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CM156, a high affinity sigma ligand, attenuates the stimulant and neurotoxic effects of methamphetamine in mice

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ABSTRACT

Methamphetamine (METH) is a highly addictive psychostimulant drug of abuse. Low and high dose administration of METH leads to locomotor stimulation, and dopaminergic and serotonergic neurotoxicity, respectively. The behavioral stimulant and neurotoxic effects of METH can contribute to addiction and other neuropsychiatric disorders, thus necessitating the identification of potential pharmacotherapeutics against these effects produced by METH. METH binds to σ receptors at physiologically relevant concentrations. Also, σ receptors are present on and can modulate dopaminergic and serotonergic neurons. Therefore, σ receptors provide a viable target for the development of pharmacotherapeutics against the adverse effects of METH. In the present study, CM156, a σ receptor ligand with high affinity and selectivity for σ receptors over 80 other non-σ binding sites, was evaluated against METH-induced stimulant, hyperthermic, and neurotoxic effects. Pretreatment of male, Swiss Webster mice with CM156 dose dependently attenuated the locomotor stimulation, hyperthermia, striatal dopamine and serotonin depletions, and striatal dopamine and serotonin transporter reductions produced by METH, without significant effects of CM156 on its own. These results demonstrate the ability of a highly selective σ ligand to mitigate the effects of METH.

1. Introduction

Methamphetamine (METH), a synthetic psychostimulant drug of abuse, has increased in popularity due to its ease of synthesis from over-the-counter drugs and its long duration of action (Krasnova and Cadet, 2009). The acute effects of METH, including enhanced locomotor activity, are caused by the release of dopamine in the mesolimbic brain region (Campbell et al., 1997). Moreover, the locomotor stimulant effects are also linked with the ability of psychostimulants to act as reinforcers and cause addiction (Wise and Bozarth, 1987). Chronic, high dose administration of METH results in hyperthermia, cardiac complications, neurotoxicity and several neurological disabilities including addiction (Cruickshank and Dyer, 2009). At high doses, neurotoxic cascades are activated due to excessive dopamine and serotonin (5-HT) release into the cytoplasm and synapse (Kuczenski et al., 1995; Baldwin et al., 1993; Gough et al., 2002). Neuroimaging and postmortem studies in human METH users, as well as studies performed using animal models, have shown damage to dopaminergic and serotonergic neurons measured as reductions in dopamine transporters (DAT), serotonin transporters (SERT), dopamine and 5-HT levels in several brain regions (Krasnova and Cadet, 2009). Recent studies have indicated an association between neurotoxicity and several neuropsychiatric disorders including psychosis, motor and cognitive deficits (Cruickshank and Dyer, 2009; Walsh and Wagner, 1992), conferring additional urgency to the development of effective pharmacotherapies against the effects of METH.

In the present study, the role of σ receptors in METH-induced locomotor stimulation and neurotoxicity is evaluated. We focus on σ receptors for several reasons. First, METH binds to σ receptors at physiologically relevant concentrations (Nguyen et al., 2005). Second, σ receptors are present in the basal ganglia and affect motor function (Bouchard and Quirion, 1997; Walker et al., 1993),

Abbreviations: AC927, 1-(2-phenethyl)piperidine oxalate; ANOVA, analysis of variance; BD1063, 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine; CM156, 3-(4-(4-cyclohexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione; DAT, dopamine transporters; ER, endoplasmic reticulum; MAM, mitochondria associated membrane; METH, methamphetamine; NMDA, N-methyl-D-aspartate; ROS, reactive oxygen species; SERT, serotonin transporters.

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indicating their potential involvement in the locomotor stimulant effects of METH. Third, σ receptors are present on dopamine and 5-HT neurons, which are damaged by neurotoxic doses of METH, and can modulate their function (Bastianetto et al., 1995; Derbez et al., 2002; Campbell et al., 1989). Fourth, σ receptors participate in cell death pathways in tumor cells (Bowen, 2000), suggesting their possible contribution in METH-induced neurotoxic cascades. Fifth, targeting σ receptors can attenuate chronic disabilities like cognitive deficits, psychosis, and depression, which are also caused by METH's neurotoxicity (Cruickshank and Dyer, 2009). Finally, a selective σ receptor ligand, AC927 (1-(2-phenethyl) piperidine oxalate), has been shown to prevent METH-induced locomotor activity, hyperthermia, and dopaminergic neurotoxicity (Matsumoto et al., 2008).

Recently, a novel σ receptor ligand, CM156 (3-(4-(4-cyclo hexylpiperazin-1-yl)butyl)benzo[*d*]thiazole-2(3*H*)-thione), was developed and shown to have an even better nanomolar affinity for σ receptors than AC927 (K_i of CM156: $\sigma_1 = 1.3$ nM, $\sigma_2 = 0.6$ nM; K_i of AC927: $\sigma_1 = 30$ nM, $\sigma_2 = 138$ nM), and >1000-fold selectivity over 80 other non-σ binding sites (Xu et al., 2010; Matsumoto et al., 2008). Additionally, a larger therapeutic window in comparison to AC927, makes CM156 more amenable as a potential drug candidate. In an earlier study, CM156 was shown to attenuate the toxic and behavioral effects of cocaine, a psychostimulant that acts as a putative agonist at σ receptors (Xu et al., 2010). Therefore, in the present study, CM156 was evaluated against the stimulant and neurotoxic effects of METH. Endpoints that were evaluated included locomotor stimulation, hyperthermia, as well as dopaminergic and serotonergic neurotoxicity.

2. Material and methods

2.1. Animals

Male, Swiss Webster mice (21–30 g, Harlan, Indianapolis, IN; Frederick, MD) were used. The rodents were housed 1–6 per cage with a 12:12 h light/dark cycle and *ad libitum* food and water. The animals were given one week to acclimate after their arrival before they were used in an experiment. All procedures were approved by the Institutional Animal Care and Use Committees at West Virginia University and the University of Mississippi. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Drugs and reagents

METH hydrochloride was obtained from Research Biochemicals International (Natick, MA). CM156 was synthesized as previously described (Mésangeau et al., 2008). Dopamine and 5-HT immunoassay kits were purchased from Rocky Mountain Diagnostics (Colorado Springs, CO).

2.3. Locomotor activity

Locomotor activity was measured as an index of the stimulant effects of METH using an automated activity monitoring system (San Diego Instruments, San Diego, CA). The mice were given 30–60 min to acclimate to the testing room before being habituated to the testing chambers for an additional 30 min. Each testing chamber consisted of two 16 × 16 photobeam arrays to detect the movements of the animals. Ambulatory, fine, and rearing movements were quantified to give an overall activity score. To obtain a dose response for METH-induced locomotor activity, the mice ($n = 41$) were given a 15 min pretreatment with saline followed by saline or METH (0.1–5 mg/kg, i.p.). The animals were then returned to the testing chambers and their activity was quantified for the next 30 min.

To evaluate the effects of CM156 on the locomotor stimulant effects of METH, three types of experiments were performed. For all of the studies, the pretreatment time was 15 min, which is based on effective pretreatment times for earlier pharmacological studies involving σ receptor ligands (Nguyen et al., 2005; Matsumoto et al., 2008). In addition, this pretreatment time was previously successful for CM156 in attenuating the locomotor stimulant effects of cocaine (Xu et al., 2010).

First, the effects of CM156 prior to receiving the locomotor stimulant dose of METH were determined. The mice ($n = 80$) were pretreated with saline or CM156 (0.1–20 mg/kg, i.p), followed 15 min later by either saline or a peak stimulant dose of METH (1 mg/kg, i.p.). The mice were then returned to the testing chambers and their activity was quantified for the next 30 min.

Second, to determine how CM156 affected the time course of METH-induced locomotor stimulation, the mice ($n = 78$) were given a pretreatment dose of saline or CM156 (20 mg/kg, i.p.), 15 min prior to saline or METH (1 mg/kg) administration. These doses of CM156 and METH were selected because they produced peak effects in the earlier parts of this study. The locomotor activity was recorded following the pretreatment injection of CM156 or saline for a total of 135 min (15 min pretreatment, 120 min post-treatment). A longer testing period was used in this part of the study to evaluate the effects of CM156 across the entire time window in which the locomotor stimulant effects of METH are apparent in the mice. Earlier preliminary studies confirmed the ability of σ receptor antagonists to attenuate the stimulant effects of METH after 30, 90 and 120 min; the 30 min time period was chosen for routine assessments to help increase the number of animals that could be tested in a given day.

Third, the effect of a single dose of CM156 on shifting the dose response curve for METH was determined. The mice ($n = 89$) were pretreated with saline or CM156 (20 mg/kg, i.p.), followed 15 min later by either saline or different doses of METH (0.1–50 mg/kg, i.p.). The 20 mg/kg dose of CM156 was used because it was the most effective at attenuating the peak effects of METH in the earlier dose response experiment. The treated mice were returned to the chambers and their locomotor activity quantified for the next 30 min.

2.4. Neurotoxicity studies

Male, Swiss Webster mice were randomly divided into groups that were injected with saline or CM156 (5–20 mg/kg, i.p.) 15 min prior to injection with saline or METH (1.25–10 mg/kg, i.p.). Each group of mice received their treatment a total of four times at 2 h intervals. One hour after each treatment, the body temperature of the mice was recorded. To allow sufficient time for the METH-induced degeneration of nerve terminals to occur, the animals were sacrificed and the brains removed one week following treatment (Cappon et al., 2000). One week after this neurotoxicity schedule, the striatal and cerebellar brain regions of the mice were collected on ice and evaluated for dopamine, DAT, 5-HT and SERT levels. The detailed procedure for each of the endpoints is provided below.

2.4.1. Dopamine assays

The mice ($n = 5$–9/group) were randomly assigned to one of the following treatments: (1) Saline + Saline; (2) Saline + METH (1.25, 2.5, or 5 mg/kg, i.p.); (3) CM156 (5, 10, or 20 mg/kg, i.p.) + Saline; (4) CM156 (5, 10, or 20 mg/kg, i.p.) + METH (5 mg/kg, i.p.). The striatum and cerebellum were dissected from each treated mice and then frozen in liquid nitrogen. The tissues were stored at −80 °C for later analysis of dopamine content.

Using a dopamine research enzyme immunoassay kit and protocols provided by the manufacturer (Rocky Mountain Diagnostics, Colorado Springs, CO), mouse brain striatal and cerebellar dopamine were quantified. Brain tissues were homogenized in 0.01 N HCl. Dopamine was extracted and then acylated to N-acyldopamine using the buffer and reagents provided by the ELISA kit. Acylated dopamine from the tissue samples was then incubated with solid phase bound dopamine, dopamine antiserum, and antiserum buffer to compete for a fixed number of antiserum binding sites. Free antigen and free antigen–antiserum complexes were removed via the wash buffer. The antibody bound to the solid phase dopamine was detected using an anti-rabbit IgG-peroxidase conjugate with TMB as the substrate. The amount of antibody bound to the solid phase dopamine was measured by monitoring the reaction at 450 nm. The solid phase dopamine measured was inversely proportional to the dopamine concentration of the tissue sample and was quantified relative to a standard curve of known concentrations.

2.4.2. DAT immunohistochemistry

Striatal sections were assessed for DAT expression. The mice ($n = 4$/group) were randomly assigned to one of the following treatment groups: (1) Saline + Saline; (2) Saline + METH (5 mg/kg, i.p.); (3) CM156 (10 mg/kg, i.p.) + METH (5 mg/kg, i.p.); (4) CM156 (10 mg/kg, i.p.) + Saline. One week following treatment, the mice were perfused transcardially with 0.1 M phosphate buffered saline (pH 7.4), followed by 4% paraformaldehyde. The brains were further fixed overnight in 4% paraformaldehyde. Coronal sections (50 μm) of the fixed tissue were made throughout the rostral-caudal extent of the striatum using a cryostat, and processed in a free-floating state in 0.1 M Tris–HCl buffered saline (TBS, pH 7.5). The sections were treated with 0.3% H_2O_2 in TBS for 30 min at room temperature. The sections were then treated with TBS containing 0.2% Triton X-100 and 1.5% normal goat serum for 30 min at room temperature. Incubation of the sections with rat anti-mouse DAT antibody (Chemicon International, Temecula, CA; MAB369, dilution 1:10,000) was performed for 36 h at 4 °C. The labeled sections were then washed twice in TBS and processed using Vectastain Elite ABC (Vector Laboratories, Burlingame, CA). Sections were then incubated with biotinylated secondary anti-rat antisera (diluted 1:200) in TBS-NBS for 60 min. This was then followed by incubation of the sections with avidin-biotinylated peroxidase substrate in TBS for 60 min. The staining was then visualized by reacting 3,3′-diaminobendine containing 0.01% H_2O_2 for 5 min.

The stained sections were mounted onto gelatin-coated slides and dried. The sections were then dehydrated, cleared, and coverslipped. The images were captured digitally using a Leica DMIL microscope (Leica Microsystems,