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Interactions between 3,4-methylenedioxymethamphetamine and σ_1 Receptors

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Abstract

Methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) are structurally similar and represent a serious and growing health threat. Earlier studies in our laboratory have shown that methamphetamine interacts with σ receptors and that antagonism of these receptors can attenuate methamphetamine-induced locomotor stimulation and neurotoxicity. However, no research exists which characterizes the interaction between σ receptors and MDMA. Therefore, the goal of the present study was to determine whether σ receptors are involved in the actions of MDMA. In the first part of the study, competition and saturation binding assays were performed to measure the interaction of MDMA with σ receptors. The receptor binding assays revealed that MDMA interacts preferentially with the σ_1 subtype, as compared to the σ_2 subtype, and that this interaction occurs in a competitive manner. The second part of the study focused on behavioral measurements in male, Swiss Webster mice to determine whether a selective σ_1 receptor antagonist, BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, 0-30 mg/kg, i.p.) could attenuate the locomotor stimulant actions of MDMA (0-50 mg/kg, i.p.). BD1063 alone had no effect on locomotor activity, but dose-dependently attenuated the locomotor stimulant effects of (+)-MDMA and produced a significant shift to the right in the MDMA dose response curve. Together, the data support the functional relevance of the interaction of MDMA with σ_1 receptors, and suggest that these receptors are involved in the stimulant actions of MDMA.

Keywords

σ receptor; MDMA; BD1063; locomotor

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that is abused in the United States, often by teens and young adults (Freese et al., 2002;National Institute on Drug Abuse, 2004). It is known on the street by many nicknames, such as ecstasy, XTC, E, X, Adam, and the love drug (Freese et al., 2002;National Institute on Drug Abuse, 2004).

MDMA has both hallucinogenic and stimulant properties (National Institute on Drug Abuse, 2004). It is thought to produce its stimulant and hallucinogenic effects by increasing the activity of monoamines, particularly serotonin (Green et al., 2003;Lyles and Cadet, 2003;National Institute on Drug Abuse, 2004). However, the pharmacology of MDMA is complex, involving interactions with many neurotransmitter receptors and transporters (Battaglia et al.,

1988;Green et al., 2003;Lyles and Cadet, 2003). Among these interactions is the ability of MDMA to bind to σ receptors (Kopajtic et al., 1986), which also serves as a target for other drugs of abuse, such as cocaine and methamphetamine (Matsumoto et al., 2003;Nguyen et al., 2005).

σ Receptors are recognized as unique proteins with a drug selectivity pattern, amino acid sequence, and anatomical distribution that is distinct from other known mammalian proteins (Guitart et al., 2004;Matsumoto et al., 2003). The endogenous ligand(s) for these receptors have not yet been determined, although there are a number of candidates, including some neuroactive steroids (Guitart et al., 2004;Monnet and Maurice, 2006). Biochemical and pharmacological studies indicate the existence of multiple σ receptor subtypes, the best characterized being the σ_1 and σ_2 receptors (Guitart et al., 2004;Matsumoto et al., 2003;Su and Hayashi, 2003). The σ_1 receptor has been cloned with high homology and identity from several species, including rodents and humans (Mei and Pasternak, 2001;Prasad et al., 1998). The σ_1 receptor is a highly conserved 223 amino acid protein that appears to participate in protein-protein interactions to modulate the activity of ion channels (Aydar et al., 2002), or signaling molecules including inositol phosphates, protein kinases, and calcium (Su and Hayashi, 2003). In contrast to σ_1 receptors, the σ_2 receptor has not yet been cloned. It is thought to be a 25-29 kDa protein that is enriched in lipid rafts, whereby it affects calcium signaling via sphingolipid products (Gebreselassie and Bowen, 2004;Hellewell et al., 1994).

Many drugs of abuse, including cocaine and methamphetamine, produce effects which can be mitigated through σ receptors, particularly the σ_1 subtype. Both cocaine and methamphetamine exhibit significant affinities for σ receptors, and about a 10- to 20-fold preference for the σ_1 subtype (Matsumoto et al., 2003;Nguyen et al., 2005). These interactions appear physiologically relevant because treatment of animals with selective σ receptor antagonists significantly attenuate cocaine-induced locomotor activity, conditioned place preference, behavioral sensitization, convulsions, lethality, and changes in gene expression (Liu et al., 2005;Matsumoto et al., 2003;Romieu et al., 2004). The importance of the σ_1 subtype is supported by the ability of antisense oligonucleotides against them to prevent a number of cocaine-induced behaviors including locomotor hyperactivity, conditioned place preference, and convulsions (Matsumoto et al., 2003;Romieu et al., 2004). Antagonism of σ receptors, using either putative antagonists or antisense oligos, also reduces methamphetamine-induced locomotor activity and behavioral sensitization (Nguyen et al., 2005;Takahashi et al., 2000;Ujike et al., 1992). In addition, σ_1 receptor proteins levels become up-regulated in the brains of rodents who self administer or are repeatedly injected with methamphetamine (Itzhak, 1993;Stefanski et al., 2004). Despite the known interactions between σ receptors and psychostimulants such as cocaine and methamphetamine, other than an early abstract reporting the binding of MDMA to σ receptors (Kopajtic et al., 1986), no other studies to investigate this interaction have been conducted.

Therefore, in the present study, the interaction of MDMA with σ receptors was characterized. Radioligand binding studies were conducted to determine the affinity of and manner in which MDMA binds to σ receptors, particularly the σ_1 subtype. Behavioral pharmacological studies were then performed to characterize the ability of the selective and well established σ_1 receptor antagonist BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine, Matsumoto et al., 1995) to attenuate the locomotor stimulant actions of MDMA. The present report focuses on σ_1 receptors because truly selective pharmacological tools to target σ_2 receptors have not yet been developed, and earlier investigations show that this subtype is important in the actions of other psychostimulant drugs (Matsumoto et al., 2003;Nguyen et al., 2005).

2. Materials and methods

2.1. Subjects

The subjects of this experiment were 25-34 g male, Swiss Webster mice and 200-250 g male, Sprague Dawley rats (Harlan, Indianapolis, IN). Experimental procedures were conducted in accordance with guidelines set forth by the Institutional Animal Care and Use Committee at the University of Oklahoma Health Sciences Center. The animals were maintained in an AAALAC-approved facility which provided a 12 h reversed light/dark cycle and 24 h food and water access.

2.2. Drugs and chemicals

The following is a list of drugs used and their respective suppliers: BD1063 (Tocris, Ballwin, MO), (+)-3,4-methylenedioxymethamphetamine (Sigma, St. Louis, MO). All radioligands were purchased from Perkin Elmer (Boston, MA). All other chemicals were obtained from Sigma (St. Louis, MO).

2.3. Radioligand binding studies

Competition binding studies were performed in rat brain homogenates to determine the affinities of MDMA and BD1063 for σ receptors and monoamine transporters using methods previously published (Matsumoto et al., 2003; Nguyen et al., 2005). Briefly, σ_1 receptors were labeled in homogenates from rat brain minus cerebellum using 5 nM [3 H](+)-pentazocine; σ_2 receptors were labeled with 3 nM [3 H]DTG in the presence of 300 nM (+)-pentazocine to mask σ_1 receptors. Non-specific binding at σ receptors was determined in the presence of 10 μ M haloperidol. Dopamine transporters were assayed in 2 mg wet weight rat striatal tissue using 0.5 nM [3 H]WIN35,428 [(-)-3 β -(4-fluorophenyl)tropane-2 β -carboxylic acid methyl ester]; non-specific binding was determined with 50 μ M cocaine. Serotonin transporters were labeled in 1.5 mg wet weight rat brainstem tissue using 0.2 nM [3 H]paroxetine; non-specific binding was determined with 1.5 μ M imipramine. Norepinephrine transporters were assayed in 8 mg wet weight rat cerebral cortical tissue using 0.5 nM [3 H]nisoxetine; non-specific binding was determined with 4 μ M desipramine. For each assay, 12 concentrations of MDMA or BD1063 were tested to evaluate their ability to displace the binding of the radioligand. The incubation conditions were as follows: 120 min at 25° C for σ receptors, 120 min at 4° C for dopamine transporters, 90 min at 25° C for serotonin transporters, and 60 min at 4° C for norepinephrine transporters. The IC₅₀s from the assays were used to calculate apparent K_i values using the Cheng-Prusoff equation and previously determined K_d values.

In addition, saturation binding assays were performed to define the interaction of MDMA and BD1063 with the σ_1 receptor, using methods previously described with slight modifications (Nguyen et al., 2005). Briefly, [3 H](+)-pentazocine (0.125-100 nM) was used to label σ_1 receptors in rat brain homogenates in the absence and presence of 3 μ M MDMA or 6 nM BD1063. The concentrations of MDMA and BD1063 that were used in the saturation binding assays represented the K_i values of the compounds for σ_1 receptors in the competition binding assays. Non-specific binding was determined in the presence of 10 μ M haloperidol. Incubations occurred for 120 min at 25° C. GraphPad Prism (San Diego, CA, USA) was used to calculate the K_d and B_{max} values using nonlinear regression, and Student's t-tests were performed to evaluate the data.

2.4. Behavioral pharmacological studies

Horizontal locomotor activity was measured using an automated activity monitoring system (San Diego Instruments, San Diego, CA) consisting of a 4 x 4 photobeam grid surrounding the testing chambers. Beam breaks were recorded as the quantifiable measure of locomotor

activity. To begin, a dose response curve for the locomotor stimulant effects of MDMA was determined. Mice (n=29) were acclimated to the testing enclosures for 30 min. Next, the mice were injected with saline or MDMA (5, 10, 20 mg/kg, i.p.). The mice were then immediately placed in the locomotor activity chambers and disruptions of the photobeams were counted for 90 min.

The approach to test the antagonistic properties of the σ_1 selective antagonist BD1063 was taken in two parts. First, varying doses of BD1063 were given to determine its ability to attenuate a single locomotor stimulant dose of MDMA. Mice were habituated to the locomotor activity chambers for 15 min, then pretreated with a dose of BD1063 (0-30 mg/kg, i.p., n=36). After an additional 15 min, the mice were challenged with saline or the dose of MDMA that produced peak locomotor stimulation during the dose response study (20 mg/kg, i.p.). The second part of the antagonism study involved producing a shift in the MDMA dose response curve. Habituated mice were pretreated with saline or a single dose of BD1063 (20 mg/kg, i.p.), and then challenged 15 min later with MDMA (0-50 mg/kg, i.p.). In both parts of the study, locomotor activity was quantified for 90 min after the second injection.

Analysis of variance was performed on data from the dose response curve study and the antagonism study. Post-hoc Dunnett's tests compared dose response curve data to control. Post-hoc Student-Neuman-Keuls multiple comparison tests evaluated the data between groups from the antagonism studies.

3. Results

3.1. Radioligand binding studies

Apparent K_i values of MDMA and BD1063 for α receptors and monoamine transporters in the rat brain are summarized in Table 1. The affinities of MDMA for the two α receptor subtypes were in the micromolar range. Most notably, these values were comparable to the affinities of MDMA for serotonin and dopamine transporters, two well established targets for MDMA actions. Earlier published affinities of MDMA for the monoamine transporters were in the high nanomolar to low micromolar range, which is consistent with the values reported herein (Battaglia et al., 1988; Han and Gu, 2006; Verrico et al., 2005). The data also confirmed that the α receptor antagonist BD1063 had nanomolar and preferential affinity for σ_1 receptors, as compared to σ_2 receptors. In addition, BD1063 had poor to negligible affinity for the monoamine transporters, thus extending earlier reports of the selectivity of BD1063 for α receptors.

The results of the σ_1 receptor saturation binding assays demonstrated that there was no change in B_{max} when the assays were conducted in the presence of MDMA, compared to when no MDMA was added to the assay ($t=2.51$, n.s.). However, there was a statistical difference in K_d ($t=6.37$, $P<0.005$) which suggested that MDMA competitively interacts with the [3H](+)-pentazocine binding site. The σ_1 receptor antagonist BD1063 produced a similar pattern, exhibiting a significant change in K_d ($t=3.06$, $P<0.05$), but not B_{max} ($t=0.13$, n.s.) when included in the assay. These data are summarized in Table 2 and representative Scatchard plots are shown in Fig. 1.

3.2. Behavioral pharmacological studies

The dose response curves for MDMA and BD1063 when they were administered alone showed a significant difference between the treatment groups ($F[6,40]=9.70$, $P<0.0001$; Fig. 2). Post-hoc Dunnett's tests confirmed that the locomotor stimulatory effects of MDMA were dose dependent with the two higher doses of MDMA statistically different from the saline control: 10 mg/kg, i.p. ($q=3.63$, $P<0.01$), 20 mg/kg, i.p. ($q=5.25$, $P<0.01$). BD1063 by itself did not

alter basal locomotor activity, as Dunnett's tests confirmed that there were no significant differences between the BD1063 doses and saline control (n.s.).

In contrast, BD1063 pretreatment altered the locomotor activity of mice subsequently challenged with a 20 mg/kg dose of MDMA ($F[4,33]=12.49$, $P<0.0001$). BD1063 pretreatment dose dependently attenuated MDMA-induced locomotor stimulation (Fig. 3) and post-hoc Student-Newman-Keuls multiple comparisons demonstrated that the difference in locomotor activity produced by MDMA with saline vs. BD1063 pretreatment was statistically significant for all three doses of BD1063: 10 mg/kg ($q=3.90$, $P<0.01$), 20 mg/kg ($q=7.67$, $P<0.001$), 30 mg/kg ($q=8.38$, $P<0.001$).

Analysis of variance revealed that a single dose of the σ_1 receptor antagonist BD1063 (20 mg/kg, i.p.) elicited a statistically significant shift to the right in the MDMA dose response curve ($F[9,69]=9.93$, $P<0.0001$; Fig. 4). The ED_{50} for MDMA was 8 mg/kg, i.p. in the absence of BD1063, and 24 mg/kg in its presence. Student-Newman-Keuls post-hoc comparisons confirmed that there was a significant difference between the following doses of MDMA in the absence vs. presence of BD1063: 10 mg/kg ($q=6.41$, $P<0.001$), 20 mg/kg ($q=7.07$, $P<0.001$). In addition, there appeared to be no change in the maximal efficacy of MDMA in the absence and presence of BD1063 since post-hoc comparisons revealed no significant differences between the following comparisons: saline + MDMA (20 mg/kg) vs. BD1063 + MDMA (40 mg/kg), and saline + MDMA (30 mg/kg) vs. BD1063 + MDMA (50 mg/kg).

4. Discussion

This is the first report of the functional relevance of α receptors in the actions of MDMA. The data demonstrate the ability of MDMA to interact with α receptors with similar affinities to its better recognized interactions with serotonin and dopamine transporters. Similar to other psychomotor stimulants such as cocaine and methamphetamine, MDMA has a better affinity for the σ_1 compared to the σ_2 subtype (Matsumoto et al., 2003; Nguyen et al., 2005). However, MDMA discriminates between the two α receptor subtypes to a lesser degree than the other psychostimulant drugs.

The interaction of MDMA with σ_1 receptors appears important because administration of the σ_1 receptor antagonist BD1063 significantly attenuated the locomotor stimulant actions of MDMA. The ability of BD1063 and other σ_1 receptor antagonists to also reduce the locomotor stimulant actions of methamphetamine and cocaine in earlier studies (Matsumoto et al., 2003; Nguyen et al., 2005), suggests that the σ_1 receptor may be a common site that can be targeted to lessen the stimulant actions of a number of abused substances.

In both the receptor binding and pharmacological studies, the actions of MDMA at the σ_1 receptor appear competitive in nature. In saturation binding studies, the presence of MDMA in the assays produced a change in the K_d , but not B_{max} , of brain σ_1 receptors. BD1063 also exhibited a similar pattern, suggesting competitive interactions with σ_1 receptors. In behavioral studies, BD1063 produced a shift to the right in the MDMA dose response curve with no significant change in maximal response, providing further indication of competitive interactions. The shift in the MDMA dose response curve by BD1063 most likely reflects actions through σ_1 receptors since earlier studies, combined with the present data, demonstrate that BD1063 has >400-fold better affinity for σ_1 receptors compared to 5-HT₂ receptors, and >1000-fold better affinity for σ_1 receptors compared to the following binding sites: opiate receptors; NMDA receptors; dopamine D₂ receptors; 5-HT₁ receptors; σ_1 , σ_2 and β -adrenergic receptors; muscarinic receptors; dopamine, serotonin and norepinephrine transporters (Matsumoto et al., 1995). Additional studies to further investigate the involvement of the σ_2 subtype in MDMA's actions will need to await the development of selective experimental tools.

In conclusion, the data support the functional relevance of the interaction of MDMA with α receptors, particularly the σ_1 subtype. The results suggest that σ_1 receptors are involved in the stimulant actions of MDMA, and antagonists that act through these sites have therapeutic potential for mitigating the effects of MDMA.

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Fig 1.

Scatchard plots of representative saturation assays, in dpm units. [³H]-(+)-Pentazocine was used to label σ_1 receptors in brain homogenates in the absence (control) and presence of MDMA (3 μ M) or BD1063 (6 nM). The concentration of MDMA and BD1063 used in the assays corresponded to their K_i values in competition binding studies. There was a significant change in K_d, but not B_{max} when MDMA or BD1063 was present.

Fig 2. Swiss Webster mice were treated (i.p.) with saline, MDMA (5-20 mg/kg) or BD1063 (10-30 mg/kg). Horizontal locomotor activity was quantified as disruptions in a 4 x 4 photobeam grid for 90 min. MDMA dose-dependently increased locomotor activity, while BD1063 produced effects that were not statistically different from the saline controls. ** $P < 0.01$, post-hoc Dunnett's tests.

Fig 3.

Swiss Webster mice were pretreated (i.p.) with saline or BD1063 (10-30 mg/kg) followed 15 min later with MDMA (20 mg/kg). Horizontal locomotor activity was quantified as disruptions in a 4 x 4 photobeam grid for 90 min. BD1063 pretreatment significantly attenuated MDMA-induced locomotor activity in a dose dependent manner. ** $P < 0.01$, *** $P < 0.001$, post-hoc Student-Newman-Keuls tests.

Fig 4.

Swiss Webster mice were pretreated (i.p.) with saline or BD1063 (20 mg/kg) followed 15 min later by MDMA (0-50 mg/kg). Horizontal locomotor activity was quantified as disruptions in a 4×4 photobeam grid for 90 min. BD1063 pretreatment significantly shifted the MDMA dose response curve to the right. MDMA ED_{50} = 8 mg/kg, i.p. in the absence of BD1063, and 24 mg/kg in its presence. Sal=saline.

Table 1
Binding affinities of MDMA and BD1063

	MDMA	BD1063
<i>Sigma receptors:</i>		
σ_1 receptors	3057 ± 45	6 ± 0.3
σ_2 receptors	8889 ± 500	440 ± 18
<i>Monoamine transporters:</i>		
Serotonin transporters	4095 ± 195	$>100,000$
Dopamine transporters	$10,320 \pm 319$	$16,820 \pm 6680$
Norepinephrine transporters	667 ± 25	$>100,000$

Competition binding assays were performed in rat brain homogenates using standard methods. The K_i values represent the mean $\pm$ S.E.M. from at least three independent assays. Values of $>100,000$ indicate that there was less than 30% displacement of the radioligand at that concentration.

Table 2Binding parameters for σ_1 receptors in the absence and presence of MDMA or BD1063

Assay condition	K_d (nM)	B_{max} (fmol/mg protein)
No additional compound	14 $\pm$ 2	357 $\pm$ 30
+ MDMA	54 $\pm$ 6 ^a	497 $\pm$ 47
+BD1063	30 $\pm$ 5 ^b	362 $\pm$ 23

Saturation binding assays for σ_1 were conducted using [³H](+)-pentazocine as the radioligand. The assays were performed in the absence or presence of either MDMA (3 μ M) or BD1063 (6nM). The concentration of MDMA or BD1063 used in the assay was equivalent to the K_i of the compound for σ_1 receptors. The K_d and B_{max} values were determined using nonlinear regression.

^a $P < 0.005$,

^b $P < 0.05$.