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Reinforcing and neurochemical effects of cannabinoid CB1 receptor agonists, but not cocaine, are altered by an adenosine A2A receptor antagonist

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Abstract

Several recent studies suggest functional and molecular interactions between striatal adenosine A_{2A} and cannabinoid CB_1 receptors. Here we demonstrate that A_{2A} receptors selectively modulate reinforcing effects of cannabinoids. We studied effects of A_{2A} receptor blockade on the reinforcing effects of delta-9-tetrahydrocannabinol (THC) and the endogenous CB_1 receptor ligand anandamide under a fixed-ratio (FR) schedule of intravenous drug injection in squirrel monkeys. A low dose of the selective adenosine A_{2A} receptor antagonist MSX-3 (1 mg/kg) caused downward shifts of THC and anandamide dose-response curves. In contrast, a higher dose of MSX-3 (3 mg/kg) shifted THC and anandamide dose-response curves to the left. MSX-3 did not modify cocaine or food-pellet self-administration. Also, MSX-3 neither promoted reinstatement of extinguished drug-seeking behavior nor altered reinstatement of drug-seeking behavior by non-contingent priming injections of THC. Finally, using in-vivo microdialysis in freely-moving rats, a behaviorally active dose of MSX-3 significantly counteracted THC-induced, but not cocaine-induced, increases in extracellular dopamine levels in the nucleus accumbens shell. The significant and selective results obtained with the lower dose of MSX-3 suggest that adenosine A_{2A} antagonists acting preferentially at presynaptic A_{2A} receptors might selectively reduce reinforcing effects of cannabinoids that lead to their abuse. However, the appearance of potentiating rather than suppressing effects on cannabinoid reinforcement at the higher dose of MSX-3 would likely preclude the use of such a compound as a medication for cannabis abuse. Adenosine A_{2A} antagonists with more selectivity for presynaptic versus postsynaptic receptors could be potential medications for treatment of cannabis abuse.

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Authors Contribution

ZJ, SF, and SRG were responsible for the study concept and design. GHR carried out primate experiments. ZJ undertook the statistical analysis of primate data and wrote the first draft of the manuscript. PM, JS, and DQ contributed to the acquisition of rodent data. PM and SF analyzed the rodent data. CEM provided MSX-3 for the experiments. SF, SY, CEM, RF, and SRG provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Keywords

Adenosine A2A receptor; anandamide; dopamine; reinforcement; reinstatement; THC

INTRODUCTION

Adenosine plays a very important modulatory role in striatal function. Adenosine A_{2A} receptors are concentrated in the striatum more than anywhere else in the brain and are strategically located, both presynaptically and postsynaptically, to modulate glutamatergic neurotransmission (Ciruela *et al.* 2006; Ferré *et al.* 2005; Ferré *et al.* 2007; Hettinger *et al.* 2001; Quiroz *et al.* 2009). Similarly, cannabinoid CB_1 receptors, which mediate the central effects of Δ^9-tetrahydrocannabinol (THC) and endocannabinoids, are also abundantly expressed in the striatum (Herkenham *et al.* 1991) and are located both presynaptically and postsynaptically, to modulate glutamatergic neurotransmission (Ferré *et al.* 2009; Fusco *et al.* 2004; Hohmann & Herkenham 2000; Kofalvi *et al.* 2005; Pickel *et al.* 2004; Pickel *et al.* 2006).

Several studies have suggested functional and molecular interactions (receptor heteromerization) between striatal adenosine A_{2A} and cannabinoid CB_1 receptors (for recent reviews, see Ferré *et al.* 2009; Ferré *et al.* 2010). We recently found that the motor depressant effects of intra-striatal administration of the cannabinoid CB_1 receptor agonist WIN 55,212-2 were counteracted by A_{2A} receptor blockade (Carriba *et al.* 2007). Moreover, Soria and colleagues (2004) reported decreased development of conditioned place preferences with THC in A_{2A} receptor-deficient mice, which suggests that rewarding effects of cannabinoids might also depend on striatal A_{2A}-CB_1 receptor interactions. Yao *et al.* (2006) showed that μ-opiate and CB_1 receptors act synergistically in nucleus accumbens-striatal neurons and that this synergy is regulated by A_{2A} receptors. In that study, pretreatment with the A_{2A}-receptor antagonist MSX-3 eliminated heroin-induced reinstatement of extinguished drug-seeking behavior in rats that had previously self-administered heroin. However, the role of adenosine A_{2A} receptors in the reinforcing effects of cannabinoids has not been investigated. Thus, it is unknown whether adenosine A_{2A} antagonists have any potential as a treatment of cannabis abuse.

The aim of this study was to investigate the role of A_{2A}-CB_1 receptor interactions in the reinforcing effects of cannabinoid CB_1 receptor agonists in squirrel monkeys. The squirrel monkey (*Saimiri sciureus*) is a primate species we have used extensively to investigate the reinforcing effects of cannabinoids (Justinová *et al.* 2003; Justinová *et al.* 2004; Justinová *et al.* 2008b; Justinová *et al.* 2005; Tanda, Munzar & Goldberg 2000). In the present study, we examined whether the selective A_{2A}-receptor antagonist MSX-3 would alter self-administration maintained by THC, the endogenous CB_1-receptor agonist anandamide, cocaine, or food under a fixed-ratio (FR) schedule. Also, to assess the potential of MSX-3 to precipitate relapse to abuse in abstinent individuals, we studied whether it would reinstate extinguished drug-seeking behavior or alter the reinstatement of extinguished drug-seeking behavior produced by THC or cocaine. Finally, we studied whether MSX-3 can alter THC- or cocaine-induced increases in extracellular dopamine levels in the nucleus accumbens shell in rats.

MATERIALS AND METHODS

Subjects

Sixteen adult male squirrel monkeys (Saimiri sciureus) weighing 0.8 to 1.1 kg were housed in individual cages in a temperature- and humidity-controlled room with unrestricted access

to water. Monkeys were fed (approximately two hours after the session) a daily food ration consisting of five biscuits of high protein monkey diet (Lab Diet 5045, PMI Nutrition International, Richmond, Indiana) and two pieces of Banana Softies (Bio-Serv, Frenchtown, NJ) that maintained their body weights at a constant level throughout the study. Fresh fruits, vegetables and environmental enrichment were provided daily. *In vivo* microdialysis experiments were performed in male Sprague Dawley rats, weighing 300-350 g. Animals, housed in groups of three, had unlimited access to water and food. Animals were maintained in facilities fully accredited by AAALAC and experiments were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of the Intramural Research Program, NIDA, NIH, DHHS.

One group of four monkeys was used for experiments with the anandamide self-administration baseline: all monkeys had a history of anandamide self-administration (6754, 67F4, 70F4, 1568). Another group of four monkeys was used for experiments with the THC self-administration baseline: three monkeys had a history of THC self-administration (434, 37B, 453) and one monkey had a prior history of anandamide self-administration (66B2). Another group of four monkeys was used for experiments with the cocaine self-administration baseline; all monkeys had a prior history of cocaine self-administration (70F7, 01714B, 5045, 39B). A group of five monkeys was used for studying responding reinforced by food pellets (34A, 27B, 30A, 1549, 39B). Monkey 39B was first trained to respond for food pellets. After testing of an MSX-3 dose-response curve was finished, monkey 39B was switched to cocaine self-administration in order to increase the size of this experimental group of monkeys to four, as with the anandamide and THC groups.

Apparatus

Experimental chambers and other apparatus used in this study were the same as previously described (Justinová *et al.* 2003). Monkeys were surgically prepared with chronic indwelling venous catheters (polyvinyl chloride) (Goldberg 1973). The catheters were connected to polyethylene tubing, which passed out of the isolation chambers where they attached to motor-driven syringe pumps. The syringe pumps were calibrated so that duration of each injection was 0.2 s and injection volume was 0.2 ml. Before the start of each session, monkeys were placed into Plexiglas chairs and restrained in the seated position by waist locks. Before the start of each session, catheters were flushed with 1 ml of saline and one injection was delivered (calculated to fill the dead space of the catheter which was about 0.2 ml).

Self-administration procedure

Fixed-ratio schedule of drug injection—A fixed-ratio schedule of intravenous drug injection was used to evaluate drug reinforcement because it provides reliable and reproducible positive reinforcing effects with cannabinoids, including THC and endocannabinoids (Justinová *et al.* 2003; Justinová *et al.* 2005; Tanda *et al.* 2000) and is a valid procedure for evaluating abuse liability of cannabinoids (Justinová *et al.* 2008a; Panlilio & Goldberg 2007; Panlilio, Justinová & Goldberg 2010). At the start of the session, a white house light was turned off and a green stimulus light was turned on. In the presence of the green light, monkeys were required to make 10 responses on the lever (10-response, fixed-ratio schedule of reinforcement; FR10) to produce an injection of anandamide, THC or cocaine. The completion of 10 responses on the lever turned off the green light and produced an intravenous (i.v.) injection of 40 μg/kg of anandamide, 4 μg/kg of THC, or 30 μg/kg of cocaine, and each injection was paired with a 2-s illumination of an amber stimulus light. Each injection was followed by a 60-s timeout period, during which the chamber was dark and lever presses had no programmed consequences. One-hour sessions were conducted five days per week (typically Monday to Friday).

All monkeys had learned to respond under the FR10 schedule for the particular training drug prior to the beginning of this study. When responding for the training dose of drug, which maintained maximal rates of responding (40 μg/kg/injection anandamide, 4 μg/kg/injection THC, 30 μg/kg/injection cocaine), was stable for at least five consecutive sessions (less than 15% variability), testing with different doses of the A_{2A} receptor antagonist MSX-3 was then conducted, with vehicle or different MSX doses (vehicle, 0.1, 0.3, 1 and 3 mg/kg; order of testing was 1, 0.3, 0.1, and 3 mg/kg; MSX-3 always administered 5 min before the session i.m.) tested by pretreatment before each of three consecutive sessions followed by recovery of stable THC, anandamide, or cocaine baseline responding. After testing these different doses of MSX, monkeys were allowed to self-administer the training dose of THC, anandamide, or cocaine for 4-5 sessions followed by saline substitution. After reaching a stable extinction baseline, pretreatment with vehicle or MSX-3 (1 and 3 mg/kg) was again tested for three sessions. After completing this step, monkeys were returned to baseline self-administration of the training dose of THC, anandamide, or cocaine, followed by vehicle extinction. Next, vehicle was replaced by a low dose of THC, anandamide, or cocaine (2.5 μg/kg/injection anandamide, 1 μg/kg/injection THC, 3 μg/kg/injection cocaine) and vehicle or MSX-3 (1 and 3 mg/kg) was tested by pretreatment. Following further saline extinction for four to five sessions, monkeys were allowed to self-administer a high dose of the anandamide (80 μg/kg/injection), THC (8 μg/kg/injection), or cocaine (100 μg/kg/injection) and MSX-3 was tested by pretreatment for three sessions (1 and 3 mg/kg). Following saline extinction for four to five sessions, monkeys from the anandamide and THC groups were allowed to self-administer a lower dose of the drug (1 μg/kg/injection anandamide, 0.5 μg/kg/injection THC), and MSX-3 was then tested by pretreatment for three sessions (3 mg/kg). Finally, following saline extinction for four to five sessions, monkeys from the anandamide and THC groups were allowed to self-administer an intermediate dose of the drug (10 μg/kg/injection anandamide, 2 μg/kg/injection THC) and MSX-3 was then tested by pretreatment for three sessions (1 mg/kg).

Before reinstatement testing began, monkeys were again allowed to self-administer the training dose of THC (4 μg/kg/injection) or cocaine (30 μg/kg/injection) until they reached a stable baseline. Saline was then substituted for THC or cocaine. After three days of stable saline extinction, we tested for drug-induced reinstatement of extinguished drug-seeking behavior by administering pre-session priming injections of MSX-3 (1 or 3 mg/kg, i.m. 5 min before the session) or its vehicle, THC (10 or 40 μg/kg, i.v. immediately before the session) or its vehicle, or combinations of THC and MSX-3. Each reinstatement test was a single session and each test was preceded and followed by saline extinction session(s). Reinstatement tests were conducted in a counterbalanced manner in the THC- and cocaine-trained monkeys.

In vivo microdialysis experiments

Concentric microdialysis probes with 2 mm-long dialysis membranes were prepared as described previously (Pontieri, Tanda & Di Chiara 1995). Animals were anesthetized with Equithesin (NIDA Pharmacy, Baltimore, MD, USA) and probes were implanted in the shell of the nucleus accumbens (coordinates with respect to bregma: anterior, +2.2; lateral, -1.0; ventral, 7.7 from the dura). A Ringer solution (in mM) of 147 NaCl, 4 KCl, and 2.2 $CaCl_2$ was pumped through the dialysis probe at a constant rate of 1 μl/min. A dose of 3 mg/kg MSX-3 was given i.p. 20 min before the administration of THC (3 mg/kg, i.p.) or cocaine (3 mg/kg, i.p.). As previously reported, theses doses of THC and cocaine are equipotent in increasing extracellular dopamine levels (Solinas *et al.* 2007; Tanda *et al.* 2005). The dose of MSX-3 chosen (3 mg/kg) was the minimal dose previously shown to produce significant motor activation in the same rat strain (Antoniou *et al.* 2005). After a washout period of 90 min, samples were collected at 20 min intervals and dopamine content was measured. Each

animal was used to study the effects of one treatment. At the end of the experiment, rats were killed with an overdose of Equithesin and methylene blue was perfused through the probe. The brain was removed and placed in a 10% formaldehyde solution and coronal sections were cut to verify the probe location. Dopamine content was measured by reverse high-performance liquid chromatography (HPLC) coupled to electrochemical detector, as described in detail previously (Pontieri *et al.* 1995)

Drugs

Anandamide (Arachidonylethanolamide; Tocris Cookson, Inc., Ellisville, MO, USA) and Δ^9-tetrahydrocannabinol (THC; National Institute on Drug Abuse, Bethesda, MD, USA) were dissolved in a vehicle containing 1% ethanol and 1% Tween 80 and saline. THC was administered i.v. immediately before the session in reinstatement tests. (-)-Cocaine HCl was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in saline. MSX-3 free acid [phosphoric acid mono-(3-{8-[2-(3-methoxy-phenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl}propyl) ester] (Hockemeyer, Burbiel & Müller 2004) was synthesized at the Pharmaceutical Institute, University of Bonn, Bonn, Germany. MSX-3 was dissolved in sterile water with addition of NaOH to obtain the disodium salt and to correct pH and was administered i.m. (0.3 ml/kg) to monkeys 5 min before each session in all tests. MSX-3 has been extensively characterized as a very selective A_{2A} receptor antagonist in rodents (Karcz-Kubicha *et al.* 2003; Solinas *et al.* 2005). Although, to our knowledge, there is no pharmacological data available about MSX-3 in non-human primates, binding studies indicate a very similar affinity of rodent and human A_{2A} receptors for this compound (Müller & Ferré 2007). Furthermore, MSX-3 is the only hydrosoluble A_{2A} receptor antagonist so far available, making it more convenient for systemic administration. In rat microdialysis experiments, THC, cocaine and MSX-3 were administered at a dose of 3 mg/kg i.p. (2 ml/kg) and MSX-3 was injected 20 min before the administration of THC or cocaine. A shorter MSX-3 pretreatment time in monkeys compared to rats was chosen mostly because of the route of administration (i.m. versus i.p., respectively) and the time parameters of the two different types of experiments.

Statistical analyses

Cumulative-response records were obtained during all sessions in order to assess within-session patterns of responding. Rates of responding during self-administration sessions are expressed as responses per sec averaged over the one-hr session, with responding during time-outs not included in calculations. Injections or pellets per session represent total number of injections or pellets delivered per session. All data are presented as mean ± S.E.M. For the dose-effect curves (Figure 1 and 2), the last three sessions under each condition were averaged for each subject and used for analysis. Reinstatement data in Figure 3 are averaged from single session tests.

Statistical analyses were done using one-way and two-way repeated measures ANOVA (data met the assumptions of the test) to assess differences between vehicle and MSX-3-pretreatment conditions. Pairwise multiple comparisons were done using Tukey's or Dunnett's procedures. For the *in vivo* microdialysis data, two-way ANOVA, followed by repeated measures ANOVA, and Dunnett's procedure were used. Statistical significance was accepted at the $P < 0.05$ level. SigmaStat software (http://www.systat.com) was used for all statistical analyses.

RESULTS

Effects of MSX-3 pretreatment on responding reinforced by anandamide, THC, cocaine and food pellets under FR schedule

Maximal responding was maintained by injection doses of 40 μg/kg of anandamide, 4 μg/kg of THC, and 30 μg/kg of cocaine, as in previous studies under the same conditions (Justinová *et al.* 2003; Justinová *et al.* 2008a; Tanda *et al.* 2000), and these injection doses were first selected for studying the effects of MSX-3 in the present experiments. In this study, at the 40 μg/kg/injection dose of anandamide, monkeys self-administered an average of 47.67 ± 1.62 injections per session at a rate of 0.87 ± 0.21 response per sec. At the 4 μg/kg/injection dose of THC, monkeys self-administered 46.69 ± 2.62 injections per session at a rate of 0.78 ± 0.23 response per sec. At the 30 μg/kg/injection dose of cocaine, monkeys self-administered 49.64 ± 2.12 injections per session at a rate of 1.04 ± 0.28 responses per sec. Monkeys self-administered on average 51.65 ± 0.60 food pellets per session at a rate of 1.31 ± 0.14 responses per sec. There were no statistical differences in rates of responding as a function of reinforcer (Figure 1; One-way ANOVA: $F_{(3,11)} = 1.22$, $p = 0.35$).

Effects of i.m. pretreatment with vehicle or MSX-3 at doses 0.1, 0.3, 1.0, and 3.0 mg/kg on number of reinforcements and rates of responding with food and with doses of the self-administered drugs that maintained maximal responding (are shown in Figure 1. MSX-3 doses of 1.0 and 3.0 mg/kg significantly and dose-dependently decreased the number of self-administered injections of anandamide ($F_{(4,12)} = 10.396$, $p < 0.001$) and THC ($F_{(4,8)} = 34.66$, $p < 0.001$), as well as rates of responding (anandamide: $F_{(4,12)} = 5.12$, $p = 0.012$; THC: $F_{(4,8)} = 10.14$, $p = 0.003$). MSX-3 did not alter either cocaine or food self-administration at any dose tested (Cocaine injections: $F_{(4,8)} = 0.76$, $p = 0.58$, rates: $F_{(4,8)} = 0.92$, $p = 0.50$; food pellets: $F_{(4,16)} = 0.86$, $p = 0.51$, rates: $F_{(4,16)} = 0.03$, $p = 0.99$).

Effects of pretreatment with MSX-3 on dose-response curves for anandamide, THC, and cocaine self-administration

When the injection dose of anandamide, THC or cocaine was varied, classic, inverted, U-shaped, dose-effect curves were obtained (Figure 2). Anandamide maintained significantly higher numbers of self-administered injections per session ($F_{(5,14)} = 94.91$, $p < 0.001$; Figure 2, left panel) than vehicle at doses of 10, 40 and 80 μg/kg/injection. THC maintained significantly higher numbers of self-administered injections per session ($F_{(5,10)} = 38.06$, $p < 0.001$; Figure 2, middle panel) than vehicle at doses of 1, 2, 4 and 8 μg/kg/injection. Cocaine maintained significantly higher numbers of self-administered injections per session ($F_{(3,6)} = 62.85$, $p < 0.001$; Figure 2, right panel) than vehicle at doses of 30 and 100 μg/kg/injection.

We next tested the effects of pretreatment with 1.0 and 3.0 mg/kg doses of MSX-3 on dose-response curves for self-administration of anandamide, THC, and cocaine. Pretreatment with 1 or 3 mg/kg doses of MSX-3 did not significantly alter responding for vehicle. A low dose of MSX-3 (1 mg/kg) caused downward shifts of the anandamide and THC dose-response curves in monkeys. However, a higher dose 3 mg/kg of MSX-3 shifted the THC and anandamide dose-response curves to the left. Two-way ANOVA analysis indicated that there was a significant interaction between anandamide dose and dose of MSX-3 (Figure 2, left panel; MSX-3 1 mg/kg: $F_{(3,6)} = 4.68$, $p = 0.05$; MSX-3 3 mg/kg: $F_{(3,9)} = 54.94$, $p < 0.001$), as well as between THC dose and dose of MSX-3 (Figure 2, middle panel; MSX-3 1 mg/kg: $F_{(3,6)} = 6.27$, $p = 0.03$; MSX-3 3 mg/kg: $F_{(3,6)} = 18.19$, $p = 0.002$). Post-hoc multiple pairwise comparisons revealed that there were statistically significant differences in the effects of an anandamide dose of 1 μg/kg/injection after 3 mg/kg of MSX-3 ($p < 0.001$), an anandamide dose of 2.5 μg/kg/injection after 3 mg/kg of MSX-3 ($p < 0.001$), an anandamide dose of 40 μg/kg/injection after both 1 mg/kg MSX-3 ($p = 0.002$) and 3 mg/kg MSX-3 ($p <$

0.001), and an anandamide dose 80 μg/kg/injection after 3 mg/kg MSX-3 ($p = 0.004$). In THC-self-administering monkeys, there were statistically significant differences in the effects of a THC dose of 0.5 μg/kg/injection after 3 mg/kg of MSX-3 ($p = 0.005$), a THC dose of 1 μg/kg/injection after 3 mg/kg of MSX-3 ($p = 0.015$), a THC dose of 2 μg/kg/injection after 1 mg/kg of MSX-3 ($p = 0.031$), a THC dose of 4 μg/kg/injection after both 1 mg/kg MSX-3 ($p = 0.006$) and 3 mg/kg MSX-3 ($p < 0.001$), and a THC dose of 8 μg/kg/injection after 3 mg/kg of MSX-3 ($p = 0.05$). Neither dose of MSX-3 altered self-administration of any dose of cocaine (Figure 2, right panel; no significant interaction between cocaine dose and dose of MSX-3: $F_{(4,8)} = 1.31$, $p = 0.35$).

Effects of pretreatment with MSX-3 on reinstatement of extinguished THC- or cocaine-seeking behavior by a priming injection of THC

The effects of pretreatment with vehicle or MSX-3 (1.0 or 3.0 mg/kg, i.m.) on the reinstatement of extinguished THC- (Figure 3, panels a, b, c) or cocaine-seeking (Figure 3, panels d, e, f) behavior by priming injections of vehicle or THC (10 or 40 μg/kg, i.v.) were studied in a counterbalanced manner in both groups of monkeys. Pretreatment with either dose of MSX-3 had no effect on drug-seeking behavior in both groups of monkeys after a priming injection of THC vehicle (Figure 3, panels a and d; compared with MSX-3 vehicle + THC vehicle prime combination).

The 10 μg/kg priming dose of THC produced a significant reinstatement of drug-seeking behavior in both groups of monkeys (Figure 3; THC group, panel b: $F_{(3,9)} = 24.84$, $p < 0.001$; cocaine group, panel e: $F_{(3,6)} = 28.02$, $p < 0.001$; compared with MSX-3 vehicle + THC vehicle prime combination). Pretreatment with 1.0 or 3.0 mg/kg of MSX-3 did not alter the reinstatement of drug-seeking behavior by 10 μg/kg THC in either group of monkeys (Figure 3, panels b and e: compared with MSX-3 vehicle + THC 10 μg/kg prime combination).

The 40 μg/kg priming dose of THC also produced a significant reinstatement of drug-seeking behavior in both groups of monkeys (Figure 3; THC group, panel c: $F_{(3,6)} = 14.33$, $p = 0.004$; cocaine group, panel f: $F_{(3,6)} = 12.20$, $p = 0.006$; compared with MSX-3 vehicle + THC vehicle prime combination). Pretreatment with 1.0 or 3.0 mg/kg of MSX-3 also did not alter the reinstatement of drug-seeking behavior by 40 μg/kg THC in either group of monkeys (Figure 3, panels c and f: compared with MSX-3 vehicle + THC 40 μg/kg prime combination).

Effects of pretreatment with MSX-3 on THC- and cocaine-induced increases in extracellular levels of dopamine in the nucleus accumbens shell of rats

THC (3 mg/kg, i.p.) produced a significant increase (of almost 100% increase versus basal values) in extracellular levels of dopamine in the shell of the nucleus accumbens in Sprague-Dawley rats (Figure 4, upper panel; $F_{(5,25)} = 5.58$ $p = 0.0001$). A 3 mg/kg dose of MSX-3 (i.p.), which is the minimal dose that produces a significant motor activation in Sprague-Dawley rats (Karcz-Kubicha *et al.* 2003), produced a small but significant decrease in extracellular dopamine levels (Figure 4, upper panel; $F_{(5,53)} = 3.03$ $p = 0.021$). After pretreatment with MSX-3 (3 mg/kg, i.p.), THC (3 mg/kg, i.p.) also elevated dopamine levels (Figure 4, upper panel; $F_{(5,35)} = 2.74$ $p = 0.042$), but the effect was significantly attenuated compared with the effect of THC alone (Two-way ANOVA; treatment effect: $F_{(1,40)} = 7.517$ $p = 0.021$). On the other hand, the increase in extracellular dopamine levels induced by an equipotent dose of cocaine (3 mg/kg, i.p.) (Figure 4, lower panel; $F_{(6, 30)} = 5.465$ $p = 0.001$) was not attenuated after pretreatment with MSX-3 (3 mg/kg, i.p.) (Two-way ANOVA; treatment effect: $F_{(1, 81)} = 0.99$ $p = 0.32$).

DISCUSSION

The present results show that the systemic administration of a low dose (1 mg/kg) of the adenosine A_{2A} receptor antagonist MSX-3 can selectively reduce the reinforcing effects of doses of THC and anandamide that maintain peak rates of responding in squirrel monkeys under a FR schedule. This was demonstrated by downward shifts of the anandamide and THC dose-response curves. However, a higher dose (3 mg/kg) of MSX-3 produced a shift to the left of the dose-response curves for THC and anandamide. In contrast, over a range of doses, MSX-3 did not alter the reinforcing effects of cocaine or food under an identical FR schedule, which indicates that our findings with MSX-3 are pharmacologically selective. Although peak rates of responding for THC, anandamide, cocaine, and food were similar under the FR schedule, it has been suggested that responding for THC and anandamide might be more sensitive to disruption than responding for cocaine or food. This does not seem to be the case, because we have seen in the same subjects that MPEP, an antagonist at metabotropic glutamate receptors subtype 5 ($mGlu_5$), selectively reduces responding for cocaine and food pellets at a dose that does not affect responding for THC (unpublished observations). MPEP has been previously shown to produce similar reductions in responding for cocaine under the same conditions in squirrel monkeys ((Platt, Rowlett & Spealman 2008). Finally, the present study shows that A_{2A} receptor blockade neither reinstates extinguished drug-seeking behavior nor alters THC-induced reinstatement of drug-seeking behavior in monkeys with cannabinoid- or cocaine-self-administration histories. Thus, our results suggest that adenosine A_{2A}-receptor antagonists – which have demonstrated anti-parkinsonian and neuroprotective effects in rodent models (for review see Takahashi, Pamplona & Prediger 2008) – can reduce the reinforcing effects of cannabinoids under certain conditions, without risk of provoking relapse to drug use in abstinent individuals.

The main anatomical target for the reinforcing effects of cannabinoid CB_1-receptor agonists is still a matter of debate, with some studies favoring the ventral tegmental area and others the ventral striatum (for reviews, see Gardner 2005; Lupica, Riegel & Hoffman 2004). In view of the preferential expression of A_{2A} receptors in the striatum, the present results strongly support the striatal hypothesis. A_{2A} receptors are preferentially localized in the dendritic spines of striatopallidal GABAergic enkephalinergic neurons, but also presynaptically in glutamatergic terminals that make contact with GABAergic dynorphinergic neurons (Ciruela *et al.* 2006; Ferré *et al.* 2007; Hettinger *et al.* 2001). In the striatum, CB_1 receptors are also located in the dendritic spines of GABAergic neurons, including striatopallidal neurons, and on glutamatergic (and GABAergic) nerve terminals (Fusco *et al.* 2004; Hohmann & Herkenham 2000; Kofalvi *et al.* 2005; Pickel *et al.* 2004; Pickel *et al.* 2006). Postsynaptic A_{2A}-CB_1 receptor interactions in GABAergic enkephalinergic neurons cannot readily explain the counteraction by the lower dose of MSX-3 (1 mg/kg) of the effects of THC and anandamide observed in the present study. If anything, postsynaptic A_{2A} receptor blockade would be expected to potentiate the reinforcing effects of cannabinoids, by its ability to potentiate the effects of endogenous dopamine on dopamine D_2 receptors, by means of antagonistic A_{2A}-D_2 receptor interactions (Ferré *et al.* 1997; Ferré *et al.* 2007). However, a postsynaptic A_{2A}-CB_1 receptor interaction could be involved in the potentiation by the higher dose of MSX-3 (3 mg/kg) of the effects of THC and anandamide, as it is also probably involved in the previously described ability of MSX-3 to counteract the motor depression induced by CB_1 receptor agonists (Andersson *et al.* 2005; Carriba *et al.* 2007).

A common molecular mechanism contributing to the development of addiction, that is shared by drugs of abuse (including cannabinoids) is their ability to increase levels of extracellular dopamine in the shell of the nucleus accumbens (Di Chiara 2002; Koob 1992;

Robbins & Everitt 1996). The systemic administration of THC induces dopamine release in the nucleus accumbens (Chen *et al.* 1990; Solinas *et al.* 2007; Tanda, Pontieri & Di Chiara 1997), although the underlying mechanism has not yet been well established. Although CB_1 receptors in the ventral tegmental area (VTA) have been suggested to be involved, *in vivo* administration of THC directly into the VTA does not induce dopamine release in the nucleus accumbens, while direct infusion of THC in the nucleus accumbens does (Gardner 2005; Lupica *et al.* 2004; Schlicker & Kathmann 2001). It has been suggested that presynaptic CB_1 receptors that control striatal glutamate release (Robbe *et al.* 2001), are main targets for the dopamine-releasing effects of cannabinoids, by decreasing the excitability of the striatal GABAergic dynorphinergic neurons that project to the mesencephalon and tonically inhibit dopaminergic cells in the VTA (Schlicker & Kathmann 2001). Therefore, presynaptic A_{2A} receptors localized in striatal glutamatergic terminals could be responsible for the counteracting effects of the A_{2A} receptor antagonist on the reinforcing effects of cannabinoids. In that case, A_{2A} receptor antagonists should also counteract THC-induced, but not cocaine-induced, dopamine release in the nucleus accumbens shell. In fact, by using *in vivo* microdialysis in freely-moving rats, we found that a behaviorally active dose of MSX-3 significantly counteracted THC-induced increases in extracellular dopamine levels in the nucleus accumbens shell induced by THC. These effects of MSX-3 on reinforcement and dopamine release were selective, occurring with cannabinoids but not with cocaine. The lack of effect of the A_{2A} receptor antagonist in cocaine-induced dopamine release could not be explained by a ceiling effect of the dose of cocaine used (3 mg/kg, i.p.), since higher cocaine doses can produce much more pronounced effects (see e.g., Tanda *et al.* 2005). The finding that an A_{2A} receptor antagonist was able to reduce cannabinoid-induced self-administration (in monkeys) and dopamine release (in rats), yet failed to attenuate cannabinoid-induced reinstatement is intriguing. It reinforces the hypothesis that different mechanisms and brain circuits are involved in these phenomena; that dopamine is involved in cannabinoid self-administration, but its involvement in the reinstatement of cannabinoid drug-seeking may not be as pronounced (Kalivas & Volkow 2005; Koob & Volkow 2010; Robbins & Everitt 1996).

In the squirrel monkeys, a low 1 mg/kg dose of MSX-3 significantly attenuated self-administration of training doses of THC or anandamide that maintained maximal responding under a FR schedule but did not potentiate reinforcing effects of lower threshold doses of both drugs. A higher dose (3 mg/kg) of MSX-3 further attenuated self-administration of training doses of THC or anandamide under the FR schedule, but it potentiated the effect of threshold doses of both drugs. These opposite effects obtained with the 1 and 3 mg/kg doses of MSX-3 could be due to a preferential presynaptic effect with the lower dose of MSX-3, with the appearance of a postsynaptic effect with higher doses. Importantly, MSX-3 (at any dose tested) did not alter the reinforcing effects of cocaine or food pellets, and did not reinstate extinguished drug-seeking behavior in monkeys that had previously self-administered THC or cocaine under a FR schedule. These findings demonstrate that adenosine A_{2A} receptors selectively modulate the reinforcing effects of cannabinoids. The significant and selective results obtained with the lower dose of MSX-3 suggest that adenosine A_{2A} antagonists acting preferentially at presynaptic A_{2A} receptors might selectively reduce the reinforcing effects of cannabinoids that lead to their abuse. However, the appearance of potentiating rather than suppressing effects on cannabinoid reinforcement at the higher dose of MSX-3 would likely preclude the use of such a compound as a medication for cannabis abuse. Adenosine A_{2A} receptor antagonists with more selectivity for presynaptic receptors could have a potential as medications for treatment of cannabis abuse.

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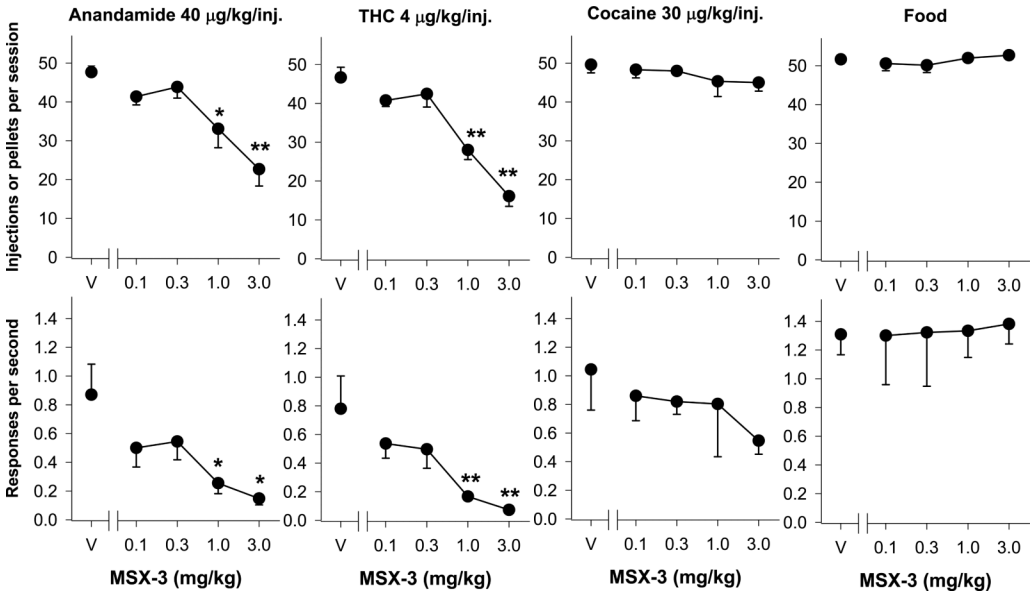

Figure 1.

Effects of different doses of MSX-3 on peak self-administration responding maintained by 40 μg/kg injections of anandamide, 4 μg/kg injections of THC, 30 μg/kg injections of cocaine or food pellets. Numbers of injections or pellets per session (upper panels) and rates of responding (lower panels) are presented as a function of dose of MSX-3 (0.1, 0.3, 1.0, and 3.0 mg/kg; i.m. 5 min before the session). Each point represents the mean (± S.E.M.) of three sessions under each MSX-3 dose condition from 3 (THC and cocaine), 4 (anandamide) or 5 (food) monkeys per each group. * $P<0.05$, ** $P<0.01$, post-hoc comparisons with the vehicle (V) conditions (Dunnett's test).

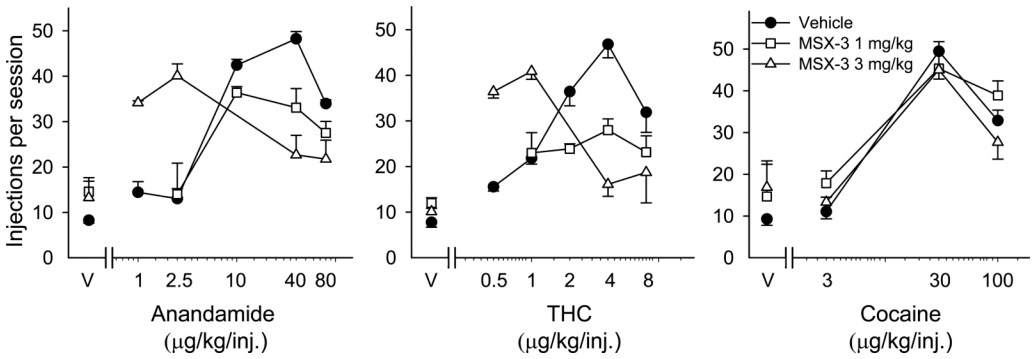

Figure 2.

Effects of pretreatment with vehicle or MSX-3 (1 and 3 mg/kg; i.m. 5 min before the session) on anandamide (left panel), THC (middle panel) or cocaine (right panel) dose-response curves. Numbers of injections per session after i.m. pretreatment with vehicle or MSX-3 are shown. Each point represents the mean (± S.E.M) from 3 (THC and cocaine) or 4 (anandamide) monkeys over three sessions under each condition.

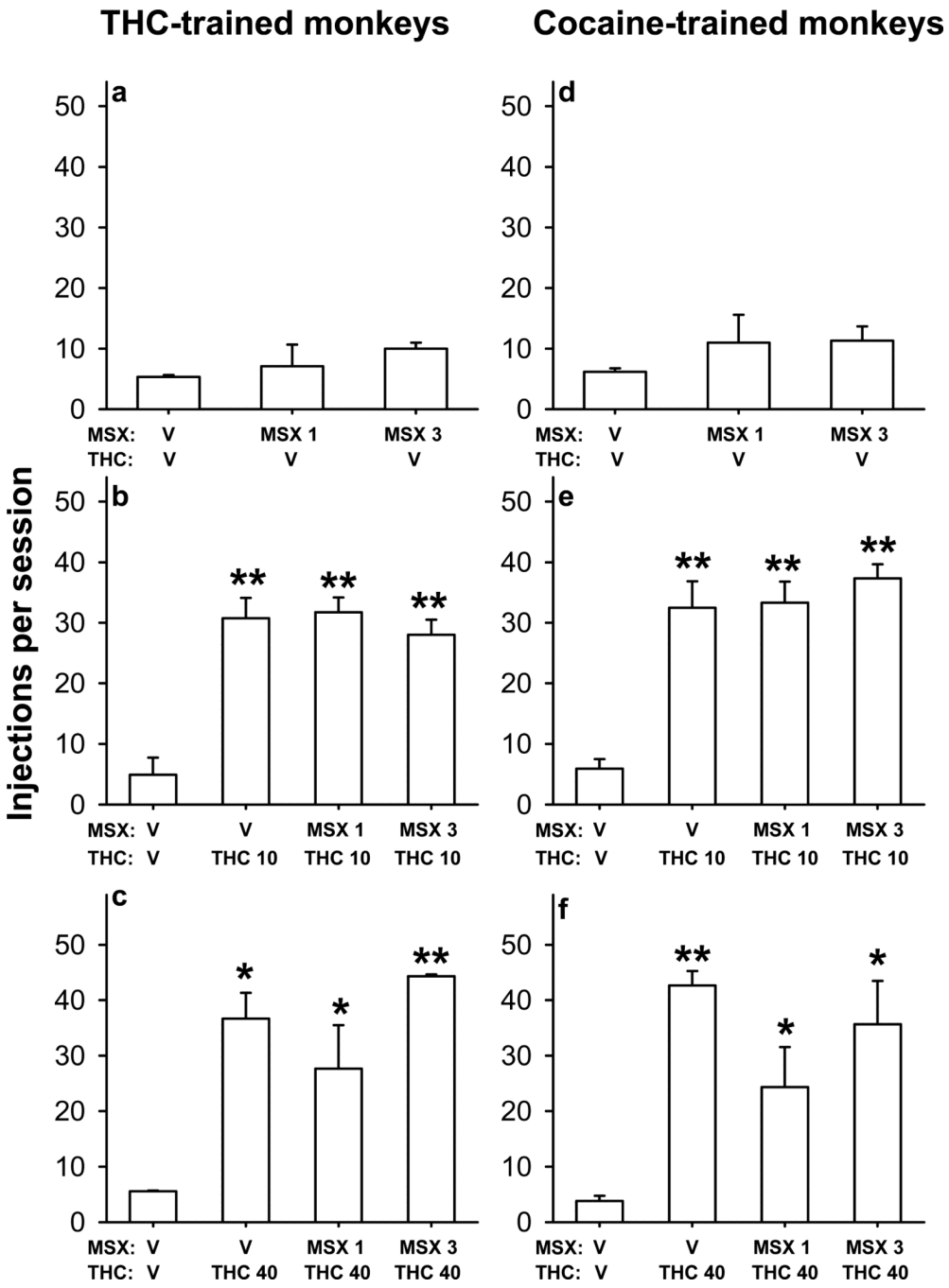

Figure 3.

Effects of pretreatment with vehicle or MSX-3 (1 or 3 mg/kg; i.m. 5 min before the session) on reinstatement of extinguished THC- (panels a, b, c) or cocaine-seeking (panels d, e, f) behavior by a priming injection of vehicle or THC (10 or 40 μg/kg; i.v. immediately before session). Each bar (number of injections per session) represents the mean ± S.E.M. from 3-4 monkeys for a single session at each condition. * $P<0.05$, ** $P<0.01$ - post-hoc pairwise comparisons (Tukey test) with combination of i.p. MSX-3 vehicle (V) + i.v. THC vehicle (V) combination.

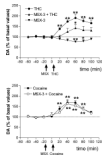

Figure 4.

Effects of pretreatment with MSX-3 on THC- or cocaine-induced increases in the extracellular concentrations of dopamine in the nucleus accumbens shell. An MSX-3 dose of 3 mg/kg was given i.p. 20 min before the administration of THC (3 mg/kg, i.p., upper panel) or cocaine (3 mg/kg i.p., lower panel). Data represent means ± S.E.M. (n = 6-9 per group, male Sprague-Dawley rats); * $P<0.05$, ** $P<0.01$, compared to the basal values (average of the values from the -60, -40 and -20 periods) (ANOVA and Dunnett's test).