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Serotonergic and dopaminergic distinctions in the behavioral pharmacology of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and lysergic acid diethylamide (LSD)☆

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ABSTRACT

Rationale: After decades of social stigma, hallucinogens have reappeared in the clinical literature demonstrating unique benefits in medicine. The precise behavioral pharmacology of these compounds remains unclear, however.
Objectives: Two commonly studied hallucinogens, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and lysergic acid diethylamide (LSD), were investigated both *in vivo* and *in vitro* to determine the pharmacology of their behavioral effects in an animal model.
Method: Rabbits were administered DOI or LSD and observed for head bob behavior after chronic drug treatment or after pretreatment with antagonist ligands. The receptor binding characteristics of DOI and LSD were studied *in vitro* in frontocortical homogenates from naïve rabbits or *ex vivo* in animals receiving an acute drug injection.
Results: Both DOI- and LSD-elicited head bobs required serotonin$_{2A}$ (5-HT_{2A}) and dopamine$_1$ (D_1) receptor activation. Serotonin$_{2B/2C}$ receptors were not implicated in these behaviors. *In vitro* studies demonstrated that LSD and the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, bound frontocortical 5-HT_{2A} receptors in a pseudo-irreversible manner. In contrast, DOI and the 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, bound reversibly. These binding properties were reflected in *ex vivo* binding studies. The two hallucinogens also differed in that LSD showed modest D_1 receptor binding affinity whereas DOI had negligible binding affinity at this receptor.
Conclusion: Although DOI and LSD differed in their receptor binding properties, activation of 5-HT_{2A} and D_1 receptors was a common mechanism for eliciting head bob behavior. These findings implicate these two receptors in the mechanism of action of hallucinogens.

1. Introduction

Although the mechanism of action of hallucinogens is incompletely understood, serotonin (5-HT) and the serotonin$_{2A}$ (5-HT_{2A}) receptor are thought to play a significant role in mediating their effects (Nichols, 2004). There are two major chemical classes fitting this profile — the phenethylamines (*e.g.* mescaline) and the tryptamines (*e.g.* lysergic acid diethylamide, psilocybin). Hallucinogens have significant value as pharmacological agents. They have been used to model psychosis and to better understand human cognition and perception (Nichols, 2004). Their role in the discovery of the serotonergic system has also proven invaluable (Nichols, 2004; Passie et al., 2008). These compounds have also demonstrated clinical utility in pain, drug addiction, headache, depression, and anxiety disorders (Griffiths et al., 2006, 2008; Grob et al., 2011; Kast and Collins, 1964; Mangini, 1998; Sewell et al., 2006). While the psychedelic effects of hallucinogens may be essential for some types of therapy, these and other physiologic side effects may preclude widespread clinical use of these drugs. As opposed to other recreational drugs, however, hallucinogens are not habit-forming in humans, nor are they reinforcing in animals (Chilcoat and Schutz, 1996; Passie et al., 2008). Deciphering the mechanisms by which hallucinogens exert their various effects will significantly benefit areas of basic and clinical science (Vollenweider and Kometer, 2010).

Drug-elicited head movement is a widely used behavioral model with which to investigate hallucinogens and there is a strong correlation between the dose of hallucinogens used to elicit mouse head twitch behavior and that used recreationally in humans (Corne and Pickering, 1967). Serotonin$_{2A}$ receptors have been implicated in head movements elicited by the phenethylamine hallucinogen, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Darmani et al., 1990; Dave et al., 2002, 2007; Schreiber et al., 1995; Willins and Meltzer, 1997), but a role for this receptor in the action of the indoleamine hallucinogen,

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lysergic acid diethylamine (LSD), which also elicits head movements, is not as clear. Serotonergic antagonists such as cyproheptadine (Vetulani et al., 1980), methysergide (Yamamoto and Ueki, 1981), and bromo-LSD (Sloviter et al., 1980) block LSD-elicited rodent head movements, but these antagonists are relatively non-selective for the 5-HT_{2A} receptor.

The report of the absence of head twitch behavior elicited by LSD in mice lacking 5-HT_{2A} receptors suggests that the 5-HT_{2A} receptor is necessary for LSD mediation of this behavior in this species (González-Maeso et al., 2007). The pharmacology that characterizes the head movement response in mice may be more complex than that for other animals, however. For example, 5-HT_{2C} receptors were not implicated in either DOI-elicited head shakes in rat (Schreiber et al., 1995) or head bobs in rabbit (Dave et al., 2002). In contrast, 5-HT_{2C} receptors contributed significantly to DOI-elicited head twitch behavior in mice (Canal et al., 2010). More precisely, Fantegrossi et al. (2010) demonstrated that 5-HT_{2C} receptor antagonism right shifted the descending limb of the DOI dose response curve in mice. These findings support an inhibitory role of 5-HT_{2C} activation at high doses of DOI (Fantegrossi et al., 2010). Further investigation with non-DOI hallucinogens, such as LSD, may help further explain the contribution of 5-HT_{2C} receptors in hallucinogen-elicited behavior.

Although 5-HT_{2A} receptors are found in many brain regions, direct infusion of the hallucinogen, DOI, into the frontal cortex of rats (Willins and Meltzer, 1997) or rabbits (Dave et al., 2007) has been shown to elicit head shakes and head bobs, respectively. Previous studies have also demonstrated that repeated systemic administration of DOI or LSD robustly down-regulates frontocortical 5-HT_{2A} receptors in both rats and rabbits (Aloyo et al., 2001; Smith et al., 1999). Thus, the frontocortical area is an appropriate brain region in which to investigate the role of 5-HT_{2A} receptors in mediating the effects of hallucinogens.

The dopaminergic system is believed to play a major role in human psychosis, a condition that hallucinogens have been shown to mimic (Nichols, 2004). Hallucinogens differ in their dopaminergic pharmacology, however. For example, LSD binds dopamine receptors, but DOI does not (Burt et al., 1976; Watts et al., 1995). Investigating the role of dopaminergic receptors in the animal head movement model would not only improve our understanding of hallucinogen pharmacology, but might also offer new insight into human psychosis. Presently, dopamine$_1$ (D_1) receptor antagonists are known to block DOI-elicited head shakes in rats (Schreiber et al., 1995), but the role of D_1 receptors in LSD-elicited head movement behavior has not been studied.

The present study compares and contrasts the 5-HT_{2A} and D_1 receptor actions of hallucinogens, represented by two chemical classes, the phenethylamines (DOI) and the indoleamines (LSD). The experiments include receptor binding properties and behavioral actions. The goal of these experiments is to identify the essential pharmacological components shared among hallucinogenic agents.

2. Materials and methods

2.1. Animals

Adult male New Zealand White rabbits (Covance; Devon, PA), weighing 1.8–2.2 kg upon arrival, were housed individually under a standard light–dark cycle of 12 h in an AAALAC-approved colony maintained at 22 ± 1 °C. Rabbits were fed 2/3 cup of rabbit chow daily and had unlimited access to water. Rabbits were adapted to the colony room and the experimenter (*via* handling) for several days before the initiation of experiments. Experiments were carried out in accordance with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research of the National Research Council" (2003) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Drexel University College of Medicine.

2.2. Drugs and solutions

Ritanserin (FW 477.6), SCH23390-HCl (FW 324.2), prazosin-HCl (FW 419.86), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-HCl (FW 357.6), and lysergic acid diethylamide (LSD base; FW 323.4) were purchased from Sigma-Aldrich (St. Louis, MO). Ketanserin tartrate (FW 554.5), SB206553-HCl (FW 328.8), and RS102221-HCl (FW 649.08) were purchased from Tocris Bioscience (Ellisville, MO). [^{3}H]ketanserin and [^{3}H]SCH23390 were purchased from Perkin–Elmer (Boston, MA). [^{3}H]mesulergine was purchased from GE Healthcare (Arlington Heights, IL) or American Radiolabeled Chemicals (St. Louis, MO). All other reagents and supplies were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO). For *in vivo* injections: DOI was dissolved in physiological saline. LSD and ritanserin were dissolved in 2% tartaric acid, pH adjusted to approximately 6.5 with NaOH, and diluted with deionized water. All other drugs were dissolved in deionized water. All drugs were injected subcutaneously at a volume of 1 mL/kg, except the high dose of ketanserin (10 μmol/kg), which required an injection volume of 3.5 mL/kg. The literature primarily reports intravenous administration of LSD (Harvey and Gormezano, 1981; Welsh et al., 1998), but in order to match mode of administration of all other drugs, LSD was also administered subcutaneously. Preliminary studies demonstrated that LSD-elicited head bob behavior was independent of route of administration (subcutaneous or intravenous; unpublished data). LSD was injected at 30 nmol/kg because this was the maximally effective dose (Romano et al., 2010). DOI was injected at 300 nmol/kg because the response at this dose was less variable and equal to that of the maximally effective dose of 1000 nmol/kg (Dave et al., 2002). All pretreatment drugs (ketanserin, ritanserin, SB206553, SCH23390, or vehicle) were injected subcutaneously 1 h prior to hallucinogen administration. Doses of pretreatment drugs were chosen based on previously used doses from both rabbit (Dave et al., 2002; Simansky et al., 1998; Welsh et al., 1998) and rat (Schreiber et al., 1995) head movement studies. For membrane binding studies, all test compounds were dissolved in ethanol and diluted into assay buffer for a final alcohol concentration of 0.025%.

2.3. Experimental procedure

For all behavioral studies, animals were weighed, injected, and immediately replaced in their home cage, and their head bob behavior recorded for 60 min for later analysis. A head bob is a sequential down-up motion of the head without intervening behaviors (*e.g.* sniffing, chewing, hopping; Dave et al., 2002). Food and water were removed from the cage for behavioral recording. In antagonist pretreatment studies, a reduction in head bobs was considered an inhibition or rightward shift of the agonist response. Both DOI and LSD produced inverted "U" dose response curves in the rabbit (Dave et al., 2002; Romano et al., 2010). Behaviors such as lying down and staring increased with higher doses of hallucinogens (unpublished data). This downward turn of the curves likely reflects increasing systemic effects. Such behaviors were not seen when hallucinogens were preceded by antagonist administration (unpublished data). Furthermore, at the doses used, no antagonist produced head bobs or other behaviors alone (unpublished data). For chronic studies, DOI (3 μmol/kg) was injected once daily for 8 days. Twenty four hours following the last DOI treatment, rabbits were challenged by administering either DOI (300 nmol/kg) or LSD (30 nmol/kg) and their behavioral activity recorded for 1 h. Animals were then sacrificed and frontal cortex tissue harvested for later analysis of receptor density as described below. For acute studies, rabbits were administered antagonist (or vehicle) and replaced in their home cage for 60 min. Subsequently, DOI (300 nmol/kg), LSD (30 nmol/kg), or vehicle was administered, the rabbits were replaced in their home cage, and their behavior recorded for 60 min. Where indicated, animals were then sacrificed 10 min later *via* decapitation and frontal cortex