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Role of adenosine receptor subtypes in methamphetamine reward and reinforcement

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ABSTRACT

The neurobiology of methamphetamine (MA) remains largely unknown despite its high abuse liability. The present series of studies explored the role of adenosine receptors on MA reward and reinforcement and identified alterations in the expression of adenosine receptors in dopamine terminal areas following MA administration in rats. We tested whether stimulating adenosine A_1 or A_{2A} receptor subtypes would influence MA-induced place preference or MA self-administration on fixed and progressive ratio schedules in male Sprague–Dawley rats. Stimulation of either adenosine A_1 or A_{2A} receptors significantly reduced the development of MA-induced place preference. Stimulating adenosine A_1, but not A_{2A}, receptors reduced MA self-administration responding. We next tested whether repeated experimenter-delivered MA administration would alter the expression of adenosine receptors in the striatal areas using immunoblotting. We observed no change in the expression of adenosine receptors. Lastly, rats were trained to self-administer MA or saline for 14 days and we detected changes in adenosine A_1 and A_{2A} receptor expression using immunoblotting. MA self-administration significantly increased adenosine A_1 in the nucleus accumbens shell, caudate-putamen and prefrontal cortex. MA self-administration significantly decreased adenosine A_{2A} receptor expression in the nucleus accumbens shell, but increased A_{2A} receptor expression in the amygdala. These findings demonstrate that MA self-administration produces selective alterations in adenosine receptor expression in the nucleus accumbens shell and that stimulation of adenosine receptors reduces several behavioral indices of MA addiction. Together, these studies shed light onto the neurobiological alterations incurred through chronic MA use that may aid in the development of treatments for MA addiction.

1. Introduction

The acute rewarding and reinforcing effects of many drugs of abuse, including methamphetamine (MA) result from elevations in dopamine (DA) in the mesocorticolimbic system, which is comprised of DA cells in the ventral tegmental area (VTA) that terminate in forebrain regions such as the nucleus accumbens (NAc). MA has multiple, well-defined actions that amplify synaptic activity of DA in the mesocorticolimbic system (McCann et al., 2008; Pereira et al., 2002, 2006). Importantly, MA potently reverses the activity of both the DA transporter and the intracellular vesicular monoamine transporter 2. This results in high intracellular concentration of DA that is transported through non-vesicular transport from the cytoplasm into the synaptic cleft via reverse action of the DA transporter (Vergo et al., 2007; Volz et al., 2007).

MA induces robust neurobiological changes in the mesolimbic system. For example, MA abuse in humans is associated with striatal DA D_1 receptors upregulation and striatal DA D_2 receptor downregulation (Volkow et al., 2001a, 2001b, 2001c; Worsley et al., 2000). Animal studies show somewhat different effects following MA administration in that MA reduces both DA D_1 and D_2 receptor expression with seemingly greater reductions in DA D_1 receptors following repeated experimenter-delivered MA (McCabe et al., 1987; Segal et al., 2005; Stefanski et al., 1999). More recent data suggests that DA receptor downregulation is offset by increases in

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high affinity DA D_1 and D_2 receptors following chronic MA treatment (Shuto et al., 2008). Together, these findings suggest that both acute and chronic actions of MA alter the mesocorticolimbic system to produce the behavioral effects of MA.

There has been recent interest in pursuing adenosine as a negative modulator of DA receptor signaling. Adenosine is a nucleoside neurotransmitter found ubiquitously in the brain. Under basal conditions, adenosine levels are quite low (nM range), but sufficient for tonic receptor binding and observable physiological effects (Ballarin et al., 1991; Dunwiddie and Masino, 2001; Snyder et al., 1981). Phasic increases in adenosine levels can arise from increased neuronal metabolic activity and co-release of adenosine triphosphate (ATP) with vesicular neurotransmitter release (Cass et al., 1987; Fredholm et al., 1982; Thorn and Jarvis, 1996; White, 1977). Vesicular release of DA, for example, is accompanied by the release of ATP that can be metabolized to adenosine and act on postsynaptic adenosine receptors (Cass et al., 1987; Fredholm et al., 1982; Thorn and Jarvis, 1996; White, 1977). Under physiological conditions, DA and adenosine receptor subtypes have antagonistic interactions through the formation of receptor–receptor complexes (i.e. heteromeric receptors) and/or opposing G protein mediated signaling cascades. However, these antagonistic receptor interactions may not be fully appreciated in the presence of MA. Thus, non-vesicular release of DA by MA can potently and aberrantly stimulate postsynaptic DA receptors in the absence of important regulators such as adenosine. This lack of complementary regulation by adenosine may promote the development and persistence of MA-induced neurobiological changes and subsequent abuse.

Here, we explore how the stimulation of the two primary neuronal adenosine receptor subtypes (A_1 and A_{2A}) affects the development of a conditioned place preference for MA and affects MA intake using self-administration procedures. Additionally, we identify how experimenter-delivered MA or MA self-administration alters the expression of these adenosine receptor subtypes that are robustly expressed in DA terminal areas such as the NAc, prefrontal cortex and amygdala (Dixon et al., 1996).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 275–325 g were individually housed with *ad libitum* food and water upon arrival. All experiments were conducted during the light period of a (12:12) light/dark cycle. All procedures were completed in accordance with the Guide for the Care and Use of Animals and approved the Institutional Animal Care and Use Committee at the University of Colorado Boulder. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize available alternatives to *in vivo* techniques.

2.2. Place conditioning

Place conditioning was conducted according to previously published procedures (Merritt and Bachtell, 2013). A custom-built, unbiased three chambered apparatus was used for place conditioning procedures. The two conditioning chambers (15 cm × 25 cm × 35 cm) were distinct in wall patterns (gray vs. vertical white and black stripes) and floor textures (grid vs. hole). The center compartment (15 cm × 10 cm) had white walls and a plexiglass floor. Chambers were equipped with infrared photocells to detect animal position and movement in the apparatus. A three-phase procedure was conducted as follows: Day 1–20 min pre-conditioning session, Days 2–4- six 30 min conditioning sessions (3 AM saline; 3 PM MA) and Day 5–20 min post-conditioning session. Locomotor activity during the conditioning sessions was measured by photocell beam breaks. During the pre- and post-conditioning session, time spent in each compartment was also measured by calculating the duration of photocell beam breaks within each chamber. The animal's preference was determined using a conditioned place preference (CPP) score that was calculated by subtracting the time in the saline-paired compartment from the time in drug-paired compartment. We tested the effects of adenosine A_1 agonist (CPA: 0.03 and 0.1 mg/kg, i.p., $n = 9$/dose) and A_{2A} receptor agonist (CGS 21680: 0.01 and 0.03 mg/kg, i.p., $n = 9$–10/dose) on the development of a place preference induced by MA (1.0 mg/kg, i.p.) by administering the agonist 5 min prior to MA during conditioning. Vehicle pretreatment ($n = 16$) served as a control. Dosing and timing of injections was determined by previous studies examining the behavioral effects of adenosine agonists on psychostimulant-induced behaviors (Bachtell and Self, 2009; Golembiowska and Zylewska, 1998, 2000; Hobson et al., 2013; O'Neill et al., 2012; Shimazoe et al., 2000; Yoshimatsu et al., 2001).

2.3. Methamphetamine treatments for tissue collection

We tested the effects of both experimenter-delivered MA and self-administered MA on the expression of adenosine receptors in dopamine terminal areas. For the experimenter-delivered experiment, rats randomly divided into two treatment groups (saline or 1.5 mg/kg MA, ip). Rats were treated for 7 consecutive days in the home cage. Twenty-four hours after the last injection, animals were sacrificed by rapid decapitation and tissue was processed and analyzed as described below. For the self-administration experiment, animals were trained to lever-press for sucrose pellets in standard operant test chambers (Med Associates Inc, St. Albans, VT) under food-restricted conditions. Rats were then fed *ad libitum* and surgically implanted with chronic indwelling intra-jugular catheters (O'Neill et al., 2012). Rats were randomly assigned to either a saline ($n = 6$) or MA ($n = 10$) self-administration group following recovery from surgery. MA self-administering animals were able to lever press for MA (0.05 mg/kg/injection) on an fixed ratio 1:time-out 15 s (FR1:TO15) schedule in daily 2 h sessions over 14 days. Saline self-administering animals were treated identically, however, saline was substituted for MA. Twenty-four hours after the last self-administration session, animals were sacrificed by rapid decapitation and tissue was processed and analyzed as described below.

2.4. Methamphetamine self-administration behavioral procedures

Separate groups of animals were run through the self-administration procedure to test the effects of adenosine receptor stimulation on FR and progressive ratio (PR) responding. To facilitate acquisition of MA self-administration, rats were trained to lever-press for sucrose pellets in standard operant test chambers under food-restricted conditions. Rats were then fed *ad libitum* and surgically implanted with chronic indwelling intra-jugular catheters. Following recovery, rats self-administered MA (FR1:TO20 s) in daily 2 h sessions. Separate groups of animals were trained with either 0.05 mg/kg/infusion MA or 0.1 mg/kg/infusion MA. After 1 week of FR1:TO20 s responding, rats were advanced to an FR5:TO20 s schedule until stable (MA intake varies <10% over 3 consecutive days). On the test day, animals received an adenosine agonist (CPA: 0.03 & 0.1 mg/kg, ip; CGS 21680: 0.01 & 0.03 mg/kg, ip) pretreatment 5 min prior to the start of the session. The breakdown of the groups administering 0.05 MA mg/kg/infusion on an FR5 schedule was as follows: Veh ($n = 12$), 0.01 CGS ($n = 8$), 0.03 CGS ($n = 6$), 0.03 CPA ($n = 9$), 0.1 CPA ($n = 10$). The breakdown of the groups administering 0.1 MA mg/kg/infusion on an FR5 schedule was as follows: Veh ($n = 7$), 0.01 CGS ($n = 9$), 0.03 CGS ($n = 6$), 0.03 CPA ($n = 9$), 0.1 CPA ($n = 7$). Dosing and timing of injections was determined by previous studies examining the behavioral effects of adenosine agonists on psychostimulant-induced behaviors (Bachtell and Self, 2009; Golembiowska and Zylewska, 1998, 2000; Hobson et al., 2013; O'Neill et al., 2012; Shimazoe et al., 2000; Yoshimatsu et al., 2001). Rats were tested in counterbalanced fashion across all doses and baseline performance in the absence of a pretreatment served as a control for repeated testing. Thus, between each treatment animals were re-stabilized on the FR5:TO20 schedule prior to receiving the next treatment. All animals received at least 1 treatment and up to 4 treatments total, but no animals were successfully administered all test doses.

Testing on the PR schedule was conducted identically to the FR5 testing except that after being advanced to an FR5:TO20 s schedule and achieving stability (MA intake varies <10% over 3 consecutive days) rats were advanced to the PR schedule. The progression for response/injection ratios was determined according to $[5e^{(\text{injection number} \times 0.2)}]-5$ (e.g. 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50 etc.). Separate groups of animals were trained with either 0.05 mg/kg/infusion MA or 0.1 mg/kg/infusion MA. Stable baseline performance on the PR schedule was evaluated over several days prior to administration of CPA (0, 0.03, 0.1 mg/kg, ip) or CGS 21680 (0, 0.01, 0.03 mg/kg, ip). On the test day, animals received a pretreatment 5 min prior to the start of the session. The breakdown of the groups administering 0.05 MA mg/kg/infusion on an PR schedule was as follows: Veh ($n = 11$), 0.01 CGS ($n = 7$), 0.03 CGS ($n = 6$), 0.03 CPA ($n = 6$), 0.1 CPA ($n = 16$). The breakdown of the groups administering 0.1 MA mg/kg/infusion on an PR schedule was as follows: Veh ($n = 10$), 0.01 CGS ($n = 10$), 0.03 CGS ($n = 16$), 0.03 CPA ($n = 10$), 0.1 CPA ($n = 16$). Rats were tested in counterbalanced fashion across all doses and baseline performance in the absence of a pretreatment served as a control for repeated testing. Thus, between each treatment animals were re-stabilized on the PR schedule prior to receiving the next treatment. All animals received at least 1 treatment and up to 4 treatments total, but no animals were successfully administered all test doses.

2.5. Sucrose self-administration procedures

The effects of adenosine receptor agonists were also tested on fixed ratio in separate groups of rats. Rats were initially trained to lever-press for 45 mg sucrose pellets (Bio-Serv, Flemington, NJ) in standard operant test chambers on an FR1:TO20 s schedule of reinforcement under food-restricted conditions. Self-