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The glial cell modulators, ibudilast and its amino analog, AV1013, attenuate methamphetamine locomotor activity and its sensitization in mice

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ABSTRACT

Over 800,000 Americans abuse the psychomotor stimulant, methamphetamine, yet its abuse is without an approved medication. Methamphetamine induces hypermotor activity, and sensitization to this effect is suggested to represent aspects of the addiction process. Methamphetamine's regulation of 3′–5′-cyclic adenosine monophosphate (cAMP) levels may be partially responsible for its behavioral effects, and compounds that inhibit phosphodiesterase (PDE), the enzyme that degrades cAMP, can alter methamphetamine-induced behaviors. Methamphetamine also activates glial cells and causes a subsequent increase in pro-inflammatory cytokine levels. Modulation of glial cell activation is associated with changes in behavioral responses, and substances that oppose inflammatory activity can attenuate drug-induced behaviors. Ibudilast (aka AV411; 3-isobutyryl-2-isopropylpyrazolo-[1,5-a]pyridine), inhibits both PDE and glial pro-inflammatory activity. Ibudilast's amino analog, AV1013, modulates similar glial targets but negligibly inhibits PDE. The present study determined whether ibudilast and AV1013 would attenuate methamphetamine-induced locomotor activity and its sensitization in C57BL/6J mice. Mice were treated b.i.d. with ibudilast (1.8–13 mg/kg), AV1013 (10–56 mg/kg) or their vehicles intraperitoneally for 7 days, beginning 48 h before 5 days of daily 1-h locomotor activity tests. Each test was initiated by either a methamphetamine (3 mg/kg) or a saline injection. Ibudilast significantly ($P<0.05$) reduced the acute, chronic, and sensitization effects of methamphetamine's locomotor activity without significantly affecting activity by itself. AV1013 had similar anti-methamphetamine effects, suggesting that glial cell activity, by itself, can modulate methamphetamine's effects and perhaps serve as a medication target for its abuse.

1. Introduction

Methamphetamine is a psychomotor stimulant that increases activity and feelings of euphoria, and can lead to drug-seeking and chronic abuse (Everitt and Robbins, 2005; Peachey et al., 1976; Vanderschuren and Everitt, 2005; Winslow et al., 2007). Methamphetamine abuse is associated with many untoward effects such as hallucinations, pulmonary and cardiac problems, dental disease, and suppressed immunity (Hamamoto and Rhodus, 2009; Hauer, 2010; Srisurapanont et al., 2003). Currently, there are no approved pharmacotherapies for treating methamphetamine abuse, and conventional, receptor-mediated approaches have not been successful (Karila et al., 2010). As such, a fuller understanding of less conventional and less studied mechanisms mediating methamphetamine's effects may lead to improved pharmacotherapeutic approaches.

Methamphetamine is well-known for its effects on the monoamine neurotransmitters, dopamine, serotonin, and norepinephrine (Cho and Segal, 1994; Creese, 1983). Methamphetamine impedes the uptake of these monoamines into the pre-synaptic neuron while also reversing their transporter actions (Cho and Segal, 1994; Creese, 1983). Methamphetamine-induced dopamine D_1 and D_2 receptor activation (Sonsalla et al., 1986) results in alterations of cyclic adenosine 3′,5′-monophosphate (cAMP) levels via coupling to adenylate cyclase (Kebabian et al., 1984). In addition, methamphetamine activates glial cells (both astrocytes and microglia) (Caporaso et al., 2000; Hebert and O'Callaghan, 2000; Iyo et al., 1995) to increase pro-inflammatory cytokine production and immune reaction (Goncalves et al., 2008; Loftis et al., 2010; Nakajima et al., 2004; Yamaguchi et al., 1991). Methamphetamine's regulation of cAMP levels and induction of inflammation are thought to be involved in its behavioral effects including hyperactivity, sensitization, and its discriminative stimulus effects (Iyo et al., 1996b; Miguel-Hidalgo,

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2009; Mori et al., 2000; Niwa et al., 2007, 2008; Yan et al., 2006, 2007), and substances that oppose its cAMP modulation or inflammatory activity have been reported to attenuate methamphetamine-induced behaviors (Iyo et al., 1996b; Niwa et al., 2007; Yan et al., 2006; Zhang et al., 2006).

Given these observations, both PDE-inhibition and glial cell modulation are potential mechanisms for reducing methamphetamine-induced behaviors. Ibudilast (aka, AV411; 3-isobutyryl-2-isopropylpyrazolo-[1,5-a]pyridine) is a non-selective PDE inhibitor, glial cell modulator and anti-inflammatory agent (Gibson et al., 2006; Kishi et al., 2001). Ibudilast is marketed in Japan to treat bronchial asthma and ischemic stroke (Kishi et al., 2001), and is being clinically evaluated for treating neuropathic pain (Ledeboer et al., 2006) and opioid dependency (Hutchinson et al., 2009). Because other inhibitors of PDE activity and glial activation attenuate methamphetamine's effects (see above), and because we previously reported ibudilast to attenuate stress- and prime-induced reinstatement of methamphetamine drug-seeking in rats (Beardsley et al., 2010), we examined the ability of ibudilast to attenuate the acute and chronic effects of methamphetamine-induced hyperactivity and sensitization in mice. Additionally, we tested the amino analog of ibudilast, AV1013, which retains ibudilast's ability to inhibit glial cell activation but has minimal PDE inhibitory effects (Cho et al., 2010), to determine whether PDE inhibition was essential for the initial effects we observed with ibudilast.

2. Materials and methods

2.1. Subjects

Male adult C57BL/6J mice were obtained at approximately 8 weeks of age (The Jackson Laboratory, Bar Harbor, ME) and were allowed to acclimate to the vivarium for approximately one week prior to commencement of testing. The mice were housed at a maximum of four per cage in an AAALAC-accredited animal facility with food (7012 Teklad LM-485 Mouse/Rat Sterilizable Diet, Harlan Laboratories, Inc., Indianapolis, IN) and water available ad libitum under a 12-h/12-h light/dark cycle (lights illuminated from 0600-h to 1800-h) with all testing occurring during the light phase. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Apparatus

Locomotor activity tests were conducted in eight commercially obtained, automated activity monitoring devices each enclosed in sound- and light-attenuating chambers that recorded distance traveled in cm in 10-min bins via computer-controlled circuitry (AccuScan Instruments, Columbus OH). The interior of each device was divided into separate $20 \times 20 \times 30$ cm arenas permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors per axis were spaced 2.5 cm apart along the walls of the chamber and were used to detect movement.

2.3. Locomotor activity procedure

One hundred and twenty-eight mice were randomly assigned into 16 groups of eight mice each. Eight groups were treated b.i.d. for 7 days with subcutaneous (s.c.) injections of either 0 (vehicle; VEH1), 1.8, 7.5, or 13 mg/kg ibudilast, with two groups of eight at each dose. Eight other groups were similarly treated but with 0 (vehicle; VEH2), 10, 30, or 56 mg/kg AV1013. Both ibudilast and AV1013 injections occurred twice daily separated approximately 7 hours apart (0900-h and 1600-h). During the last five days of these seven-day regimens (Days 3–7), the mice were given locomotor activity tests. Two, 1-h locomotor activity sessions (Baseline and Test) were given on Days 1 and 5. Single locomotor activity sessions were given on Days 2–4 to minimize the occurrence of extinction of any conditioned locomotor activity effects in methamphetamine treated mice. On days when locomotor activity sessions were administered (Days 3–7), morning ibudilast and AV1013 injections were given 1 h prior to the first session. Immediately prior to Baseline and Test sessions on Days 1 and 5, all mice were injected intraperitoneally (i.p.) with saline or 3 mg/kg methamphetamine, respectively. On Days 2–4, half of all mice in the ibudilast and AV1013 groups received 3 mg/kg i.p. methamphetamine (METH) before all locomotor activity sessions (IBUD + METH and AV1013 + METH groups), while the other half received saline injections (IBUD + SAL and AV1013 + SAL groups). Thus, the mice were distributed across groups as shown in Table 1 and treated as shown in Table 2.

2.4. Drugs

(±)-Methamphetamine (National Institute on Drug Abuse, Rockville, MD) was prepared in 0.9% saline stock solutions sterilized by filtration through 0.2 μm filtration disks. Working methamphetamine solutions were dissolved in sterile 0.9% saline and injected i.p. Ibudilast (3-isobutyryl-2-isopropylpyrazolo[1,5-a]pyridine) and AV1013 ((R)-2-amino-1-(2-isopropylpyrazolo[1,5-a]pyridin-3-yl) propan-1-one hydrochloride) were received as a gift from MediciNova, Inc., San Diego, CA). Ibudilast was prepared in 35% polyethylene glycol (PEG) in saline vehicle and administered s.c. (referred to below as "VEH1"). Doses of AV1013 were administered s.c. and prepared in sterile 0.9% saline (referred to below as "VEH2"), with the exception of the highest dose (56 mg/kg) that was solubilized in a 35% PEG in saline vehicle (i.e., VEH1) because of its incomplete dissolution in 0.9% saline. All injections were given in a volume equivalent to 10 ml/kg body weight.

2.5. Data analysis

Distance traveled (cm) was subjected to analysis by a mixed-model ANOVA (repeated measures on Testday test and between comparisons on drug condition) for the chronically administered methamphetamine and vehicle groups separately for each drug (i.e., 2 drugs × 2 methamphetamine treatment conditions = 4 ANOVAs). Comparisons between ibudilast or AV1013-treated mice to their respective vehicle condition were made using Bonferroni Multiple Comparisons Tests. AD50 (CI) values for attenuating methamphetamine hyperactivity by 50% relative to vehicle controls were estimated by first converting distance traveled scores for each mouse to percent of its respective mean vehicle control, logarithmically transforming dose, and using nonlinear regression assuming a normalized response. All statistical tests were conducted using computer

Table 1
Distribution of mice in chronically and acutely treated methamphetamine (METH) groups.

Chronic METH	Acute METH
Ibudilast groups	
VEH1 + METH	VEH1 + SAL
1.8 IBUD + METH	1.8 IBUD + SAL
7.5 IBUD + METH	7.5 IBUD + SAL
13 IBUD + METH	13 IBUD + SAL
AV1013 groups	
VEH2 + METH	VEH2 + SAL
10 AV1013 + METH	10 AV1013 + SAL
30 AV1013 + METH	30 AV1013 + SAL
56 AV1013 + METH	56 AV1013 + SAL