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The effect of the sigma-1 receptor selective compound LS-1-137 on the DOI-induced head twitch response in mice

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ABSTRACT

Several receptor mediated pathways have been shown to modulate the murine head twitch response (HTR). However, the role of sigma receptors in the murine (±)-2,5-dimethoxy-4-iodoamphetamine (DOI)-induced HTR has not been previously investigated. We examined the ability of LS-1-137, a novel sigma-1 *vs.* sigma-2 receptor selective phenylacetamide, to modulate the DOI-induced HTR in DBA/2J mice. We also assessed the *in vivo* efficacy of reference sigma-1 receptor antagonists and agonists PRE-084 and PPCC. The effect of the sigma-2 receptor selective antagonist RHM-1-86 was also examined. Rotarod analysis was performed to monitor motor coordination after LS-1-137 administration. Radioligand binding techniques were used to determine the affinity of LS-1-137 at 5-HT2A and 5-HT2C receptors. LS-1-137 and the sigma-1 receptor antagonists haloperidol and BD 1047 were able to attenuate a DOI-induced HTR, indicating that LS-1-137 was acting *in vivo* as a sigma-1 receptor antagonist. LS-1-137 did not compromise rotarod performance within a dose range capable of attenuating the effects of DOI. Radioligand binding studies indicate that LS-1-137 exhibits low affinity binding at both 5-HT2A and 5-HT2C receptors. Based upon the results from these and our previous studies, LS-1-137 is a neuroprotective agent that attenuates the murine DOI-induced HTR independent of activity at 5-HT2 receptor subtypes, D2-like dopamine receptors, sigma-2 receptors and NMDA receptors. LS-1-137 appears to act as a sigma-1 receptor antagonist to inhibit the DOI-induced HTR. Therefore, the DOI-induced HTR can be used to assess the *in vivo* efficacy of sigma-1 receptor selective compounds.

1. Introduction

Previous behavioral pharmacology studies suggest that hallucinogenic drugs are capable of inducing psychotic-like episodes and cognitive deficits in humans that resemble the disordered thoughts, auditory and/or visual hallucinations (positive symptoms) associated with schizophrenia (Moreno and González-Maeso, 2013; Corne and Pickering, 1967; Arnedo et al., 2014). Stimulation of serotonin 5-HT2 receptor subtypes has been implicated in the mechanism of action of several hallucinogenic drugs (Glennon et al., 1984). In addition, a number of known hallucinogenic compounds, including the ergoline lysergic acid diethylamide (LSD) (Maj et al., 1978; Halberstadt and Geyer, 2013; Moreno et al., 2013a, b), the arylcyclohexylamine phencyclidine (PCP) (Nabeshima et al., 1987), the psychedelic tryptamine 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT) (Fantegrossi et al., 2006) and the substituted amphetamine 2,5-dimethoxy-4-iodoamphetamine (DOI) (Canal and Morgan, 2012) induce a head twitch response (HTR), which manifests as a rapid side-to-side head movement in mice.

The DOI-induced HTR has been discussed as an animal model for examining 5-hydroxytryptamine 5-HT2 receptor activity *in vivo* (Canal and Morgan, 2012). DOI binds with high affinity at 5-HT2 receptors (Knight et al., 2004; McKenna and McKenna and Peroutka, 1989; Shannon et al., 1984) and has been reported to be a full agonist at 5-HT2A receptors and a partial agonist at 5-HT2C receptors (Porter et al., 1999). However, DOI is likely functionally selective (Urban et al., 2007) because it has been reported to be either a partial or a full agonist for 5-HT2A, 5-HT2B and 5-HT2C receptors, depending on the signaling pathway that is used for the evaluation (Marek and Aghajanian, 1996; Porter et al., 1999; Berg et al., 1998, 2001; Cussac et al., 2002; Moya et al., 2007).

The symptoms of schizophrenia have been historically categorized as positive (abnormal thoughts/perceptions, auditory hallucinations, delusions) and negative (decreased social functions) symptoms (Kay et al., 2004). However, cognitive measures have also been documented to be impaired in schizophrenia, including a) attention, b) working memory, c) verbal and visual learning, d) reasoning/problem solving and e) social cognition (*i.e.*, emotion perception and social cue

Abbreviations: DOI, 2,5-dimethoxy-4-iodoamphetamine; HTR, Head twitch response; TS, Tourette Syndrome; 5-hydroxytryptamine, 5-HT2; Dimethyl sulfoxide, DMSO.

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interpretation) (Michalopoulou et al., 2013; Rodriguez-Jimenez et al., 2012). Cognitive impairment represents a major impediment to the recovery of schizophrenic patients. One might anticipate that the administration of a hallucinogen, such as DOI, might also affect cognitive performance.

At a low dose (0.1 mg/kg) DOI has been reported to enhance conditioned response-based memory formation in rats (Meneses, 2002), while at a similar dose DOI interfered with a delayed non-matching to position working memory task in 5,7-dihydroxytryptamine lesioned rats (Ruotsalainen et al., 1998). At doses of 0.1 mg/kg and 0.25 mg/kg, DOI did not affect rat learning or the error rate in an "alleys and door" Morris water maze test (Kant et al., 1998). At moderate doses (0.25 to 0.625 mg/kg) DOI was found to increase temporal discrimination, where the length of time for a stimulant is the controlling variable, which the authors suggest may reflect an impairment of sustained attention (Hampson et al., 2010). However, at a higher dose, DOI (2.0 mg/kg) was reported to reduce accuracy and impaired responding in a n-back working memory task in rodents (Ko and Evenden, 2009).

Antipsychotic drugs, which are used clinically to attenuate the frequency and intensity of the positive symptoms of schizophrenia, are also capable of attenuating the HTR in rodents (Hayslett and Tizabi, 2005; Moreno et al., 2013b). For example, the butyrophenone haloperidol, which is a typical antipsychotic used for the treatment of Tourette Syndrome (TS), schizophrenia and obsessive compulsive disorder, has been shown to inhibit the murine DOI-dependent HTR in a dose-dependent manner (Hayslett and Tizabi, 2005; Rangel-Barajas et al., 2014). Although haloperidol is generally thought to mediate its antipsychotic activity primarily by antagonizing dopamine D2 receptors, it also binds with high (nM) affinity at the D3 dopamine receptor subtype (Luedtke et al., 2012). In addition, the affinity of haloperidol at sigma-1 receptors is comparable to its affinity at D2 and D3 dopamine receptors (Largent et al., 1984; Tam and Cook, 1984; Su et al., 1986; Weissman et al., 1990; Luedtke et al., 2012). Several other neuroleptics, including chlorpromazine and pimozide, have appreciable affinity for sigma-1 receptors and have also been shown to attenuate the DOI-induced HTR (Deutsch et al., 1988; Tam and Cook, 1984; Walker et al., 1990; Rangel-Barajas et al., 2014).

The studies in this communication were performed to further investigate the *in vivo* pharmacological properties of LS-1-137, a sigma-1 *vs.* sigma-2 receptor selective phenylacetamide that we developed (Huang et al., 2001), by examining its ability to attenuate the DOI-induced HTR in mice. We recently reported studies on the characterization of the DOI-induced HTR in male DBA/2J mice and the ability of a panel of D2-like dopamine receptor selective ligands, that we developed, to attenuate the murine HTR. That panel of compounds included a) haloperidol, b) D2 *vs.* D3 dopamine receptor selective compounds (Rangel-Barajas et al., 2014) and c) D3 *vs.* D2 dopamine receptor selective compounds (Rangel-Barajas et al., 2015). Although haloperidol is a high affinity D2 dopamine, D3 dopamine and sigma-1 receptor antagonist, the D2 and D3 receptor selective compounds that we used to inhibit the HTR bind with low affinity at both sigma-1 and sigma-2 receptors.

In this communication we investigated whether LS-1-137, a sigma-1 selective compound devoid of D2-dopaminergic and 5-HT2 binding activity, could modulate the DOI-induced HTR in DBA/2J mice. We previously reported that LS-1-137 has neuroprotective properties *in vivo* using a transient middle cerebral artery occlusion (t-MCAO) model of stroke (Schetz et al., 2007; Luedtke et al., 2012). More recently we reported that LS-1-137 could a) partially reverse the cognitive deficits associated with muscarinic antagonist administration in mice and b) trigger the release of brain-derived neurotrophic factor (BDNF) from astrocytes (Malik et al., 2015). These effects of LS-1-137 appear to be independent of the involvement of NMDA and muscarinic receptors. We now report that LS-1-137 can inhibit the DOI-dependent HTR in a dose-dependent manner. We compared its inhibitory activity to reference sigma-1 receptor compounds to determine if LS-1-137 was acting *in vivo* as an agonist or antagonist at sigma-1 receptors.

2. Methods

2.1. Animals

All animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) at University of North Texas Health Science Center. Male DBA/2J (6–8 weeks) mice were obtained from Jackson Laboratories. The weight of the animals ranged from 20 to 28 g. Mice were habituated for 1 week in the animal facility before behavioral studies were initiated. Mice were housed at ≤5 animals per cage under standard 12 h light/12 h dark conditions with free access to food and water. The animals were randomly assigned into different groups. Animal care and housing were in adherence with the conditions set forth in the "Guide for the Care and Use of Laboratory Animals".

2.2. Preparation of drugs

LS-1-137 was synthesized by NIMH Chemical Synthesis. DOI, 4-[4-(p-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone (haloperidol) and 2-morpholin-4-ylethyl 1-phenylcyclohexane-1-carboxylate (PRE-084) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (1*R*,2*S*/1*S*,2*R*)-2-[(4-Hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate ((±)-PPCC) and N′-[2-(3,4-dichlorophenyl)ethyl]-*N*,*N*,*N*′-trimethylethane-1,2-diamine (BD 1047) were purchased from Tocris Bioscience (Bristol, UK).

DOI and all test drugs for animal experiments were dissolved in sterile, Millipore filtered distilled water containing 5% dimethyl sulfoxide (DMSO). Test drugs were prepared on the day of the experiment. All drugs were administered *via* intraperitoneal (i.p.) injection. Test drugs were given 5 min prior to the DOI injection. The quantification of the HTR was started immediately following DOI administration. Animals were allowed a 6 day drug-free period of time between experiments.

2.3. Binding assays

The binding properties of membrane-associated receptors were characterized using a competitive radioligand filtration binding assay (Clarke et al., 2001). Human 5-HT2A receptors were expressed in HEK 293 cells and human 5-HT2C receptors were expressed in Chinese hamster ovary (CHO) cells. For 5-HT2A receptor binding studies, cells were incubated with [^3H]ketanserin and 1 μM of methysergide was used to define the non-specific binding. For 5-HT2C receptor binding studies, cells were incubated with [^3H]mesulergine and 1uM of mianserin was used to define the non-specific binding. The cells were incubated at 37 °C for 60 min and the final assay volume for both the 5-HT2A and 5-HT2C receptor binding assays was 300 μl.

D2long, D3 and D4.4 dopamine receptors were expressed in HEK 293 cells. Membrane homogenates (50 μl) expressing the D2-like dopamine receptors were suspended in 50 mM Tris–HCl/150 mM NaCl/10 mM EDTA buffer, pH = 7.4 and incubated with 50 μl of ^{125}I-IABN (Luedtke et al., 2000) in the presence or absence of the competitive inhibitor (50 μl) at 37 °C for 60 min, using 2.5 M (+)-butaclamol to define the non-specific binding.

For each competition curve, two concentrations of inhibitor per decade were used and each assay was performed in triplicate. Binding was terminated by the addition of cold wash buffer (10 mM Tris–HCl/150 mM NaCl, pH = 7.4) and filtration over a glass-fiber filter (Pall A/B filters, #66198). For these binding studies the IC50 values were determined using a one site fit analysis. The IC50 values were converted to equilibrium dissociation constants (Ki values) using the Cheng and Prusoff equation (Cheng and Prusoff, 1973). Mean Ki values ± S.E.M. are reported for at least three independent experiments. The Kd values that were used to calculate the Ki values were a) 1.1 nM for 5HT2A receptors and b) 0.56 nM for 5-HT2C receptors.