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ORIGINAL INVESTIGATION

Effects of adenosine A_{2A} receptor stimulation on cocaine-seeking behavior in rats

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Abstract
Rationale Dopamine (DA) receptor stimulation in the nucleus accumbens (NAc) plays an important role in regulating cocaine-seeking behavior. Adenosine receptors antagonize the effects of DA receptor stimulation on intracellular signaling, neuronal output, and behavior.
Objectives The goal of the present study is to determine the effects of adenosine A_{2A} receptor stimulation on reinstatement of cocaine-seeking behavior in rats.
Methods Rats were trained to lever press for cocaine in daily self-administration sessions on a fixed-ratio 1 schedule for 3 weeks. After 1 week of abstinence, lever pressing was extinguished in six daily extinction sessions. We subsequently assessed the effects of the adenosine A_{2A} receptor agonist, CGS 21680, on cocaine-, quinpirole (D_2 agonist)-, and cue-induced reinstatement to cocaine seeking. We also assessed the effects of CGS 21680 on sucrose seeking in rats extinguished from sucrose self-administration.
Results Pretreatment of CGS 21680 dose-dependently blunted cocaine-induced reinstatement (15 mg/kg, i.p.). Pretreatment with CGS 21680 (0.03 mg/kg, i.p.) also attenuated quinpirole- and cue-induced reinstatement. A minimally effective dose of CGS 21680 failed to alter cocaine-induced locomotor activity or sucrose seeking.
Conclusions Stimulation of adenosine A_{2A} receptors antagonizes reinstatement of cocaine seeking elicited by cocaine, DA D_2-receptor stimulation, and cocaine-conditioned cues. These findings suggest that adenosine A_{2A} receptor stimulation may oppose DA D_2 receptor signaling in the NAc that mediates cocaine relapse.

Keywords A_{2A} receptor · D_2 receptor · Self-administration · Craving · Relapse · Reinstatement · Reward · Incentive motivation

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Introduction

Relapse to drug seeking is induced by exposure to drug-associated cues and pharmacological stimuli that activate the mesolimbic dopamine (DA) system (Shalev et al. 2002). The mesolimbic DA system is composed of DA cells in the ventral tegmental area (VTA) that terminate in forebrain regions such as the nucleus accumbens (NAc). DA release in the NAc targets two major classes of DA receptors that are differentiated by their opposing effects on intracellular signaling cascades. DA D_1 receptors increase adenylyl cyclase activity, while DA D_2 receptors decrease activity of this enzyme (Lachowicz and Sibley 1997). These DA receptor subtypes are also distinguished by differential expression patterns on distinct subtypes of NAc neurons. Although there is evidence for co-localization in the same neurons (Aizman et al. 2000; Schwartz et al. 1998), D_1 receptors exist primarily on neurons expressing substance P and dynorphin, whereas D_2 receptors exist primarily on neurons expressing enkephalin. These two distinct populations of NAc neurons differ in their projection targets and reflect the direct and indirect striatal output pathways, respectively (Aubert et al. 2000; Lu et al. 1998; Steiner and Gerfen 1998).

Chronic cocaine self-administration increases behavioral responses mediated by DA D_2 receptors. Thus, repeated

cocaine administration produces cross-sensitization with DA D_2 receptor agonists (Ujike et al. 1990) and animals with high self-regulated cocaine intake patterns display greater D_2 receptor-induced locomotion (Edwards et al. 2007). In addition, reinstatement of cocaine seeking is elicited by systemic and intra-NAc stimulation of D_2 receptors (Bachtell et al. 2005; De Vries et al. 1999; Dias et al. 2004; Khroyan et al. 2000; Schmidt et al. 2006; Schmidt and Pierce 2006; Self et al. 1996). Thus, amplification of D_2-mediated behaviors following chronic cocaine administration may enhance relapse elicited by cocaine-conditioned cues and pharmacological stimuli by targeting D_2 receptors (Cervo et al. 2003; Gal and Gyertyan 2006). Tempering enhancements in D_2 receptor-mediated behaviors following cocaine administration may provide effective treatments for curbing relapse vulnerability.

Adenosine functions as a neuromodulator of dopamine neurotransmission and recent studies suggest that stimulation of adenosine A_{2A} receptors oppose many behavioral effects of cocaine. Thus, stimulation of A_{2A} receptors reduces both the development and expression of cocaine sensitization (Filip et al. 2006) and impairs the initiation of cocaine self-administration (Knapp et al. 2001). Adenosine A_{2A} receptor antagonists, on the other hand, exacerbate cocaine sensitization (Filip et al. 2006) and enhance cocaine-evoked discriminative stimulus effects (Justinova et al. 2003). While the exact mechanisms of these A_{2A} receptor effects are not known, they may involve reciprocal regulation of DA D_2 receptors through receptor heteromerization and/or opposing intracellular signaling cascades (Fuxe et al. 2003).

Adenosine A_{2A} receptors are primarily localized to striatal regions in the brain where they are co-expressed with DA D_2 receptors on enkephalin-containing neurons (Dixon et al. 1996; Fink et al. 1992; Svenningsson et al. 1998). Interestingly, A_{2A} and D_2 receptors exert a number of effects that oppose one another on intracellular signaling cascades, cellular functioning, and behavioral responses. Stimulation of A_{2A} receptors decreases the affinity of D_2 receptors for dopamine (Ferre et al. 1991b), counteracts D_2 receptor-mediated signal transduction (Yang et al. 1995), opposes the effects of DA receptor stimulation on immediate early gene expression in the striatum (Morelli et al. 1994; Svenningsson et al. 1999a), and reverses D_2-induced inhibition of GABA output to the pallidum (Ferre et al. 1993). Consequently, adenosine A_{2A} receptor stimulation exerts behavioral effects that are functionally similar to DA receptor antagonists (Barraco et al. 1993; Brown et al. 1991; Heffner et al. 1989; Rimondini et al. 1997; Zarrindast et al. 1993).

Based on the aforementioned relationship between A_{2A} and D_2 receptors, stimulation of A_{2A} receptors would decrease DA neurotransmission and consequently relapse behaviors mediated by D_2 receptor signaling. In the present set of experiments, we tested the hypothesis that stimulation of adenosine A_{2A} receptors will blunt cocaine seeking using an animal model of relapse. We trained animals to self-administer cocaine and tested the effects of adenosine A_{2A} receptor stimulation on cocaine seeking following extinction training. The effects of adenosine A_{2A} receptor stimulation were evaluated on cocaine seeking elicited by cocaine, the D_2 receptor agonist, quinpirole, and cocaine-associated cues. We used the A_{2A} agonist, CGS 21680, to stimulate A_{2A} adenosine receptors since the specificity of CGS 21680 binding to A_{2A} receptors in nucleus accumbens is abolished in adenosine A_{2A} but not A_1 receptor knockout mice (Halldner et al. 2004), and is widely used as a ligand for stimulating striatal adenosine A_{2A} receptors (Cunha et al. 1996; Jarvis et al. 1989). In addition, recent work has demonstrated that motor depression induced by the A_{2A} agonist, CGS 21680, but not the A_1 agonist, N^6-cyclopentyladenosine, was specifically blocked by the A_{2A} antagonist, MSX-3, and not an A_1-specific antagonist (Karcz-Kubicha et al. 2003).

Materials and methods

Animals and housing conditions

Male Sprague–Dawley rats initially weighing 275–325 g (Charles River Laboratories, Kingston, NY, USA) were individually housed in wire cages with food and water available ad libitum. Experiments were conducted during the light cycle of a 12:12-h light:dark cycle (lights on at 0700 hours) in accordance with guidelines established by the National Institute of Health and the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Cocaine self-administration procedure

Self-administration and reinstatement testing were performed in operant conditioning chambers (Med-Associates, St. Albans, VT, USA) equipped with two response levers and an infusion pump system as previously described (Edwards et al. 2007). Animals were initially trained to lever press for sucrose pellets to facilitate acquisition of cocaine self-administration. These rats were food restricted to prevent weight gain, and trained to lever press for sucrose pellets on a fixed ratio 1 (FR1) reinforcement schedule until acquisition criteria had been achieved (100 sucrose pellets in two consecutive sessions). After lever-press training, animals were fed ad libitum for at least 1 day prior to surgical implantation with a chronic intrajugular catheter as previously described (Self et al. 1998).

Following at least 5–7 days' recovery from surgery, animals were allowed to self-administer intravenous cocaine (0.5 mg/kg/50 μl injection) on a fixed ratio 1 (FR1) reinforcement schedule in daily 4-h sessions for 5–6 days/week. Cocaine injections were delivered over 2.5 s concurrent with the illumination of a cue light above the active lever, and followed by an additional 12.5-s time out period (TO 15 s) when the house light remained off and responding was without consequence. Inactive lever responses produced no consequence throughout testing.

Extinction/reinstatement testing

After a minimum of 15 cocaine self-administration sessions, animals remained in their home cages for 7 days of abstinence. On days 8–13 of abstinence, animals returned to the operant conditioning chambers for extinction training in the absence of cocaine reinforcement in 4-h test sessions. Responses on the lever that previously delivered cocaine injections during self-administration (drug-paired lever) and on the inactive lever were recorded but had no programmed drug or cue delivery.

Cocaine-primed reinstatement

The effects of adenosine A_{2A} receptor stimulation on cocaine-primed reinstatement was tested over repeated reinstatement sessions to allow for testing of several doses of the A_{2A} agonist (0.01, 0.03, 0.1, and 0.3 mg/kg, i.p.). Each test session was initiated with 3 h of extinction conditions followed by a 1-h reinstatement test period. A pretreatment of the A_{2A} receptor agonist, CGS 21680 (vehicle, 0.01, 0.03, 0.1, 0.3 mg/kg, i.p.), was administered 5 min prior to a priming injection of cocaine (15 mg/kg, i.p.), which was followed by a 1-h reinstatement test. Animals received a maximum of four treatments in a randomized order although different dose ranges were tested spanning the five doses. All animals were tested under the vehicle pretreatment/cocaine reinstatement condition to provide a baseline of cocaine-primed reinstatement. However, all animals did not receive all doses of CGS 21680 due to concerns of residual testing and weakening of reinstatement responding over repeated trials. Responses at both drug-paired and inactive levers were recorded but produced no cue or drug delivery during testing.

Cue-induced reinstatement

In a separate set of animals, we tested the effects of adenosine A_{2A} receptor stimulation on reinstatement elicited by cocaine-associated cues. Cue-induced reinstatement of cocaine-seeking behavior was measured in a 4-h reinstatement session consisting of 3 h of extinction conditions followed by a 1-h cue-primed reinstatement test period. A pretreatment of vehicle or 0.03 mg/kg CGS 21680 was administered 5 min prior to the cue reinstatement test. This dose was used because it proved most effective in blunting cocaine-induced reinstatement, while having little sedative effects. The cue-induced reinstatement test was initiated with non-contingent (priming) presentation of the cocaine injection cues delivered every 2 min for the first 10 min. During the entire session, responding at the drug-paired lever resulted in response-contingent cue delivery (2.5 s illumination of cue light and infusion pump, 15 s termination of house light).

Quinpirole-induced reinstatement

In separate study groups, the effect of adenosine A_{2A} receptor stimulation on dopamine D_2 receptor-primed relapse behavior was assessed. Given the longer duration of quinpirole action relative to cocaine, priming injections of quinpirole (0, 0.1, 0.3, and 1.0 mg/kg, s.c.) were given before the final 2 h of the session immediately after 2 h of extinction conditions. A pretreatment of CGS 21680 (0.03 mg/kg, i.p.) was administered 5 min prior to quinpirole treatment. Quinpirole doses were administered in randomized order across test days. Responses at both levers were recorded, but resulted in no cue or cocaine delivery.

Sucrose reinstatement

Animals were trained to self-administer sucrose pellets on an FR1:TO 15-s schedule as described above. After 15 daily sessions (100 pellets/session), animals remained in their home cages for 7 days of "abstinence", and were then subjected to extinction training in six daily 4-h sessions. Following extinction training, animals were tested for reinstatement of sucrose seeking. A pretreatment of CGS 21680 (0.03 mg/kg, i.p.) was administered 5 min prior to sucrose reinstatement testing. Reinstatement testing was initiated by non-contingent sucrose pellet delivery in a single 1-h test immediately following 1 h of extinction conditions. During the reinstatement phase, animals were presented with the non-contingent delivery of a sucrose pellet every 2 min for 1 h (30 pellets/1 h). Responding at both levers was recorded, but resulted in no cues or sucrose pellet delivery.

Locomotor testing

Locomotor activity was recorded in darkened circular test chambers with a 12-cm-wide runway, equipped with four pairs of photocells located at 90° intervals around the 1.95-m perimeter. All locomotor tests were performed

during the light phase of the light:dark cycle. Five days following cocaine self-administration and reinstatement procedures, animals were habituated to the locomotor testing apparatus for 2 h on the day prior to cocaine-induced locomotor activity testing. On the test day, animals were again habituated for 2 h, given a pretreatment of vehicle or CGS 21680 (0.03 mg/kg, i.p.) and administered a cocaine challenge (15 mg/kg, i.p.) 5 min later. Locomotor activity was assessed for 2 h.

Data analysis

Cocaine-induced reinstatement data (lever presses) were analyzed with a mixed design two-factor ANOVA with lever (within) and CGS 21680/cocaine treatment (between) as the factors. Quinpirole-induced reinstatement data (lever presses) were analyzed with a two-factor ANOVA with quinpirole and CGS pretreatment as the factors. Cue- and sucrose-induced reinstatement data (lever presses) were analyzed with a mixed design two-factor ANOVA with the within factor, reinstatement (extinction vs. cue/sucrose), and the between factor, pretreatment. The effect of CGS 21680 pretreatment on cocaine-induced locomotor activity (beam breaks) was analyzed by an unpaired *t* test. All interactive effects of the ANOVAs were followed by simple main effects analyses (one-way ANOVA) and post hoc tests (Bonferroni's comparisons, Dunnett's test or *t* test). Statistical significance was preset at $p<0.05$.

Drugs

CGS 21680 [4-[2-[[6-amino-9-(*N*-ethyl-b-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride] was purchased from Tocris Bioscience (Ellisville, MO, USA). Quinpirole [(−)-quinpirole hydrochloride] was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC, USA). All drugs were dissolved in sterile-filtered physiological saline.

Results

Adenosine A_{2A} receptor stimulation dose-dependently blocks cocaine-induced reinstatement

Figure 1 illustrates that administration of the adenosine A_{2A} agonist CGS 21680 dose-dependently reduced cocaine-induced drug seeking. A significant treatment×lever interaction ($F_{5,50}=5.47$, $p<0.001$) and significant main effects of treatment ($F_{5,50}=5.07$, $p<0.001$) and lever ($F_{1,50}=26.32$, $p<0.001$) were observed. Subsequent analysis of the interaction found that cocaine treatment significantly induced drug-paired lever pressing, which was dose-dependently decreased by pretreatment with CGS 21680 ($F_{5,50}=5.29$, $p<0.001$). A statistical trend for reduced inactive lever pressing following the treatments was observed ($F_{5,50}=2.25$, $p=0.06$).

Because systemic administration of CGS 21680 produces sedation and reduced sensitized locomotor activity to psychostimulants (Filip et al. 2006; Rimondini et al. 1997), we tested the effects of the minimally effective dose of CGS 21680 (0.03 mg/kg, i.p.) on cocaine-stimulated locomotor activity. These tests were performed in the same animals that had self-administered cocaine and were tested

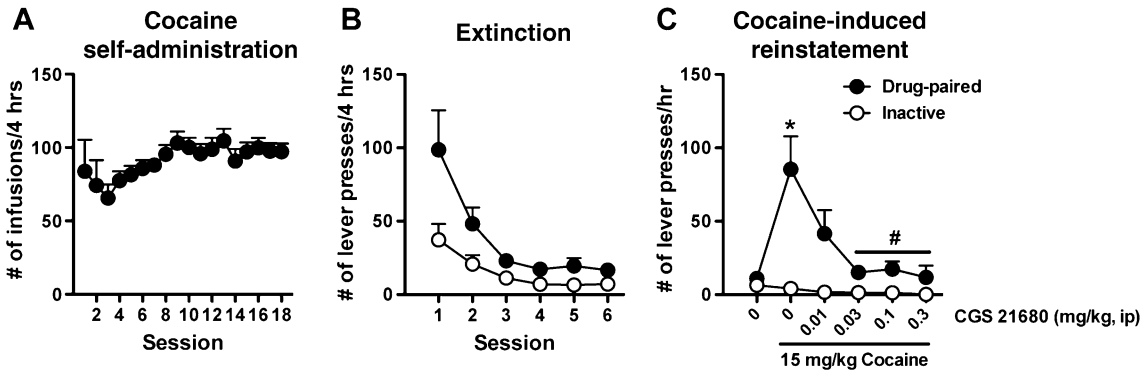

Fig. 1 Administration of the adenosine A_{2A} receptor agonist CGS 21680 dose-dependently blocked cocaine-induced reinstatement. **a** Number of cocaine infusions in each 4-h session during the cocaine self-administration phase. **b** Extinction training was performed in six daily sessions 1 week following the last self-administration session. Responses on the previously drug-paired lever were reduced to levels comparable to inactive lever responses. **c** Cocaine-induced reinstatement testing conducted across 5 days following extinction training. Each reinstatement session included an initial extinction phase (2 h) that preceded the reinstatement phase. The A_{2A} agonist, CGS 21680, dose-dependently reduced cocaine-induced drug-paired lever responding. The numbers of animals in each treatment group is as follows: 0 CGS/saline=7, 0 CGS/cocaine=10, 0.01 CGS/cocaine=10, 0.03 CGS/cocaine=14, 0.1 CGS/cocaine=11, 0.3 CGS/cocaine=4. *Significant from vehicle ($p<0.05$, Bonferroni's post test), #significant from 15 mg/kg cocaine with 0 CGS 21680 pretreatment ($p<0.05$, Bonferroni's post test)

for cocaine-induced reinstatement. As shown in Fig. 2, pretreatment of CGS 21680 had no effect on cocaine-induced locomotion at the same dose (15 mg/kg) used for cocaine priming in reinstatement ($t_{22}<1$, NS).

Adenosine A_{2A} receptor stimulation blunts quinpirole-induced reinstatement

Systemic administration of DA D_2 receptor agonists robustly stimulates reinstatement of cocaine seeking in extinguished animals. Therefore, we tested whether a pretreatment of CGS 21680 (0.03 mg/kg, i.p.) would attenuate quinpirole-induced reinstatement using the minimum dose effective at blocking cocaine priming. Figure 3 illustrates the dose-dependent increase in drug-paired lever pressing resulting from quinpirole administration, which was attenuated by a pretreatment with 0.03 mg/kg CGS 21680. Significant main effects of pretreatment ($F_{1,44}=8.44$, $p<0.006$) and quinpirole ($F_{3,44}=14.29$, $p<0.001$) were observed; however, the interaction was not significant ($F_{3,44}=1.68$, $p=0.18$). No effects of quinpirole or pretreatment were observed in inactive lever pressing (data not shown).

Adenosine A_{2A} receptor stimulation reduces cue-induced reinstatement

Presentation of cocaine-associated cues is sufficient to elicit reinstatement of cocaine seeking in extinguished animals. Because this is mediated in part by DA transmission in the striatum, we tested whether a pretreatment of CGS 21680 (0.03 mg/kg, i.p.) would block cue-induced reinstatement. Figure 4 illustrates that a CGS 21680 pretreatment significantly blunts cue-induced reinstatement. While both pretreatment groups displayed significant increases in drug-paired lever pressing during the cue presentations compared to the preceding hour of extinction (Cue—$F_{1,15}=40.61$, $p<0.001$), the CGS 21680 group responded at significantly lower levels (Pretreatment—$F_{1,15}=7.49$, $p<0.02$; Cue×Pretreatment—$F_{1,15}=8.28$, $p<0.02$). No effects of the cue presentation or CGS 21680 pretreatment were observed on inactive lever responding (data not shown).

Adenosine A_{2A} receptor stimulation has no effect on sucrose seeking

Finally, we incorporated a procedural control to account for any generalized performance effects of CGS 21680 (0.03 mg/kg, i.p.) on reinstatement using non-contingent delivery of sucrose pellets in animals trained to self-administer sucrose pellets. Figure 5 illustrates that a pretreatment of CGS 21680 had no effect on sucrose reinstatement (Pretreatment—$F_{1,13}<1$, NS; Sucrose×Pretreatment—$F_{1,13}<1$, NS), despite the significant levels of sucrose-induced lever pressing (Sucrose—$F_{1,13}=11.97$, $p<0.005$), which was comparable to cue-induced reinstatement following cocaine self-administration.

Discussion

These findings demonstrate for the first time that pharmacological activation of adenosine A_{2A} receptors attenuates cocaine-seeking behavior. We show that A_{2A} receptor activation reduces cocaine seeking induced by pharmacological stimuli such as cocaine and quinpirole and also by discrete cocaine-associated cues. These findings agree with previous work demonstrating that A_{2A} stimulation attenuated the development and expression of behavioral sensitization to cocaine and methamphetamine (Filip et al. 2006; Shimazoe et al. 2000), the expression of cocaine place conditioning (Poleszak and Malec 2002), and the initiation

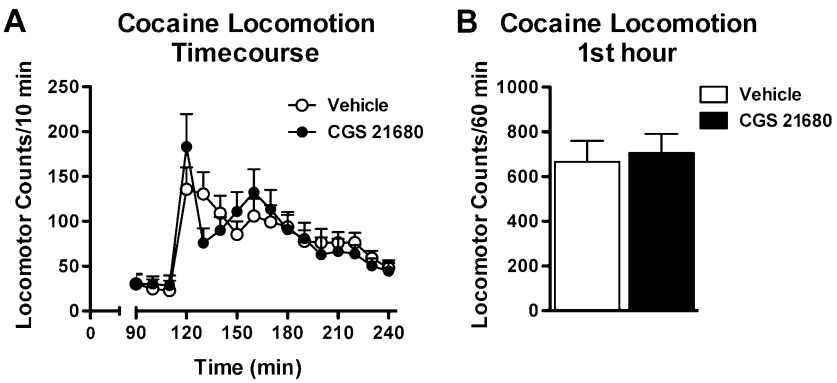

Fig. 2 Cocaine-induced locomotor activity was unaltered by a pretreatment with CGS 21680. **a** Time course of locomotor activity illustrating the last 30 min of the habituation period (90–110 min) followed by the effects of 15 mg/kg cocaine (i.p.) with and without a pretreatment of 0.03 mg/kg CGS 21680 (i.p.). This dose was chosen since it was the lowest dose that was effective in reducing cocaine-induced reinstatement (Fig. 1). **b** Cocaine-induced locomotor activity over the first hour in animals pretreated with vehicle or 0.03 mg/kg CGS 21680 (i.p.). No significant changes in locomotor activity were observed, $n=12$/group

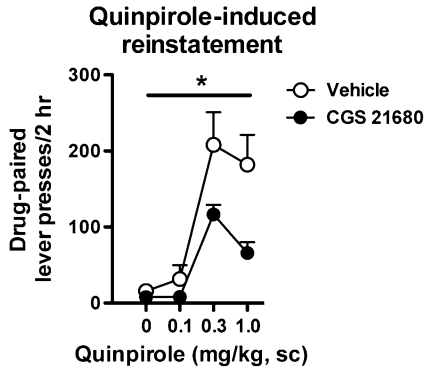

Fig. 3 Administration of the adenosine A_{2A} receptor agonist CGS 21680 blunted dopamine D_2 receptor-induced reinstatement. Animals were trained to self-administer cocaine in 4-h sessions over 3 weeks and extinguished following a week of abstinence. On the subsequent 5 days, animals were tested for D_2 agonist (quinpirole)-induced reinstatement. As can be seen, pretreatment with 0.03 mg/kg CGS 21680 (i.p.) blunted quinpirole-induced reinstatement at the two highest doses. *Significant main effect of the CGS 21680 pretreatment, n=5–8/group

of cocaine self-administration (Knapp et al. 2001). Other complementary work utilizing pharmacological blockade of adenosine A_{2A} receptors also supports an antagonistic effect of A_{2A} receptors on cocaine-mediated behaviors. Thus, antagonists of A_{2A} receptors enhanced the acute locomotor effects of cocaine, the development, and the subsequent expression of cocaine sensitization (Filip et al. 2006). Previous studies also showed a reversal in intracranial self-stimulation current threshold impairments observed during cocaine withdrawal, further suggesting that A_{2A} receptor antagonism alters behavioral indices of withdrawal observed during abstinence (Baldo et al. 1999; Filip et al. 2006). Together, these findings suggest that adenosine A_{2A} receptor stimulation oppose the effects of cocaine and cocaine-associated cues. Our findings that CGS 21680 completely abolished drug-paired lever pressing establish the potential beneficial effects of adenosine A_{2A} receptor stimulation on cocaine-induced cocaine-seeking behavior, although cocaine seeking elicited by the D_2 agonist, quinpirole, or cocaine-associated cues was only partially attenuated.

Other work utilizing genetic deletion of the adenosine A_{2A} receptor report conflicting evidence and paradoxically display behavioral effects similar to those utilizing pharmacological stimulation. Thus, mice lacking the A_{2A} receptor display attenuated locomotor responses to cocaine, impaired development of amphetamine sensitization, and reductions in the reinforcing efficacy of self-administered cocaine (Chen et al. 2000; Chen et al. 2003; Soria et al. 2006). These findings may result from compensatory changes during development or may reflect the lack of neuroanatomical specificity of A_{2A} receptor knockdown in neural circuits regulating these behaviors. Recent work supports the latter since extra-striatal (forebrain) knockdown of A_{2A} receptors reduced psychostimulant-induced locomotion, while striatal-specific knockdown of A_{2A} receptors enhanced psychostimulant-induced locomotion akin to pharmacological antagonism (Shen et al. 2008). These findings further suggest an inhibitory role for adenosine A_{2A} receptors specifically in the striatum; however, further study will be required to ascertain the site of action for reducing cocaine seeking.

Stimulation of the adenosine A_{2A} receptors is known to activate dopamine D_2- and enkephalin-containing neurons in the striatum that form the indirect pathway (Karcz-Kubicha et al. 2006). Thus, local stimulation of A_{2A}

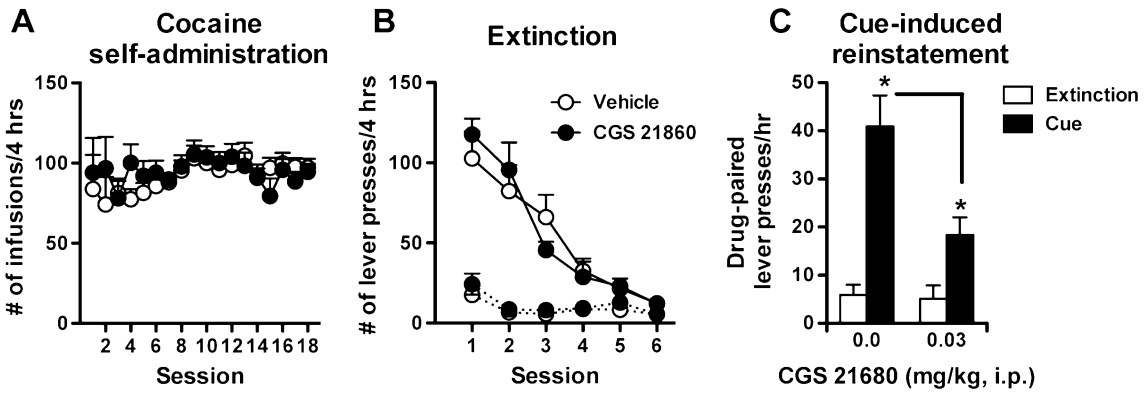

Fig. 4 Administration of the adenosine A_{2A} receptor agonist CGS 21680 blunted reinstatement induced by cocaine-associated cues. **a** Number of cocaine infusions in each 4-h session during the cocaine self-administration phase. **b** Extinction training was performed in six daily sessions 1 week following the last self-administration session. Responses on the previously drug-paired lever (*solid lines*) were reduced to levels comparable to inactive lever responses (*dotted lines*). **c** Cue-induced reinstatement testing was conducted in a 4-h reinstatement session that included an initial extinction phase (3 h) that preceded the reinstatement phase (1 h). Shown in the figure is the third hour of the extinction phase and the following hour of cue testing. The A_{2A} agonist, CGS 21680, significantly reduced cue-induced drug-paired lever responding. *Significant from extinction (p<0.05, Bonferroni post test). *Bar—significant from 0 CGS 21680 pretreatment (t_{15}= 3.12, p< 0.01), Vehicle N= 8; CGS 21680 N= 9*

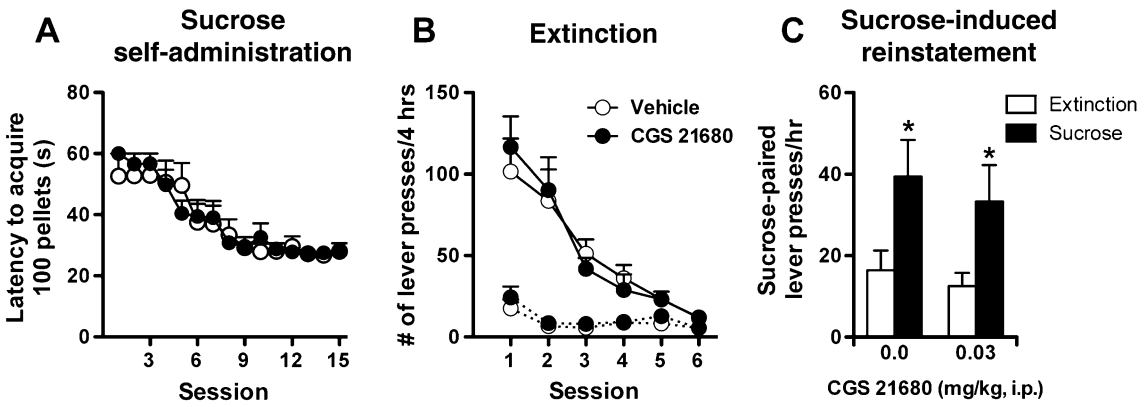

Fig. 5 Administration of the adenosine A_{2A} receptor agonist CGS 21680 had no effect on sucrose seeking. **a** Sucrose self-administration was conducted over 3 weeks. **b** Extinction training was performed in six daily sessions 1 week following the last self-administration session. Responses on the previously sucrose-paired lever (*solid line*) were reduced to levels comparable to inactive lever responses (*dotted line*). **c** Sucrose reinstatement testing was conducted in a 2-h reinstatement session that included an initial extinction phase (1 h) that preceded the reinstatement phase (1 h). The A_{2A} agonist, CGS 21680, failed to alter sucrose seeking despite significant responding on the lever previously paired with sucrose delivery. *Significant from extinction ($p<0.05$, Dunnett's test), Vehicle $N=7$, CGS 21680 $N=8$

receptors in the dorsal and ventral striatum enhances GABA input to the globus pallidus and ventral pallidum, respectively (Mingote et al. 2008; Ochi et al. 2000). It is plausible that A_{2A} receptor stimulation antagonizes the heightened sensitivity of dopamine D_2 receptors in the striatum following long-term cocaine administration. In this manner, A_{2A} receptor stimulation acts similarly to a D_2 receptor antagonist in blocking the functional inhibition of indirect striatal GABA output mediated by D_2 receptors.

Not only are A_{2A} and D_2 receptors co-localized on the enkephalin-containing neurons as previously described, the receptors play an antagonistic and reciprocal role in modulating cellular function (Ferre 1997; Ferre et al. 1991a). The inhibitory role of adenosine A_{2A} receptor stimulation on dopamine D_2 receptors may reflect opposing intracellular signaling cascades mediated by A_{2A} and D_2 receptors acting independently, or may involve the formation of A_{2A}/D_2 heteromers. Stimulation of A_{2A} receptors counteracts D_2 receptor-mediated signal transduction (Yang et al. 1995) and opposes the effects of DA receptor stimulation on immediate early gene expression in the striatum (Morelli et al. 1994; Svenningsson et al. 1999a). Thus, A_{2A} receptor-induced activation of stimulatory G proteins and increases in cAMP production would consequently increase neuron excitability and effectively offset D_2 receptor effects mediated by inhibitory G proteins (Colwell and Levine 1995; Schiffmann et al. 2007; Svenningsson et al. 1999a; Tozzi et al. 2007). Some of the opposing effects also may be dictated by the formation of heteromeric receptor complexes between postsynaptic A_{2A} and D_2 receptors (Canals et al. 2003; Fuxe et al. 2003; Hillion et al. 2002). The formation of A_{2A}/D_2 receptor complexes provides inhibitory regulation over dopamine D_2 receptor binding and inhibitory G-protein coupling (Ferre et al. 1991a; Fuxe et al. 1998; Hillion et al. 2002; Torvinen et al. 2005). The relative contribution of heteromerized and non-heteromerized A_{2A} and D_2 receptors to counteract D_2-mediated signaling remains unclear, especially in the context of addiction.

Alternatively, it is possible that presynaptic A_{2A} receptors located on glutamate terminals in striatum indiscriminately alter the striatal neuronal function since local injections of CGS 21680 have been shown to increase striatal glutamate release (Corsi et al. 1999; Rodrigues et al. 2005). However, previous work has demonstrated that stimulation of AMPA glutamate receptors in the NAc induces drug seeking (Cornish et al. 1999) while blockade of AMPA receptors attenuates cocaine- and cue-induced drug seeking (Backstrom and Hyytia 2007; Cornish et al. 1999). It therefore does not seem likely that presynaptic A_{2A} receptor stimulation resulting in striatal glutamate release would mediate a reduction in cocaine seeking.

It is also possible that the effects of systemic CGS 21680 administration on the reinstatement behavior shown here are mediated by A_{2A} receptors located outside of the striatum. While adenosine A_{2A} receptors are most abundant in striatal regions, low to moderate levels of A_{2A} receptors are found in other regions such as the medial prefrontal cortex and the amygdala (Svenningsson et al. 1999b). These two regions also receive dopaminergic innervation that is known to be involved in cocaine seeking (McFarland and Kalivas 2001), and A_{2A} receptor stimulation within these structures may similarly modulate dopamine transmission. Stimulation of A_{2A} receptors in the prefrontal cortex decreased sedation time and electroencephalographic activity, both measures of increased arousal (Van Dort et al. 2009), which were not observed in the present study with the most effective dose of CGS 21680. The effect of A_{2A}

receptor stimulation in the amygdala is unclear. Given the amygdala's involvement in cue-induced cocaine seeking, one may predict that stimulation of A_{2A} receptors in the amygdala would blunt cue-induced cocaine seeking perhaps through similar antagonism of D_2 receptors as found in the striatum. Future studies will be necessary to fully elucidate the involvement of A_{2A} receptors in specific brain circuits regulating drug seeking.

In conclusion, our findings suggest an important antagonistic role for adenosine A_{2A} receptor stimulation in mediating cocaine relapse behaviors. We demonstrate that stimulation of A_{2A} receptors blunts cocaine seeking induced by pharmacological and conditioned stimuli in an animal model of relapse. These findings support the notion that this effect involves a negative interaction between adenosine A_{2A} receptors and relapse mediated by D_2 receptor stimulation. Together, these findings suggest that enhancing the inhibitory regulation of dopamine D_2 receptors may provide an effective pharmacological treatment strategy.

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