Pharmacology, Biochemistry and Behavior 102 (2012) 257–263

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Time and sex-dependent effects of an adenosine A2A/A1 receptor antagonist on motivation to self-administer cocaine in rats

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ARTICLE INFO

Article history:
Received 13 January 2012
Received in revised form 26 April 2012
Accepted 1 May 2012
Available online 10 May 2012

Keywords:
Cocaine
Self-administration
Adenosine receptors
Sex difference

ABSTRACT

Adenosine is an important neuromodulator, known to interact with both dopaminergic and glutamatergic systems to influence psychostimulant action. In the present study, we examined the effects of ATL444, a novel adenosine receptor antagonist, on motivation for cocaine in male and female rats. Adult male and female Sprague–Dawley rats were trained to self-administer cocaine (1.5 mg/kg/infusion) on a fixed-ratio 1 schedule with a daily maximum of 20 infusions. Following 5 consecutive sessions during which all 20 available infusions were obtained, motivation for cocaine (0.5 mg/kg/infusion) was assessed under a progressive ratio (PR) schedule, and once responding stabilized, the effect of treatment with ATL444 (0, 15, and 30 mg/kg, i.p.) was examined. As a control, we also assessed its effects on PR responding for sucrose. Binding studies revealed that ATL 444 was 3-fold, 25-fold, and 400-fold more selective for the A2A receptor as compared to A1, A2B, and A3 receptors, respectively. ATL444 produced a significant increase in motivation for cocaine on the day of treatment in females with a trend for an increase in males. In addition, over the two PR sessions following ATL444 treatment a significant decrease in responding was observed in males but not females. Responding for sucrose was unaffected by ATL444 treatment. Our results reveal that adenosine receptor blockade may mediate both acute increases in the reinforcing effects of cocaine, and longer term inhibitory effects on cocaine reinforcement that differ according to sex.

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1. Introduction

Adenosine is a purine nucleoside that is widely distributed throughout the central nervous system and is recognized as a modulator of neurotransmitter release and neuronal excitability. Its physiological effects are mediated through the activation of four receptor types (A1, A2A, A2B, and A3). Antagonistic interactions exist between different subtypes of adenosine and dopamine receptors (Ferré et al., 1997). Adenosine, acting on both A1 and A2A receptors modulates dopaminergic neurotransmission through its effects on dopamine release and functional interactions between adenosine and dopamine receptors. Adenosine A1 receptors, which are expressed widely throughout the brain, co-localize with dopamine D1 receptors (Ferré et al., 1994; Ginés et al., 2000). The A2A receptor is highly expressed in the striatum, primarily in GABAergic striato-pallidal projection neurons that also express dopamine D2 receptors (Augood and Emson, 1994; Fink et al., 1992; Pollack et al., 1993; Schiffmann et al., 1991) and to a lesser extent in excitatory synapses of cortico-striatal terminals (Svenningsson et al., 1999). A2A receptors have been shown to interact with several neurotransmitter receptors, including dopamine D2 and metabotropic glutamate subtype 5 (mGluR5) receptors (Ferré et al., 1991, 2002; Fink et al., 1992), with evidence for both antagonistic and synergistic effects. Although little is known regarding the role of adenosine A1 receptors in psychostimulant action and addiction-related behaviors, a large number of studies have demonstrated a role for A2A receptors. Baldo et al. (1999) have demonstrated that A2A agonists elevate brain stimulation reward thresholds, while antagonists reverse this effect, suggesting that A2A receptors are involved in the mesolimbic system regulation of reward, and signaling at this receptor is increasingly recognized as a possible therapeutic target for addiction (for reviews, see Brown and Short, 2008; Ferré et al., 2007; Shen and Chen, 2009).

A2A-D2 receptor heterodimers, through which A2A receptors might act to antagonize D2 receptor signaling, have been hypothesized to mediate A2A receptor effects on psychostimulant reward. A2A-D2 receptor interactions have been demonstrated in vitro (Canals et al., 2003; Fuxe et al., 1998; Hillion et al., 2002; Marcellino et al., 2010), and functionally antagonistic interactions between A2A receptors and cocaine-mediated behaviors involving D2 receptors (Adams et al., 2001; Kita et al., 1999; Ushijima et al., 1995) have been reported. Given findings showing that D2 receptor antagonism attenuates cocaine's reinforcing and addiction-related properties (Anderson et al., 2006; Mantsch et al., 2010; Milivojevic et al., 2004; Xi and Gardner, 2007; but see Xue et al., 2011), adenosine A2A receptors may be a potential therapeutic target for cocaine addiction treatment. Several pharmacological studies

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0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2012.05.001

indicate that adenosine A2A receptors influence the behavioral response to cocaine, although the direction of these effects has been inconsistent. For example, A2A receptor antagonists have been shown to increase cocaine sensitization and enhance discriminative-stimulus effects of cocaine (Filip et al., 2006; Justinova et al., 2003), whereas agonists reduce cocaine sensitization (Filip et al., 2006). Stimulation of A2A receptors also reduces reinstatement of cocaine seeking elicited by cocaine and cocaine-conditioned cues (Bachtell and Self, 2009) while an A1/A2A receptor antagonist has been shown to reinstate cocaine-seeking behavior (Weerts and Griffiths, 2003). However, there have been very few studies characterizing the effects of adenosine receptor antagonism on ongoing cocaine self-administration. Three studies have addressed the role of A2A receptors in self-administering animals, and they have yielded mixed results. Justinova et al. (2011) found that in squirrel monkeys, cocaine self-administration on a fixed-ratio 10 schedule was not affected by treatment with the adenosine A2A receptor antagonist MSX-3. Soria et al. (2006) reported that motivation for cocaine was decreased in A2A receptor knockouts, while Knapp et al. (2001) found that initiation of cocaine self-administration on a fixed-ratio 5 (FR5) schedule was reduced by treatment with adenosine A2A receptor agonists. This latter experiment is furthermore difficult to interpret due to the possibility that the agonist may have mimicked the effects of drug to produce satiation. A primary goal of our study was therefore to more fully characterize the effects of adenosine receptor antagonism in self-administering animals by examining its effects on motivation for cocaine using a progressive ratio (PR) schedule which is believed to be a more sensitive measure of changes in reinforcement efficacy than the fixed-ratio schedule.

A second goal of this work was to compare the effects of adenosine receptor antagonism on motivation for cocaine between males and females. The vast majority of treatment studies for cocaine addiction in animals have focused exclusively on males. However, sex differences have been demonstrated in many behavioral measures of cocaine addiction, including the motivation to self-administer low doses of cocaine as assessed by responding on a PR schedule (Carroll et al., 2002; Lynch and Taylor, 2004; Roberts et al., 1989). Moreover, dopaminergic transmission has been shown to differ according to sex. Release of dopamine in the striatum following cocaine is greater in females (Walker et al., 2006), and differential effects of both dopamine D1 and D2 receptor manipulation with respect to acute cocaine induced behaviors have been reported in male and female rats (Festa et al., 2006; Schindler and Carmona, 2002; Walker et al., 2006). Given the role that adenosine receptors play in modulating dopamine signaling, it is likely that the effects of adenosine receptor antagonism will also vary according to sex. Although sex differences in the effects of A1 antagonism have been reported following withdrawal from ethanol (Butler et al., 2008, 2009), to date no studies have examined sex differences in the effects of A2A receptor antagonism in addiction models.

In the present study, we examined the effect of a preferential A2A receptor antagonist on motivation to obtain cocaine infusions in actively self-administering animals. Specifically, the effects of pre-treatment with systemic injection of the adenosine receptor antagonist ATL444 were tested in Sprague–Dawley rats responding for cocaine under a PR schedule. In addition, we evaluated the effects of ATL444 in both males and females in order to determine the existence of sex differences in the response to adenosine receptor inhibition. Separate groups of male and female rats responding for sucrose pellets were used to test for behavioral specificity.

2. Methods

2.1. Subjects

Male and female Sprague–Dawley rats (approximately 90 days old and weighing 380–410 g (males) or 280–310 g (females) were obtained from Charles River Laboratories. Animals were housed in operant conditioning chambers (Med-Associates, Inc., St. Albans, VT) in a temperature (20–22 °C) and humidity (40–70%) controlled vivarium, and were maintained in a 12-hour light: 12-hour dark cycle (lights on 0700, off 1900 h). Food (Purina rat chow) and water were available ad libitum for the duration of the study. In order to facilitate acquisition of cocaine self-administration, after an acclimation period of at least 3 days following arrival, rats were briefly trained to lever press for sucrose pellets on a fixed-ratio 1 schedule. Training was considered to be complete after two consecutive 24-hour sessions during which 100 or more sucrose pellets were obtained. Following training, rats were anesthetized with a combination of ketamine (60 mg/kg) and pentobarbital (Nembutal, 5 mg/kg) and implanted with a silicone catheter into the right jugular vein as previously described (Lynch, 2008). During 3 days of recovery from surgery, animals received intravenous gentamicin (2 mg) followed by 0.1 ml heparinized saline (8.3 IU heparin/ml 0.9% physiological saline) to prevent infection and ensure catheter patency. Throughout the self-administration period animal health was monitored daily, and rats were weighed and catheters flushed with heparinized saline 3 times per week. All procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and protocols were approved by the University of Virginia's Animal Care and Use Committee.

2.2. Drugs

Cocaine HCl was provided by the National Institute on Drug Abuse (Research Triangle Park, NC) and was dissolved to a concentration of 0.7 mg/ml in sterile 0.9% saline and delivered at a constant rate of 0.025 ml/s through a 10 ml syringe housed in a motorized syringe pump (Med-Associates, Inc., St. Albans, VT). The dose of cocaine/infusion, either 1.5 mg/kg or 0.5 mg/kg, was held constant across subjects while infusion duration varied according to body weight (1 s/100 g). Cocaine solutions were made fresh weekly and refrigerated, but were delivered from the syringes at room temperature. ATL444, a preferential antagonist of A2A receptor adenosine receptors developed by Dogwood Pharmaceuticals, Inc., was dissolved in a 1.0 ml solution containing 10% DMSO/10% cremophor/80% saline.

2.3. Chemistry

ATL444 was synthesized using Comparative Molecular Field Analysis (CoMFA; Tripos, Inc.), a widely used 3D qualitative structure–activity relationship (QSAR) methodology, along with a host of similar compounds designed as agonists and antagonists of the A2A receptor over a wide range of K_Ds (Sun et al., 2007). The structure of ATL444 and the general synthetic scheme for this class of substituted adenine compounds are shown in Fig. 1. Briefly, guanosine, 4.1, is acetylated to protect the ribose during reductive chlorination by $POCl_3$/diethylaniline to form 6-chloroguanosine, 4.3. Non-aqueous diazotization in the presence of elemental iodine in diiodomethane is a standard route to the protected 6-chloro-2-iodonebularine, 4.4. Heating in methanolic ammonia deprotects the sugar and displaces the 6-chloro substituent to form 2-iodoadensoine, 4.5. Palladium-catalyzed coupling of 4.5 with a terminal alkyne generates 2-alkynyladenosine, 4.6. The sugar moiety is cleaved with acid to form the 9 H-adenine, 4.7. Alkylation with a halide completes the synthesis of target 2,9-disubstituted adenine, 4.8. The requisite alkyne synthesis (Fig. 1, bottom panel) occurs as follows: starting from the Boc-protected methanol compound, the acetylene group is installed by displacing the tosylated alcohol using lithium acetylide. The Boc group is then removed using TFA and ATL444 is realized by treating the cycloalkylketone with ethynylmagnesium bromide. The structural features of ATL444 include the lack of a 7-ribose moiety normally required for agonist activity. ATL444 has also been shown to cross the blood-brain barrier and was originally identified as a drug candidate for Parkinson's disease (Adenosine Therapeutics, LLC internal