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Effects of adenosine A_{2A} receptor antagonists on cocaine-induced locomotion and cocaine seeking

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Abstract

Rationale and Objectives—Adenosine signaling through adenosine A_{2A} receptors (A_{2A}R) is known to influence cocaine-induced behaviors. These studies sought to elucidate how two A_{2A}R antagonists distinguished by their antagonist effects at presynaptic and postsynaptic A_{2A}R influence cocaine-induced locomotion and cocaine seeking.

Methods—Sprague-Dawley rats were used to assess the differential effects of SCH 442416 and istradefylline that antagonize presynaptic and postsynaptic A_{2A}R, respectively. We evaluated the effects of these antagonists on both basal and cocaine-induced locomotion in cocaine-naïve rats and rats that received 7 daily cocaine treatments. The effects of SCH 442416 or istradefylline on cocaine seeking was measured in animals extinguished from cocaine self-administration. We assessed the effects of the A_{2A}R antagonists to induce cocaine seeking when administered alone and their effects on cocaine seeking induced by a cocaine priming injection. Lastly, we evaluated the effects of the antagonists on sucrose seeking in animals extinguished from sucrose self-administration.

Results—Neither istradefylline nor SCH 442416 significantly altered basal locomotion. Istradefylline enhanced acute cocaine-induced locomotion but had no effect on the expression of locomotor sensitization. SCH 442416 had no effect on acute cocaine-induced locomotion but inhibited the expression of locomotor sensitization. Istradefylline was sufficient to induce cocaine seeking and augmented both cocaine-induced seeking and sucrose seeking. SCH 442416 inhibited cocaine-induced seeking, but had no effect on sucrose seeking and did not induce cocaine seeking when administered alone.

Conclusions—These findings demonstrate differential effects of two A_{2A}R antagonists distinguished by their effects at pre- and postsynaptic A_{2A}R on cocaine-induced behaviors.

Keywords

Purinergic Receptors; Psychostimulant; Relapse; Behavioral Sensitization; KW 6002

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Conflict of interest

The authors declare that there are no conflicts of interest.

Introduction

Cocaine addiction affects millions of people around the world, yet no accepted pharmacological treatment exists. Finding neurobiological targets for potential pharmacological intervention could provide a means by which people could more effectively recover from addiction. One such target for treatment may lie within the striatum, a component of the basal ganglia circuitry that is affected by cocaine administration (Kalivas 2009; Gerfen and Surmeier 2011). Here, multiple neurotransmitter systems interact during chronic cocaine administration and are thought to underlie many behavioral characteristics of addiction (Self et al. 2004; Kalivas 2009). A more detailed understanding of these neurotransmitter systems and their target receptors may help uncover new treatment approaches.

Striatal neurons integrate several neurotransmitter systems through the cellular co-localization and synaptic localization of receptor subtypes. One particularly interesting neurotransmitter is adenosine, a nucleoside produced by the breakdown of adenosine triphosphate (ATP) (Dunwiddie and Masino 2001; Schiffmann et al. 2007). Adenosine has the capacity to modulate the functioning of other neurotransmitters, such as dopamine and glutamate, that are known to be involved in the acute and chronic effects of cocaine (Ferré et al. 2007). The modulatory functions of adenosine are largely attributed to the synaptic co-localization of adenosine receptor subtypes with other receptors within the striatum. The two primary adenosine receptor subtypes found in the striatum are the $G_{i/o}$ protein-coupled adenosine A_1 receptor (A_1R), and the $G_{s/olf}$ protein-coupled adenosine A_{2A} receptor ($A_{2A}R$) (Dunwiddie and Masino 2001). There is substantial evidence demonstrating that $A_{2A}Rs$ play a role in cocaine-induced locomotion, reward, and relapse (Knapp et al. 2001; Poleszak and Malec 2002a, b; Filip et al. 2006; Bachtell and Self 2009; O'Neill et al. 2012; Wydra et al. 2015a). The recent development of pharmacological tools to examine different $A_{2A}R$ subpopulations that are expressed at pre- and postsynaptic sites is particularly interesting since these tools may allow for more specific modulation of dopamine and/or glutamate neurotransmission (Rosin et al. 2003; Ciruela et al. 2006; Quiroz et al. 2009; Farrar et al. 2010; Orru et al. 2011a, b).

$A_{2A}Rs$ are co-expressed with A_1Rs on presynaptic glutamate terminals where they bi-directionally affect glutamate release (Ciruela et al. 2006; Quiroz et al. 2009). Under basal conditions, stimulation of the higher affinity A_1R inhibits glutamate release (Barrie and Nicholls 1993; Ciruela et al. 2006). However, in the presence of increased cellular activity and higher phasic adenosine release that may occur during cocaine administration, the lower affinity $A_{2A}R$ is stimulated causing glutamate release into the synapse (Popoli et al. 1995; Marchi et al. 2002; Rodrigues et al. 2005; Ciruela et al. 2006; Shen et al. 2013; Matsumoto et al. 2014). Increased glutamate neurotransmission in the nucleus accumbens (NAc) is associated with increased cocaine seeking and locomotor activation (Schmidt and Pierce 2010; Steketee and Kalivas 2011). The facilitation of glutamate transmission produced by presynaptic $A_{2A}R$ stimulation would therefore be expected to enhance cocaine-mediated behaviors, while blockade of presynaptic $A_{2A}R$ may reduce cocaine seeking and cocaine-induced locomotion.

Stimulation of A_{2A}Rs is largely associated with a reduction in cocaine-mediated behaviors (Knapp et al. 2001; Poleszak and Malec 2002a, b; Bachtell and Self 2009; O'Neill et al. 2012; Wydra et al. 2015a). These effects are attributed to the postsynaptic expression of A_{2A}Rs on striatopallidal (indirect pathway) medium spiny neurons (MSNs) in the striatum (Svenningsson et al. 1999; Ferre et al. 2008; Azdad et al. 2009; Higley and Sabatini 2010). Activation of postsynaptic A_{2A}Rs on these neurons counteracts the inhibitory cellular effects of the G_{i/o} protein-coupled D₂R stimulation via a heteromeric receptor complex with adenylyl cyclase 5 (Hakansson et al. 2006; Harper et al. 2006; Ferre et al. 2008; Farrar et al. 2010; Ferré et al. 2018). Vesicular release of dopamine is accompanied by the release of ATP that can be catabolized to adenosine within a few hundred milliseconds that subsequently acts on adenosine receptors (Poelchen et al. 2001; Ho et al. 2015). Initial D₂R stimulation is subsequently counteracted by the latent adenosine signal to restore D₂R-induced cellular effects (Chen et al. 2001; Ferre et al. 2008; Ferré et al. 2018). This process is best observed behaviorally following the application of an A_{2A}R antagonist which results in increased locomotor activity due to its permissive effect on D₂R activity (Chen et al. 2001; Orru et al. 2011a, b; Ferré 2016). Stimulation of A_{2A}Rs with agonists generally inhibits D₂R-induced behaviors (Chen et al. 2001; Bachtell and Self 2009; O'Neill et al. 2012; Hobson et al. 2012). Because pre- and postsynaptic expression of A_{2A}Rs may have unique functional roles, preferential antagonism of pre- versus postsynaptic A_{2A}Rs may result in distinct behavioral effects when administered alone or when combined with cocaine.

Utilizing pharmacological tools previously shown to preferentially antagonize pre- versus postsynaptic A_{2A}Rs (Orru et al. 2011a, b), we determined the differential effects of A_{2A}R antagonists on cocaine-induced behaviors in rats. Specifically, we investigated how the putative presynaptic antagonist, SCH 442416, and postsynaptic antagonist, istradefylline, affected cocaine-induced locomotion and cocaine seeking. We hypothesized that administration of the presynaptic A_{2A}R antagonist would blunt cocaine-induced locomotion and cocaine seeking, while administration of the postsynaptic A_{2A}R antagonist would induce locomotor activity and enhance cocaine seeking. Ultimately, these results shed light on specific roles of the synaptic localization of A_{2A}Rs and may lead to novel pharmacotherapeutic approaches.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (Envigo, Indianapolis, USA) weighing 300 ± 30g were housed individually with *ad libitum* access to water and food throughout the experiments. Rats were housed under 12:12 light/dark cycle conditions. Animals were allowed at least 7 days after arrival to habituate to the new setting before starting experiments. All procedures complied with the Guide for Care and Use of Animals as adopted and promulgated by the U.S. National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at the University of Colorado Boulder.

Drugs

The selective A_{2A}R antagonists, SCH 442416 (2-(2-Furanyl)-7-[3-(4-methoxyphenyl)propyl]-7H-pyrazolo[4,3-c][1,2,4]triazolo[1,5-c]pyrimidin-5-amine) and istradefylline, also known as KW 6002 (8-[(E)-2-(3,4-dimethoxyphenyl)ethyl]-1,3-diethyl-7-methylpurine-2,6-dione), were purchased from SigmaAldrich (St. Louis, MO, USA). Cocaine hydrochloride was also purchased from Sigma-Aldrich (St. Louis, MO, USA). Both A_{2A}R antagonist drugs were dissolved in 50% DMSO and 50% sterile-filtered physiological (0.9%) saline. Cocaine hydrochloride was dissolved in sterile-filtered physiological (0.9%) saline.

Locomotor testing apparatus and general procedure

Rats were individually placed into plexiglass chambers sized 16×16×15 inches (San Diego Instruments, San Diego, USA) with pairs of infrared beams spaced one inch apart to record horizontal locomotion. All tests occurred in a dark room during the light phase of the light/dark cycle. Rats were habituated to the test room in their home cages for 1 hour prior to each locomotor test. Prior to testing, animals were habituated to the chambers for 2 hours the day prior to experimental testing before returning to their home cages. On test days, animals were habituated to the chambers for 1 hour before receiving intraperitoneal (ip) treatments.

Chronic cocaine administration procedure

Following the first day of habituation, animals were tested in the locomotor boxes as described above. Chronic cocaine (15 mg/kg) or saline was administered with seven daily ip injections. Locomotor testing was conducted on days 1, 7, and 14. Drug administration on days 2 through 6 occurred in home cages. Only animals meeting criterion for locomotor sensitization (day 7 cocaine-induced activity > 1.25 times greater than day 1 cocaine-induced locomotor activity) were subsequently challenged and tested on day 14 after a 7 day drug-free period. The effects of an A_{2A}R antagonist pretreatment, SCH 442416 and istradefylline, were tested on day 14 with either a saline or cocaine (15 mg/kg) challenge (see details below).

Cocaine self-administration and extinction procedures

Self-administration procedures were performed in operant conditioning chambers (Med-Associates, St. Albans, VT, USA) equipped with two retractable levers (an active and an inactive lever), stimulus light cues above the levers, a sound-attenuating fan in each chamber, and an infusion pump system. Animals were initially trained on a fixed ratio 1 (FR1) schedule of reinforcement where pressing the active lever resulted in sucrose pellet delivery. Following adequate training to respond for a sucrose reward (100 sucrose pellets in one session), rats underwent surgery in which an intravenous catheter was implanted into the jugular vein under halothane anesthesia (1–2.5%). Following recovery from surgery, animals were allowed to administer intravenous cocaine (0.5 mg/kg/100 µl injection) over 15 2-h sessions using an FR1 schedule of reinforcement. During these sessions, pressing the drug-paired lever resulted in a 5-s infusion of cocaine and the presentation of the stimulus cue light, while pressing the inactive lever had no consequence. After drug delivery, the house light remained off, and pressing the drug-paired lever did not result in a drug infusion for 20-

s. Following self-administration, rats underwent seven 2-hr extinction training sessions where pressing the drug-paired lever no longer resulted in the administration of cocaine or stimulus light illumination.

Experiment 1: Effects of systemic A_{2A}R antagonist administration on locomotion

Following habituation to the locomotor chambers, rats received ip injections of vehicle, istradefylline (0, 0.1, 1.0, or 3.0 mg/kg), or SCH 442416 (0, 0.1, 1.0, or 3.0 mg/kg). Locomotor activity was recorded for 2 hours immediately following antagonist administration. Animals were repeatedly tested such that the dosing order was counterbalanced, rats were treated once every 48 hours, and all rats received each dose of either istradefylline or SCH 442416.

Experiment 2: Effects of A_{2A}R antagonists on acute cocaine-induced locomotion

Rats received chronic saline for 7 days. After 7 days of home cage withdrawal, rats were administered a pretreatment of either vehicle, istradefylline (1 mg/kg, ip), or SCH 442416 (1 mg/kg, ip) 10 minutes prior to either a saline or cocaine (15 mg/kg, ip) challenge on day 14. Rats were immediately placed in the locomotor chambers, and locomotor activity was monitored for 2 hours.

Experiment 3: Effects of A_{2A}R antagonists on locomotion in cocaine-sensitized rats

Rats received chronic cocaine for 7 days. After 7 days of home cage withdrawal, rats were administered a pretreatment of either vehicle, istradefylline (1 mg/kg, ip), or SCH 442416 (1 mg/kg, ip), 10 minutes prior to a saline or cocaine (15 mg/kg, ip) challenge on day 14. Rats were immediately placed in the locomotor chambers, and locomotor activity was monitored for 2 hours.

Experiment 4: Effects of A_{2A}R antagonists on reinstatement of cocaine seeking

After extinguishing drug-paired lever presses, animals underwent a 2-hr reinstatement test. For these tests, animals were pretreated with vehicle, istradefylline (0.3, 1.0, or 3.0 mg/kg, ip), or SCH 442416 (1.0 or 3.0 mg/kg, ip) 10 minutes prior to a systemic saline challenge before being placed into the operant chamber. The number of active and inactive lever presses was then recorded and analyzed, though neither lever resulted in a drug infusion.

Experiment 5: Effects of A_{2A}R antagonists on cocaine-primed reinstatement of drug seeking

Once rats had completed extinction, they underwent a 2-hr reinstatement test. Here, animals were pretreated with vehicle, istradefylline (0.3 mg/kg, ip), or SCH 442416 (1.0 mg/kg, ip), 10 minutes prior to a cocaine challenge. Rats pretreated with istradefylline were primed with both a subthreshold dose (5 mg/kg, ip) and maximal dose (15 mg/kg, ip) of cocaine to reveal the potential enhancement of cocaine seeking. Rats pretreated with SCH 442416 were primed with only a maximal dose (15 mg/kg, ip) of cocaine to reveal potential inhibitory effects on cocaine seeking. Following the cocaine primes, rats were immediately placed back into operant chambers, and the number of active and inactive lever presses was recorded and analyzed. During the test, neither lever resulted in a drug infusion.

Experiment 6: Effects of A_{2A}R antagonists on reinstatement of sucrose seeking

Sucrose self-administration procedures occurred in operant boxes as described above. Food restricted rats were trained to lever press for 45 mg banana-flavored sucrose pellet (Bio-Serv, Flemington, NJ) into a food magazine located between the retractable levers on an FR1 schedule of reinforcement. Correct lever responses also resulted in the presentation of a stimulus cue light (5-s) followed by a 20-s time-out period when active lever pressing no longer resulted in sucrose pellet delivery. After 10 sessions and stabilization of sucrose self-administration, lever pressing was extinguished in seven 120-min sessions. Animals then underwent a reinstatement protocol similar to that described above. Animals were pre-treated with vehicle, istradefylline (1.0 mg/kg, ip), or SCH 442416 (1.0 mg/kg, ip) 10-min prior to being placed into the chambers for a 120-min reinstatement session. During the reinstatement session, 5 sucrose pellets were delivered noncontingently every 120-s during the first 10-min of the session. Throughout the session, responses on the previously sucrose-paired lever resulted in the presentation of stimulus cue light but not sucrose pellets. Active and inactive lever presses were recorded and analyzed. Responses on the inactive lever had no programmed consequences.

Statistical analyses

Total number of beam breaks per session was used to measure overall locomotion. For Experiments 1–3, the effect of the A_{2A}R antagonists on beam breaks was analyzed using a between subjects one-way ANOVA with either antagonist dose or antagonist drug as the between subjects variable. Total active and inactive lever presses were used to evaluate the effects of the antagonists on cocaine seeking. In experiment 4, the effect of A_{2A}R antagonist pretreatment dose on reinstatement of lever pressing was analyzed using a between subjects 2×4 factorial ANOVA with lever (active and inactive) and istradefylline (0.0, 0.3, 1.0, and 3.0 mg/kg) as the variables. A separate 2×3 factorial ANOVA with lever (active x inactive) and SCH 442416 (0.0, 1.0, and 3.0 mg/kg) was also utilized.

Experiment 5 was analyzed with between subjects 2×2 factorial ANOVAs with lever (active and inactive) and cocaine dose (5 or 15 mg/kg) to evaluate istradefylline, or lever (active and inactive) and treatment (vehicle, cocaine, or SCH 442416) to evaluate SCH 442416. A between subjects one-way ANOVA was used to analyze Experiment 6 with pretreatment drug as the between subjects variable to assess the effects of antagonist pretreatment on reinstatement of sucrose seeking. Significant main effects and interactive effects were subsequently analyzed by a one-way ANOVA and/or Bonferroni's test for multiple comparisons. Significance level was set at $p < 0.05$.

Results

The A_{2A}R antagonist, istradefylline, increases locomotor activity

Experiment 1 investigated the effects of various doses of the A_{2A}R antagonists on locomotor activity in male rats (Fig. 1). We found that administration of the postsynaptic A_{2A}R antagonist, istradefylline, increased locomotion, although this did not reach statistical significance ($F_{3,31} = 2.77$, $p = 0.06$) (Fig. 1A). The presynaptic antagonist, SCH 442416, did not significantly alter locomotor activity at any of the selected doses compared to vehicle-

treated rats (Fig. 1B). Given these and previous findings, the dose of 1.0 mg/kg was chosen for both A_{2A}R antagonists in all subsequent studies (Harper et al. 2006; Orru et al. 2011a, b).

The A_{2A}R antagonist, istradefylline, increases cocaine-induced locomotion

Experiment 2 investigated the effects of A_{2A}R antagonism on locomotor activity in drug-naïve animals to understand how the antagonists affected acute cocaine-induced locomotion. We first tested the effects of the A_{2A}R antagonists on animals having experienced a protocol in which they received repeated saline administration (Fig. 2A). There was a significant effect of antagonist pretreatment where animals receiving istradefylline treatments had significantly higher locomotor activity than vehicle- or SCH 442416-treated animals ($F_{2,37} = 6.37$, $p = 0.0042$) (Fig. 2B). We next wanted to characterize how these antagonists affected cocaine-induced locomotion (Fig. 2C). Here, animals pretreated with istradefylline had elevated locomotor activity compared to vehicle- and SCH 442416-pretreated animals ($F_{2,21} = 6.22$, $p = 0.0076$) (Fig. 2C). These results suggest that postsynaptic A_{2A}R antagonism enhances locomotor activity while presynaptic A_{2A}R antagonism is without effect, substantiating findings from Experiment 1 and supporting previous findings (Harper et al. 2006; Orru et al. 2011a, b).

The A_{2A}R antagonist, SCH 442416, decreases expression of cocaine sensitization

After studying the effects of the A_{2A}R antagonists on acute cocaine treatments, Experiment 3 assessed the effects of these antagonists following repeated cocaine administration. Rats that reached the criterion for locomotor sensitization were counterbalanced and assigned to a pretreatment group (vehicle, istradefylline, or SCH 442416) and either the saline or cocaine challenge treatment group. On day 14 of testing, rats received either a vehicle, istradefylline, or SCH 442416 injection prior to the saline/cocaine challenge (Fig. 3A). Prior to a saline challenge, there was a main effect of antagonist pretreatment ($F_{2,15} = 3.951$, $p = 0.042$), although a post hoc test did not reveal a significant difference between individual pretreatment groups (Fig. 3B). Interestingly, the A_{2A}R antagonists had a much different locomotor activity profile prior to a cocaine challenge in rats chronically treated with cocaine. We observed a main effect of antagonist pretreatment (Fig. 3C, $F_{2,27} = 4.26$, $p = 0.025$) with the pretreatment of SCH 442416 significantly reducing locomotor activity compared to vehicle-pretreated animals.

Differential effects of A_{2A}R antagonists on cocaine seeking

To augment the above results utilizing non-contingent drug delivery, we used a cocaine self-administration paradigm. Animals quickly learned to self-administer cocaine on an FR1 schedule and responded consistently after 10 days of testing (Fig. 4A). Prior to reinstatement testing, lever responding was extinguished over 8 sessions resulting in a progressive decrease in responding at the formerly cocaine-paired lever (Fig. 4B). To evaluate the ability of the A_{2A}R antagonists to reinstate non-reinforced lever responding, a dose-response curve was generated for both istradefylline and SCH 442416. Istradefylline significantly increased responding on the previously drug-paired lever ($F_{3, 162} = 9.685$, $p < 0.0001$) with the higher doses of (1.0 and 3.0 mg/kg, ip) having the most robust effects (Fig 4C). Conversely, SCH 442416 was ineffective at reinstating lever responding at any of the doses tested (Fig. 4D).

These results are consistent with our observations in locomotor testing following experimenter-administered A_{2A}R antagonist treatments.

We next tested the effects of the A_{2A}R antagonists in combination with a cocaine priming injection. A separate cohort of animals was trained to self-administer cocaine (Fig. 5A) and was extinguished (Fig. 5B) prior to reinstatement testing. Administration of istradefylline at a dose that was insufficient to reinstate cocaine seeking alone (0.3 mg/kg, ip) was tested on two doses of cocaine (5 or 15 mg/kg, ip). We found a significant main effect of pretreatment with istradefylline ($F_{1,30} = 6.91$, $p = 0.013$) and a significant main effect of cocaine dose ($F_{1,30} = 9.79$, $p = 0.0039$) (Fig. 5C). Interactive effects were not significant. Administration of SCH 442416 prior to a cocaine priming injection significantly reduced cocaine-induced reinstatement of cocaine seeking (Fig. 5D; $F_{2,42} = 22.45$, $p < 0.0001$). These findings are congruent with the locomotor studies and demonstrate that istradefylline enhances cocaine seeking, while SCH 442416 reduces cocaine seeking.

Effects of A_{2A}R antagonists on reinstatement of sucrose seeking

In order to evaluate the potential motor suppressant and/or motivational effects of the adenosine A_{2A}R antagonists, we conducted an experiment using a sucrose seeking paradigm. Thus, we investigated the effects of istradefylline and SCH 442416 on sucrose seeking in rats that were extinguished from sucrose pellet self-administration. Animals learned to self-administer sucrose pellets during ten 120-min sessions (Fig. 6A) and sucrose-paired lever pressing was then extinguished. As shown in Fig. 6B, all rats displayed sucrose seeking compared with the final day of extinction responding ($F_{3,32} = 8.37$, $p < 0.001$). Interestingly, rats treated with istradefylline showed enhanced sucrose seeking compared with vehicle, suggesting that the enhancement in cocaine seeking observed with istradefylline generalizes to seeking of natural rewards. Rats treated with SCH 442416 displayed a trend toward enhanced sucrose seeking, although this did not reach statistical significance. These findings suggest that the suppressive effects of SCH 442416 on cocaine locomotor sensitization and cocaine seeking are not due to a generalized suppression of activity or motivation.

Discussion

The goal of the present study was to elucidate the effects of two A_{2A}R antagonists, SCH 442416 and istradefylline, that were previously shown to inhibit pre- and postsynaptic A_{2A}R, respectively, on cocaine-induced behavior (Orzu et al. 2011a). We found that SCH 442416 had no effect on locomotion in cocaine naïve animals but significantly decreased cocaine-induced locomotion in cocaine-sensitized animals. Similarly, SCH 442416 did not induce cocaine seeking when administered alone but blunted cocaine seeking when administered prior to a cocaine challenge. Istradefylline, however, modestly increased locomotor activity and induced cocaine seeking when administered alone. Although istradefylline had no effect on locomotion in sensitized rats, istradefylline enhanced cocaine seeking when combined with both a subthreshold and maximal cocaine-priming injection. Finally, we showed that istradefylline also enhanced sucrose seeking, while SCH 442416 had no effect. These results suggest that presynaptic A_{2A}R antagonism may decrease

cocaine-induced behavior in animals with a cocaine history, while postsynaptic A_{2A}R antagonism increases behavioral activation and exacerbates cocaine-induced behaviors.

Our results demonstrate distinct qualitative behavioral differences following the administration of two A_{2A}R antagonists. Recent evidence suggests that the A_{2A}R antagonists bind selectively to different subpopulations of A_{2A}Rs based on the formation of specific heteromeric receptors (Orru et al. 2011a, b). *In vitro* binding assays demonstrate that SCH 442416 has lower affinity for A_{2A}Rs that are transfected into D₂R-expressing cells, while istradefylline has high affinity for A_{2A}Rs transfected into D₂R-expressing cells (Orru et al. 2011b). Very different binding characteristics are observed when A_{2A}Rs are transfected into A₁R-expressing cells, where SCH 442416 demonstrates high-affinity binding, while istradefylline has relatively low-affinity binding. These findings suggest that SCH 442416 binds preferentially to A₁R-A_{2A}Rs receptor complexes while istradefylline preferentially binds to A_{2A}R-D₂R complexes (Orru et al. 2011b). These A_{2A}R heteromeric complexes are important given their synaptic localization where A₁R-A_{2A}Rs are localized to presynaptic glutamate terminals and A_{2A}R-D₂R are localized at postsynaptic sites on indirect pathway MSNs (Ciruela et al. 2006; Ferre et al. 2008; Azdad et al. 2009). While these findings remain speculative, our findings suggest that qualitatively different behavioral effects arise from the administration of these antagonists having differential binding profiles at presynaptic and postsynaptic A_{2A}Rs.

There is growing evidence suggesting that the localization of A_{2A}R is an important factor in determining the behavioral effects of A_{2A}R antagonism. A_{2A}R stimulation impairs many behavioral effects of cocaine, including cocaine-induced locomotion, cocaine reward, and cocaine seeking during periods of abstinence, while A_{2A}R blockade generally enhances these effects (Knapp et al. 2001; Poleszak and Malec 2002a, b; Justinova et al. 2003; Filip et al. 2006; Bachtell and Self 2009; O'Neill et al. 2012; Doyle et al. 2012; Hobson et al. 2012; Wydra et al. 2015a, b). Our findings support these observations while also suggesting that the synaptic localization of A_{2A}R may be an important consideration. Similar findings were observed in another study investigating the rewarding properties of the psychoactive cannabinoid compound, tetrahydrocannabinol (THC). Here, SCH 442416 reduced the rewarding effects of THC, while istradefylline enhanced the rewarding effects of THC (Justinová et al. 2014). We also find that istradefylline enhances sucrose seeking, while SCH 442416 does not influence sucrose seeking. These findings suggest that blocking postsynaptic A_{2A}Rs may increase motivation and/or behavioral activation and that presynaptic A_{2A}R blockade may counteract the effects of drugs of abuse to reduce drug dependence.

The ability of the presynaptic A_{2A}R antagonism to inhibit cocaine-sensitized locomotion and drug seeking may arise from the unique A_{2A}R expression profile in the striatum. A_{2A}Rs colocalize with A₁Rs on presynaptic glutamate terminals (Hettinger et al. 2001; Rosin et al. 2003; Ciruela et al. 2006; Quiroz et al. 2009; Farrar et al. 2010; Orru et al. 2011a, b). Stimulation of A₁Rs and A_{2A}Rs has opposing effects on glutamate release, with A₁R stimulation inhibiting and A_{2A}R stimulation enhancing glutamate release (Popoli et al. 1995; Karcz-Kubicha et al. 2003; Ciruela et al. 2006; Quiroz et al. 2009; Shen et al. 2013). The inhibitory effects of presynaptic A_{2A}R antagonism on cocaine-induced behavior may be

especially relevant when coupled with the underlying cocaine-induced changes in glutamate neurotransmission observed following repeated cocaine exposure. While acute cocaine has little effect on extracellular glutamate levels, cocaine re-exposure produces a large surge in glutamate that is critical for the expression of cocaine sensitization and reinstatement (Pierce et al. 1996; McFarland et al. 2003). Evidence suggests that SCH 442416 blunts striatal glutamate release induced by cortical stimulation in both rats and mice (Orru et al. 2011b; Ferré et al. 2018). Thus, presynaptic A_{2A}R antagonism may also blunt the cocaine-induced glutamate surge in the striatum of animals following exposure to repeated cocaine that is critical for the sensitized locomotor response and cocaine-induced reinstatement (Steketee and Kalivas 2011). Additional support for this notion is derived from our observation that SCH 442416 does not influence acute cocaine-induced locomotion or sucrose seeking, two behaviors not associated with changes in glutamate signaling (Pierce et al. 1996; Bobadilla et al. 2017). The ability of presynaptic A_{2A}R antagonism to inhibit cocaine seeking is also supported by recent work demonstrating that SCH 442416 administered during extinction training produces a lasting reduction in cocaine-induced drug seeking (O'Neill et al. 2014). The lasting effects of SCH 442416 occur in the absence of any overt behavioral suppression during the extinction training when the drug was administered. These effects of presynaptic A_{2A}R antagonism mimic those observed when an A₁R agonist is administered during extinction training, suggesting that presynaptic A_{2A}R antagonism with SCH 442416 facilitates A₁R activity to reduce glutamate transmission. Future research should be aimed at determining whether SCH 442416 alters enhanced glutamate transmission that is associated with cocaine seeking.

Both human and animal studies indicate that cocaine craving and relapse is associated with increased activity of the prefrontal cortex, amygdala, hippocampus, ventral mesencephalon, and thalamus, all of which send glutamate projections to the striatum (Kalivas and Volkow 2011).

Given the diversity of glutamate projections to the striatum that contribute to cocaine relapse, it is difficult to determine which of these glutamate inputs express A_{2A}R and may be important for the observed findings. Presynaptic A_{2A}R expression has been best characterized in the cortical glutamate terminals where its physical presence has been shown with electron microscopy, and its functional importance has been observed on postsynaptic excitatory synaptic transmission, intracellular signaling, and glutamate release in the striatum (Ciruela et al. 2006; Quiroz et al. 2009; Orru et al. 2011a; Ferré et al. 2018). Though other presynaptic populations of A_{2A}R may exist and play a role in our observations, A_{2A}R expressed on cortical glutamatergic terminals have been well-characterized and likely predominate in these effects. Future research using circuit-specific molecular approaches will aid in uncovering specific pathways that mediate these effects.

Unlike SCH 442416, istradefylline enhanced sucrose and cocaine seeking, and induced behavioral activation in cocaine-naïve animals. These observations are congruent with previous studies using A_{2A}R antagonists having either a preferentially postsynaptic or a mixed pre- and postsynaptic binding profile. For example, both systemic and intra-NAc administration of A_{2A}R antagonists having greater activity at postsynaptic A_{2A}R, such as istradefylline, MSX-3 and SCH 58261, augment cocaine and sucrose seeking (O'Neill et al.

2012; Wydra et al. 2015b). It is presumed that postsynaptic A_{2A}R antagonist effects predominate over presynaptic A_{2A}R mechanisms since D₂R stimulation is necessary to reveal A_{2A}R antagonist-induced behavioral activation (Harper et al. 2006; Orru et al. 2011a, b; O'Neill et al. 2012; Wydra et al. 2015b). Blockade of A_{2A}Rs also prevents behavioral deficits induced by a D₂R antagonist and mimics the cellular signaling effects of a D₂R agonist (Hakansson et al. 2006; Farrar et al. 2010; Nunes et al. 2010; Orru et al. 2011a). In fact, postsynaptic A_{2A}R antagonists are a therapeutic approach for the motor disturbances observed in Parkinson's Disease due to their ability offset D₂R deficiencies and induce movement without worsening dyskinesias observed after long-term levodopa treatments (Navarro et al. 2016). Istradefylline, in particular, has been approved in Japan as an adjunct therapy with levodopa (Dungo and Deeks 2013). Together, these findings demonstrate that postsynaptic A_{2A}R antagonism disinhibits indirect pathway MSNs resulting in enhanced behavioral outputs including cocaine-induced locomotion, and cocaine and sucrose seeking.

These studies sought to determine how two A_{2A}R antagonists having differential binding profiles affected cocaine seeking and locomotor activation in rats. We show that antagonism of postsynaptic A_{2A}Rs induced locomotor activation, cocaine seeking, and sucrose seeking. Blockade of presynaptic A_{2A}Rs reversed the expression of cocaine sensitization and inhibited cocaine seeking. These findings suggest that SCH 442416 may prohibit some aspects of neurotransmission that contribute to cocaine-induced behaviors in animals with a history of cocaine administration. Given that A_{2A}Rs are most densely expressed in the striatal regions (Svenningsson et al. 1997, 1999), we speculate that the A_{2A}R antagonists are having these differential effects based on their unique expression profiles on presynaptic glutamate terminals and postsynaptic MSNs. These studies represent an initial step in identifying a novel treatment strategies for psychostimulant use disorders. It will be important for future work to more fully characterize the functional activity of the presynaptic A_{2A}R antagonists within specific neurocircuits.

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Figure 1.

Dose-dependent effects of the two adenosine $A_{2A}R$ antagonists on locomotion in habituated rats. A) Systemic administration of SCH 442416 had no effect on locomotor activity at any of the tested doses. B) Systemic administration of istradefylline dose-dependently increased locomotor activity, although no statistical significance was detected.

Figure 2.

Locomotor activity was significantly enhanced by the postsynaptic $A_{2A}R$ antagonist, istradefylline, but not the presynaptic $A_{2A}R$ antagonist, SCH 442416. A) Timeline of experiment depicting that rats received 7 daily injections of saline (0.9%, i.p.), followed by 7 days in home cages without treatment. Locomotion was tested on Day 14 following both an $A_{2A}R$ antagonist pretreatment (Vehicle, 1.0 mg/kg istradefylline, or 1.0 mg/kg SCH 442416) and challenge (Saline or 15 mg/kg, i.p. cocaine). B) Rats receiving a pretreatment of istradefylline showed heightened locomotor activity compared to vehicle-treated animals when challenged with saline on Day 14. C) A pretreatment of istradefylline increased cocaine-induced locomotor activity compared to vehicle- and SCH 442416pretreated animals. * indicates significance between both Vehicle and SCH 442416 ($p < 0.05$)

Figure 3.

The presynaptic A_{2A}R antagonist, SCH 442416, prevents expression of cocaine-sensitized locomotion, while the postsynaptic A_{2A}R antagonist, istradefylline, increases locomotion in cocaine-sensitized animals prior to a saline challenge. A) Timeline of experiment depicting that rats received 7 daily injections of cocaine (15 mg/kg, i.p.), followed by 7 days in home cages without treatment. Locomotion was tested on Day 14 following both an A_{2A}R antagonist pretreatment (vehicle, 1.0 mg/kg istradefylline, or 1.0 mg/kg SCH 442416) and challenge (saline or 15 mg/kg, i.p. cocaine). B) Cocaine-sensitized rats that were challenged with saline on Day 14 displayed an increase in locomotor activity following a pretreatment of istradefylline, although this did not reach statistical significance. C) Cocaine-sensitized rats that were challenged with cocaine following a pretreatment of SCH 442416 displayed overall decreased locomotor activity compared to vehicle-pretreated rats. * indicates significance from vehicle pretreatment ($p < 0.05$)

Figure 4.

Postsynaptic $A_{2A}R$ antagonism with istradefylline dose-dependently reinstates cocaine seeking. A) Average number of cocaine infusions during each 2-hr cocaine self-administration session on an FR1 schedule. B) Lever responding was extinguished over 8 consecutive 2-hr sessions during which lever responding had no programmed consequences. C) Administration of istradefylline dose-dependently increased previously drug-paired lever responses with the higher doses (1.0 and 3.0 mg/kg, ip), demonstrating robust cocaine seeking. D) Administration of SCH 442416 had no effect on cocaine seeking at any of the tested doses. * indicates significance from vehicle ($p < 0.05$)

Figure 5.

Presynaptic A_{2A}R antagonism with SCH 442416 reduces cocaine-induced seeking, while postsynaptic antagonism with istradefylline enhances cocaine-induced seeking. A) Average number of cocaine infusions during each 2-hr self-administration session on an FR1 schedule. B) Lever responding was extinguished over 8 consecutive 2-hr sessions during which lever responding had no programmed consequences. C) Administration of istradefylline at a dose (0.3 mg/kg, i.p.) ineffective at inducing cocaine seeking on its own enhanced cocaine seeking when administered prior to a low (5 mg/kg, i.p.) and high (10 mg/kg, i.p.) priming dose of cocaine. Solid lines indicate drug-paired lever responding while dashed lines indicate inactive lever responding. D) Cocaine seeking resulting from a cocaine challenge (15 mg/kg, i.p.) was significantly reduced following a pretreatment of SCH 442416. Solid bars indicate drug-paired lever responses and white overlaid bars indicate

inactive lever responses. * indicates significance from vehicle pretreatment ($p < 0.05$), ~ indicates significance from 5 mg/kg cocaine ($p < 0.05$), # indicates significance between vehicle-cocaine group

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Figure 6.

Postsynaptic A_{2A}R antagonism with istradefylline enhances sucrose seeking. A) Average number of sucrose deliveries during ten daily 2-hr sessions. B) Sucrose seeking on the previously sucrose-paired lever was enhanced compared to extinction responding in all groups. Istradefylline produced a significant enhancement in sucrose seeking compared to vehicle treated animals. Solid bars indicate sucrose-paired lever responses and white overlaid bars indicate inactive lever responses. * indicates significance from vehicle pretreatment ($p < 0.05$)