Neuropsychopharmacology (2008) 33, 1735–1745

www.neuropsychopharmacology.org

Cannabinoid CB1 Receptor Antagonists Attenuate Cocaine's Rewarding Effects: Experiments with Self-Administration and Brain-Stimulation Reward in Rats

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Previous studies suggest that cannabinoid CB1 receptors do not appear to be involved in cocaine's rewarding effects, as assessed by the use of SR141716A, a prototypic CB1 receptor antagonist and CB1-knockout mice. In the present study, we found that blockade of CB1 receptors by AM 251 (1–10 mg/kg), a novel CB1 receptor antagonist, dose-dependently lowered (by 30–70%) the break point for cocaine self-administration under a progressive-ratio (PR) reinforcement schedule in rats. The same doses of SR141716 (freebase form) maximally lowered the break point by 35%, which did not reach statistical significance. Neither AM 251 nor SR141716 altered cocaine self-administration under a fixed-ratio (FR2) reinforcement schedule. AM 251 (0.1–3 mg/kg) also significantly and dose-dependently inhibited (by 25–90%) cocaine-enhanced brain stimulation reward (BSR), while SR141716 attenuated cocaine's BSR-enhancing effect only at 3 mg/kg (by 40%). When the dose was increased to 10 or 20 mg/kg, both AM 251 and SR141716 became less effective, with AM 251 only partially inhibiting cocaine-enhanced BSR and PR cocaine self-administration, and SR141716 having no effect. AM 251 alone, at all doses tested, had no effect on BSR, while high doses of SR141716 alone significantly inhibited BSR. These data suggest that blockade of CB1 receptors by relatively low doses of AM 251 dose-dependently inhibits cocaine's rewarding effects, whereas SR141716 is largely ineffective, as assessed by both PR cocaine self-administration and BSR. Thus, AM 251 or other more potent CB1 receptor antagonists deserve further study as potentially effective anti-cocaine medications.
Neuropsychopharmacology (2008) **33,** 1735–1745; doi:10.1038/sj.npp.1301552; published online 29 August 2007

Keywords: cocaine; cannabinoid; AM 251; SR141716; self-administration; brain reward

INTRODUCTION

Converging evidence suggests that endocannabinoids are critically involved in drug addiction, particularly to marijuana, opiates, nicotine or ethanol (see reviews by Cohen *et al*, 2005; Lupica *et al*, 2004; Tanda and Goldberg, 2003). However, the role of endocannabinoids in cocaine addiction remains largely undetermined. It has been heretofore generally believed that endocannabinoids may be involved in relapse to cocaine-seeking behavior, but not in cocaine's direct rewarding effects (see review by Arnold, 2005). This belief derives from evidence that the cannabinoid receptor agonist HU 210 reinstates, while the CB1 receptor antagonists SR141716A or AM 251 inhibit cocaine or cocaine-associated cue-induced reinstatement of cocaine-seeking behavior (De Vries *et al*, 2001; Xi *et al*, 2006a). However, studies in other animal models have produced conflicting results. Activation of cannabinoid receptors by WIN55,212-2 decreased intravenous (i.v.) cocaine self-administration and inhibited cocaine-enhanced brain stimulation reward (BSR) (Fattore *et al*, 1999; Vlachou *et al*, 2003). In contrast, the cannabinoid receptor agonists WIN55,212-2 and CP55940 and the CB1 receptor antagonist SR141716A have been reported to inhibit electrical BSR in rats (Arnold *et al*, 2001; Deroche-Gamonet *et al*, 2001; Vlachou *et al*, 2003, 2005). Adding to these conflicting findings, other studies demonstrate that neither SR141716A nor deletion of CB1 receptors alters cocaine self-administration in mice, rats or squirrel monkeys (Cossu *et al*, 2001; De Vries *et al*, 2001; Tanda *et al*, 2000), cocaine-induced conditioned place preference (Martin *et al*, 2000) or cocaine-induced behavioral sensitization (Martin *et al*, 2000). In addition, neither SR141716A nor deletion of CB1 receptors altered cocaine-induced increases in extracellular dopamine (DA) in the nucleus accumbens (Caillé and

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Received 16 February 2007; revised 18 July 2007; accepted 26 July 2007

Parsons, 2006; Soria *et al*, 2005). Such findings have been interpreted as indicating that CB1 receptors are not critically involved in cocaine's rewarding effects (Arnold, 2005; Lesscher *et al*, 2005). However, it is not yet convincingly established whether blockade of CB1 receptors inhibits cocaine self-administration under progressive-ratio (PR) reinforcement conditions or cocaine-enhanced BSR in rats, arguably the most reliable models to directly evaluate brain reward function or brain reward responses to drugs of abuse (O'Brien and Gardner, 2005).

The reasons for the conflicting reports in the published literature are unclear. Given that WIN55,212-2 is a non-selective cannabinoid receptor agonist (Wiley and Martin, 2002), and that SR141716A is not highly selective as a CB1 receptor antagonist (Beardsley and Thomas, 2005; Pertwee, 2005), it seems likely that actions on other receptors may be involved. Further, the findings from CB1-knockout mice may be not conclusive because of neuroadaptations, which may alter the effects produced by gene mutation (Crawley, 1999; Phillips *et al*, 1999). Based on these considerations, we hypothesized that a more potent and selective CB1 receptor antagonist would be needed to adequately assess the role of CB1 receptors in cocaine's rewarding effects. Therefore, in the present study, we examined and compared the effects of AM 251 (a novel CB1 receptor antagonist) and SR141716 on cocaine self-administration under both fixed-ratio (FR) and PR reinforcement schedules and on cocaine-enhanced BSR in rats. AM 251 is both more potent ($K_i = 7.49$ *vs* 11.5 nM) and more selective (1:306 *vs* 1:143) than SR141716 as an antagonist at CB1 *vs* CB2 receptors (Lan *et al*, 1999; Krishnamurthy *et al*, 2004).

MATERIALS AND METHODS

Animals

Experimentally naive male Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA) weighing 250 to 300 g were used for all experiments. They were housed individually in a climate-controlled animal colony room on a reversed light–dark cycle (lights on at 1900, lights off at 0700) with free access to food and water. The animals were maintained in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the US National Academy of Sciences, and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the US National Institutes of Health.

Experiment 1: Cocaine Self-Administration

Surgery. All animals were prepared for experimentation by surgical catheterization of the right external jugular vein. The venous catheters were constructed of microrenathane (Braintree Scientific Inc., Braintree, MA, USA), and catheterization was performed under sodium pentobarbital anesthesia (65 mg/kg, i.p.) with standard aseptic surgical techniques. After exiting the jugular, the catheter passed subcutaneously to the top of the skull, where it exited into a connector (a modified 24 g cannula; Plastics One, Roanoke, VA, USA) mounted to the skull with jeweler's screws and dental acrylic. During experimental sessions, the catheter was connected to the injection pump via tubing encased in a protective metal spring from the head-mounted connector to the top of the experimental chamber. To help prevent clogging, the catheters were flushed daily with a gentamicin-heparin-saline solution (30 IU/ml heparin; ICN Biochemicals, Cleveland, OH, USA).

Apparatus. Intravenous self-administration experiments were conducted in operant response test chambers ($32 \times 25 \times 33$ cm) from MED Associates Inc. (Georgia, VT, USA). Each test chamber had two levers: one active and one inactive, located 6.5 cm above the floor. Depression of the active lever activated the infusion pump; depression of the inactive lever was counted but had no consequence. A cue light and a speaker were located 12 cm above the active lever. The house light was turned on at the start of each 3 h test session. To aid acquisition and maintenance of drug self-administration behavior, each drug infusion was always paired with a conditioned cue light and a cue sound (tone). Scheduling of experimental events and data collection were accomplished using MED Associates software.

General procedure. After recovery from surgery, each rat was placed into a test chamber and allowed to lever-press for i.v. cocaine (1 mg/kg/infusion) delivered in 0.08 ml over 4.6 s, on an FR1 reinforcement schedule. During the 4.6 s infusion time, additional responses on the active lever were recorded but did not lead to additional infusions. Each session lasted 3 h. FR1 reinforcement was used for 3–5 days until stable cocaine self-administration was established. The initial cocaine dose of 1 mg/kg/infusion was chosen on the basis of our previous experience that this dose produces rapid and facile acquisition of cocaine self-administration behavior. Subsequently, subjects were randomly assigned to one of the following two experiments: (1) cocaine self-administration under an FR2 reinforcement schedule or (2) cocaine self-administration under a PR reinforcement schedule. In all experiments, AM 251 or SR141716 was given 30 min prior to testing because our previous data showed that onset of behavioral effects occurred approximately 30 min after systemic administration of these compounds (Xi *et al*, 2006a).

Cocaine self-administration under FR2 reinforcement. After transition from FR1 reinforcement, subjects were allowed to continue cocaine (0.5 mg/kg/infusion) self-administration under FR2 reinforcement until the following criteria for stable cocaine-maintained responding were met: less than 10% variability in the inter-response interval and less than 10% variability in number of presses on the active lever for at least 3 consecutive days. The dose of cocaine was chosen based on previous findings that rats self-administering cocaine at 0.5 mg/kg/infusion display highly stable self-administration behavior. In addition, previous studies have shown that 0.5 mg/kg/infusion of cocaine lies within the range of the descending limb of the cocaine dose–response self-administration curve, where stable and reliable dose-dependent effects are observed (Xi *et al*, 2005). Furthermore, we chose 0.5 mg/kg, rather than 1 mg/kg of

cocaine in order to increase the work demand (ie lever presses) of the animals for the same amount of drug intake. In our previous experience, this approach increases the sensitivity of measuring changes in drug-taking or drug-seeking behavior. To avoid cocaine overdose during the self-administration period, each animal was limited to a maximum of 50 cocaine injections per 3 hr session. After stable rates of responding were established, each subject randomly received one of three doses of AM 251 (1, 3, 10 mg/kg, i.p.), one of four doses of SR141716 (0.3, 1, 3, 10 mg/kg), or vehicle (1 ml of 25% 2-hydroxypropyl-β-cyclodextrin solution) 30 min prior to the test session. Animals then received an additional 5–7 days of self-administration of cocaine alone until baseline response rate was reestablished prior to testing the next dose of drug. The order of testing for the various doses of AM 251 or SR141716 was counterbalanced according to a Latin square design.

Cocaine self-administration under progressive-ratio reinforcement. Initial cocaine self-administration under FR1 and FR2 reinforcement schedules was identical to that outlined above. After stable cocaine self-administration under FR2 reinforcement was established, the subjects were switched to cocaine self-administration (0.5 mg/kg/infusion) under a PR schedule, during which the work requirement of lever presses needed to receive a single i.v. cocaine infusion was progressively raised within each test session (see details in Richardson and Roberts, 1996) according to the following PR series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492 and 603 until the break point was reached. The break point was defined as the maximal workload (ie number of lever presses) completed for the last cocaine infusion prior to a 1-h period during which no infusions were obtained by the animal. Animals were allowed to continue daily sessions of cocaine self-administration under PR reinforcement conditions until day-to-day variability in break point fell within 1–2 ratio increments for 3 consecutive days. Once a stable break point was established, subjects were assigned to eight subgroups to determine the effects of four different doses of AM 251 (1, 3, 10, 20 mg/kg, i.p.), five doses of SR141716 (0.3, 1, 3, 10, 20 mg/kg) or vehicle (1 ml of 25% 2-hydroxypropyl-β-cyclodextrin solution) on PR break point for cocaine self-administration. Since it is relatively difficult to reachieve basal break point levels after each drug test, we chose to use a between-subjects design rather than a within-subjects design to determine the dose–response effects of AM 251 and SR141716 on break point.

Experiment 2: Intracranial Electrical Brain Stimulation Reward

Surgery. Under the same anesthesia as used in Experiment 1, rats were placed in a stereotaxic frame, and a unilateral monopolar stainless-steel stimulating electrode (Plastics One, Roanoke, VA, USA) was placed into the medial forebrain bundle at the anterior-posterior level of the lateral hypothalamus, using standard aseptic surgical and stereotaxic techniques. The implant coordinates for the tips of the electrodes were AP-2.56, ML ± 1.9, and DV −8.6, according to the rat brain stereotaxic atlas of Paxinos and Watson (1998). The electrode was attached to the skull with jeweler's screws and dental acrylic. A wire leading from the electrode was wrapped around a skull screw to serve as a current return.

Apparatus. The experiments were conducted in standard MED Associates operant chambers (32 × 25 × 33 cm). Each operant chamber had a lever located 6.5 cm. above the floor, connected to an electrical stimulator.

General procedure. The general procedures for electrical BSR were the same as we have reported previously (Xi *et al*, 2006b). Briefly, after 7 days of recovery from surgery, rats were allowed to self-train (autoshape) to lever-press for rewarding BSR. Each press on the lever resulted in a 500-ms train of 0.1-ms rectangular cathodal pulses through the electrode in the rat's medial forebrain bundle, followed by a 500 ms 'timeout' in which further presses did not produce brain stimulation. The initial stimulation parameters were 72 Hz and 200 μA. If the animal did not learn to lever-press, the stimulation intensity was increased daily by 50 μA until the animal learned to press (45–60 responses/30 s) or a maximum of 800 μA was reached. Animals that did not lever-press at 800 μA or in which the stimulation produced unwanted effects (eg head or body movements or vocalization) were removed from the experiment.

Rate-frequency BSR procedure. Following establishment of lever-pressing for BSR, animals were presented with a series of 16 different pulse frequencies, ranging from 141 to 25 Hz in descending order. At each pulse frequency, animals responded for two 30-s time periods ('bins'), after which the pulse frequency was decreased by 0.05 log units. Following each 30-s bin, the lever retracted for 5 s. Throughout the experiment, animals were run for three sessions a day. The response rate for each frequency was defined as the mean number of lever responses during two 30-s bins. Since lever-pressing behavior was variable during the first session (the 'warm-up' session), but was stable during the second and third sessions, the data from the first session were discarded, and the data from the second and third sessions were designated as the baseline session data and test session data, respectively. The BSR threshold (θ_0) was defined as the minimum frequency at which the animal responded for rewarding stimulation. In addition, M_{50}, ie stimulation frequency for half maximal reward efficacy, was also used to evaluate the effects of drugs on BSR itself or on cocaine-enhanced BSR. BSR threshold (θ_0) and M_{50} were mathematically derived for each 'baseline' run and each 'drug' run by analyzing each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies using 'best-fit' mathematical algorithms. Specifically, each rate-frequency BSR function was mathematically fitted, by iterative computer programs derived from the Gauss–Newton algorithm for nonlinear regression, to three different sigmoid curve-fitting mathematical growth models that appear to accurately fit rate-frequency brain-stimulation reward functions (Coulombe and Miliaressis, 1987)—the Gompertz model ($Y' = ae^{-e(b-cX)}$), the logistic model ($Y' = a/[1 + e^{(b-cX)}]$), and the Weibull function ($Y' = a[1-e^{-(bX)c}]$); where Y' is the rate of response

(number of lever presses for rewarding brain stimulation per unit of time), X is the pulse frequency, and a, b, and c are parameters approximated from each empirical rate-frequency data curve (a representing the asymptotic response rate value, b relating to the intercept of the rate-frequency curve with the y axis, and c representing the rate at which Y increments). From each curve-fitting model, a solution for θ_0 and a solution for M_{50} were obtained. Thus, for each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies, three solutions for θ_0 and three solutions for M_{50} were obtained. The three solutions for θ_0 were averaged, to produce a mean θ_0 for each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies. Similarly, the three solutions for M_{50} were averaged, to produce a mean M_{50} for each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies. The mean θ_0 values and mean M_{50} values were expressed as means ± SEM. Data analyses were performed on percent changes from baseline levels.

Testing the effects of cocaine, AM 251 or SR141716 on BSR. Once a baseline θ_0 value or M_{50} value was achieved (<15% variation over 5 continuous days), the effects of cocaine and/or AM 251 or SR141716 on BSR were assessed. On test days, animals randomly received one of five different doses of AM 251 (0.1, 0.3, 1, 3, 10 mg/kg i.p.), SR141716 (0.1, 0.3, 1, 3, 10 mg/kg i.p.) or vehicle (1 ml of 25% 2-hydroxypropyl-β-cyclodextrin) 30 min prior to a cocaine injection (2 mg/kg i.p.). After each test, animals received an additional 5–7 days of BSR restabilization until a new baseline θ_0 or M_{50} was established. The order of testing for various doses of AM 251 or SR141716 was counterbalanced according to a Latin square design. The effect of AM 251 or SR141716 on cocaine-enhanced BSR was evaluated by comparing cocaine-induced alterations in θ_0 or M_{50} value in the presence or absence of each dose of drug pretreatment.

Drugs

Cocaine HCl (Sigma Chemical Co., St Louis, MO, USA) was dissolved in physiological saline. SR141716 (free base form) was obtained from Research Triangle Institute (Research Triangle Park, NC, USA). AM 251 was purchased from Tocris (Ellisville, MO, USA). For i.p. injections, 25% 2-hydroxypropyl-β-cyclodextrin (Sigma/RBI, St Louis, MO, USA) was used as vehicle.

Data Analyses

All data are presented as means (± SEM). One-way analysis of variance (ANOVA) was used to analyze the effects of AM 251 or SR141716 on cocaine self-administration under FR or PR conditions. Two-way ANOVA with repeated measurements was used to analyze the effects of AM 251 or SR141716 on cocaine-enhanced BSR. Post-ANOVA individual group comparisons were carried out using the Bonferroni t-test procedure.

RESULTS

Effects of AM 251 or SR141716 on Cocaine Self-Administration

Figure 1 shows the effects of AM 251 (1–10 mg/kg, i. p.) or SR141716 (0.3–10 mg/kg, i.p.) on cocaine self-administration under a FR2 reinforcement schedule, illustrating that neither AM 251 ($F_{3,18} = 0.74$, p = NS) nor SR141716 ($F_{3,24} = 1.25$, p = NS) altered the cocaine self-administration rate, when administered 30 min prior to the beginning of daily cocaine self-administration sessions. However, pretreatment with AM 251 significantly and dose-dependently inhibited cocaine self-administration under PR reinforcement conditions (Figures 2b and c), while SR141716 did not produce a statistically significant reduction of break point for cocaine self-administration (Figure 2d and e). Figure 2a shows representative individual responses, illustrating that 3 mg/kg AM 251 lowered the PR break point (ie number of lever presses for the last cocaine infusion) from 77 after vehicle (Figure 2a, upper trace) to 40 after AM 251 administration (Figure 2a, lower trace). One-way ANOVA for the data shown in Figure 2b indicates a statistically

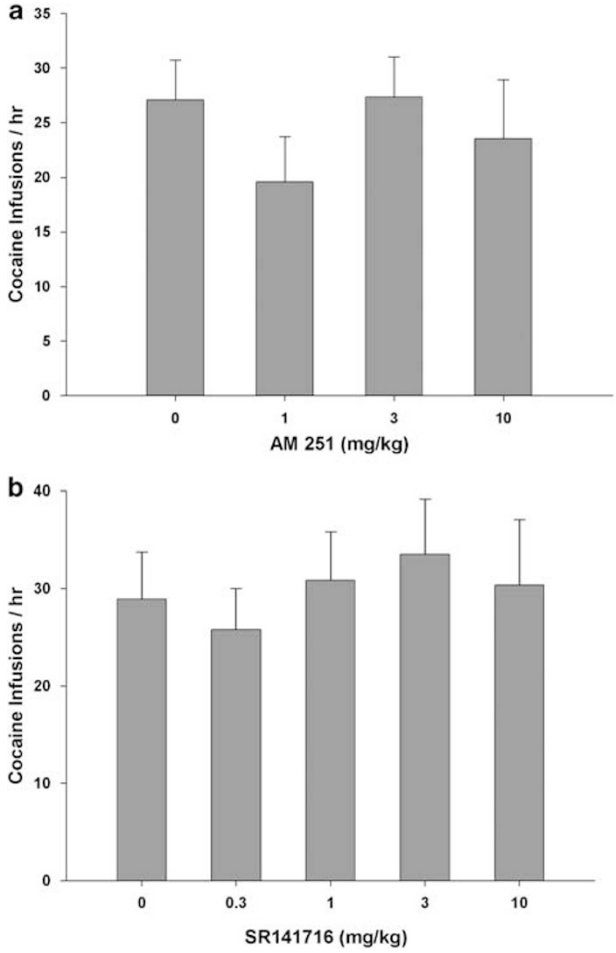

Figure 1 Effects of AM 251 or SR141716 on cocaine self-administration under a fixed-ratio 2 (FR2) reinforcement schedule. When administered 30 min prior to testing, neither AM 251 (1–10 mg/kg, i.p.) nor SR141716 (0.3–10 mg/kg, i.p.) significantly altered FR2 cocaine self-administration behavior.

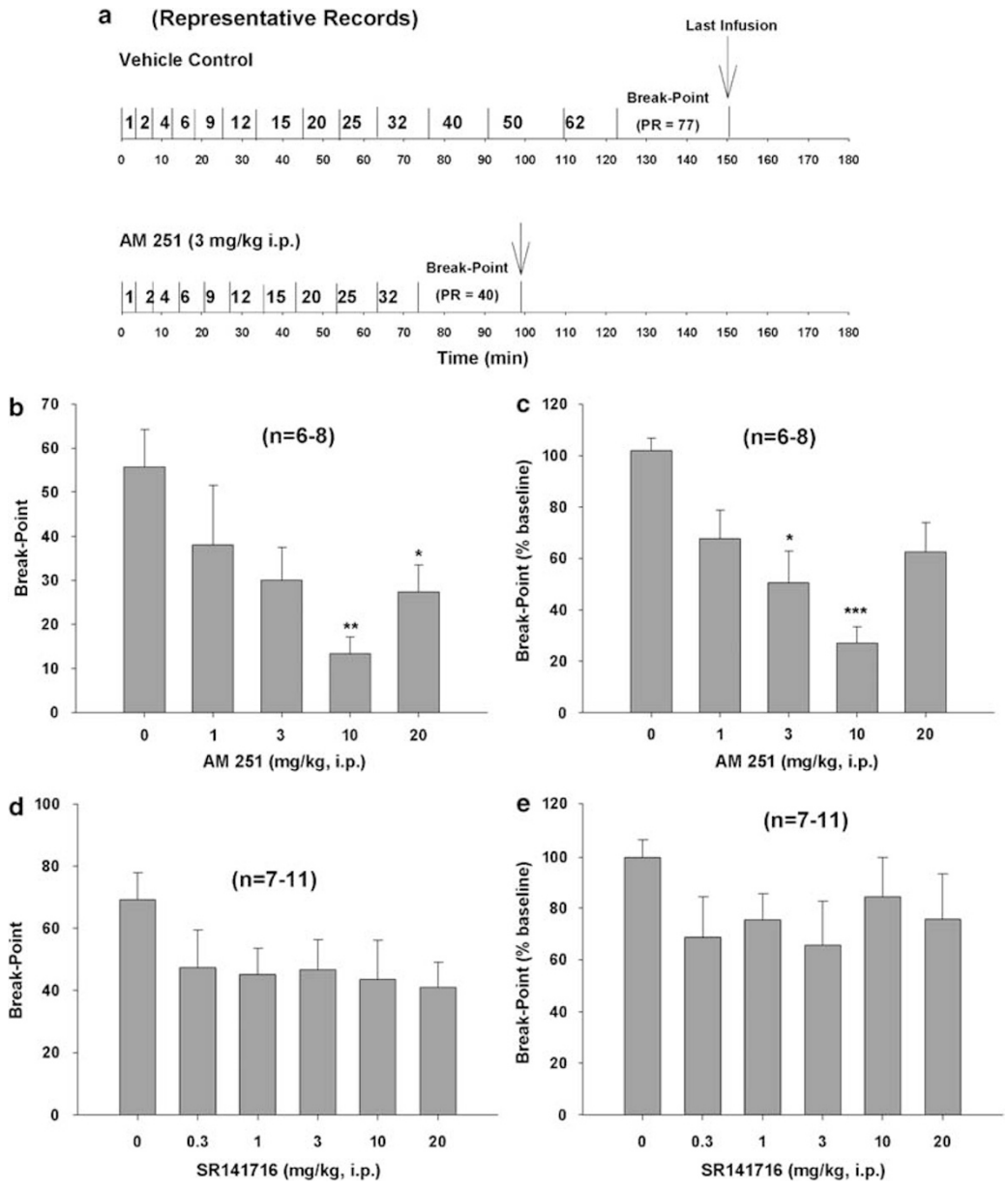

Figure 2 Effects of AM 251 or SR141716 on cocaine self-administration under a progressive-ratio (PR) reinforcement schedule. (a) Representative records of an individual animal illustrating a reduction in the PR break point for cocaine self-administration from 77 after vehicle (upper trace) to 40 after 3 mg/kg AM 251 (lower trace) pretreatment. Each vertical line indicates one cocaine infusion (0.5 mg/kg/infusion). The number between the vertical lines indicates the work demand (ie progressively increased number of lever presses) for a subsequent cocaine infusion. (b) Mean effects of AM 251 (1–20 mg/kg) on break point levels for cocaine self-administration under PR reinforcement conditions. (c) Percentage changes in break point after AM 251 administration. (d) SR141716's effect on PR break point for cocaine self-administration, and (e) Percentage changes in break point after SR141716 administration. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, when compared with the vehicle treatment group.

significant reduction by AM 251 on break point for cocaine self-administration under PR reinforcement schedule ($F_{4,28}=3.52$, $p<0.05$). Post-ANOVA individual group comparisons using the Bonferroni *t*-test reveals a statistically significant reduction in break point for cocaine self-administration after 10 mg/kg AM 251 ($t=3.63$, $p<0.01$) or 20 mg/kg AM 251 ($t=3.30$, $p<0.05$), but not after 1 mg/kg ($t=1.54$, $p=$NS) or 3 mg/kg ($t=1.71$, $p=$NS), when compared with the vehicle treatment group. One-way ANOVA for the data shown in Figure 2c revealed a statistically significant treatment main effect ($F_{4,28}=5.32$, $p<0.01$). Post-ANOVA individual group comparisons indicated a significant reduction in break point after 3 mg/kg ($t=3.44$, $p<0.05$) or 10 mg/kg ($t=4.41$, $p<0.01$) AM 251, but not after 1 mg/kg AM251 ($t=2.34$, $p=$NS) or 20 mg/kg ($t=2.96$, $p=$NS) AM251, when compared with the vehicle pretreatment group.

In contrast to AM251, SR141716 appears to have no effect on PR cocaine self-administration, as assessed either by the break point levels (Figure 2d: $F_{5,52}=1.56$, $p=$NS) or the percent changes of break point compared to baseline (Figure 2e: $F_{5,52}=1.96$, $p=$NS).

Effects of AM 251 on Cocaine-Enhanced BSR

Figure 3a shows representative rate-frequency function curves for BSR, indicating the BSR threshold (θ_0, Hz), the stimulation frequency for half maximal reward efficacy (M_{50}, Hz), the maximal work amount (Ymax, lever presses), and the effects of cocaine and/or AM251 on BSR. Cocaine (2 mg/kg i.p.) produced a significant leftward shift in the rate-frequency function curve, reflecting lowered BSR threshold (θ_0) and M_{50} values. Pretreatment with AM 251 (3 mg/kg, i.p., administered 30 min prior to the test session) attenuated the cocaine-induced decrease in θ_0 and M_{50} values. Figures 3b and c show the group mean data of the changes (%) in BSR after cocaine and/or AM251 administration, indicating that cocaine significantly enhanced brain reward (vehicle *vs* 2 mg/kg cocaine, Figure 3b: $t=8.59$, $p<0.001$; Figure 3c: $t=6.13$, $p<0.001$), which was significantly attenuated by AM 251 (0.3–10 mg/kg, i.p.). Two-way ANOVA for repeated measurements on the data shown in Figure 3b (θ_0 data, 0–3 mg/kg AM251) revealed a statistically significant treatment main effect ($F_{1,11}=43.19$, $p<0.001$), dose main effect ($F_{4,44}=8.25$, $p<0.001$), and treatment × dose interaction ($F_{4,44}=11.77$, $p<0.001$). Individual group comparisons revealed a statistically significant reduction in cocaine-enhanced BSR after 0.3 mg/ kg ($t=2.99$, $p<0.05$), 1 mg/kg ($t=4.04$, $p<0.001$), or 3 mg/kg AM 251 ($t=4.69$, $p<0.001$), but not after 0.1 mg/kg AM 251 ($t=0.80$, $p=$NS). Similarly, two-way ANOVA for repeated measurements on the data shown in Figure 3c (M_{50} data, 0–3 mg/kg) also revealed a statistically significant treatment main effect ($F_{1,11}=29.01$, $p<0.001$), dose main effect ($F_{4,44}=4.91$, $p<0.01$) and treatment × dose interaction ($F_{4,44}=5.35$, $p<0.001$). Individual group comparisons revealed a statistically significant reduction in cocaine-enhanced BSR after 0.3 mg/kg AM 251 ($t=3.73$, $p<0.01$), 1 mg/kg AM 251 ($t=3.59$, $p<0.01$) or 3 mg/kg AM 251 ($t=4.67$, $p<0.001$), but not after 0.1 mg/kg AM 251 ($t=1.52$, $p=$NS). When the dose was further increased to

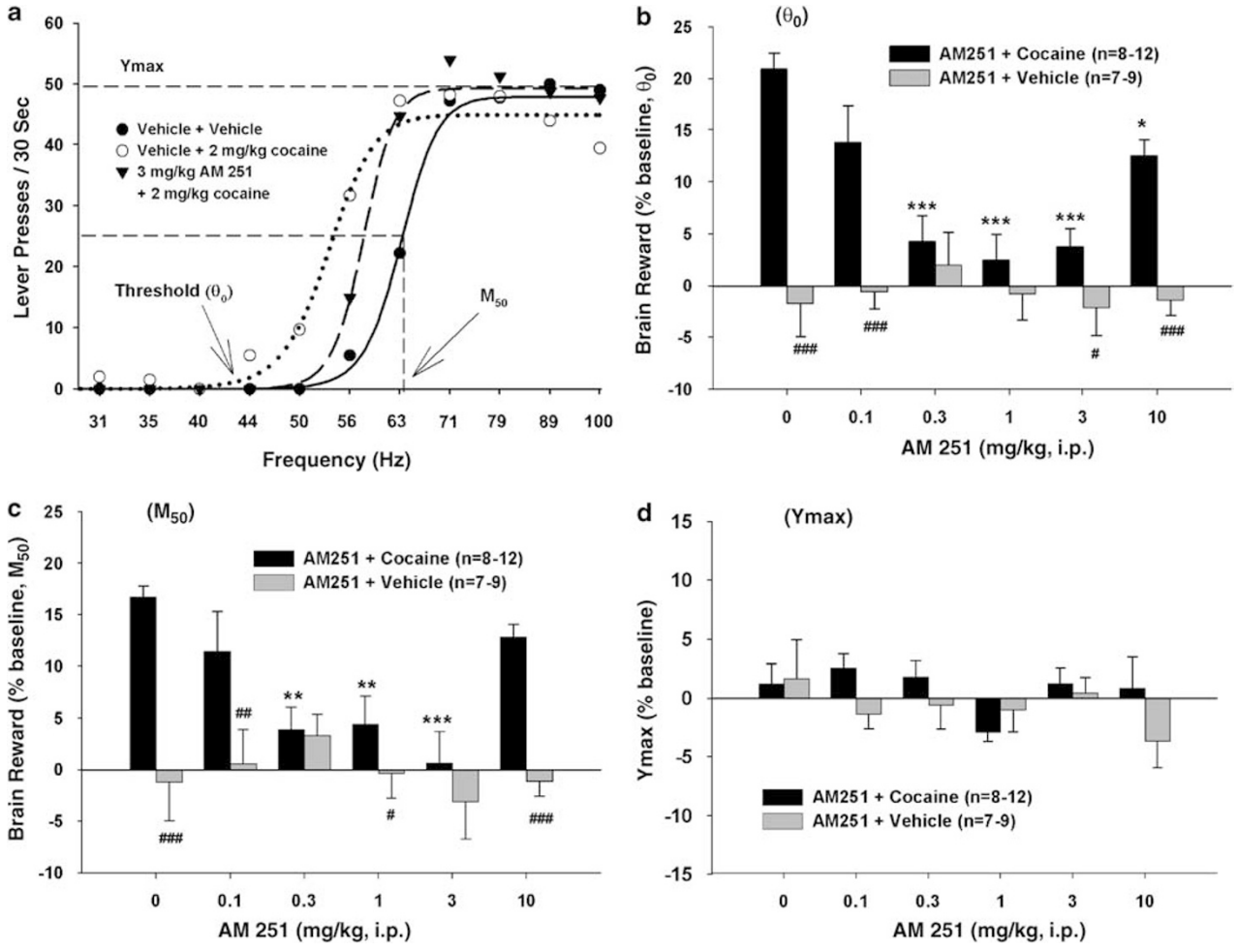

Figure 3 Effects of cocaine and/or AM 251 on electrical brain stimulation reward (BSR). (a) Representative rate-frequency curves for BSR, indicating BSR threshold (θ_0), M_{50}, Ymax, and the effects of AM251 and/or cocaine on BSR. Cocaine (2 mg/kg i.p.) produced a leftward shift in the rate-frequency function, lowering the BSR θ_0 and M_{50} values, which was significantly attenuated by AM 251 (3 mg/kg, i.p.). (b and c) Mean percent changes in BSR θ_0 and M_{50} values, indicating that AM 251 (0.1–10 mg/kg, i.p.) significantly inhibited the enhanced BSR produced by 2 mg/kg cocaine. AM 251 alone had no significant effect on BSR, at any doses tested, as assessed by either θ_0 or M_{50}. (d) AM 251 had no effect on Ymax levels in the absence or presence of cocaine. $^{\#}p<0.001$, $^{\#\#}p<0.001$, $^{\#\#\#}p<0.001$, comparisons between AM 251 + Cocaine and AM251 + Vehicle groups at each different AM 251 dose. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, when compared with the cocaine-alone treatment group (0 mg/kg AM 251).

10 mg/kg in an additional group of rats, AM 251 became less effective at attenuating cocaine-enhanced BSR. Individual group comparisons (10 mg/kg AM 251 *vs* 0 mg/kg AM251) revealed a significant reduction in cocaine-enhanced BSR as assessed by threshold (θ_0) (Figure 3b: $t = 2.17$, $p < 0.05$), but not by M_{50} (Figure 3c: $t = 1.04$, $p =$ NS). AM 251 alone, at any dose tested, failed to alter either threshold (θ_0) or M_{50} values for BSR, when compared with the vehicle (0 mg/kg AM251) treatment group. Figure 3d shows that AM251, at any dose tested, had no effect on Ymax levels in the absence or presence of cocaine (two-way ANOVA: $F_{1,5} = 5.55$, $p =$ NS).

Effects of SR141716 on Cocaine-Enhanced BSR

In contrast to AM251, SR141716 (0–3 mg/kg i.p.) appeared to be less effective at reducing cocaine-enhanced BSR (Figure 4). Two-way ANOVA for repeated measurements for the data shown in Figure 4a (θ_0 data, 0–3 mg/kg SR141716) revealed a statistically significant treatment main effect ($F_{1,12} = 97.55$, $p < 0.001$). Individual group comparisons revealed a statistically significant reduction in cocaine-enhanced BSR only after 3 mg/kg ($t = 3.52$, $p < 0.05$), but not after any other doses of SR141716 tested. Similarly, two-way ANOVA with repeated measurements for the data shown in Figure 4b (M_{50} data, 0–3 mg/kg SR141716) also revealed a statistically significant treatment main effect ($F_{1,12} = 111.90$, $p < 0.001$), dose main effect ($F_{4,48} = 2.93$, $p < 0.05$) and treatment × dose interaction ($F_{4,48} = 7.57$, $p < 0.001$). Post-ANOVA individual group comparisons indicate a significant reduction in cocaine-enhanced BSR only after 3 mg/kg ($t = 3.17$, $p < 0.05$), but not after any other doses of SR141716 tested. SR141716 alone, in the dose range of 0.1–3 mg/kg, had no effect on BSR itself. However, when the dose was increased to 10 mg/kg in an additional group of animals, SR141716 became ineffective on cocaine-enhanced BSR (Figure 4a: $t = 2.61$, $p =$ NS; Figure 4b: $t = 0.04$, $p =$ NS), while significantly inhibiting BSR by itself (Figure 4a, $t = 7.51$, $p < 0.001$; Figure 4b, $t = 8.74$, $p < 0.001$). Figure 4d shows that SR141716, at any dose tested, had no effect on Ymax levels in the absence or presence of cocaine (two-way ANOVA: $F_{1,5} = 4.38$, $p =$ NS).

DISCUSSION

The present study demonstrates that blockade of CB1 receptors by relatively low doses of AM 251 significantly inhibits cocaine self-administration under PR reinforcement and also attenuates cocaine-enhanced BSR. In contrast, SR141716 only produces a moderate inhibition of cocaine-enhanced BSR at 3 mg/kg, and had no effect on PR cocaine self-administration at any dose tested. When doses were increased to 10 or 20 mg/kg, both AM 251 and SR141716 became less effective in attenuating cocaine-enhanced BSR and cocaine self-administration under PR reinforcement. These data suggest that blockade of CB1 receptors produces an inhibitory effect on cocaine's rewarding effects, and that AM 251 is more effective than SR141716 in antagonizing cocaine's rewarding actions. Further, AM 251 alone, at all doses tested, failed to alter BSR itself, while high-dose SR141716 inhibited BSR. These

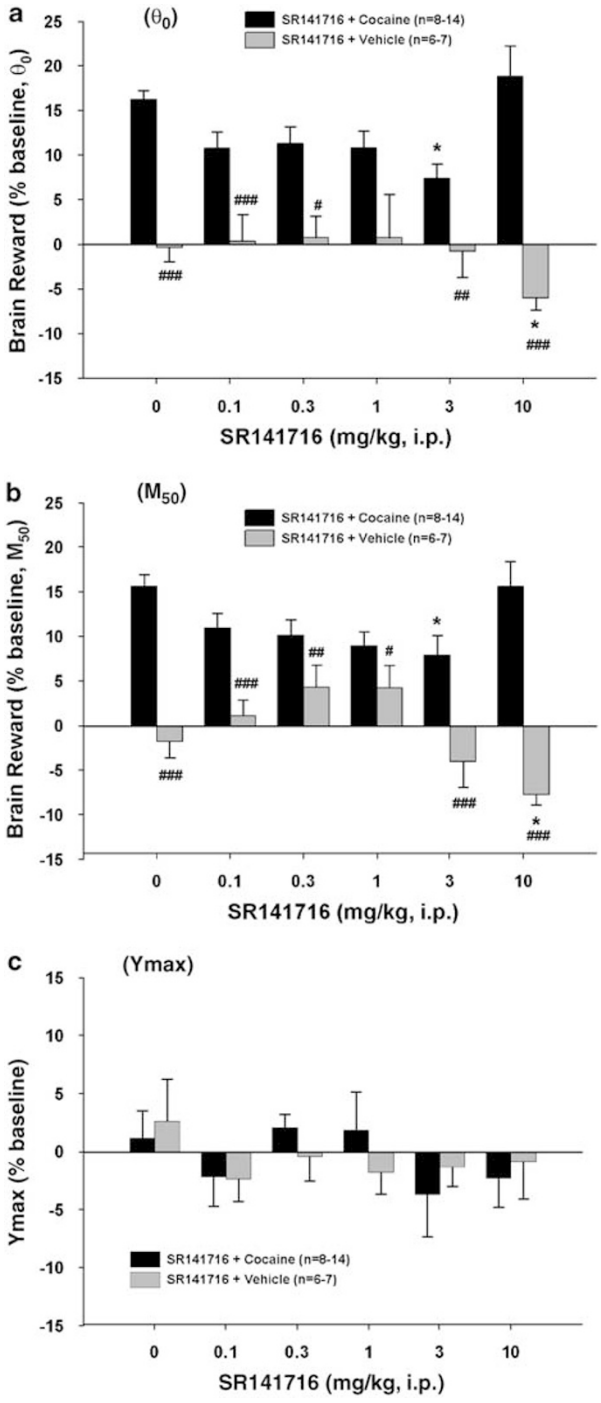

Figure 4 Effects of cocaine and/or SR141716 on electrical brain stimulation reward (BSR). (a) Mean percentage change in BSR threshold (θ_0) after SR141716 pretreatment, indicating that SR141716, at 3 mg/kg, but not at other doses, produced a statistically significant inhibition of cocaine-enhanced BSR. (b) SR141716, at 3 mg/kg, but not at other doses, produced a statistically significant attenuation of cocaine-enhanced BSR as assessed by M_{50} value. SR141716 alone, at 10 mg/kg, significantly inhibited BSR itself. (c) SR141716 had no effect on Ymax levels in the absence or presence of cocaine. $^{\#}p < 0.001$, $^{\#\#}p < 0.001$, $^{\#\#\#}p < 0.001$, comparisons between SR141716 + Cocaine and SR141716 + Vehicle groups at each different SR141716 dose. $^{*}p < 0.05$, when compared with the cocaine-alone treatment group (0 mg/kg SR141716).

data suggest that SR141716 itself may produce aversive-like effects *in vivo*, while AM 251 does not.

CB1 receptors have gained interest in the study of drug abuse because of the rewarding and psychostimulating effects of cannabinoids (see reviews by Tanda and Goldberg, 2003; Beardsley and Thomas, 2005). CB1 agonists activate the mesolimbic DA system (Chen *et al*, 1990; Tanda *et al*, 1997), produce significant enhancement of BSR (Gardner *et al*, 1988), and are self-administered i.v. in mice (Martellotta *et al*, 1998; Ledent *et al*, 1999), rats (Fattore *et al*, 2001) and squirrel monkeys (Tanda *et al*, 2000). Conversely, it has been reasoned that CB1 receptor antagonists might attenuate brain reward functions and thus have potential as pharmacotherapies for treatment of drug addiction (Le Foll and Goldberg, 2005). However, investigation of this suggestion was hampered by lack of selective CB1 receptor antagonists, until the development of SR141716 (Rinaldi-Carmona *et al*, 1994). Accumulating evidence has indicated that blockade of CB1 receptors by SR141716 significantly inhibits the rewarding effects of a variety of addictive drugs, including Δ^9-tetrahydrocannabinol (Δ^9-THC) (Tanda and Goldberg, 2003), opiates (Fattore *et al*, 2004), nicotine (Cohen *et al*, 2005), and ethanol (Vinod and Hungund, 2005).

However, the role of CB1 receptors in mediating cocaine's rewarding effects has been controversial. Early studies suggested that CB1 receptors do not play a significant role in cocaine's rewarding effects, because SR141716A had no effect on FR cocaine self-administration in rats (De Vries *et al*, 2001; Lesscher *et al*, 2005) or monkeys (Tanda *et al*, 2000). In addition, CB1 receptor-deficient mice learned to self-administer cocaine (Cossu *et al*, 2001). In the conditioned place preference (CPP) paradigm, SR141716A inhibited the acquisition, but not the expression of cocaine-induced CPP (Chaperon *et al*, 1998). Also, CB1 receptor deletion did not prevent the development of cocaine-induced CPP or behavioral sensitization (Martin *et al*, 2000).

However, the opinion that CB1 receptors play no role in cocaine's rewarding effects has been challenged recently by evidence demonstrating that SR141716A appears to inhibit PR cocaine self-administration in mice (Soria *et al*, 2005), and that the novel selective CB1 receptor antagonist AM 251 inhibits methamphetamine self-administration in rats (Vinklerova *et al*, 2002). These data suggest that the ineffectiveness of SR141716A on FR cocaine self-administration or other behavioral measurements of cocaine's reward efficacy could be related to SR141716's potency and/or selectivity *in vivo*. To test this hypothesis, we observed and compared the effects of both AM 251 and SR141716 on cocaine self-administration under PR and FR2 reinforcement conditions and on cocaine-enhanced BSR in the present study. We found that AM 251 (1–10 mg/kg) dose-dependently inhibited cocaine self-administration under PR conditions ($\sim$75% at 10 mg/kg, $p<0.01$), while the same doses of SR141716 only produced a modest overall inhibition ($\sim$30%, $p=$NS). When the dose was further increased to 20 mg/kg, AM 251 became less effective at inhibiting cocaine self-administration, while SR141716 still failed to significantly inhibit PR cocaine self-administration. This latter finding appears to contradict a previous report that SR141716A (1–3 mg/kg) produced a significant inhibition ($\sim$55%) of PR cocaine self-administration in mice (Soria *et al*, 2005). This difference could be related to the use of different species in the two studies. In addition, we also found that neither AM 251 nor SR141716 inhibited FR cocaine self-administration, which is in agreement with previous reports (De Vries *et al*, 2001; Tanda *et al*, 2000). These data suggest that blockade of CB1 receptors by AM 251 significantly inhibits cocaine's rewarding efficacy, as assessed by PR cocaine self-administration, while SR141716 is considerably less effective.

There are several possible explanations for AM251's inhibition of cocaine self-administration under PR, but not under FR conditions. First, animals may compensate for AM251's action by increasing cocaine intake under FR2 conditions. We consider this possibility unlikely, because no such increase in cocaine self-administration rate was observed in the present study (Figure 1). Second, the FR2 reinforcement schedule demands less work to obtain a much higher cumulative cocaine dose than does the PR reinforcement schedule. Thus, the stronger rewarding effects produced by the higher cumulative dose of cocaine may counteract the antagonism by AM 251 of cocaine's actions. This may, at least in part, explain why the PR break point shift paradigm is more sensitive to changes in a drug's rewarding efficacy than FR self-administration (Richardson and Roberts, 1996; Arnold and Roberts, 1997; Gardner, 2000; Rowlett, 2000). Given that PR break point levels for cocaine self-administration are cocaine dose-dependent, AM251-induced reduction in break point for cocaine self-administration may suggest a reduction in both cocaine's rewarding efficacy and incentive motivational properties for drug-taking and drug-seeking behavior (Richardson and Roberts, 1996; Arnold and Roberts, 1997; Rowlett, 2000; Xi *et al*, 2005). This is consistent with our previous finding that AM 251 also significantly inhibits cocaine-triggered reinstatement (relapse) of drug-seeking behavior in rats (Xi *et al*, 2006a).

Our conclusion with respect to AM251's attenuation of cocaine's reward efficacy is further supported by our finding that AM 251 also significantly inhibited cocaine-enhanced BSR, another commonly used paradigm to study brain reward function (Wise 1996). The rewarding effects of brain stimulation are thought to involve the same reward circuits as, and to summate with, the rewarding effects of addictive drugs (Wise, 1996). Cocaine significantly shifts the stimulation-response curve to the left, ie decreases BSR threshold (θ_0) or M_{50}, indicating summation of the reward produced by the electrical brain stimulation and the cocaine (Bauco and Wise, 1997). In the present study, pretreatment with AM 251 (0.1–3 mg/kg) produced a significant and dose-dependent inhibition (maximally by 90%) of cocaine-enhanced BSR, while SR141716 produced a modest inhibition of cocaine-enhanced BSR only at 3 mg/kg, but not by lower doses (0.1–1 mg/kg). This is consistent with our findings with cocaine self-administration—showing that AM 251 is more effective than SR141716 at antagonizing cocaine's rewarding effects. Again, when the dose was further increased to 10 mg/kg, both AM 251 and SR141716 became less effective. At 10 mg/kg, AM 251 only partially attenuated cocaine-enhanced BSR, while SR141716 had no effect at all. These data suggest that other non-CB1 receptor mechanisms may be involved in the actions produced by

such high doses of CB1 receptor antagonists (Beardsley and Thomas, 2005; Pertwee, 2005).

Further, AM 251 alone, at all doses tested, failed to alter BSR, while SR141716 produced an inhibition of BSR at high dose. This is consistent with previous studies demonstrating that high doses of SR141716A significantly inhibit BSR (Arnold *et al*, 2001; Deroche-Gamonet *et al*, 2001), raising the possibility that SR141716 may be dysphorigenic at the human level.

We should note that the effective doses of AM 251 in the BSR paradigm are much lower than those in cocaine self-administration, suggesting that the BSR paradigm is more sensitive than self-administration paradigms to changes in brain reward function. This could be related to the fact that cumulative doses of drug (such as cocaine) during self-administration obviously reach much higher levels than those reached following a single injection of drug in the BSR paradigm (Xi and Gardner, 2007). This is also consistent with our previous findings demonstrating that DA D3 receptor antagonists are more effective at attenuating cocaine's actions in the BSR paradigm than in cocaine self-administration (Xi *et al*, 2005).

We should also point out that the reduction in PR cocaine self-administration and cocaine-enhanced BSR by AM 251 is unlikely due to a nonspecific inhibition of locomotion or locomotor ability, because the same doses of AM 251 altered neither cocaine self-administration under FR2 reinforcement conditions (Figure 1) nor Ymax levels in the BSR paradigm (Figures 3d and 4c). In addition, we have reported that AM 251 does not alter sucrose-triggered reinstatement of sucrose-seeking behavior (Xi *et al*, 2006a). All of these are behaviors that are known to be sensitive to motoric inhibition.

The mechanisms underlying the differences between the pharmacological actions of the two CB1 antagonists are unclear. The present findings demonstrate that AM 251 is more potent in attenuating cocaine's rewarding efficacy than SR141716. This is consistent with their *in vitro* binding properties, ie that AM 251 is roughly twofold more potent ($K_i = 7.49$ *vs* 11.5 nM) and more selective (1:306 *vs* 1:143) than SR141716 for CB1 over CB2 receptors (Lan *et al*, 1999; Krishnamurthy *et al*, 2004). In addition, growing evidence demonstrates that SR141716 is not as highly selective a CB1 receptor antagonist *in vivo* as previously believed: (1) SR141716 and its analogs significantly alter locomotion behaviors, actions that are poorly correlated to their binding affinities to the CB1 receptor (Bass *et al*, 2002); (2) acute or chronic administration of SR141716 produce many nonspecific 'side effects', such as grooming, intense scratching, forepaw fluttering, wet-dog shaking, ultrasonic vocalization, and in some species, emesis, which are not correlated to its action on CB1 receptors or on post-receptor intracellular G protein–mediated signaling (Rubino *et al*, 1998, 2000; Beardsley and Thomas, 2005); (3) SR141716 produces similar biological effects in *in vitro* cell lines expressed with intact or mutant CB1 receptors (Pertwee, 2005); (4) SR141716 significantly elevates extracellular glutamate levels in the nucleus accumbens similarly in both wild-type and CB1-knockout mice (Xi *et al*, 2006c), and also reverses the inhibition of WIN55,212-2 on hippocampal glutamate transmission in both wild-type and CB1-knockout mice (Hajos *et al*, 2001). These data suggest that SR141716 may have nonspecific binding properties on other non-CB1 and/or non-cannabinoid receptors *in vivo*, which may contribute to its relative ineffectiveness in attenuating cocaine's rewarding efficacy. Such nonspecific binding potential may also partially explain why SR141617 or AM251, at the high doses (10–20 mg/kg), lost the pharmacological potency observed at lower doses. Finally, the pharmacokinetics and brain penetration properties of both antagonists *in vivo* may be different, thereby contributing to the different pharmacological actions observed in the present study.

The mechanisms by which CB1 receptors are involved in mediating cocaine's rewarding effects are unclear. Previous studies show that Δ^9-THC enhances NAc extracellular DA (Chen *et al*, 1990; Tanda *et al*, 1997), suggesting that a DA-related mechanism may underlie the antagonism of CB1 receptor antagonists on cocaine's rewarding efficacy. On the other hand, growing evidence demonstrates that genetic mutation or pharmacological blockade of CB1 receptors by either AM 251 or SR141716A fails to alter basal or cocaine-enhanced NAc DA (Tanda *et al*, 1997; Soria *et al*, 2005; Caillé and Parsons, 2006; Xi *et al*, 2006a), pointing to a DA-independent mechanism. This is consistent with anatomical evidence demonstrating that CB1 receptors are not located on brain DA neurons (Herkenham *et al*, 1991; Mailleux and Vanderhaeghen, 1992; Freund *et al*, 2003; but see Wenger *et al*, 2003). It is well documented that cocaine or DA produces inhibitory effects on NAc GABAergic neurons (White *et al*, 1993; Nicola and Malenka, 1997; Centonze *et al*, 2002), and that GABAergic inhibition has been proposed to be critical to drug reward (Carlezon and Wise, 1996). In addition, recent studies demonstrate that cocaine or DA significantly increases endocannabinoid release in the striatum (Giuffrida *et al*, 1999; Patel *et al*, 2003; Centonze *et al*, 2004). Given that high densities of CB1 receptors are expressed on medium spiny GABAergic neurons and glutamatergic terminals in striatum (Herkenham *et al*, 1991; Mailleux and Vanderhaeghen, 1992; Matyas *et al*, 2006; Corbillé *et al*, 2007), it is suggested that an increase in endocannabinoid tone may mediate cocaine's inhibition of medium-spiny GABAergic neurons via activation of CB1 receptors on GABAergic neurons and/or glutamatergic terminals (in the latter case, decreasing glutamate input onto medium spiny neurons) (Robbe *et al*, 2001). Our previous studies have shown that blockade of CB1 receptors by AM251 significantly elevates NAc extracellular glutamate levels (Xi *et al*, 2006a). This finding may constitute a possible explanation for the present results. This is, an increase in NAc glutamate may counteract cocaine- or DA-induced inhibition of the medium spiny GABAergic neurons, thereby producing antagonism of cocaine's rewarding effects.

Whatever the underlying mechanisms, the present study demonstrates that blockade of CB1 receptors by AM 251 (and to a lesser extent by SR141716) inhibits cocaine's rewarding effects as assessed by PR cocaine self-administration and cocaine-enhanced BSR. SR141716's lesser potency in this regard may relate to its lower specificity and selectivity for CB1 receptors as compared to AM251. The intermodel consistency of the present findings (PR break point and BSR) strengthens the conclusion that CB1 receptor blockade inhibits cocaine's rewarding effects. This

is also consistent with human studies showing that smoked cannabis tends to enhance cocaine's euphorigenic effects (Foltin *et al*, 1993; Lukas *et al*, 1994).

In sum, the present data suggest that AM 251 or other more potent CB1 receptor antagonists may be effective in the treatment of cocaine addiction.

ACKNOWLEDGEMENTS

This research was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services.

DISCLOSURE/CONFLICT OF INTEREST

We hereby declare that, except for income received from their respective primary employers, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional services. There are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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