Brief Communications

Cannabinoid CB₁ Receptor Antagonist AM251 Inhibits Cocaine-Primed Relapse in Rats: Role of Glutamate in the Nucleus Accumbens

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Blockade of cannabinoid CB₁ receptors has been reported to inhibit cocaine- or cocaine cue-induced reinstatement of drug seeking. However, the mechanisms underlying this action are poorly understood. Given the importance of dopamine, glutamate, and GABA in cocaine reward and relapse, we studied the effects of AM251 [N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide], a novel highly selective CB₁ receptor antagonist, on cocaine-primed reinstatement of drug-seeking behavior and on cocaine-induced changes in extracellular DA, glutamate, and GABA in the nucleus accumbens (NAc) under reinstatement conditions. We found that systemic administration of AM251 selectively inhibited cocaine-induced, but not sucrose plus sucrose cue-induced, reinstatement of reward-seeking behavior. AM251 alone did not trigger reinstatement. Local perfusion of AM251 into the NAc or the dorsal striatum also inhibited cocaine-triggered reinstatement. AM251 alone dose dependently elevated NAc glutamate in a voltage-dependent Na⁺ channel-dependent manner. AM251 did not affect NAc DA or GABA. Pretreatment with AM251 dose dependently inhibited cocaine-induced increases in NAc glutamate but not in DA. Blockade of NAc metabotropic glutamate mGluR2/3 receptors by LY341495 [(2S)-2-amino-2-[(15S,2S)-2-carboxycycloprop-1-yl]-(xanth-9-yl) propanoic acid] slightly facilitated cocaine-enhanced glutamate release but blocked the antagonism of cocaine-induced reinstatement by AM251. These data suggest the following: (1) CB₁ receptors exert tonic inhibition over NAc glutamate release under cocaine-extinction conditions; (2) blockade of CB₁ receptors by AM251 inhibits cocaine-enhanced NAc glutamate release and cocaine-triggered reinstatement; and (3) these effects appear to be mediated by activation of presynaptic mGluR2/3 autoreceptors secondary to AM251-induced increase (disinhibition) of NAc glutamate release.

Key words: cocaine; dopamine; glutamate; mGluR2/3; AM251; cannabinoid; relapse

Introduction

Cocaine addiction is a chronic, relapsing disorder with no effective medications available for treatment despite extensive effort. The endocannabinoid system appears critically involved in relapse to drug seeking for many addictive drugs. Activation of cannabinoid CB₁ receptors by HU 210 [(6αR,10αR)-trans-3-(1,1'-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methyl] or WIN55,212-2 [R(+)-(2,3-dihydro-5-methyl-3-[4-morpholinyl]methyl)[pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl) methanone mesylate] dose dependently reinstates cocaine or heroin seeking, whereas CB₁ receptor blockade by SR141716A [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] inhibits reinstatement (relapse) of drug-seeking behavior (De Vries et al., 2001; Anggadiredja et al., 2004; Spano et al., 2004; Fattore et al., 2005). Thus, it is suggested that CB₁ receptors may be a useful target for anti-relapse medication development (De Vries et al., 2001). However, the mechanisms by which cannabinoids modulate relapse are poorly understood.

Enhanced dopamine (DA) transmission in the nucleus accumbens (NAc), a critical brain region for drug reward (Wise, 1996), plays a role in cocaine-primed relapse. Systemic administration or local NAc microinjection of DA agonists reinstates, whereas DA antagonists block, reinstatement of cocaine-seeking behavior (Shalev et al., 2002; Anderson et al., 2003, 2006; Bachtell et al., 2005). Endocannabinoids appear uninolved in such DA-dependent drug-primed relapse, because CB₁ receptor blockade by SR141716A fails to block cocaine- or heroin-induced increases in NAc DA in drug-naïve rats (Tanda et al., 1997; Caillé and Parsons, 2006). The CB₁ receptor appears located predominantly on glutamatergic and/or GABAergic afferents in the NAc (Mailleux and Vanderhaeghen, 1992; Pickel et al., 2004), suggesting that cannabinoids may affect cocaine-primed relapse by action on glutamatergic or GABAergic NAc terminals. In support of this hypothesis, cocaine priming elevates NAc DA and glutamate (Neisewander et al., 1996; Pierce et al., 1996; McFarland et al.,...
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Materials and Methods

Animals. Male Long–Evans rats (Charles River Laboratories, Raleigh, NC) weighing 250 –300 g were used. They were housed individually in a climate-controlled animal room on a reversed light/dark cycle with access to food and water ad libitum. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the United States National Academy of Sciences and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse.

Cocaine self-administration and reinstatement of drug-seeking behavior. Animals were surgically implanted with intravenous catheters (for cocaine self-administration) and/or intracranial guide cannulas (for microinjection) under sodium pentobarbital anesthesia as reported previously (Xi et al., 2004). After recovery from surgery, animals were placed into standard operant chambers for cocaine self-administration and reinstatement as reported previously (Xi et al., 2006). Initially, animals pressed the active lever for cocaine (1 mg/kg per infusion) for 3 h/d under fixed ratio 1 (FR1) reinforcement and were then switched to 0.5 mg/kg per infusion under FR2 reinforcement. Cocaine infusions were associated with light and sound cues. Inactive lever presses were counted but had no consequence.

After stable self-administration was established, animals were exposed to extinction conditions: cocaine was replaced by saline, and the cocaine-associated cue light and tone were turned off. Daily extinction sessions continued until lever pressing was <10 per 3 h session for 3 consecutive days. Then, animals were divided into several experimental groups, and reinstatement testing was begun 24 h later. On the reinstatement test day, each group of rats received either vehicle (2% 2-hydroxypropyl-

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cure plus cue-triggered reinstatement of sucrose-seeking behavior. The procedures for oral sucrose self-administration, extinction, and reinstatement testing were identical to the procedures for cocaine self-administration, extinction, and reinstatement testing except for the following: (1) no surgery was performed on the animals in the sucrose experiment; (2) active lever presses led to delivery of 0.1 ml of 5% sucrose solution into a liquid food tray on the operant chamber wall; and (3) reinstatement was triggered initially by two “free” sucrose deliveries, and subsequent lever presses led to the presentation of the conditioned cue light and tone. Because sucrose-triggered reinstatement is significantly weaker than cocaine-triggered reinstatement, we used sucrose plus cue priming to facilitate reinstatement of sucrose-seeking behavior.

In vivo brain microdialysis. Microdialysis experiments were performed in additional groups of rats under identical cocaine self-administration and extinction conditions. Five groups of rats were used to evaluate the effects of AM251 (0, 1, 3, and 10 mg/kg) on NAc DA, glutamate, and GABA in the presence or absence of tetrodotoxin (voltage-dependent Na+ channel blocker). Another five groups of rats were used to evaluate the effects of the same doses of AM251 or LY341495 [(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid] (metabotropic glutamate receptor mGluR2/3 antagonist), given as pre-

treatments, on cocaine-induced changes in NAc DA, glutamate, and GABA. Microdialysis was performed in drug-unpaired microdialysis chambers to prevent the potential effects of drug-paired environmental cues or subsequent drug-seeking (lever presses) behavior on extracellular neurotransmitter levels. Microdialysis protocols and probe construction were as reported previously (Xi et al., 2003). Guide cannulas (20 gauge; Plastics One, Roanoke, VA) were surgically implanted into the NAc (anteroposterior, +1.6 mm; mediolateral, ±1.8 mm; dorsoventral, –4.3 mm, angled 6° from vertical). Microdialysis probes were inserted into the NAc 12 h before the experiment to minimize damage-induced neurotransmitter release. During the experiment, microdialysis buffer was perfused through the probe (2.0 µl/min) for at least 2 h before sampling started. Samples were collected every 20 min into 10 µl of 0.5 M perchloric acid to prevent neurotransmitter degradation. After 1 h baseline collection, one of three doses of AM251 (1, 3, or 10 mg/kg, i.p.) or vehicle (1 ml of 2-hydroxypropyl-β-cycloextrin) were administered 60 min before cocaine priming. All samples were frozen at –80°C until analyzed.

Microdialysate DA was measured by HPLC with an ESA (Chelmsford, MA) electrochemical (EC) detection system as described previously (Xi et al., 2003), upgraded by a Coulochem III EC detector. Concentrations of glutamate and GABA were determined using HPLC with fluorometric detection (Xi et al., 2003). Different excitation (ExA) and emission (EmA) wavelengths were used to measure glutamate (ExA, 314 nm; EmA, 394 nm) and GABA (ExA, 336 nm; EmA, 420 nm) in the same samples. Areas under the curve (AUCs) for DA, glutamate, or GABA were measured.

Drugs. Cocaine HCl was from the National Institute on Drug Abuse and was dissolved in physiological saline. AM251 was purchased from Tocris Cookson (Ellisville, MO) and dissolved in 25% 2-hydroxypropyl-

β-cycloextrin (Sigma, St. Louis, MO). The dose of AM251 for intracranial microinjections was chosen on the basis of a ratio of ~1000:1 for systemic/intracranial microinjection dosing that we successfully used.
previously (Xi et al., 2004). The microinjection volume was 1.0 μl bilaterally.

Histology. After microdialysis, rats were killed by pentobarbital overdose (>100 mg/kg, i.p.) and perfused transcardially with 0.9% saline, followed by 10% Formalin. Brains were removed and placed in 10% Formalin for 1 week. The tissue was blocked around the NAc, and coronal sections (100 μm thick) made by vibratome. The sections were stained with cresyl violet.

Data analyses. All data are presented as means ± SEM. AUC data were used for quantifying AM251 effects on basal and cocaine-induced changes in neurotransmitter levels. One-way ANOVA was used to analyze the effects of AM251 on cocaine- or sucrose plus cue-induced reinstatement (see Fig. 1) and on basal and cocaine-induced neurochemical events (see Figs. 2–4). Two-way ANOVA with repeated measures over time was used to analyze the effects of AM251 alone or of AM251 pretreatment on cocaine-induced changes in NAc neurotransmitter levels (see Figs. 2–4). Post-ANOVA individual group comparisons were by the Bonferroni’s procedure.

Results

AM251 on cocaine-triggered reinstatement of drug seeking

A single, noncontingent cocaine injection (10 mg/kg, i.p.) produced robust reinstatement of lever pressing within 30–60 min, with responding reextinguishing gradually thereafter. AM251 pretreatment (1, 3, and 10 mg/kg, i.p.) dose dependently inhibited cocaine-induced reinstatement of drug-seeking behavior (Fig. 1a, $F_{(3,36)} = 4.64$, $p < 0.01$). Individual group comparisons revealed statistically significant decreases in cocaine-induced reinstatement after 1 mg/kg ($t = 2.82, p < 0.05$), 3 mg/kg ($t = 3.76, p < 0.001$), or 10 mg/kg ($t = 3.40, p < 0.001$) AM251. In contrast, the same doses of AM251 neither altered sucrose plus cue-induced reinstatement of sucrose-seeking behavior (Fig. 1b, $F_{(2,18)} = 1.419, p > 0.05$) nor reinstated drug-seeking behavior by itself (Fig. 1c, $F_{(2,14)} = 1.65, p > 0.05$). When administered locally into the NAc or the dorsal striatum, AM251 (5 and 10 μg/μl) similarly inhibited cocaine-triggered reinstatement of drug-seeking behavior but only at the high dose (Fig. 1d, $F_{(2,22)} = 3.57, p < 0.05$; Fig. 1e, $F_{(2,27)} = 3.59, p < 0.05$). Because systemic administration of AM251 selectively elevated NAc extracellular glutamate, but not DA or GABA (see results below), it is suggested that the antagonism of cocaine-triggered relapse by AM251 could be mediated indirectly by activating presynaptic mGluR2/3 receptors. In support of this hypothesis, Figure 1f shows that intra-NAc microinjection of LY341495 (mGluR2/3 antagonist, 0.2 μg/1 μl, bilaterally) attenuated AM251-induced inhibition of cocaine-triggered relapse ($F_{(2,24)} = 3.66, p < 0.05$).

AM251 on cocaine-induced increases in NAc DA

AM251 alone (1, 3, and 10 mg/kg, i.p.) failed to alter NAc DA (Fig. 2a) within 5 h (Fig. 2b, $F_{(3,22)} = 1.09, p > 0.05$). Cocaine priming (10 mg/kg, i.p.) produced a significant increase (~300%) in NAc DA (Fig. 2c). AM251 pretreatment (1, 3, and 10 mg/kg) had no effect on cocaine-augmented NAc DA (Fig. 2d, $F_{(3,25)} = 1.21, p > 0.05$). Two-way ANOVA for repeated measures over time for the data shown in Figure 2c revealed a statistically significant main effect over time ($F_{(14,350)} = 22.53, p < 0.001$) but no significant drug treatment main effect ($F_{(2,25)} = 1.19, p > 0.05$) or treatment × time interaction ($F_{(12,350)} = 0.88, p > 0.05$). Individual group comparisons revealed that the cocaine-induced increases in NAc DA in each group were statistically significant.

AM251 on cocaine-induced increases in NAc glutamate

AM251 by itself produced a small elevation in NAc glutamate levels within 2–3 h after administration (Fig. 3a), which then grew with passage of additional time into a statistically significant elevation (Fig. 3b, $F_{(4,27)} = 9.16, p < 0.001$) with a prolonged time course (Fig. 3a). Two-way ANOVA for repeated measures over time for the data shown in Figure 3a revealed a statistically significant treatment main effect ($F_{(4,28)} = 5.98, p = 0.001$), time main effect ($F_{(17,476)} = 7.66, p < 0.001$), and treatment × time interaction ($F_{(68,476)} = 3.29, p < 0.001$). Cocaine priming produced a significant increase (~200%) in NAc glutamate in rats subjected to reinstatement test conditions, lasting ~60 min. AM251 pretreatment dose dependently attenuated cocaine-induced increases in NAc glutamate (Fig. 3d, $F_{(3,32)} = 2.55, p < 0.05$). Two-way ANOVA for repeated measures over time for the data shown in Figure 3c revealed a statistically significant treatment main effect ($F_{(3,32)} = 2.59, p < 0.05$), time main effect ($F_{(14,308)} = 3.65, p < 0.001$), and treatment × time interaction ($F_{(42,308)} = 2.17, p < 0.001$). Individual group comparisons revealed that the cocaine-induced increase in glutamate was statistically significant in the vehicle treatment group, and that this
cocaine-induced increase was significantly attenuated by 3 mg/kg (t = 2.22, p < 0.05) or 10 mg/kg (t = 3.13, p < 0.05) but not 1 mg/kg (t = 1.84, p = NS) AM251 (Fig. 3d). Intra-NAc perfusion of LY341495 failed to inhibit, but slightly prolonged, cocaine-induced increases in glutamate.

**AM251 or cocaine on extracellular NAc GABA**
AM251 by itself failed to significantly alter extracellular NAc GABA (Fig. 4a) (Fig. 4b, F(3,24) = 1.23, p = NS). Acute cocaine priming did not significantly alter extracellular NAc GABA in the presence of vehicle or the various AM251 doses (Fig. 4d, F(3,23) = 0.75, p = NS). Two-way ANOVA for repeated measures over time for the data shown in Figure 4e revealed no significant treatment main effect (F(3,23) = 0.81, p = NS), time main effect (F(14,322) = 0.91, p = NS), or treatment × time interaction (F(42,322) = 0.73, p = NS).

**Histological examinations**
Figure 4e shows the loci of intracranial microinjections of AM251 in rat brain. Microinjection tips aimed at the NAc were found to be within the NAc shell and core. Microinjection tips aimed at the dorsal striatum were found to be in the dorsal striatum, near the border of the corpus callosum. Figure 4f shows the locations of microdialysis probes in the NAc. The membrane portions of the probes were primarily in the NAc core. Some were at the interface between the core and shell, and a few were in the shell.

**Discussion**
The present study, for the first time, measured changes in extracellular NAc DA, glutamate, and GABA in rats in the same time frame and under the similar conditions as in cocaine-primed reinstatement of cocaine-seeking behavior. The findings are, first, that cocaine priming-triggered reinstatement of cocaine-seeking behavior parallels increases in NAc DA and glutamate but not GABA, suggesting an important role for enhanced DA and glutamate in cocaine-triggered relapse, congruent with previous suggestions (Kalivas, 2004). Second, systemic administration of AM251 does not significantly augment cocaine-induced DA, but not sucrose plus cue-induced, relapse, confirming previous findings with the CB1 receptor antagonist SR141716A (De Vries et al., 2001; Fattore et al., 2005). Third, local administration of AM251 into the NAc or the dorsal striatum similarly inhibited cocaine-triggered relapse, suggesting an important role for the whole striatum in habit learning of drug-seeking behavior (Vanderschuren et al., 2005). Fourth, AM251 inhibited cocaine-induced increases in NAc glutamate but not DA, suggesting a glutamate-related mechanism underlying the antagonism of AM251 of cocaine-primed relapse. Fifth, AM251 alone significantly increased extracellular NAc glutamate but not DA or GABA, suggesting that CB1 receptors exert tonic inhibition on glutamate release (Robbe et al., 2001). Finally, blockade of NAc mGlur2/3 receptors prevented the antagonism of cocaine-triggered relapse by AM251, suggesting a critical role for mGlur2/3 receptors in cocaine-triggered reinstatement of drug-seeking behavior (Xi et al., 2002; Baptista et al., 2004).

**DA mechanisms in cocaine-primed relapse**
Impressive evidence suggests that cocaine-triggered relapse to cocaine seeking is mediated by NAc DA mechanisms (Shalev et al., 2002). Among such evidence is that most drugs that trigger
reinstatement are DA agonists, either direct or indirect (Shalev et al., 2002). For example, intra-NAc microinjection of DA itself or DA D1-like receptor agonists triggers reinstatement, whereas DA D1- or D2-like receptor antagonists attenuate cocaine-triggered reinstatement (Cornish and Kalivas, 2000; Anderson et al., 2003, 2006; Bachtell et al., 2005). Although SR141716A blocks cocaine-triggered relapse (De Vries et al., 2001), it fails to block cocaine- or heroin-induced NAc DA release in drug-naive rats (Tanda et al., 1997; Caille and Parsons, 2006). Similarly, AM251 also inhibits cocaine-triggered relapse but fails to alter cocaine-induced increases in NAc DA in cocaine-treated rats (present study). Therefore, additional mechanisms underlying relapse must be sought.

Glutamate mechanisms in cocaine-primed relapse

Relevant to the present findings, glutamate has been proposed to be crucial to cocaine-triggered reinstatement (Kalivas, 2004).

Systemic cocaine injections or intra-NAc microinjections of either DA or AMPA produce reinstatement of drug-seeking behavior. Intra-NAc AMPA blocks reinstatement by all tested compounds, whereas intra-NAc DA antagonism only blocks reinstatement by intra-NAc DA (Cornish and Kalivas, 2000; Park et al., 2002). These data suggest that, although NAc DA mechanisms may be involved in relapse to cocaine seeking, glutamate-mediated NAc mechanisms may constitute a final common pathway into which such DA mechanisms feed. Consistent with this hypothesis, the present study found that blockade of CB1 receptors by AM251 produced a significant increase in extracellular glutamate and also antagonized cocaine-induced increase in glutamate release, suggesting that a glutamate-related mechanism may underlie the antagonism of cocaine-triggered relapse by AM251. Because an increase in NAc glutamate after AM251 leads to increased binding to presynaptic metabotropic glutamate (mGluR2/3) autoreceptors, it is hypothesized that such mGluR2/3-mediated inhibition of glutamate release may underlie the antagonism of cocaine-triggered reinstatement by AM251 of drug-seeking behavior. This is supported by the evidence demonstrating that the mGluR2/3 antagonist LY341495 significantly blocked the antagonism of cocaine-triggered relapse by AM251 but did not block cocaine-induced increases in NAc glutamate. This is consistent with a recent finding demonstrating that mGluR2/3 agonists inhibit cocaine-triggered relapse (Baptista et al., 2004). In addition, mounting evidence demonstrates that repeated cocaine administration produces long-term reduction in basal NAc glutamate (Baker et al., 2003) or long-term depression of excitatory synaptic transmission (Thomas et al., 2001). The present findings suggest that AM251-elevated NAc glutamate may normalize such repeated cocaine-induced reduction in NAc glutamate transmission. Furthermore, neuroimaging studies in human cocaine addicts suggest reduced neural activity in the orbitofrontal cortex and cingulate gyrus, which has been hypothesized to correlate with drug craving and vulnerability to relapse (Volkow et al., 2004). Because high densities of CB1 receptors are found in the orbitofrontal and cingulate cortices (Mailleux and Vanderhaeghen, 1992), the present findings also suggest that CB1 receptor blockade in these brain regions may normalize neural activity, thereby antagonizing cocaine craving and relapse.

GABA mechanisms in cocaine-primed relapse

In addition to actions on DA and glutamate, CB1 receptor activation also inhibits NAc GABAergic transmission, as evidenced by electrophysiological experiments (Hoffman and Lupica, 2001;
Manzoni and Bockaert, 2001). However, in the present study, AM251 did not affect NAc GABA in cocaine-abstinent rats, suggesting that NAc GABAergic transmission is not involved in AM251-inhibited induction of cocaine-prime relapse. This is consistent with a recent report that SR141716A fails to block cocaine-induced decreases in extracellular GABA in the ventral pallidum of drug-naive rats (Caillé and Parsons, 2006).

In conclusion, the present study, for the first time, demonstrates that the novel selective CB1 receptor antagonist AM251 inhibits cocaine-triggered reinstatement of drug-seeking behavior by a glutamate-dependent mechanism: CB1 receptor-mediated disinhibition of NAc glutamate release may activate presynaptic mGlur2/3 receptors, which then inhibits cocaine-enhanced NAc glutamate release and cocaine-triggered reinstatement of drug-seeking behavior. Given the paucity of effective medications to treat cocaine addiction, the present findings suggest that CB1 receptor antagonists may be promising in relapse prevention for cocaine addiction.

References


Baptista MA, Martin-Fardon R, Weiss F (2004) Preferential effects of the CB1 antagonist SR141716A on conditioned reinforcement and reinstatement of drug-seeking behavior. Given the paucity of effective medications to treat cocaine addiction, the present findings suggest that CB1 receptor antagonists may be promising in relapse prevention for cocaine addiction.