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CRF_1 receptor antagonists attenuate escalated cocaine self-administration in rats

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Abstract

Rationale—Previous work suggests a role for stress-related corticotropin-releasing factor (CRF) systems in cocaine dependence. However, the involvement of activation of CRF_1 receptors in rats self-administering cocaine with extended access is unknown.

Objective—The current study examined whether CRF_1 receptor antagonist administration alters cocaine self-administration in animals given extended access.

Materials and methods—Wistar rats (*n*=32) acquired cocaine self-administration (0.66 mg/kg per infusion) in 1 h sessions for up to 11 days. Rats then were assigned to receive either daily short (1 h, ShA) or long (6 h, LgA) access to cocaine self-administration (*n*=7–9 per group). Following escalation of intake, animals received one of two selective CRF_1 antagonists: antalarmin (6.3–25 mg/kg, i.p.) or *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo [1,5a]pyrimidin-7-amine (MPZP; 3.6–27.5 mg/kg, s.c.).

Results—By day 11 of the escalation period, LgA rats increased their cocaine intake, reaching an intake level of 15.1 mg/kg, compared to 11.1 mg/kg in ShA rats, during the first hour of sessions. Antalarmin reduced cocaine self-administration at the highest dose selectively in the LgA group but not the ShA group. MPZP reduced cocaine intake both in LgA and ShA rats. However, MPZP did so at a lower dose in LgA rats than in ShA rats. Within the LgA group, MPZP decreased cocaine intake in the first 10 min (loading phase) as well as in the latter session intake (maintenance phase).

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Conclusion—The data suggest that hypersensitivity of the CRF system occurs with extended access to cocaine self-administration and that this altered CRF system may contribute to the increased motivation to self-administer cocaine that develops during psychostimulant dependence.

Keywords

Cocaine; Self-administration; Escalation; Rats; Corticotropin-releasing factor; Addiction; Antalarmin; MPZP

Introduction

Corticotropin-releasing factor (CRF) is a 41-amino-acid peptide identified as a hypothalamic releasing factor (Vale et al. 1981). CRF stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary into the bloodstream, which releases glucocorticoids from the adrenal gland. These hypothalamic–pituitary–adrenal (HPA) hormones play an important role in physiological responses to stress (for reviews, see De Souza 1995; Koob 1999a). In addition, the central nervous system contains CRF in several extrahypothalamic brain regions where it coordinates behavioral and autonomic responses to stressors (Cummings et al. 1983; Erb et al. 2001). Extracellular CRF levels and CRF synthesis increase in the amygdala in response to physical, physiological, and social/psychological stressors (Merlo-Pich et al. 1995; Makino et al. 1999; for reviews, see Zorrilla and Koob 2004; Bale and Vale 2004). To date, two genes encoding mammalian CRF receptors (CRF_1 and CRF_2) have been identified (Hauger et al. 2006; Bale and Vale 2004; Chang et al. 1993; Lovenberg et al. 1995). While evidence indicates a role of CRF_1 receptors in anxiety-like behaviors in laboratory animals (Zorrilla and Koob 2004; McElroy et al. 2002), CRF_2 receptors appear to be related to appetite regulation and possibly modulation of anxiety-like behavior (for review, see Fekete and Zorrilla 2007).

The development of drug dependence has been hypothesized to involve allostatic dysregulation of brain reward function (Koob and Le Moal 1997, 2005). In addition to disruption of reward neurotransmission, the recruitment of brain stress systems, such as noradrenergic and CRF neurocircuitry, may contribute to the onset of drug dependence and addiction (Koob 2003). Dysphoria and anxiety are reported among the major drug-withdrawal symptoms in drug-dependent humans (Kampman et al. 1998), and CRF and norepinephrine may possess a feed-forward interaction in driving anxiety-like responses (Koob 1999b).

The hypothesis that CRF may be involved in withdrawal-induced anxiety-like responses is supported by research showing an association between cocaine withdrawal and increased extracellular CRF levels in the amygdala, as well as depleted amygdala CRF tissue content (Richter and Weiss 1999; Zorrilla et al. 2001). Intracranial administration of a peptide CRF receptor antagonist blocks cocaine withdrawal-induced anxiety-like behavior in rats (Basso et al. 1999). An interplay between cocaine and the brain stress systems is demonstrated by the facilitation of cocaine self-administration in rats that are high-responding to novelty (Piazza et al. 2000) and the blockade of acquisition and maintenance of cocaine self-administration following bilateral adrenalectomy (Goeders and Guerin 1996a). Additionally, a specific stressor, such as noncontingent footshock stress, increases corticosterone and is correlated with an increased acquisition rate of cocaine self-administration (Goeders and Guerin 1994, 1996b). Similarly, repeated corticosterone treatments promote the acquisition of cocaine self-administration (Mantsch et al. 1998). There also is downregulation of CRF receptors in the medial prefrontal cortex, nucleus accumbens, olfactory tubercle, and amygdala following cocaine administration (Goeders et al. 1990). Pretreatment with the CRF_1 antagonist CP-154,526 results in dose-dependent decreases in cocaine self-administration in rats with limited daily drug access (1 h/day) yet does not affect food-reinforced responding (Goeders and Guerin 2000). The CRF system involvement is not limited to the acquisition and

maintenance of cocaine self-administration. It was shown that the CRF system stimulation induces reinstatement of cocaine-seeking behavior in rats during withdrawal (Erb et al. 2001, 2006). Taken together, these data suggest that activation of CRF systems in the brain may be involved in the development of the emotional dysregulation hypothesized to motivate drug intake in cocaine dependence.

In our laboratory, daily, extended access to cocaine has been shown to produce an increase in self-administration of the drug over sessions (termed “escalation”; Ahmed and Koob 1998; Wee et al. 2007). The increased drug intake with extended access in rats provides some face validity for compulsive drug intake in humans (American Psychiatric Association 2000). Furthermore, the increased cocaine self-administration with extended access strongly correlates with an increasing threshold for intracranial self-stimulation (Ahmed et al. 2002), suggesting decreased brain reward function during the escalation of cocaine self-administration. This finding, in turn, supports the construct validity of this model for the development of drug dependence (Koob et al. 1997; Koob and Le Moal 1997; Sinha et al. 2000).

Previous studies have shown that CRF_1 antagonists have differential effects on ethanol self-administration in dependent vs nondependent rats (Funk et al. 2007; Sabino et al. 2006; Heilig and Koob 2007). Therefore, the present study tested the hypothesis that CRF_1 receptor blockade differentially affects rats with a history of short and extended access to cocaine. To test the hypothesis, we examined the effect of two non-peptide CRF_1 receptor antagonists, antalarmin and *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5a] pyrimidin-7-amine (MPZP), on cocaine self-administration under short-access (ShA, 1 h/day) and long-access (LgA, 6 h/day) conditions. We report that CRF_1 receptor antagonists more effectively decrease the increased cocaine intake of LgA rats than the intake of ShA rats.

Materials and methods

Animals

Thirty-two male Wistar rats (Charles River, Kingston, NY, USA), weighing 250–300 g at the start of the experiment, were group-housed (two to three per cage) on a reverse light/dark cycle (lights off 0800 to 2000 hours), in a climate-controlled vivarium. All behavioral testing occurred during the dark cycle. Food and water were freely available unless otherwise specified. All procedures met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (The National Academies Press 1996).

Drugs

Cocaine hydrochloride (National Institute on Drug Abuse, Rockville, MD, USA) was dissolved in sterile physiological saline to 0.25 mg/0.1 ml per infusion. *N*-butyl-*N*-ethyl-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolol[2,3-*d*]pyrimidin-4-amine hydrochloride (antalarmin hydrochloride, Sigma, St. Louis, MO, USA) was initially dissolved in 1 M HCl (a volume equal to 5% of the final volume), then suspended in a 0.5% (*w*/*v*) low-viscosity carboxymethyl-cellulose (Sigma) saline solution. This solution was back-titrated with 1 M NaOH to a pH of ~4 and injected at a volume of 5 ml/kg. MPZP was synthesized by Dr. Peter Wirsching (Department of Chemistry, The Scripps Research Institute). The methoxy substituents in the “top” MPZP alkyl unit confer increased hydrophilicity compared to the parent compound or antalarmin, yielding a CRF_1 antagonist with lipophilicity more typical of central nervous system-acting drug-like molecules (Zorrilla and Koob 2004). MPZP was solubilized in 1 M HCl, diluted in hydroxypropyl β-cyclodextrin (20% *w*/*v* final concentration, Cavitron 82004, Cargill, Wayzata, MN, USA) saline solution, back-titrated with NaOH to a

final pH of 4.5, and injected at a volume of 2 ml/kg. Figure 1 presents the CRF_1 antagonists under study.

Both MPZP and antalarmin have high affinity for rat CRF_1 receptors (Table 1). Like antalarmin, MPZP is a selective CRF_1 antagonist. MPZP has a 5–10 nM affinity for the CRF_1 receptor and negligible activity at the CRF_2 receptor. In vitro receptor autoradiography studies have shown that MPZP does not displace [^{125}I]-Tyr^0-sauvagine binding from rat lateral septum or ventromedial hypothalamus (CRF_2-like binding) at a concentration (1 μM) that concurrently displaces the majority of [^{125}I]-Tyr^0-sauvagine from the cerebral cortex (CRF_1-like binding). Although the binding affinity of MPZP for CRF_1 receptors is slightly less potent than that of antalarmin, MPZP has lipophilicity 3.5 to 4 times lower than that of antalarmin and in a range more typical of central nervous system-acting therapeutics (compare cLog*P* and cLog*D* in Table 1; Zorrilla and Koob 2004). The molecular volume and polar surface area of MPZP also are consistent with an absorbable, blood–brain barrier-penetrating molecule (Kelder et al. 1999;Zhao et al. 2007;Fu et al. 2005;Liu et al. 2004).

Apparatus

Behavioral training occurred in operant-conditioning chambers (Coulbourn Instruments, Allentown, PA, USA) housed in sound-attenuating cubicles. All chambers were equipped with two retractable levers, a dispenser for food pellets (P.J. Noyes, Lancaster, NH, USA), and a syringe pump (Model A, Razel Scientific Instruments, Stamford, CT, USA) delivering 0.1 ml of cocaine solution over 4 s via Tygon tubing attached to liquid swivels (Model 375, Instech Labs, Plymouth Meeting, VA, USA). A time-out (20 s) followed each infusion, during which a cue light above the active lever was illuminated. At the start of a session, two levers were presented. Responding on the active lever resulted in reinforcement, whereas responding on the inactive lever resulted in no consequences but was recorded. Sessions were controlled and recorded by a personal computer with a custom interface and software.

Intravenous surgery

Rats were implanted with an indwelling catheter into the right jugular vein under 1–3% isoflurane as described by Caine et al. (1993). Catheters were flushed daily with 0.2 ml of sterile antibiotic solution containing Timentin (100 mg/ml; SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) and heparin (30 USP units/ml). Catheter patency was checked by briefly aspirating blood from the catheter.

Self-administration procedure

Initially, rats were food-restricted (15 g per rat per day) and trained to press a lever for a food pellet (45 mg Formula A/I, Research Diets, New Brunswick, NJ, USA) under a fixed-ratio (FR)1 schedule in 30-min sessions, twice daily for a total of 5 days before intravenous catheterization. During this period, the length of time-out following reinforcement was gradually increased (1, 5, 10, and 20 s). After the animal reached the 20-s time-out, food was available *ad libitum* for the remainder of the study. The rats then were implanted with intravenous catheters as described above.

After recovery from surgery, rats self-administered 0.25 mg per infusion (0.66 mg/kg per infusion) of cocaine in daily 1-h sessions under an FR1 schedule for a maximum of 11 days. Following these baseline sessions, animals were separated into two groups balanced for body weight and cocaine intake. The session length was kept to 1 h for one group (short access, ShA, n=16) and was increased to 6 h for the other group (long access, LgA, n=16; escalation period). Sessions in this escalation period lasted for 11 to 15 days before testing with CRF_1 receptor antagonists. Following the escalation period, the effects of antalarmin or MPZP on cocaine self-administration were tested in separate groups of ShA and LgA rats under an FR1 schedule.

The antalarmin (6.3–25 mg/kg) pretreated animals were injected intraperitoneally 80 min before a test session. The MPZP (3.6–27.5 mg/kg) pretreated animals were injected subcutaneously 45 min before a test session. These doses and pretreatment time intervals were chosen based on previous studies with the anti-stress time course and potencies of these compounds (Zorrilla et al. 2002; Fekete et al. 2003). Doses of each drug were tested in a Latin square design. Test sessions were 1 h long and separated by one to two treatment-free daily escalation sessions.

Data analyses

Data were expressed as the first hour and total session cocaine intake (milligram per kilogram). To analyze changes in cocaine intake during the escalation period, a repeated-measures two-way analysis of variance (ANOVA) was used (access × session). Post-hoc comparisons were performed using the Student Neuman Keuls test. The effects of the CRF_1 receptor antagonists on cocaine intake were evaluated using a repeated-measures two-way ANOVA (dose × access). In addition to a two-way ANOVA, we performed a dose-by-access linear trend contrast analysis. We subsequently identified the source of the interaction with dose using simple main effects (within subject) and individual means comparisons with Dunnett's test. Regarding the error bars in the figures, they reflect between-subject variability, whereas the statistical test used every animal as its own control. With dose–response functions, a powerful way of using analysis of variance is to do linear trend analysis (Bewick et al. 2004; Bretz et al. 2004; Rosner 1995; Sheskin 2004). With a significant dose-by-access linear trend contrast, a simple main effect of dose on cocaine intake within each group was performed using the error term, $MS_{B\times subjw.groups}$ [$F=MS_{b \text{ at } a1}/MS_{B\times subjw.groups}$, b=dose, a=access, Winer 1962) with a Dunnett's post-hoc test for individual mean comparisons.

Previous research in our laboratory demonstrated that rats self-administer more drug during the first 10 min (loading phase) than in any other 10-min period of a 1-h session (Kitamura et al. 2007; Wee et al. 2007). Therefore, we further analyzed the effect of MPZP and antalarmin on the pattern of cocaine intake within a test session only in LgA rats. Data in each test session were divided into the loading phase intake (first 10 min) and the maintenance-phase intake (the average for the 10-min bin from 10–60 min) and subjected to a two-way ANOVA and a two-way linear contrast (dose × phase). The statistical packages used were Statview (SAS Institute, Cary, NC, USA), Statistical Package for the Social Sciences (Chicago, IL, USA), Instat, and Prism (GrapPad, San Diego, CA, USA).

Results

Figure 2a and b illustrate cocaine intake (milligram per kilogram) for ShA and LgA groups during the entire session and first hour, respectively. Data analyses of cocaine intake for the ShA group and the first hour of the LgA group during the first 11 days of escalation revealed a main effect of daily sessions ($F_{10,300}$=9.031, p<0.0001), main effect of access ($F_{1,30}$=7.347, p<0.05), and an overall interaction ($F_{10,300}$=2.996, p<0.01). Compared to escalation session 1, the ShA group exhibited increased responding in sessions 10–11, whereas the LgA group exhibited increased 1 h responding earlier, beginning in session 3. LgA animals also significantly increased total session responding by session 3. Cocaine intake within the first hour by LgA rats significantly exceeded that of ShA rats on day 5 (p<0.01), continuing through sessions 6 to 11 (p< 0.001). Separation of the cocaine intake data into the respective antagonist treatment groups yielded the following results: The Antalarmin groups (ShA vs LgA) exhibited a main effect for daily sessions ($F_{10,150}$=6.091, p<0.0001), an overall interaction ($F_{10,150}$=2.632, p<0.01), but no main effect for group on cocaine intake. The antalarmin LgA group increased intake by day 2 for the first hour and by day 4 for total session intake. The MPZP groups (ShA vs LgA) exhibited a main effect of daily sessions ($F_{13,130}$= 4.465,

$p<0.0001$), a main effect for group ($F_{1,130}=5.758$, $p<0.05$), and an overall interaction ($F_{13,130}=2.343$, $p<0.05$) on cocaine intake. The MPZP LgA group increased intake by day 10 for the first hour and by day 3 for the total session intake. The separation of cocaine intake data into the respective antagonist treatment groups resulted in a lack of increased intake for either ShA group.

The two CRF_1 antagonists differentially decreased cocaine intake (milligram per kilogram) during the test sessions. Two-way ANOVA of cocaine intake following antalarmin pretreatment (Fig. 3) revealed a main effect of access (ShA $n=8$, LgA $n=9$; $F_{1,45}=5.446$, $p<0.05$) but no main effect for antalarmin dose and no interaction. However, a dose-by-access linear trend contrast analysis showed a significant dose-by-access linear contrast ($F_{1,15}= 5.05$, $p<0.05$), indicating that the strength of the log-linear relation of antalarmin dose to cocaine intake differed significantly between access conditions. Please note that the denominator degrees of freedom (*df*) for an orthogonal contrast test is calculated as *N*-*J*, where *N* is the sample size, and *J* is the number of contrast coefficients (e.g., one coefficient for the overall linear dose trend and one coefficient for the linear dose × access interaction). Additionally, there was a simple main effect of dose of antalarmin on cocaine intake in LgA rats ($MS_{b \text{ at } a1}=6.7$ ($df=3$), $MS_{B\times subjw.groups}=1.97$ ($df=45$), $F_{3,45}=3.41$, $p< 0.05$) with a significant decrease in cocaine intake at 25 mg/kg compared with the vehicle. In contrast, no simple main effect of dose on cocaine intake was found in ShA rats ($MS_{b \text{ at } a1}=0.52$, $MS_{B\times subjw.groups}=1.97$, $F_{3,45}=0.26$, $p> 0.05$, Fig. 3).

MPZP decreased cocaine intake in both ShA and LgA rats (Fig. 4). Two-way ANOVA revealed an overall main effect for access (ShA $n=8$, LgA $n=7$; $F_{1,39}=7.249$, $p< 0.05$), a main effect for MPZP dose ($F_{3,39}=10.076$, $p< 0.001$) but no interaction. However, a two-way linear contrast showed a significant dose × access linear contrast interaction ($F_{1,13}=5.48$, $p<0.05$). Additionally, there was a simple main effect of dose on cocaine intake in both LgA and ShA rats [LgA, $MS_{b \text{ at } a1}=17.56$ ($df=3$), $MS_{B\times subjw.groups}=1.89$ ($df=39$), $F_{3,39}=9.29$, $p<0.01$; ShA, $MS_{b \text{ at } a1}= 7.72$ ($df=3$), $MS_{B\times subjw.groups}=1.89$ ($df=39$), $F_{3,39}=4.01$, $p <0.05$]. Post hoc Dunnett's tests showed a significant decrease in cocaine intake at 10 mg/kg ($p<0.05$) and 27.5 mg/kg ($p<0.01$) in LgA rats and at 27.5 mg/kg in ShA rats ($p<0.05$).

Cocaine intake during the loading phase was higher than that of the maintenance phase (Antalamin group, 5.2±0.6 vs 2.0±0.1; MPZP group, 5.9±0.5 vs 2.6±0.2 mg/10 min after the vehicle pretreatment). When the effect of CRF_1 antagonists on cocaine intake was compared between the loading and maintenance phases in LgA rats, neither a two-way ANOVA nor a two-way linear contrast found a significant interaction between dose and phase for antalarmin or for MPZP (data not shown). However, a two-way ANOVA showed a significant main effect of phase for antalarmin ($F_{1,48}=52.0$, $p<0.001$) and significant main effects of phase and dose for MPZP (phase, $F_{1,36}=46.6$, $p <0.001$; dose, $F_{3,36}=13.4$, $p<0.001$).

Discussion

Anxiety and dysphoria occur during cocaine abstinence in human cocaine users (Kampman et al. 1998). This negative emotional state is hypothesized to motivate the maintenance and persistence of drug intake via negative reinforcement mechanisms, thereby playing a critical role in the development of drug dependence (Koob and Le Moal 1997). Evidence supports the notion that the CRF system mediates anxiety and other dysphoric states (for review, see Zorrilla and Koob 2004; Bale and Vale 2004), and recruitment of the CRF system has been hypothesized to be involved in drug dependence in humans (Koob 1999a). The present study tested whether rats with a history of extended access to cocaine and resultant escalation of intake showed increased sensitivity to the ability of CRF_1 antagonists to reduce cocaine self-administration, as would be predicted with increased activity of the CRF receptor system.

Consistent with previous reports, LgA rats increased cocaine intake during the first hour and across 6-h sessions (Ahmed and Koob 1998; Wee et al. 2007). In humans, drug intake increases with the development of drug dependence (American Psychiatric Association 2000), supporting the face validity of the extended-access cocaine self-administration procedure as a model of cocaine dependence in humans. Under the present conditions, when all ShA rats were combined, increased cocaine intake was observed across daily sessions but to a much smaller degree and more slowly than the increase seen in first-hour intake in LgA rats. The hypothesis of differential activity of CRF systems in rats with a history of cocaine escalation was supported by the current findings. Systemic pretreatment with the CRF_1 antagonist antalarmin decreased cocaine intake selectively in LgA rats at the 25 mg/kg dose. A previous study found that 30 min pretreatment of antalarmin did not alter the dose–response function of cocaine self-administration in rhesus monkeys with limited (2 h) daily drug access (Mello et al. 2006), which is consistent with the present finding that antalarmin had no effect in ShA rats. Similar to the present results, antalarmin (20 mg/kg, i.p.) dose-dependently reduced escalated ethanol intake in ethanol-dependent rats tested during acute withdrawal yet did not influence ethanol self-administration in nondependent rats (Funk et al. 2007). Doses of antalarmin, similar to those used in the present study (20 mg/kg, i.p.), were shown previously to inhibit spontaneous defensive withdrawal behavior as well as intracerebroventricular CRF-induced anxiogenic-like behavior in the elevated plus maze (Zorrilla et al. 2002). These data support the hypothesis that the CRF system contributes to the escalated drug intake of rats with extended drug access, a model of the development of drug dependence.

The CRF_1 antagonist MPZP decreased cocaine intake in both ShA and LgA rats. However, MPZP reduced cocaine self-administration in LgA rats at a lower dose than in ShA rats, suggesting that cocaine intake by LgA rats was more sensitive to the blockade of CRF_1 receptors than the intake by ShA rats. Doses of MPZP similar to those used in the present study reduced anxiety-like behavior in the defensive burying test (Fekete et al. 2003). Moreover, an increased time spent for defensive burying was found in LgA rats compared with ShA rats after 22 days of cocaine self-administration with extended access, which lasted over a month (Aujla et al. 2007). Thus, the data suggest that the physiological systems affected by cocaine self-administration with extended access may reflect a hypersensitive state in the CRF system, which produces an increased anxiety-like state upon the exposure to shock stimulus in the defensive burying test and perhaps contributes to an increased cocaine intake.

The decreased cocaine intake in ShA rats by MPZP pretreatment was somewhat unexpected because previous reports using various doses and regimens of antalarmin, etomide, ketoconazole, astressin, and dexamethasone in rhesus monkeys and CP-154,526 in Wistar rats did not find a relationship between the CRF system and the acute reinforcing effects of cocaine (Mello et al. 2006; Broadbear et al. 1999; Przegalinski et al. 2005). However, consistent with the present results, CP-154,526, a non-peptide CRF_1 receptor antagonist, reduced cocaine self-administration (0.5 mg/kg per injection) and cocaine-induced conditioned place preference in rats (Goeders and Guerin 2000; Lu et al. 2003). Reasons for these differences are not clear, although possible explanations might include the doses used and the session paradigms. Thus, a relationship between the CRF system and the acute reinforcing effect of cocaine remains unresolved, but the increased cocaine intake associated with extended access appears particularly sensitive to the CRF_1 antagonist effect.

The difference between the effects of antalarmin and MPZP on cocaine intake by ShA rats might be explained by pharmacokinetic variables related to the compounds' different lipophilicities or the different routes and vehicles of administration. Antalarmin, a pyrrolopyrimidine, is approximately 4.5 orders (30,000-fold) more hydrophobic than MPZP (cLog*P*=6.98 vs 2.52, respectively). The very high lipophilicity of antalarmin results in poor aqueous solubility and low bioavailability of the compounds, with a pharmacokinetic profile

unfavorable for accumulation of high central levels (Zorrilla and Koob 2004). The use of hydroxypropyl β-cyclodextrin as a vehicle excipient also may have increased central availability of MPZP by increasing solubility and distribution and, perhaps, reducing degradation (Strickley 2004).

In the present study, increased cocaine intake in LgA rats appears to be related to the increased sensitivity of the CRF system with extended access to cocaine. Thus, one may speculate that the loading phase is more sensitive than the maintenance phase of the 1-h test session, as presumably, the loading phase would reflect an attempt to reverse the hypothesized increase in CRF activity observed during cocaine withdrawal (Richter and Weiss 1999). However, further analysis of the effect of the CRF_1 antagonists on cocaine intake by LgA rats during the loading and the maintenance phases showed no significant interaction between the antagonist dose and phase intake in LgA rats. This suggests that, although the cocaine intake during the loading phase was higher than that of the maintenance phase, antalarmin and MPZP did not differentially decrease the loading- and maintenance-phase cocaine intake. On the other hand, MPZP had effects throughout the session (six 10-min bins) whereas antalarmin did not. This may reflect a longer duration of action of MPZP than antalarmin. The first half-life of antalarmin-related compound CP-154,526 was 0.9 h in rats (Keller et al. 2002), and the half-life of MPZP-related compound DMP904 was 46 h in dogs (Gilligan et al. 2000).

Neuroadaptation in the CRF system in the extended amygdala has been proposed to drive the negative motivational state associated with abstinence in drug-dependent humans (Koob 2003). Research substantiating this hypothesis includes findings that extracellular CRF levels are increased in the central amygdala during cocaine, ethanol, cannabinoid, and opioid withdrawal in rats (Richter and Weiss 1999; Merlo-Pich et al. 1995; Rodriguez de Fonseca et al. 1997; Weiss et al. 2001, respectively) and that tissue content levels of CRF in the amygdala are depleted during withdrawal from cocaine or ethanol (Zorrilla et al. 2001; Funk et al. 2006). CRF antagonists have been found to reduce negative emotional states during withdrawal from cocaine (Basso et al. 1999, Przegalinski et al. 2005), methamphetamine (Moffet and Goeders 2007), nicotine (Bruijnzeel et al. 2007), and ethanol (Baldwin et al. 1991, Rassnick et al. 1993; Menzaghi et al. 1994). Such negative emotional states may indicate decreased brain reward function, which has been observed in a previous study in LgA rats (Ahmed et al. 2002). Altogether, these results support a hypothesis that CRF system activity increases, likely in the extended amygdala, with extended access to cocaine self-administration, which in turn may contribute to an increase in cocaine intake in LgA rats.

Rather than an effect on extrahypothalamic CRF systems, a possible alternative explanation for the current findings is that antalarmin and MPZP reduced cocaine intake because of an ability of CRF_1 antagonists to reduce HPA activation. For example, the structurally related compound DMP904 inhibited ACTH release from rat pituitary corticotropes (Li et al. 2005) and dose-dependently inhibited the stress-induced increase in plasma corticosterone in rats (Lelas et al. 2004). Similarly, antalarmin (20 mg/kg, i.p.) blocked CRF-induced increases in plasma ACTH levels (Webster et al. 1996). The understanding of the relationship between the acute reinforcing effect of cocaine and the HPA axis is evolving (Marinelli et al. 1997). Mantsch et al. (1998) reported that dexamethasone, a glucocorticoid receptor agonist, inhibited the acquisition of cocaine self-administration in rats, yet corticosterone treatment promoted acquisition. Later studies found no clear relationship between glucocorticoids and stress-induced escalation of cocaine intake, cocaine-seeking behavior, or the discriminative stimulus properties of cocaine in rats (Mantsch and Katz 2006; Mantsch and Goeders 1999). Similarly, Broadbear et al. (1999) reported that cocaine-maintained responding was not altered by effective inhibitors of HPA axis hormonal responses in rhesus monkeys. Furthermore, under conditions similar to those in the present study, Mantsch et al. (2003) noted the development of tolerance in HPA axis responses to cocaine intake in LgA rats but not in ShA rats. Thus,

cocaine exposure may initially activate the HPA axis (Marinelli and Piazza 2002), whereas prolonged cocaine exposure may result in a subsequent blunting of the HPA axis, with a compensatory sensitization of extrahypothalamic CRF systems (Lee et al. 1994; Pecina et al. 2006; Shepard et al. 2006).

In conclusion, the present study demonstrates that CRF_1 receptor antagonists decreased cocaine intake in rats, especially in those with a history of extended, as opposed to brief, daily cocaine access. The data suggest that neuroadaptations in the CRF system may contribute to the increased motivation to self-administer cocaine that develops during psychostimulant dependence.

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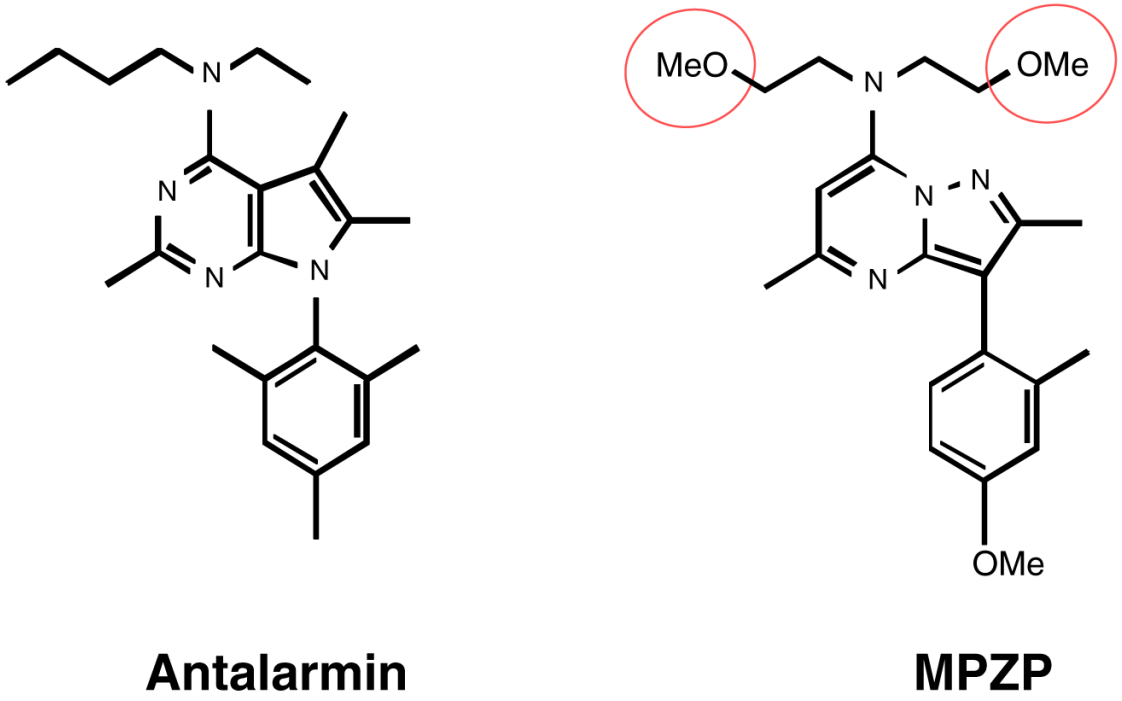

Fig. 1.
Chemical structures of antalarmin and MPZP

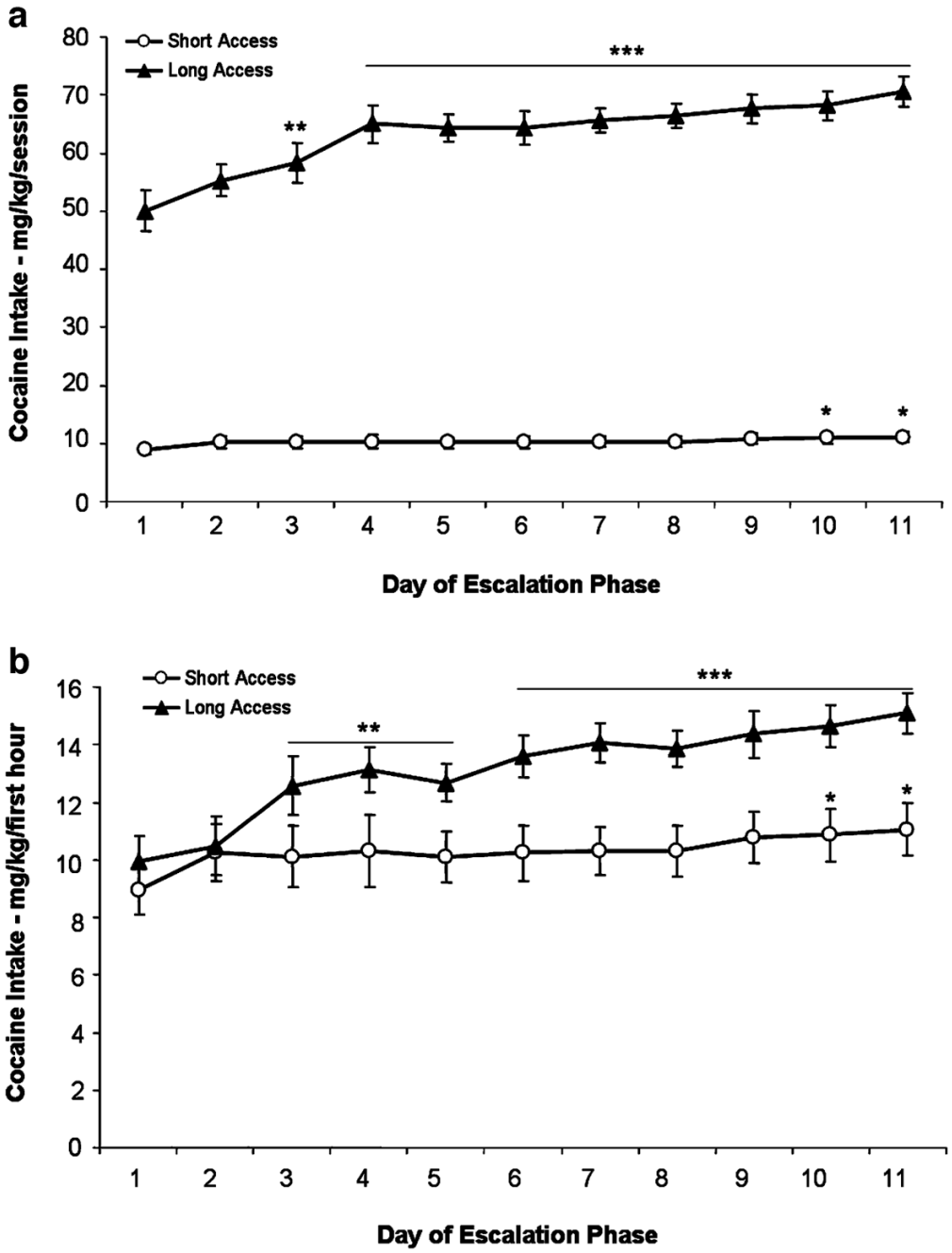

Fig. 2.

Self-administration of cocaine by rats under a fixed-ratio schedule during the escalation period. Data from entire sessions (**a**) and from the first hour of sessions (**b**). The data represent mean (+SEM) cocaine intake adjusted for body weight (milligram per kilogram). *Open symbols* are the data for rats in 1-h sessions (ShA, n=16). *Filled symbols* are the data for rats in 6-h sessions (LgA, n=16). *p<0.05, **p<0.01, ***p<0.001 compared with session 1

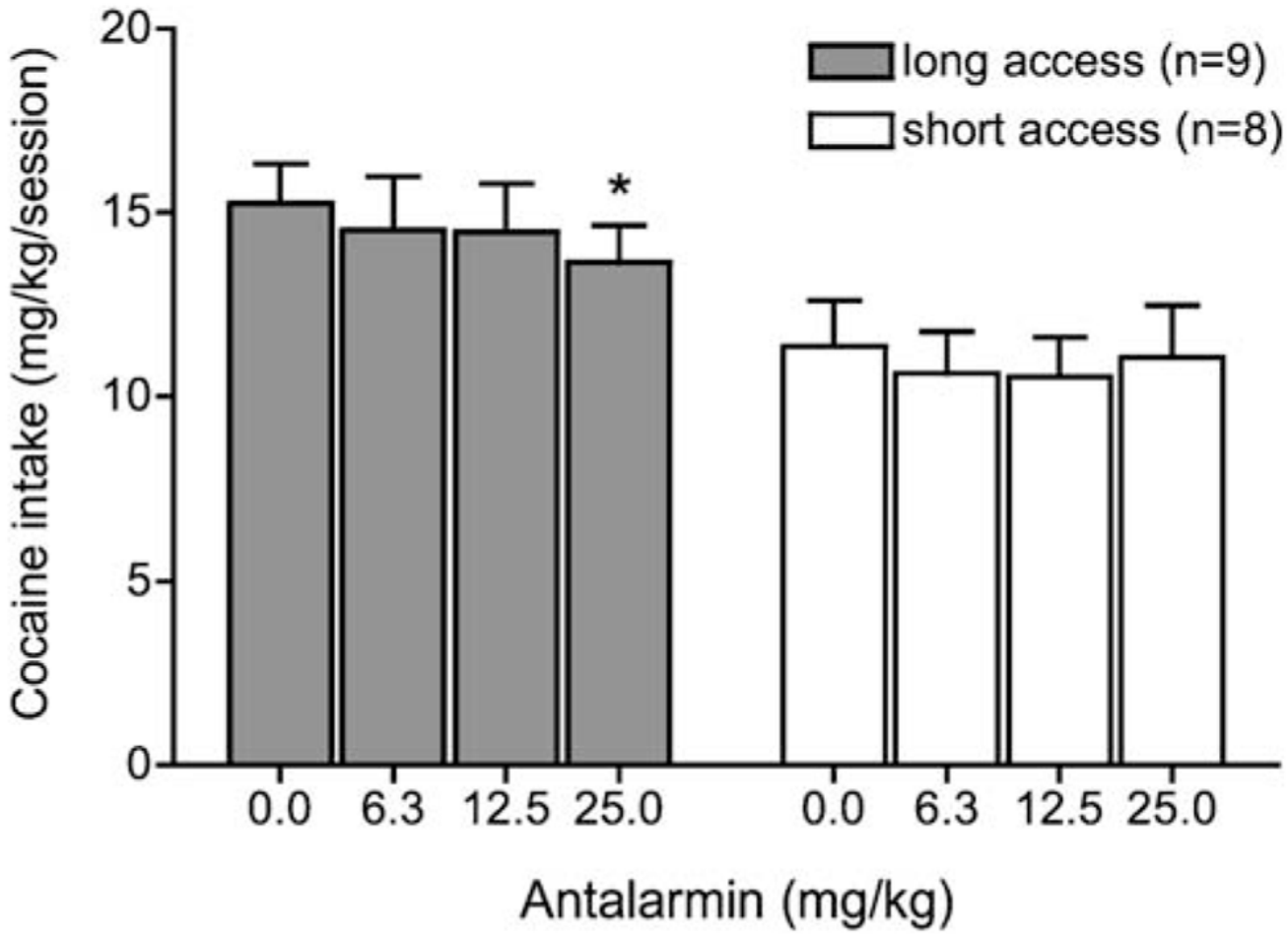

Fig. 3.

Antalarmin effects on cocaine intake in ShA and LgA rats under a fixed-ratio schedule. Antalarmin was intraperitoneally injected 80 min before a test session. Test sessions lasted 1 h and were separated by one or two treatment-free escalation sessions. Data are expressed as mean (+SEM) cocaine intake (milligram per kilogram). *p<0.05 compared with the vehicle

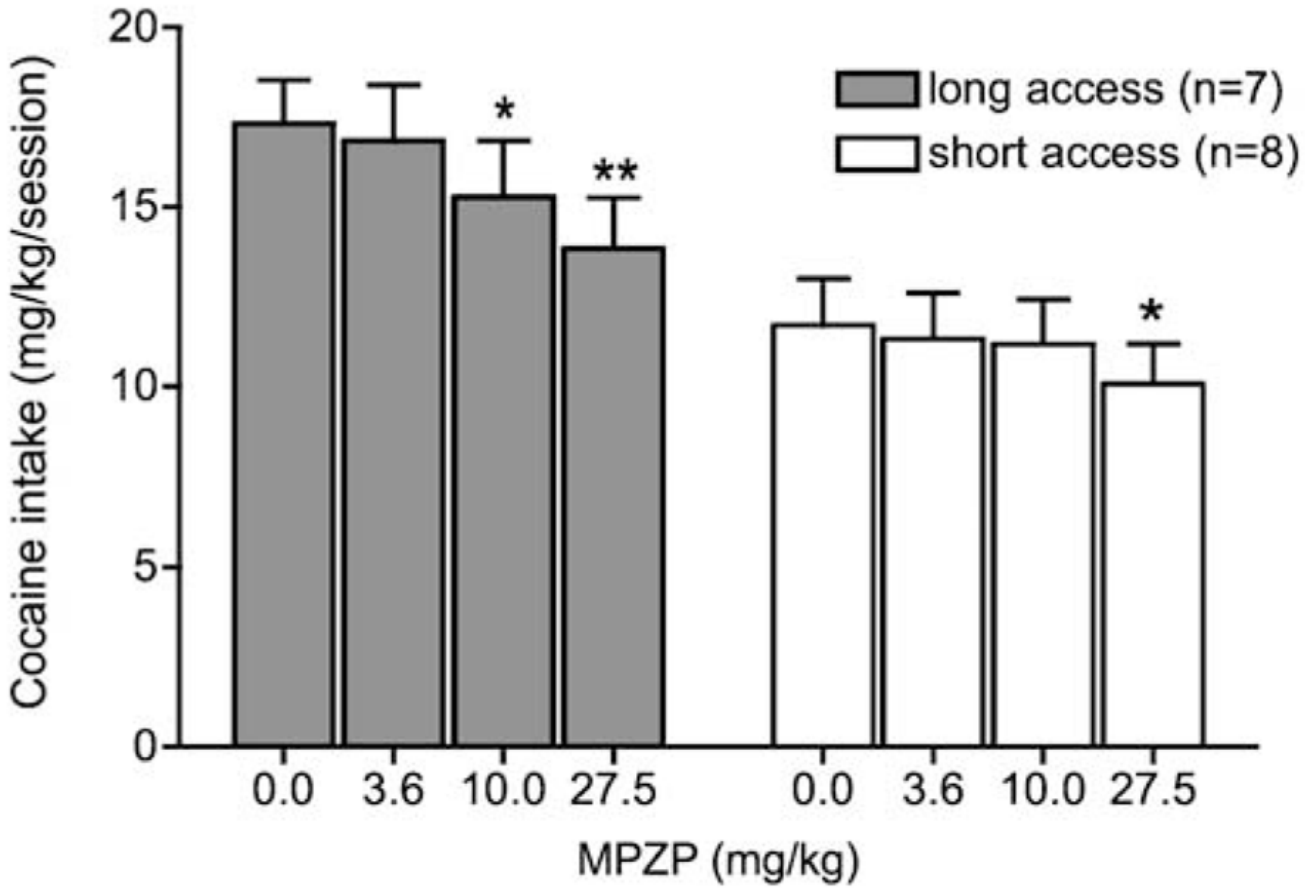

Fig. 4.

MPZP effects on cocaine intake in ShA and LgA rats under a fixed-ratio schedule. MPZP was subcutaneously injected 45 min before a test session. Test sessions lasted 1 h and were separated by one or two treatment-free escalation sessions. Data are expressed as mean (+SEM) cocaine intake (milligram per kilogram). *p<0.05, **p <0.01 compared with the vehicle

Table 1

Selected pharmacological and physiochemical properties of antalarmin and MPZP

	Antalarmin	MPZP
CAS registry number	157284-96-3	202579-79-8
Affinity at rat CRF_1 receptor (K_i, nM)	1[a]	5–10[b]
cLog*P*	7.09+1.31	2.95+1.13
cLog*D*, pH 7	6.41	2.93
Polar surface area ($Å^2$)	29	61.1
pKa	7.48+0.30	5.32+0.30
Molar volume (cm^3/mol)	357.2+7.0	346.2+7.0

Physiochemical properties were calculated using Advanced Chemistry Development (ACD/Labs) Software v8.14 for Solaris (ACD/Labs).

CAS Chemical Abstracts Service

[a] Reviewed in Zorrilla and Koob 2004

[b] Estimated from displacement of [^{125}I]-Tyr^0 -sauvaine from rat cerebellar homogenates in four independent replications