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The sigma receptor agonist SA4503 both attenuates and enhances the effects of methamphetamine

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Abstract

Background—Methamphetamine's behavioral effects have been attributed to its interaction with monoamine transporters; however, methamphetamine also has affinity for sigma receptors.

Method—The present study investigated the effect of the sigma receptor agonist SA 4503 and the sigma receptor antagonists BD-1047 and BD-1063 on methamphetamine-evoked [³H] dopamine release from preloaded rat striatal slices. The effect of SA 4503 on methamphetamine-induced hyperactivity and on the discriminative stimulus properties of methamphetamine also was determined.

Results—SA 4503 attenuated methamphetamine-evoked [³H]dopamine release in a concentration-dependent manner. BD-1047 and BD-1063 did not affect release. SA 4503 dose-dependently potentiated and attenuated methamphetamine-induced hyperactivity. SA 4503 pretreatment augmented the stimulus properties of methamphetamine.

Conclusions—Our findings indicate that SA 4503 both enhances and inhibits methamphetamine's effects and that sigma receptors are involved in the neurochemical, locomotor stimulatory and discriminative stimulus properties of methamphetamine.

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Contributors

K.R. Rodvelt and D. K. Miller designed the behavioral studies and undertook the statistical analysis. K. R. Rodvelt wrote the first draft of the manuscript. D. K. Miller wrote the animal use protocol. L. R. Blount, K.-H. Fan, J. R. Lever, and S.Z. Lever designed the synthesis of SA 4503 and prepared the ligand. K. R. Rodvelt conducted the behavioral experiments. C. E. Oelrichs and D. K. Miller conducted the [³H]overflow experiments. All authors contributed to and have approved the final manuscript.

Conflict of Interest

The authors have no other funding sources or conflicts of interest to report.

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Keywords

Sigma receptors; methamphetamine; dopamine release; locomotor activity; drug discrimination

1. Introduction

Methamphetamine has affinity for the dopamine plasmalemmal and vesicular monoamine transporters ($K_i = 5.2$ and $0.14 \mu\text{M}$, respectively), through which it increases dopamine levels in the synapse and cytosol (Eshleman et al., 1999; Peter et al., 1994). The behavioral effects of methamphetamine are believed to be related to its interaction with these primary targets. However, methamphetamine has affinity for σ_1 and σ_2 sigma receptors ($K_i = 2.2$ and $46.7 \mu\text{M}$, respectively) (Nguyen et al., 2005), suggesting that methamphetamine's effects may be partially mediated by these receptors. A role for sigma receptors also has been identified by multiple behavioral studies with subtype-selective ligands. The induction of methamphetamine locomotor sensitization was augmented by the nonselective sigma receptor agonist (+)-3-PPP in rats (Ujike et al., 1992b). Furthermore, BMY 14802, a σ_1 sigma receptor antagonist, blocked methamphetamine-induced sensitization in rats (Ujike et al., 1992a). More recently, mice pretreated with selective σ_1 sigma receptor antagonists BD-1063, BD-1047 or AC927, exhibited a reduction in methamphetamine-induced hyperactivity (Matsumoto et al., 2008; Nguyen et al., 2005). Further, σ_1 sigma receptors were upregulated in midbrain regions in rats actively self-administering methamphetamine (Stefanski et al., 2004). Overall, these studies indicate a role for sigma receptors in methamphetamine's effects.

The mechanism(s) through which sigma receptors putatively regulate methamphetamine is unknown. Sigma receptors represent a unique class of receptors distinct from other identified receptor classes, and they are found in portions of the limbic system (Walker et al., 1990). More specifically, the σ_1 sigma receptors were found to be expressed in striatum, substantia nigra, nucleus accumbens, and ventral tegmental area (Hayashi et al., 2010). Sigma receptors are thought to modulate intracellular signaling *via* several second messengers including inositol triphosphate (IP_3) (Hayashi and Su, 2001), which may alter downstream dopamine systems. Both sigma receptor agonists di-o-tolylguanidine (DTG) and pentazocine dose-dependently increased extracellular dopamine levels in striatum (Patrick et al., 1993). Interestingly, pentazocine inhibited NMDA-stimulated [^3H]dopamine release from rat striatal slices (Gonzalez-Alvear and Werling, 1994). BD-1063 inhibited NMDA-induced current in rat ventral tegmental area dopamine neurons (Yamazaki et al., 2002). Additionally, BD-1047 attenuated neuropeptide Y-induced increases in hippocampus extracellular dopamine levels (Meurs et al., 2007).

The present study focused on SA 4503, which has high affinity for sigma receptors with a preference (~15-fold) for the σ_1 sigma receptor ($K_i = 0.004 \mu\text{M}$) over the σ_2 sigma receptor ($K_i = 0.06 \mu\text{M}$) (Lever et al., 2006). SA 4503 showed no affinity ($K_i > 10 \mu\text{M}$) for 36 receptors, ion channels, and second messenger systems associated with methamphetamine's behavioral effects (Matsuno et al., 1996). Regarding its pharmacological activity, SA 4503 is considered to be a sigma receptor agonist, as it displays neuropharmacological properties similar to other known agonists (e.g. pentazocine) (Matsuno et al., 1996).

Several SA 4503 studies have aimed to elucidate the physiological functions of sigma receptors in the central nervous system. In an electrophysiological experiment, SA 4503 decreased the number of spontaneously active dopamine neurons in substantia nigra and increased the number of active dopamine neurons in ventral tegmental area (Minabe et al., 1999), suggesting that sigma receptors regulate dopamine neurons. Nicotine, but not SA

4503, produced significant place-conditioning and SA 4503 pretreatment attenuated nicotine place preference (Horan et al., 2001), suggesting SA 4503 blocks nicotine's conditioned-reinforcing properties. SA 4503 improved dizocilpine-induced working memory impairments as assessed in a radial arm maze task (Zou et al., 2000), indicating that SA 4503 ameliorates memory impairments. In these studies, SA 4503's effects were reversed by the selective σ_1 sigma receptor antagonist NE-100 (Nakazawa et al., 1998; Zou et al., 2000), indicating these effects were mediated by σ_1 sigma receptors. Additionally, SA 4503 administered repeatedly potentiated *d*-amphetamine-induced hyperactivity as assessed in a forced swimming test (Skuza and Rogoz, 2002). Further research is needed to elucidate the involvement of sigma receptors in methamphetamine-induced changes in neurochemistry and behaviors.

The focus of the study was to investigate the role of sigma receptors in the neurochemical and behavioral properties of methamphetamine. The effect of SA 4503 on methamphetamine-induced [3 H]dopamine release from preloaded rat striatal slices was determined to evaluate the pharmacological interaction between these drugs. The effect of BD-1047 and BD-1063 also were determined as a comparison of SA 4503 to receptor antagonists. As only SA 4503 altered methamphetamine-evoked [3 H]dopamine release (*vide infra*), the effects of SA 4503 on methamphetamine-induced behavioral changes were determined. The effect of SA 4503 on methamphetamine-induced hyperactivity was evaluated to explore sigma receptor involvement in the locomotor stimulatory properties of methamphetamine. Finally, a drug discrimination study determined the effect of SA 4503 on the discriminative stimulus (S^D) properties of methamphetamine to investigate the role of sigma receptors in methamphetamine's interoceptive properties.

2. Methods

2.1. Subjects

Male Sprague-Dawley rats (Harlan, Indianapolis, IN; ~200 g upon arrival to the laboratory) were used for all experiments. The colony was maintained under a 12-hr/12-hr light/dark cycle and the experiments were conducted during the light phase of the cycle. For the [3 H]overflow and locomotor activity experiments, rats were housed 2 rats per cage and received ad libitum access to standard rodent chow and tap water. For the drug discrimination study, rats were weighed and handled daily prior to the start of the experiment. Rats had ad libitum access to tap water, but were given access to a limited amount (~25 g) of chow only after the completion of the behavioral session during the entire drug discrimination study. Body weights were maintained at approximately 300 - 350 g.

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri.

2.2. Drugs and chemicals

Ascorbic acid, glucose and pargyline hydrochloride were purchased from Acros Organics (Fairlawn NJ). Radiolabeled dopamine (dihydroxyphenylethylamine 3,4-[ring-2,5,6- 3 H]) was purchased from PerkinElmer Life Sciences (Boston MA). Cocaine hydrochloride, ($\pm$)-methamphetamine hydrochloride, *d*-amphetamine sulfate, and (-)-nicotine ditartrate were purchased from Sigma Chemical Co. (St. Louis MO). SA 4503 was synthesized as the dihydrochloride salt in our laboratory as previously described (Fujimura et al., 1997) and was characterized by reverse phase HPLC (> 97% pure), 1 H NMR and 13 C NMR (Lever et al., 2006). Characterization data were consistent with those obtained with previously synthesized materials (Lever et al., 2006). BD-1047 dihydrobromide and BD-1063 di-

hydrochloride were purchased from Tocris (Ellisville MO). All other chemicals were purchased from Fisher Scientific (Pittsburgh PA).

Throughout the manuscript all drug concentrations and doses represent the free base weight. For the behavioral studies, cocaine and SA 4503 were prepared in saline (0.9% w/v) and administered IP. Methamphetamine and *d*-amphetamine were prepared in saline and administered SC. All injections were administered at a volume of 1 ml solution/kg body weight.

2.3. [³H]Dopamine overflow experiment

The first [³H] overflow experiment determined the intrinsic activity of SA 4503, BD-1047 and BD-1063 to evoke [³H] overflow from rat striatal slices preloaded with [³H]dopamine. Rats were euthanized *via* rapid decapitation and dorsal striata were dissected and sliced (750 μ m thick slices). Slices were incubated in oxygenated (95% O₂/5% CO₂) buffer (in mM, 108 NaCl, 25 NaHCO₃, 11.1 glucose, 4.7 KCl, 1.3 CaCl₂, 1.2 MgSO₄, 1.0 Na₂HPO₄, 0.11 ascorbic acid, 0.004 EDTA; pH 7.4) in a metabolic shaker at 37°C for 30 min. Slices were transferred to fresh buffer, [³H]dopamine (0.1 μ M) was added, and slices were incubated for an additional 30 min. Each slice was then transferred to 1 of 12 reaction chambers (0.2 ml) bounded by glass microfiber filters (GF/B, Whatman, Madistone England) in an automated superfusion system (Suprafusion 2500, Brandel, Gaithersburg MD). Slices were superfused with buffer containing the monoamine oxidase inhibitor pargyline (10 μ M) at a rate of 0.75 ml/min. After 60 min of equilibration, sample collection commenced at a rate of 1 sample/3 min. After the collection of 3 baseline samples, slices were superfused for 9 min with SA 4503 (0.1 nM – 10 μ M), BD-1047 (0.1 nM – 10 μ M) or BD-1063 (0.1 nM – 10 μ M). Slices were then superfused with only buffer for 9 min. One slice was superfused only with buffer and represented a control condition. At the completion, slices and filters were removed from the reaction chamber and solubilized. Radioactivity in superfusate samples and slices/filters was measured by liquid scintillation (LS 6500 Scintillation Counter, Beckman-Coulter, Fullerton CA; counting efficiency $\approx$ 45–55%).

The second [³H]overflow experiment determined the effect of sigma compounds on methamphetamine-evoked [³H] overflow, striatal slices were prepared as described and superfused with buffer for 30 min. Three baseline samples were collected and slices were superfused with SA 4503 (0.1 nM – 10 μ M), BD-1047 (0.1 nM – 1 μ M) or BD-1063 (0.1 nM – 1 μ M) for 6 min. Methamphetamine (3 μ M) was added for 9 min and then all slices were superfused with only buffer for 9 min. The methamphetamine concentration was selected from previous experiments on methamphetamine's concentration-response curve, as a concentration that consistently evoked [³H]overflow greater than that in the presence of only buffer (Miller et al., 2005). As controls, one slice was superfused only with buffer, and a second slice was superfused with methamphetamine in the absence of sigma ligand.

As SA 4503 attenuated methamphetamine (3 μ M)-evoked [³H]overflow, a third [³H]overflow experiment was conducted to determine if SA 4503 alters the effect of a higher (10 μ M) methamphetamine concentration. To determine if the inhibitory effects of SA 4503 were selective for methamphetamine, a fourth experiment was conducted to determine if SA 4503 attenuates nicotine (10 μ M)-evoked [³H]overflow. In both follow-up experiments, slices were superfused with buffer for 30 min, three baseline samples were collected, SA 4503 (0.1 nM – 10 μ M) was added for 9 min, methamphetamine (10 μ M) or nicotine (10 μ M) was added for 9 min, and then slices were superfused with only buffer for 9 min. The nicotine concentration was selected based on our previous work as a concentration that consistently evokes [³H]overflow, but is inhibited by nicotinic acetylcholine receptor antagonists (Miller et al., 2000).

Fractional release for each 3-min superfusate sample was calculated by dividing the [^3H] collected in each 3-min sample by the total [^3H] present in the tissue at the time of sample collection. Basal [^3H]outflow was calculated from the average of fractional release in the three samples just before the addition of the compound. The fractional samples greater than baseline were summed and basal outflow was subtracted to determine total [^3H]overflow. For experiments that analyzed the intrinsic activity of the sigma ligands, separate one-way analyses of variance (ANOVAs) were performed with SA 4503, BD-1047 or BD-1063 Concentration as a within-groups factor. When assessing the effect of the ligands on methamphetamine- or nicotine-evoked [^3H]overflow, separate one-way ANOVAs were performed for SA 4503, BD-1047, or BD-1063. Tukey post hoc tests were performed when appropriate ($p < 0.05$).

2.4. Locomotor activity experiment

To determine the effect of SA 4503 on methamphetamine-induced hyperactivity, rats were acclimated to standard automated activity monitors (Med Associates; Georgia VT) as described previously (Polston et al., 2006) for three consecutive days. On the fourth consecutive day, rats were injected (IP) with SA 4503 (1 – 30 mg/kg) or saline, placed in the monitor for 10 min, injected (SC) with methamphetamine (0.5 mg/kg) or saline, and returned to the monitor for 80 min. The SA 4503 doses were chosen based on previous research as doses that did not alter basal locomotor activity in rodents (Skuzza, 1999). In the design of the experiments, eight treatment conditions ($n = 6$ –10 rats/group) were formed—Saline–Saline, Saline–Methamphetamine (0.5 mg/kg), SA 4503 (1 mg/kg)–Saline, SA 4503 (1 mg/kg)–Methamphetamine (0.5 mg/kg), SA 4503 (10 mg/kg)–Saline, SA 4503 (10 mg/kg)–Methamphetamine (0.5 mg/kg), SA 4503 (30 mg/kg)–Saline, SA 4503 (30 mg/kg)–Methamphetamine (0.5 mg/kg).

Distance traveled was analyzed *via* three-way repeated measures ANOVA (RM-ANOVA) with SA 4503 Dose and Methamphetamine Dose as between-group factors and Time as a within-subjects factor. Post hoc comparisons were performed when necessary.

2.5. Drug discrimination experiment

Rats were trained to discriminate methamphetamine (0.5 mg/kg) from saline using standard operant chambers (ENV-001; Med Associates, Georgia VT). The chambers have a recessed receptacle for food reinforcer (20 mg pellet; Bio-Serv, Frenchtown, NJ) delivery, with 2 response levers located on either side. A house light was opposite the response levers and food receptacle. Responses made on the active lever were reinforced, and responses made on the inactive lever were recorded but not reinforced. All stimulus and response events were controlled and recorded by Med Associates' Med PC-IV Software.

During the initial shaping sessions, rats ($n = 10$) were placed in the operant chambers and responding on either lever was maintained by a fixed-ratio-1 schedule. Shaping sessions continued until each rat successfully completed the session and the ratio requirement for each shaping session was systematically increased to fixed-ratio-10. During each shaping session, rats were reinforced with a maximum of 30 food pellets.

During the training sessions, rats were injected with methamphetamine or saline, put back into their home cage for 10 min, and then placed in the operant chamber. After administration of methamphetamine, one lever was active (the opposite lever was inactive) and following saline administration, the other lever was active (the opposite lever was inactive), which were counterbalanced. The session was terminated after 30 min or 300 responses, whichever occurred first. Only one discrimination training session was

administered daily and the presentation of drug or saline across sessions was maintained by the following pattern – saline (S), drug (D), D, S, S, D, S, D, S, S, D, D.

Performance during the training session was assessed on the first completed ratio. A correct lever selection was recorded when the rat made 10 responses on the active lever with 5 or fewer responses on the inactive lever. The criterion for acquisition of the discrimination (stimulus control) for each rat was correct lever selection on 8 out of 10 successive daily sessions.

Substitution test sessions began after all rats reached stimulus control using a discrete-trials procedure. Only one drug dose and one post-injection interval were assessed in each test session. Additionally, only one test session was completed in a day and at least two training sessions followed each test session. In the test program, both levers were active and 10 responses on either lever delivered a food pellet. The test session was terminated after delivery of food reinforcement or 15 min, whichever occurred first.

In the substitution tests, rats were administered a single dose of methamphetamine (0.000005 – 0.5 mg/kg), cocaine (0.005 – 10 mg/kg), *d*-amphetamine (0.0003 – 0.3 mg/kg), or SA 4503 (0.3 – 30 mg/kg). Rats were returned to the home cage for a 10 min delay, and then placed in the operant chamber when a test session commenced.

In the pretreatment tests, rats were administered a single dose of SA 4503 (0.3 – 1 mg/kg), returned to the home cage for a 10 min delay, injected with methamphetamine (0.000005 – 0.5 mg/kg), cocaine (0.005 – 5 mg/kg) or *d*-amphetamine (0.0003 – 0.3 mg/kg), returned to the home cage for a 10 min delay, and then placed in the operant chamber when a test session commenced.

For the substitution and pretreatment test sessions, the percent of responses on the methamphetamine-paired lever and response rates were calculated. Response rates were determined by dividing the total number of responses on both (methamphetamine- and saline-paired) levers by the session latency.

Substitution was defined as > 80% of responses made on the methamphetamine-paired lever. A nonlinear regression analysis was performed on the mean dose response curve when substitution was observed and an ED₅₀ value was calculated. Dose-response curves were considered to be significantly different when 95% confidence intervals of their ED₅₀ values did not overlap.

For substitution tests, a one-way RM-ANOVA was performed on response rate data with Drug Dose as the within-subjects factor. For pretreatment tests, a two-way RM-ANOVA was performed on response rate data with Pretreatment Condition and Drug Dose as within-subjects factors. To determine if SA 4503 pretreatment altered selection for the methamphetamine- or for the saline-paired lever, a 2 x 2 chi-square test for differences in probability was performed at each methamphetamine, cocaine and *d*-amphetamine dose. Comparisons were made between the absence and presence of SA 4503 treatment on the number of rats that made 10 responses on either the methamphetamine-paired lever or on the saline-paired lever.

Animals that exhibited poor training (i.e. did not respond on the appropriate-paired lever during two training sessions consecutively before a test session) were tested, but their results were not included in the analyses. These circumstances are indicated in the drug discrimination figures as a ratio of the number of rats tested over the number of rats in the group (e.g., 8/10). A significance level of $p < 0.05$ was established a priori for all experiments.

3. Results

3.1. [³H]overflow experiment

Table 1 presents total [³H]overflow from superfusion with SA 4503, BD-1047 and BD-1063 in the absence of methamphetamine. Within the concentration ranges examined, none of the ligands evoked [³H]overflow significantly greater than that evoked in the absence of sigma ligand.

The effect of BD-1047 and BD-1063 on methamphetamine (3 μ M)-evoked [³H]overflow is presented in Table 2. As expected, methamphetamine evoked [³H]overflow that was greater (~13-29-fold) than that evoked in the absence of sigma ligand or methamphetamine (mean = 0.1%, S.E.M. = $\pm$ 0.1%). However, BD-1047 and BD-1063 did not significantly alter methamphetamine-evoked [³H]overflow.

Panel A of Figure 1 depicts the effect of SA 4503 on methamphetamine (3 and 10 μ M)-evoked [³H]overflow. The two higher (100 nM and 1 μ M) SA 4503 concentrations significantly attenuated (93% and 96%, respectively) [³H]overflow evoked by the lower (3 μ M) methamphetamine concentration ($F(3,54) = 4.17$, $p < 0.05$). However, there was no significant effect of SA 4503 on [³H]overflow evoked by the higher (10 μ M) methamphetamine concentration. There was no significant effect of SA 4503 on nicotine (10 μ M)-evoked [³H]overflow (Panel B of Figure 1).

3.2. Locomotor activity experiment

Locomotor activity data are presented in Figure 2. Analysis of the time course revealed a significant three-way Time x SA 4503 Dose x Methamphetamine Dose interaction [$F(19,358) = 4.59$, $p < 0.05$].

The effect of SA 4503 on basal locomotor activity was determined in rats administered SA 4503 or saline, but did not receive methamphetamine. These data are presented in Panel A of Figure 2. In rats administered 1 or 10 mg/kg SA 4503, no differences were found relative to rats administered saline only. However, rats treated with 30 mg/kg SA 4503 were less active than saline-treated rats at the 5 – 20 min time points.

Post hoc analyses indicated that rats in the Saline-Methamphetamine group were more active than those in the Saline-Saline group at all time points between 20 – 90 min (compare open squares between Panels A and B).

Rats were pretreated with SA 4503 followed by methamphetamine to determine the interaction between these drugs, and these data are depicted in Panel B of Figure 2. At time points 20 – 40 min, activity for the 1 mg/kg SA 4503-Methamphetamine group was greater than that for the Saline-Methamphetamine group. Rats in the 10 mg/kg SA 4503-Methamphetamine group were less active than rats in the Saline-Methamphetamine group at 15 – 50 and 65 min. Furthermore, rats in the 30 mg/kg SA 4503-Methamphetamine group were less active than rats in the Saline-Methamphetamine group at 15 – 90 min.

3.3. Drug discrimination experiment

3.3.1. SA 4503 Substitution Tests—Response rates for the SA 4503 substitution test are presented in Panel B of Figure 3. The 3 mg/kg SA 4503 dose produced a significant decrease in response rates [$F(3,20) = 4.18$, $p < 0.05$] relative to the saline test session. Regarding the percentage of responses on the methamphetamine-paired lever (Panel A of Figure 3), substitution was not evident at any SA 4503 dose.

3.3.2. Methamphetamine Tests—For the drug discrimination tests with methamphetamine, response rates are presented in Panel D of Figure 4 and the percent responding on the methamphetamine-paired lever are presented in Panel A of Figure 4.

On the methamphetamine substitution test, none of the methamphetamine doses significantly altered response rates relative to the saline test session (see closed symbols in Panel D of Figure 4). Substitution was evident at 0.5 mg/kg methamphetamine (see closed symbols in Panel A of Figure 4) and regression analyses determined an ED₅₀ value of 0.019 mg/kg (95% C.I. = 0.0090–0.049 mg/kg).

In pretreatment tests, SA 4503 (0.3 and 1 mg/kg) with methamphetamine did not significantly alter response rates, relative to methamphetamine in the absence of SA 4503 (compare open and closed symbols in Panel D of Figure 4).

Regarding the percentage of responses on the methamphetamine-paired lever, substitution was evident at the 0.5 mg/kg methamphetamine dose in the presence of both 0.3 and 1 mg/kg SA 4503 (see open symbols on Panel A of Figure 4). SA 4503 (1 mg/kg) pretreatment altered methamphetamine- or saline-paired lever selection at three methamphetamine doses. At the 0.000005 mg/kg methamphetamine dose, 6 rats (out of 10 rats) pretreated with 1 mg/kg SA 4503 made 10 responses on the methamphetamine-paired lever, compared to only 2 rats (out of 8 rats) when SA 4503 was not pretreated [$T(1) = 3.33$, $p < 0.05$]. At the 0.0005 mg/kg methamphetamine dose, 5 rats (out of 10 rats) pretreated with 1 mg/kg SA 4503 made 10 responses on the methamphetamine-paired lever, but only 1 rat (out of 10 rats) made 10 responses on that lever when SA 4503 was not pretreated [$T(1) = 3.809$, $p < 0.05$]. At the 0.005 mg/kg methamphetamine dose, 6 rats (out of 10 rats) made 10 responses on the methamphetamine-paired lever following 1 mg/kg SA 4503 pretreatment, but only 2 rats (out of 10 rats) made 10 responses on that lever in the absence of SA 4503 pretreatment [$T(1) = 3.33$, $p < 0.05$]. No differences between the presence and absence of 1 mg/kg SA 4503 pretreatment were evident at the 0.00005, 0.05 or 0.5 mg/kg methamphetamine doses. Furthermore, no differences were observed between pretreatment with 0.3 mg/kg SA 4503 dose and the absence of SA 4503 pretreatment at any methamphetamine dose.

Regression analyses determined an ED₅₀ value of 0.016 mg/kg (95% C.I. = 0.003–0.06 mg/kg) for the 0.3 mg/kg SA 4503 pretreatment condition, which was not different from the ED₅₀ value determined in the absence of SA 4503 (0.019 mg/kg). For the 1 mg/kg SA 4503 pretreatment condition, regression analyses determined an ED₅₀ value of 0.000134 mg/kg (95% C.I. = 0.000006–0.0019 mg/kg), which was different from the value determined in the absence of SA 4503. This indicates that 1 mg/kg SA 4503 pretreatment shifted (~144 fold) the methamphetamine substitution dose-response curve to the left.

3.3.3. Cocaine Tests—For the drug discrimination tests with cocaine, response rates are presented in Panel E of Figure 4 and the percent responding on the methamphetamine-paired lever are presented in Panel B of Figure 4.

In the cocaine substitution tests, none of the cocaine doses significantly altered response rates relative to the saline test session (see closed symbols in Panel E of Figure 4). Substitution was evident at the 5 and 10 mg/kg cocaine doses (see closed symbols in Panel B of Figure 4). The ED₅₀ value was 0.27 mg/kg (95% C.I. = 0.14–0.52 mg/kg).

In SA 4503 pretreatment tests, the addition of SA 4503 (1 mg/kg) pretreatment to cocaine did not significantly alter response rates, relative to cocaine in the absence of SA 4503 (compare open and closed symbols in Panel E of Figure 4). Regarding the percentage of responses on the methamphetamine-paired lever, in the presence of 1 mg/kg SA 4503

substitution was evident at the 5 mg/kg cocaine dose (see open symbols on Panel B of Figure 4). Analyses did not reveal any differences between the absence and presence of SA 4503 pretreatment on the number of rats making 10 responses on the drug-paired lever at any cocaine dose. Regression analyses determined an ED₅₀ value of 0.16 mg/kg (95% C.I. = 0.05–0.5 mg/kg) in the presence of 1 mg/kg SA 4503, which did not differ from the value determined in the absence of SA 4503 pretreatment (0.27 mg/kg).

3.3.4. d-Amphetamine Tests—For the drug discrimination tests with *d*-amphetamine, response rates are presented in Panel F of Figure 4 and the percent responding on the methamphetamine-paired lever are presented in Panel C of Figure 4.

In the *d*-amphetamine substitution tests, the 0.3 mg/kg *d*-amphetamine dose significantly increased response rates [$F(3,22) = 4.01$, $p < 0.05$] relative to the saline test session (see closed symbols in Panel F of Figure 4). Substitution was evident at 0.3 mg/kg *d*-amphetamine (see closed symbols in Panel C of Figure 4). Regression analyses determined an ED₅₀ value of 0.011 mg/kg (95% C.I. = 0.004–0.04 mg/kg).

In SA 4503 pretreatment tests, the addition of SA 4503 (1 mg/kg) pretreatment to *d*-amphetamine did not significantly alter response rates, relative to *d*-amphetamine in the absence of SA 4503. Regarding the percentage of responses on the methamphetamine-paired lever, in the presence of SA 4503, substitution was evident at the 0.3 mg/kg *d*-amphetamine dose (see open symbols on Panel C of Figure 4). Analyses did not reveal any differences between the absence and presence of SA 4503 treatment on the number of rats making 10 responses on the methamphetamine-paired lever at any *d*-amphetamine dose. Regression analyses determined an ED₅₀ value of 0.0074 mg/kg (95% C.I. = 0.002–0.03 mg/kg) in the presence of 1 mg/kg SA 4503, which did not differ from the value determined in the absence of SA 4503 (0.011 mg/kg).

4. Discussion

The current study examined the role of sigma receptor ligands on the neurochemical and behavioral properties of methamphetamine. The sigma receptor antagonists BD-1047 and BD-1063 did not alter methamphetamine-evoked [³H]dopamine release from rat striatal slices. However, the sigma receptor agonist SA 4503 attenuated methamphetamine-evoked [³H]dopamine release in a concentration-dependent manner. In contrast, SA 4503 dose-dependently enhanced methamphetamine's S^D properties without substituting for the methamphetamine S^D. SA 4503 both enhanced and inhibited methamphetamine's stimulatory effect on locomotor behavior in rats.

BD-1063 and BD-1047 did not evoke [³H]dopamine release or alter methamphetamine-evoked release from striatal slices in the present study. This contrasts to a previous locomotor activity study where the antagonists prevented methamphetamine's stimulatory effect (Nguyen et al., 2005). Additionally, previous research found that both antagonists produced inhibitory effects on dopamine systems in ventral tegmental area and hippocampus (Meurs et al., 2007; Yamazaki et al., 2002). However, dopamine neuron inhibition has not previously been reported in striatum. Thus, further research is needed to elucidate the mechanism for sigma receptor antagonists to diminish methamphetamine's hyperactivity.

SA 4503 attenuated methamphetamine-evoked [³H]dopamine release. This inhibition was surmountable, as increasing the methamphetamine concentration reversed SA 4503's inhibitory effect. Although methamphetamine and SA 4503 are considered to have distinct pharmacological mechanisms in brain (Eshleman et al., 1999; Matsuno et al., 1996; Peter et al., 1994), the present interaction suggests competition for a similar site (e.g. σ_1 sigma

receptors). This inhibition is in accordance with a previous study where pentazocine inhibited NMDA-stimulated [^3H]dopamine release from rat striatal slices (Gonzalez-Alvear and Werling, 1994), and SA 4503 decreased the number of spontaneously active dopamine neurons in the substantia nigra (Minabe et al., 1999), the location of the dopamine cell bodies for striatal dopamine nerve terminals.

Whereas SA 4503 attenuated methamphetamine-evoked [^3H]dopamine release, it was ineffective to block nicotine-evoked [^3H]dopamine release. This finding conflicts with a behavioral study where SA 4503 prevented nicotine-induced place conditioning (Horan et al., 2001). However, it is consistent with SA 4503's lack of affinity for nicotinic acetylcholine receptors (Kawamura et al., 2006; Matsuno et al., 1996). The dichotomy between findings could be related to SA 4503's affinity ($K_i = 0.05 \mu\text{M}$) for the vesicular acetylcholine transporter (Kawamura et al., 2006; Matsuno et al., 1996). Nicotine-evoked dopamine release is mediated primarily *via* plasmalemmal nicotinic acetylcholine receptors and not the vesicular transporter (Maskos, 2010). The impact of SA 4503's interaction with the vesicular acetylcholine transporter on methamphetamine's effects is not clear, as there are no reports of interactions between methamphetamine and this transporter in the literature.

In the present drug discrimination experiment 0.3 or 1 mg/kg SA 4503 did not substitute for the methamphetamine S^D . However, SA 4503 (1 mg/kg) pretreatment dose-dependently enhanced the S^D properties of methamphetamine. At low methamphetamine doses more rats responded on the methamphetamine-paired lever following 1 mg/kg SA 4503 pretreatment than did in the absence of SA 4503 pretreatment. Moreover, pretreatment with 1 mg/kg SA 4503 increased methamphetamine's ED_{50} value ~144-fold compared to its ED_{50} value determined in the absence of SA 4503. These findings indicate that pretreatment with SA 4503 caused lower doses of methamphetamine to produce similar interoceptive properties to the methamphetamine S^D .

While SA 4503 enhanced methamphetamine substitution, it did not alter the effect of *d*-amphetamine to substitute for the methamphetamine S^D . While methamphetamine and *d*-amphetamine are similar in many respects, they differ in their affinity for SA 4503's primary target, σ_1 sigma receptors. Methamphetamine exhibits affinity ($K_i = 2.2 \mu\text{M}$) for σ_1 sigma receptors while *d*-amphetamine lacks affinity ($K_i > 10 \mu\text{M}$) for this receptor (Nguyen et al., 2005; Walker et al., 1990). Differences in affinity and the present drug discrimination experiment suggest an important role for the σ_1 sigma receptor in methamphetamine's interoceptive properties.

SA 4503's inhibition of methamphetamine-evoked [^3H]dopamine release and enhancement of methamphetamine's S^D properties indicates a biphasic effect for SA 4503. This biphasic relationship was observed clearly in the present locomotor activity experiment: A single SA 4503 injection potentiated methamphetamine-induced hyperactivity at lower SA 4503 doses (1 mg/kg) and augmented methamphetamine-induced hyperactivity at higher SA 4503 doses (10 and 30 mg/kg). One explanation for this biphasic interaction is that SA 4503 is a mixed agonist-antagonist at sigma receptors. At lower concentrations ($\leq 1 \text{ mg/kg}$) SA 4503 might function as an agonist and enhance psychostimulant effects, while at higher concentrations ($> 1 \text{ mg/kg}$) it might function as an antagonist and block psychostimulant effects. In the drug discrimination experiment enhancement was observed at 0.3 and 1 mg/kg SA 4503, and in the [^3H]overflow experiment inhibition was observed at SA 4503 concentrations (100 nM and 1 μM) higher than its reported affinity (4.6 and 63 nM) for σ_1 and σ_2 sigma receptors, respectively (Lever et al., 2006).

A second explanation for the biphasic pattern is based on the way sigma receptors regulate dopamine neuron activity. Sigma receptors could regulate separate downstream dopamine systems that uniquely control the locomotor and interoceptive properties of psychostimulants. SA 4503 administration significantly decreased the number of spontaneously active dopamine neurons in the substantia nigra (Minabe et al., 1999), suggesting that sigma receptors play a role in the motor effects mediated by dopamine neurons in the nigrostriatal pathway. Additionally, administration of SA 4503 increased the number of spontaneously active dopamine neurons in the ventral tegmental area (Minabe et al., 1999), suggesting that sigma receptors are involved in subjective effects of psychostimulants mediated by dopamine neurons in the mesolimbic dopamine pathway.

A third explanation is the role of sigma receptors in regulating intracellular signaling pathways. Sigma receptor ligands may be increasing intracellular calcium levels and producing amplification of downstream dopamine systems (Su et al., 2009). This would explain the potentiation of methamphetamine-induced hyperactivity and the augmentation of methamphetamine to substitute for the methamphetamine S^D in the present study. In contrast, higher concentrations of sigma receptor ligands may be inhibiting plasmalemmal ion channels and blocking downstream dopamine systems (Su et al., 2009). This would clarify the differential attenuation and enhancement of methamphetamine-induced hyperactivity in the same experiment.

To summarize, the present study found that SA 4503 both enhances and inhibits methamphetamine's effects *in vitro* and *in vivo*. To the extent that sigma receptors are involved in the present findings, the data suggest that SA 4503 might be characterized as a mixed agonist-antagonist. SA 4503's biphasic effects might also be related to the activity of sigma receptors to regulate the dopamine pathways that contribute to the interoceptive and locomotor-activating effects of psychostimulants. Future research with SA 4503 and other sigma receptor ligands is necessary to understand this interaction better and to consider the potential role of sigma receptors as a target for methamphetamine addiction therapies.

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Figure 1.

SA 4503 attenuates methamphetamine-evoked [^3H]overflow (Panel A) from rat striatal slices preloaded with [^3H]dopamine, but does not alter nicotine-evoked [^3H]overflow (Panel B). Slices were superfused with buffer containing SA 4503 (0.1 nM – 1 μM) and methamphetamine (3 or 10 μM) or nicotine (10 μM) for 9 min. Data represent total [^3H]overflow (mean $\pm$ S.E.M.) and the control represents superfusion in the presence of methamphetamine or nicotine and the absence of SA 4503. Asterisks designate a significant within methamphetamine group difference from the control condition. (n = 6-9 rats/cell)

Figure 2.

SA 4503 dose-dependently potentiates and attenuates methamphetamine-induced hyperactivity. Rats were administered SA 4503 (1, 10 or 30 mg/kg) or saline, placed in the monitor for 10 min, administered methamphetamine (0.5 mg/kg) or saline, and returned to the monitor for 80 min. Panel A presents data from rats administered SA 4503 or saline followed by saline, and Panel B presents data from rats administered SA 4503 or saline followed by methamphetamine. Data represent mean ($\pm$ S.E.M.) distance travelled in 5-min intervals. Arrows designate the second injection (methamphetamine or saline). Asterisks designate a significant ($p < 0.05$) difference from rats that were not administered SA 4503 (i.e., the Saline-Saline group in Panel A and the Saline-Methamphetamine group in Panel B). ($n = 6-10$ rats/group)

Figure 3.

SA 4503 does not substitute for the methamphetamine S^D . Rats were administered SA 4503, returned to the home cage for 10 min, and were placed in the chamber where testing commenced. Data represent mean ($\pm$ S.E.M.) percent of responses on the methamphetamine-paired lever (Panel A) and the mean ($\pm$ S.E.M.) response rates (Panel B). Asterisks designate a significant ($p < 0.05$) difference from a substitution session with saline ($n = 10$ rats)

Figure 4.

SA 4503 augments the S^D properties of methamphetamine. Rats were administered SA 4503, returned to the home cage for 10 min, injected with methamphetamine, cocaine, or *d*-amphetamine and placed in the chamber 10 min later. Closed squares represent substitution tests where rats were administered methamphetamine, cocaine, or *d*-amphetamine in the absence of SA 4503. Open symbols represent pretreatment with 0.3 (triangles) or 1 mg/kg (squares) SA 4503. Data represent mean ($\pm$ S.E.M.) percent of responses on the methamphetamine-paired lever (Panels A-C) and the mean ($\pm$ S.E.M.) response rates (Panels D-F). ($n = 10$ rats)

Table 1

Sigma ligands do not evoke [^3H] overflow from rat striatal slices preloaded with [^3H]dopamine

	Sigma Ligand Concentration						
	0 M	0.1 nM	1 nM	10 nM	100 nM	1 μM	10 μM
BD-1047	0.15 $\pm$ 0.15	0.05 $\pm$ 0.05	0.08 $\pm$ 0.05	0.00 $\pm$ 0.00	0.04 $\pm$ 0.04	0.19 $\pm$ 0.19	0.08 $\pm$ 0.07
BD-1063	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
SA 4503	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

Data represent mean ($\pm$ SEM) total [^3H] overflow (as percent tissue content) for the 9 min period of superfusion with drug (n = 3–8 rats/cell).

BD-1047 and BD-1063 do not alter methamphetamine (3 µM)-evoked [³H] overflow from rat striatal slices preloaded with [³H]dopamine

Table 2

	Sigma Ligand Concentration + Methamphetamine (3 µM)					
	0 M	0.1 nM	1 nM	1 nM	100 nM	1 µM
BD-1047	2.31 ±0.52	1.94 ±0.48	2.33 ±1.28	1.31 ±0.47	1.81 ±0.53	2.23 ±0.94
BD-1063	1.38 ±0.19	1.29 ±0.49	0.87 ±0.36	1.01 ±0.18	1.92 ±0.89	1.29 ±0.40

Data represent total [³H]overflow (as percent tissue content) from the 9 min period of superfusion with sigma ligand and methamphetamine (n = 3-8 rats/cell).