dose of 0.25 mg/kg on an FR-5 schedule of reinforcement (Ahmed et al. 1996).

It is important to note however, that although changes in CRF activity occur during withdrawal (see Menzaghi et al. 1994; Heinrichs et al. 1995; Sarnyai 1995), their relevance to relapse to drug use induced by stress after prolonged drug-free periods is not obvious. There is no evidence that increased activity of the CRF system seen after withdrawal from drugs persists for more than 24–48 h. A recent study reported that behavioral anxiety exhibited 48 h after withdrawal from cocaine was accompanied by reduction in tissue levels of CRF-IR in hypothalamus, amygdala and basal forebrain, considered to reflect greater release of CRF (Sarnyai et al. 1995). In other studies, however, in which aspects of the CRF system were examined after longer periods of withdrawal, no changes were found. Ambrosio et al. (1997) reported changes in CRF_1 receptor binding in the basolateral amygdala immediately after the last injection of cocaine, but not after 10 days of withdrawal. In a study of CRF mRNA in the brain after a "binge" pattern of cocaine administration, increases were seen in the hypothalamus and the amygdala on the first 2 days of cocaine administration, but these levels returned to baseline after 10 days of withdrawal (Zhou et al. 1996). It should be noted, however, that there are no studies on the reactivity of the CRF system to stressors after prolonged drug-free periods. We suggest that the reactivity of the CRF system may change as a consequence of chronic exposure to drugs, even if there are no changes in basal measures.

Acknowledgements This study was supported by grants from the National Institute of Drug Abuse, the Medical Research Council of Canada, Fonds pour la Formation de Chercheurs et l'Aide la Recherche (FCAR, Quebec) and a non-restricted grant from Pfizer. We thank Heshmat Rajabi and Narges Joharchi for their expert technical assistance. We also thank Dr. A.D. Lê for the use of his operant chambers for the study with sucrose.

References

Ahmed SH, Koob GF (1997) Cocaine- but not food-seeking behavior is reinstated by stress after extinction. Psychopharmacology 132:289–296

Ahmed SH, Epping-Jordan MP, Griffin PS, Markou SC, Heinrichs SC, De Souza EB, Rivier J, Koob GF (1996) Maintenance of cocaine self-administration is not affected by pharmacological blockade of brain CRF receptors. Soc Neurosci Abstr 22:925

Ambrosio E, Sharpe L, Pilotte N (1997) Regional binding to corticotropin releasing factor receptors in brain of rats exposed to chronic cocaine and cocaine withdrawal. Synapse 25:272–276

Behan DP, Grigoriadis DE, Lovenberg T, Chalmers D, Heinrichs S, Liaw C, De Souza EB (1996) Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders. Mol Psychiatry 1:265–277

Carroll ME, Comer SD (1996) Animal models of relapse. Exp Clin Psychopharmacol 4:11–18

Chen YL, Mansbach RS, Winter SM, Brooks E, Collins J, Corman ML, Dunaiskis AR, Faraci SW, Gallaschum J, Schmidt A, Schulz DW (1997) Synthesis and oral efficacy of a 4-(butylethylamino)pyrrolo[2,3-d]pyrimidine: a centrally active corticotropin-releasing factor$_1$ receptor antagonist. J Med Chem 40: 1749–1754

Comer SD, Lac ST, Wyvell CL, Curtis LK, Carroll ME (1995) Food deprivation affects extinction and reinstatement of responding in rats. Psychopharmacology 121:150–157

de Souza EB (1985) Corticotropin-releasing factor receptors in the rat central nervous system: characterization and regional distribution. J Neurosci 7:88–100

de Souza EB (1995) Corticotropin-releasing factor receptors: physiology, pharmacology, biochemistry and role in central nervous system and immune disorders. Psychoneuroendocrinology 20: 789–819

de Wit H (1996) Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol 4:5–10

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75:134–143

de Wit H, Stewart J (1983) Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology 79:29–31

Dunn AJ, Berridge CM (1990) Physiological and behavioral responses to corticotropin-releasing factor: is CRF a mediator of anxiety or stress responses? Brain Res Rev 15:71–100

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology 128:408–412

Erb S, Shaham Y, Stewart J (1997) Effects of D-Phe CRF$_{12–41}$ on footshock-and cocaine-induced reinstatement of cocaine seeking in rats. Soc Neurosci Abstr 23:2146

Gerber GJ, Stretch R (1975) Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacol Biochem Behav 3:1055–1061

Heinrichs S, Menzaghi F, Schulteis G, Koob G, Stinus L (1995) Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. Behav Pharmacol 6:74–80

Jaffe JH (1990) Drug addiction and drug abuse. In: Gilman AG, Rall TW, Nies AS, Taylor P (eds) Goodman & Gilman's the pharmacological basis of therapeutics. Pergamon Press, New York, pp 522–573

Jaffe JH, Cascell NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology 97:59–64

Joseph S, Knigge K (1983) Corticotropin releasing factor: immunocytochemical localization in rat brain. Neurosci Lett 35: 135–141

Koob GF (1996) Drug addiction: the yin and yang of hedonic homeostasis. Neuron 16:893–896

Kosten TR, Rounsaville BJ, Kleber HD (1986) A 2.5-year follow-up of depression, life crises, and treatment effects on abstinence among opioid addicts. Arch Gen Psychiatry 43:733–739

Lê AD, Quan B, Juzytch W, Fletcher PJ, Joharchi N, Shaham Y (1998) Reinstatement of alcohol-seeking by priming injections of alcohol and exposure to stress in rats. Psychopharmacology 135:169–174

Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, Moreau LJ (1996) A non-peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like effects. Eur J Pharmacol 309: 195–200

Mansbach R, Brooks E, Chen Y (1997) Antidepressant-like effects of CP-154,526, a selective CRF$_1$ receptor antagonist. Eur J Pharmacol 323:21–26

Menzaghi F, Heinrichs SC, Merlo Pich E, Weiss F, Koob GF (1993) The role of limbic and hypothalamic corticotropin-releasing factor in behavioral response to stress. Ann NY Acad Sci 697:142–154

Menzaghi F, Rassnick S, Heinrichs S, Baldwin H, Merlo Pich M, Weiss F, Koob GF (1994) The role of corticotropin-releasing factor in the anxiogenic effects of ethanol withdrawal. Ann NY Acad Sci 739:176–184

Merlo Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, Weiss F (1995) Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci 15: 5439–5447

Meyer RE, Mirin SM (1979) The heroin stimulus: implications for a theory of addiction. Plenum Press, New York

Nemeroff CB (1996) The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions. Mol Psychiatry 1:336–342

Sarnyai Z, Buri E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1995) Brain corticotropin-releasing factor mediates "anxiety-like" behavior induced by cocaine withdrawal in rats. Brain Res 657:89–97

Schulz DW, Mansbach RS, Sprouse J, Braselton JP, Collins J, Corman M, Dunaiskis A, Faraci S, Schmidt AW, Seeger T, Seymour P, Tingley DF III, Winston EN, Chen YL, Heym J (1996) CP-154,526: a potent and selective non-peptide antagonist of corticotropin releasing factor receptors. Proc Natl Acad Sci USA 93:10477–10482

Shaham Y, Stewart J (1995) Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology 119:334–341

Shaham Y, Stewart J (1996) Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. Psychopharmacology 125:385–391

Shaham Y, Funk D, Erb S, Brown T, Walker C-D, Stewart J (1997) Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 17:2605–2614

Shiffman S, Wills TA (1985) Coping and substance abuse. Academic Press, Orlando

Stewart J, de Wit H (1987) Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: Bozarth MA (ed) Methods of assessing the reinforcing properties of abused drugs. Springer, New York, pp 211–227

Swanson L, Sawchenko P, Rivier J, Vale W (1983) Organization of ovine corticotropin releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397

Whitehead CC (1974) Methadone pseudowithdrawal syndrome: paradigm for a psychopharmacological model of opiate addiction. Psychosom Med 36:189–198

Zhou Y, Spangler R, LaForge K, Maggos C, Ho A, Kreek MJ (1996) Corticotropin-releasing factor and type 1 corticotropin-releasing factor receptor messenger RNAs in rat brain and pituitary during "binge"-pattern cocaine administration and chronic withdrawal. J Pharmacol Exp Ther 279:351–358