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N-phenylpropyl-*N*′-substituted piperazines occupy sigma receptors and alter methamphetamine-induced hyperactivity in mice

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ABSTRACT

This study examined the effect of the *N*-phenylpropyl-*N*′-substituted piperazine ligands SA4503 (3,4-dimethoxyphenethyl), YZ-067 (4-methoxyphenethyl), YZ-185 (3-methoxyphenethyl) and Nahas-3h (4-methoxybenzyl) on methamphetamine-induced hyperactivity in mice. In a previous study in rats, SA4503 increased methamphetamine-induced hyperactivity at a lower ligand dose and enhanced it at a higher dose. The other ligands have not been investigated in this assay. Presently, mice were administered sigma ligands, and specific [^{125}I]*E*-IA-DM-PE-PIPZE and [^{125}I]RTI-121 binding was measured to determine σ1 sigma receptor and dopamine transporter occupancy, respectively. Mice were also administered sigma ligands followed by methamphetamine, and locomotor activity was measured. Each of the ligands occupied σ1 sigma receptors (ED_{50} = 0.2–0.6 μmol/kg) with similar potency, but none occupied the transporter (ED_{50} > 10 μmol/kg). At the highest dose tested (31.6 μmol/kg) all four sigma ligands significantly attenuated methamphetamine-induced hyperactivity. Interestingly, SA4503, YZ-067 and Nahas-3h, but not YZ-185, enhanced methamphetamine-induced hyperactivity at lower ligand doses (1–3.16 μmol/kg). These results suggest that these ligands function as stimulant agonists at lower doses and as antagonists at higher does, with subtle changes in the substitution pattern at the 3- and 4-positions of the phenethyl group contributing to the nature of the interactions. Overall, these data indicate a complex role for σ1 sigma receptor ligands in methamphetamine's behavioral effects.

1. Introduction

The behavioral effects of psychostimulants, such as cocaine and methamphetamine, are related to their actions at the dopamine transporter (DAT) that increase catecholamine levels in the central nervous system (Schmitt and Reith, 2010; Uhl et al., 2002). However, the impact of this enhanced dopamine neurotransmission is also regulated by σ1 sigma receptors that are complexed with ion channels and modulate dopamine receptor intracellular signaling (Hayashi et al., 2010; Hayashi and Su, 2007; Patel et al., 2009). Sigma receptor ligands, such as *N*-phenylpropyl-*N*′-substituted piperazines (Fig. 1), have been shown to alter the behavioral effects of cocaine and methamphetamine in rodents. SA4503 is a substituted piperazine that has a methoxy group in both the 3- and 4-positions of the phenethyl moiety. For YZ-185 a single methoxy group is in the 3- position, while for YZ-067 the methoxy group is in the 4-position. A single methoxy group is in the 4-position on the benzyl group for Nahas-3h. Each substituted piperazine binds with 7- to 44-fold greater selectivity for the σ1 over σ2 sigma receptor subtype (Table 1), as assessed via in vitro binding in rodent brain preparations (Lever et al., 2006; Matsumoto et al., 2004; Nahas et al., 2008).

Acute cocaine or methamphetamine injection produces a transient increase in locomotor activity in mice and *N*-phenylpropyl-*N*′-substituted piperazine ligands alter this stimulant-induced hyperactivity (Rodvelt et al., 2011; Sage et al., 2013). SA4503 (2.7 and 27 μmol/kg), YZ-067 (10 and 31.6 μmol/kg) and Nahas-3h (0.316–10 μmol/kg) all attenuated cocaine-induced hyperactivity at ligand doses that had minimal impact on basal locomotor activity. Interestingly, a high YZ-185 dose (31.6 μmol/kg) inhibited cocaine-induced hyperactivity, while a lower YZ-185 dose (0.1 μmol/kg) enhanced cocaine-induced hyperactivity. A similar pattern on methamphetamine-induced hyperactivity in rats was observed with SA4503 (Rodvelt et al., 2011). At a low dose (2.7 μmol/kg) SA4503 enhanced the hyperactivity observed for the first 30 min after methamphetamine (0.5 mg/kg) injection. However, at higher doses (27 and 81 μmol/kg) SA4503 inhibited methamphetamine-induced hyperactivity for as long as 80 min. These findings indicate that YZ-185 and SA4503 have a mixed agonist-antagonist

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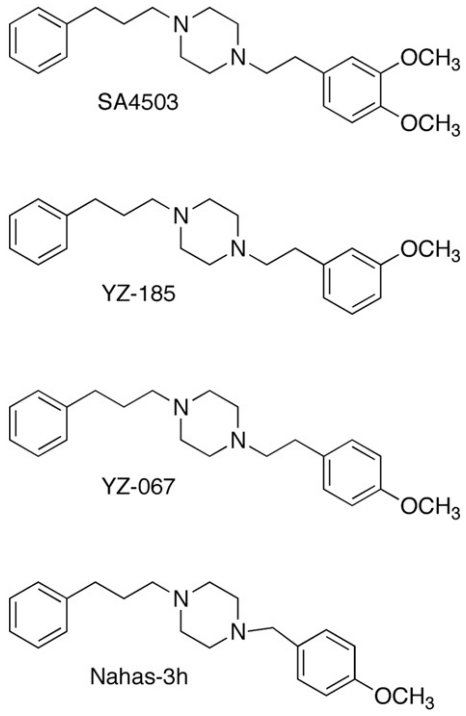

Fig. 1. Structures of the *N*-phenylpropyl-*N*′-substituted piperazine sigma receptor ligands examined.

behavioral profile on the locomotor-activating properties of cocaine and methamphetamine, respectively. The methoxy group at the 3-position may be critical to this biphasic interaction for the enhancement observed with YZ-185 and SA4503, but not YZ-067 and Nahas-3h. However, the effect of YZ-185, YZ-067 and Nahas-3h on methamphetamine-induced hyperactivity has not been reported.

SA4503, the best known ligand from this class, is considered to be a σ1 sigma receptor agonist based upon a variety of studies, including its ability to cause dissociation of immunoglobulin binding protein from the σ1 sigma receptor (Fujimoto et al., 2012). Despite the structural similarities to SA4503, the YZ compounds are generally regarded as σ1 sigma receptor antagonists based primarily upon their ability to block cocaine-induced convulsions in mice (Matsumoto et al., 2004). Thus, the overall goal of this study was to continue exploration of the mixed behavioral properties we observed previously for these ligands by determining the effect of piperazine ligand structure on σ1 sigma receptor occupancy and on the stimulatory properties of methamphetamine. Occupancy was assessed in vivo by administering mice SA4503, YZ-185, YZ-067 or Nahas-3h followed by [^{125}I]*E*-IA-DM-PE-PIPZE, a novel radioligand with high affinity and selectivity for σ1 sigma receptors in rodent brain (Lever et al., 2016). Potential DAT occupancy by the piperazine ligands was also assessed by known procedures using the radioligand [^{125}I]RTI-121 (Lever et al., 1996). In the behavioral experiments, mice were administered SA4503, YZ-185, YZ-067 or Nahas-3h prior to injection of methamphetamine at a stimulant dose that produces a transient increase in locomotor activity using procedures similar to those described for determining the interaction of these ligands with cocaine (Sage et al., 2013).

2. Material and methods

2.1. Drugs and chemicals

SA4503 dihydrochloride salt (368.5 g/mol; *N*-phenylpropyl-*N*′-(3.4-dimethoxyphenethyl)piperazine); YZ-185 dihydrochloride salt, quarter hydrate (415.9 g/mol; *N*-phenylpropyl-*N*′-(3-methoxyphenethyl)piperazine); YZ-067 dihydrochloride salt, quarter hydrate (415.9 g/mol; *N*-phenylpropyl-*N*′-(4-methoxyphenethyl)piperazine); and Nahas-3h dihydrochloride salt (397.4 g/mol; *N*-phenylpropyl-*N*′-(4-methoxybenzyl)piperazine); were synthesized as described previously (Fujimura et al., 1997; Matsumoto et al., 2004; Nahas et al., 2008) and exhibited appropriate spectral data and combustion analyses. BD-1063 (1-[2-(3.4-dichlorophenyl)ethyl]-4-methylpiperazine) dihydrochloride), GBR-12909 dihydrochloride, and (+)-methamphetamine hydrochloride ((*S*)-*N*,α-dimethylbenzeneethanamine hydrochloride) were purchased from Sigma Chemical Co. (St. Louis, MO). *E*-*N*-1-(3′-iodoallyl)-N′-4-(3″,4″-dimethoxyphenethyl)-piperazine (*E*-IA-DM-PE-PIPZE) and [^{125}I]*E*-IA-DM-PE-PIPZE (ca. 2000 Ci/mmol) were prepared as previously described (Lever et al., 2012). [^{125}I]RTI-121 (3β-(4-iodophenyl)tropan-2β-carboxylic acid isopropyl ester) was prepared (ca. 2000 Ci/mmol) as previously described (Lever et al., 1996). Other chemicals and solvents were the best available commercial grade and were used as received. All drug doses and concentrations refer to the free-base weight.

2.2. Animals

Male CD-1 mice (Charles River, approximately 42 days old at the time of testing) were housed 4 mice per cage with ad libitum access to standard rodent chow and water. The animal colony was maintained under a 12-h/12-h light/dark cycle and experiments were conducted during the light phase of the cycle. A total of 322 mice were used in these experiments and the procedures were approved by the Institutional Animal Care and Use Committees of the University of Missouri and the Harry S. Truman Memorial Veterans' Hospital. Experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

2.3. In vivo binding

Biodistribution studies of [^{125}I]*E*-IA-DM-PE-PIPZE binding to σ1 sigma receptors (Lever et al., 2012) and [^{125}I]RTI-121 binding to DAT (Desai et al., 2005; Lever et al., 1996) were conducted as described previously. Awake mice received injections (i.v.) of radioligands (2.5 μCi) prepared in saline (0.1 ml) containing ethanol (2%), and were euthanized by cervical dislocation. For σ1 receptor studies, radioligand binding was evaluated in whole brain, and data from a group treated with BD1063 (5 μmol/kg) in saline (0.1 ml, i.v.) was used to define nonspecific binding. For DAT studies, samples of striatum and cerebellum were analyzed, and cerebellar radioactivity was defined as nonspecific binding (Desai et al., 2005; Lever et al., 1996). Wet weights of tissue samples

Table 1
Binding affinities of *N*-phenylpropyl-*N*′-substituted piperazines for σ_1 and σ_2 sigma receptors.

	N′-piperazine substitution	σ_1 (nM)	σ_2 (nM)	Citation
SA4503	3,4-Dimethoxyphenethyl	4.63 (±0.21)	63.09 (±4.33)	Lever et al. (2006)
YZ-185	3-Methoxyphenethyl	1.4 (±0.2)	10.2 (±0.5)	Matsumoto et al. (2004)
YZ-067	4-Methoxyphenethyl	1.3 (±0.3)	28.6 (±1.9)	Matsumoto et al. (2004)
Nahas-3h	4-Methoxybenzyl	0.76 (±0.07)	32.8 (±2.93)	Nahas et al. (2008)
Methamphetamine		2160 (±0.25)	46,670 (±10.34)	Nguyen et al. (2005)

Values represent mean (±S.E.M.) K_i values as reported by the citations in the table.