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Short communication

Effects of CP-154,526 on responding during extinction from cocaine self-administration in rats

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Abstract

Conditioned cues associated with cocaine induce craving and relapse. Although the role of corticotropin releasing hormone (CRH) in stress- and cocaine-induced relapse has been reported, its involvement in cue-induced behavior has not been established. Using responding during extinction as a model of cue-induced craving, we tested the effects of a selective CRH1 receptor antagonist, CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-d]pyrimidin-4-yl]-amine). Rats were trained to respond on a multiple schedule of cocaine self-administration and food reinforcement. On extinction test days, saline was substituted for cocaine. Pretreatment with CP-154,526 (20 mg/kg, i.p.) decreased responding on the cocaine-associated lever during extinction, suggesting an involvement of CRH1 receptors in cue-induced craving.

Keywords: Cocaine; Self-administration; Conditioning; CRH (corticotropin releasing hormone); (Rat)

1. Introduction

The presentation of cues associated with cocaine self-administration is known to induce craving (Ehrman et al., 1992; Kilgus and Pumariega, 1994) and to precipitate cocaine-seeking behavior and relapse (Meil and See, 1996). Preventing cue-induced relapse is a promising target for the development of new pharmacotherapies for cocaine addiction. CRH1 receptor antagonists are effective in reducing cocaine self-administration (Goeders and Guerin, 2000) and in decreasing the stress (Erb et al., 1998; Shaham et al., 1998, Erb and Stewart, 1999)- and cocaine-induced (Erb et al., 1998) reinstatement of extinguished cocaine-seeking behavior in rats. However, it is unknown if CRH receptor antagonists will also reduce cue-induced cocaine seeking.

Animals will respond for days on the lever previously associated with cocaine, even if only saline is delivered (i.e., extinction), when presented with cues that had been associated with cocaine in past. It has been hypothesized that such responding is a model of cue-induced cocaine seeking (Weiss et al., 2000). We used this model to test the involvement of CRH1 receptors in cue-induced cocaine seeking by using the selective CRH1 receptor antagonist (Schulz et al., 1996) CP-154,526 (butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-d]pyrimidin-4-yl]-amine).

2. Methods

Twenty-seven male Wistar rats (Harlan Sprague–Dawley, Indianapolis, IN, USA), 80–100 days old at the start of the experiments were used. All rats were housed individually in cages equipped with a laminar flow unit and air filter in a temperature- and humidity-controlled, American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care facility on a reversed 12-h light–dark cycle (lights on at 18:00). Rats were maintained at 85–90% of their free-feeding body weights by presentations of food pellets (P.J. Noyes, Lancaster, NH, USA; 45 mg) during the behavioral sessions and by supplemental post-session feeding (Purina Rat Chow) and had access to water ad libitum. All procedures were carried out in accordance with National Institutes of Health (NIH)

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Rats were implanted with chronic indwelling jugular catheters (Goeders and Guerin, 1996) under pentobarbital anesthesia (50 mg/kg, i.p.) with methylatropine nitrate pretreatment (10 mg/kg, i.p.). The catheter (silicone tubing, 0.12 in. o.d. × 0.025 in. i.d.) was inserted into the right posterior facial vein and pushed down into the jugular vein until it terminated outside the right atrium. The catheter was attached to the area around the vein and exited posterior to the scapulae through a Marlex mesh/dental acrylic/22-gauge guide cannula (Plastic Products) assembly implanted under the skin. During experimental sessions, a stainless steel spring leash (Plastic Products) was attached to the guide cannula and to a fluid swivel suspended above the cage. Tubing connected the swivel to a 20-ml syringe in a motor-driven pump located outside the chamber. At the end of session, the leash was disconnected and a dummy cannula was inserted into the guide.

Experimental chambers were equipped with two response levers, a stimulus light located above each lever and a food pellet dispenser. One week after surgery, rats were trained to respond under a multiple, alternating schedule (Goeders and Guerin, 2000) of food reinforcement (45 mg) and cocaine self-administration (0.25 mg/kg/infusion in 0.2 ml saline delivered over 5.6 s). Each experimental session was divided into eight 15-min bins during which either food or cocaine was available. A 1-min timeout period followed each bin. Illumination of the stimulus lights above the levers indicated the availability of cocaine or food reinforcement. Animals were trained to respond under a fixed-ratio 1 (FR1) schedule of reinforcement, and a 20-s timeout period followed each reinforcer presentation. The requirement for food reinforcement was gradually increased to FR4.

After stable responding for cocaine was achieved (i.e., < 10% variation for three consecutive sessions) saline was substituted for cocaine, and the animals were tested for extinction for the first time. Animals in the experimental group received an injection of CP-154,526 (20 mg/kg, i.p.), 30 min before session, while controls received vehicle. We chose this dose since we previously reported that pretreatment with this dose of CP-154,526 decreased ongoing cocaine self-administration without affecting food-maintained responding (Goeders and Guerin, 2000). Extinction testing was repeated after an additional 3–6 days of stable cocaine self-administration. Since the effects of drugs might be different depending on the length of cocaine self-administration, extinction testing was also repeated following chronic self-administration. Rats were allowed to self-administer cocaine for 30 days, and extinction was tested twice as above with an interval of 3–6 days of stable cocaine self-administration between each test. Blood for the analysis of plasma corticosterone was taken before and after the extinction test sessions via the i.v. catheters.

Data were analyzed using a repeated-measures Analysis of Variance (ANOVA). Dunnett's Multiple Comparison Tests were used to determine significant differences between groups ($P < 0.05$).

3. Results

Rats received 45–60 cocaine infusions per session following the acquisition of self-administration. There were no differences between experimental and control groups in responding for cocaine at any time point. Fig. 1 represents the effects of CP-154,526 and vehicle on cocaine lever responding during extinction. CP-154,526-treated animals responded significantly less than vehicle-treated animals during extinction on the first day of testing [$F(1,23) = 7.79$, $P = 0.01$] and after 30 days of self-administration [$F(1,18) = 4.75$, $P = 0.043$]. However, there was no significant effect of time (i.e., first vs. second test) on the first day of extinction [$F(1,25) = 0.75$, $P = 0.3$] or after 30 days of self-administration [$F(1,14) = 2.75$, $P = 0.12$]. There were no significant differences ($P > 0.05$) in responding among the different tests in either the vehicle- or CP-154,526-treated groups.

Fig. 2 depicts responding on the cocaine-associated lever during the first extinction test across 15-min bins. CP-154,526 produced the greatest effect in the first bin (i.e., the first 15 min of the session; [$F(1,26) = 22.929$, $P = 6.45 \times 10^{-5}$]). Significant differences were also observed during the third [$F(1,26) = 6.078$, $P = 0.020$] but not the second bin. A similar dynamic of responding was observed during all extinction test days. No corticosterone

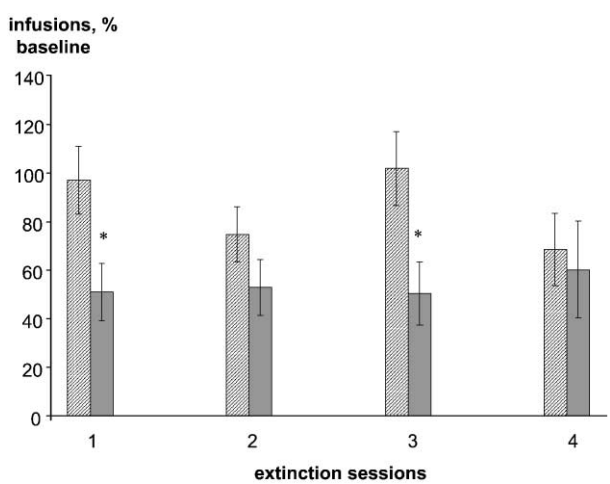

Fig. 1. Effects of pretreatment with CP-154,526 (20 mg/kg, i.p.) on cocaine lever responding during extinction. Data are presented as the percentage of responding on the day before the test (i.e., baseline). Striped bars represent responding in vehicle-pretreated animals; gray bars represent responding in CP-154,526-pretreated animals. * $P < 0.05$ CP-154,526 vs. vehicle pretreatment.

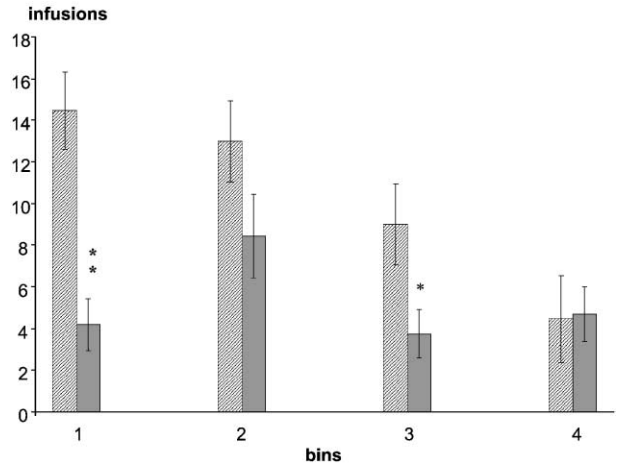

Fig. 2. Effects of CP-154,526 pretreatment (20 mg/kg, i.p.) during each of the four 15-min bins during extinction. Striped bars represent responding in vehicle-pretreated animals; gray bars represent responding in CP-154,526-pretreated animals. $^{*}P < 0.05$ CP-154,526 vs. vehicle pretreatment, $^{**}P < 0.01$ CP-154,526 vs. vehicle pretreatment.

suppression was observed in the CP-154,526-pretreated animals before or after the session at any time.

4. Discussion

Data obtained on the first day of extinction testing and following 30 days of cocaine self-administration suggest that CRH might be involved in cue-induced cocaine craving. Although there was a trend for a decrease in responding by rats in the vehicle-treated group during the second extinction test following acquisition and after chronic cocaine self-administration (Fig. 1), this effect was not statistically significant and may simply be attributed to the fact that the vehicle-treated rats rapidly learned to decrease lever pressing during repeated extinction testing. In addition, CP-154,526 did not completely block cue-induced behavior. This effect might be explained by the fact that the effects of CP-154,526 were diminished at the end of the 2-h session, as suggested by time-course studies (unpublished observations). We had expected that cue-induced cocaine seeking would have been greater after prolonged exposure to cocaine self-administration. Surprisingly, we failed to observe any significant increases in responding during extinction following 30 days of cocaine self-administration. This finding may be explained by the very rapid establishment of the conditioned effects of cocaine that reached a maximum by the first extinction test.

The finding that CRH1 receptors might be involved in cue-induced craving is not unexpected. CRH receptors have been shown to be involved in different types of conditioned behavior associated with cocaine, including conditioned anxiety (De Vries and Pert, 1998) and conditioned hypothalamo–pituitary–adrenocortical axis activation (De Vries et al., 1998) following the presentation of cocaine cues. These reports indicated that both hypothalamic and extrahypothalamic CRH can be activated by conditioned cues associated with cocaine, suggesting that at least two mechanisms can account for the observed decrease in cue-induced cocaine-seeking behavior. Given that the hypothalamo–pituitary–adrenocortical axis has been implicated in several aspects of cocaine-seeking behavior (Piazza et al., 1994; Goeders and Guerin, 1994, 1996; Deroche et al., 1997; Mantsch et al., 1998), it is possible that the blockade of CRH1 receptors might decrease cue-induced behavior via suppression of the hypothalamo–pituitary–adrenocortical axis. However, in this study CP-154,526 did not decrease plasma corticosterone, suggesting that the hypothalamo–pituitary–adrenocortical axis was not involved. Therefore, cocaine-seeking behavior was probably mediated by extrahypothalamic CRH1 receptors, as has already been shown for stress- and cocaine-induced reinstatement (Erb et al., 1998; Erb and Stewart, 1999).

The mechanisms mediating the involvement of CRH in cue-induced cocaine seeking are unclear. However, behavioral experiments have implicated the medial prefrontal cortex, anterior cingulate cortex and amygdala in this behavior (Whitelaw et al., 1996; Meil and See, 1997; Weissenborn et al., 1997; Grimm and See, 2000). Consistent with these behavioral data, cerebral blood flow is increased in the anterior cingulate and amygdala during cue-induced craving in humans (Childress et al., 1999). In rats, cocaine-associated cues also produce changes in the expression of Fos (Brown et al., 1992; Crawford et al., 1995; Baker et al., 1999; Neisewander et al., 2001; Ciccocioppo et al., 2001), Fos-related antigen (Franklin and Druhan, 2000) and gamma protein kinase C (Thomas and Everitt, 2001) in these regions.

The basolateral amygdala and prefrontal cortex contain CRH1 receptors (Smagin and Dunn, 2000), and CRH has been shown to modulate dopamine transmission in the medial prefrontal cortex (Dunn, 2000). Interestingly, cue-induced increases in Fos expression depend on dopamine in the basolateral amygdala and medial prefrontal cortex (Ciccocioppo et al., 2001), and conditioned dopamine release in the amygdala has also been reported (Weiss et al., 2000). However, CRH in the basolateral amygdala probably does not mediate these conditioned effects since dopamine transmission in the amygdala is not affected by CRH (Dunn, 2000). Taken together, these data suggest that the modulation of medial prefrontal cortex dopamine by CRH might underlie its involvement in cue-induced behavior.

In conclusion, our data suggest that CRH1 receptors are involved in cocaine-seeking behavior and may be good target for cocaine addiction therapy.

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