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Atypical binding at dopamine and serotonin transporters contribute to the discriminative stimulus effects of mephedrone

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ABSTRACT

Mephedrone (4-methylmethcathinone), a constituent of the recreational substances known as "bath salts", is a synthetic cathinone that can produce auditory and visual hallucinations, as well as problematic cardiovascular effects. This study compared the discriminative stimulus effects of mephedrone (0.32–10 mg/kg) with other prototypical drugs of abuse: cocaine (0.56–32 mg/kg), D-amphetamine (0.18–3.2 mg/kg), ketamine (1.8–18 mg/kg), phencyclidine (PCP, 1–5.6 mg/kg), heroin (1–10 mg/kg), 2,5-dimethoxy-4-iodoamphetamine (R-DOI, 0.1–1 mg/kg), Δ^9-tetrahydrocannabinol (Δ^9-THC 0.56–5.6 mg/kg), 3,4-methylenedioxyamphetamine (MDA, 0.32–5.6 mg/kg), methylphenidate (1–10 mg/kg), and 3,4-methylenedioxypyrovalerone (MDPV, 0.56–5.6 mg/kg). The discriminative stimulus effects of mephedrone were also assessed after administration of the sigma receptor antagonist rimcazole (0.32–10 mg/kg), the relatively selective norepinephrine transporter (NET) inhibitor desipramine (1.8–18 mg/kg), and the selective serotonin transporter (SERT) inhibitor fluoxetine (1–18 mg/kg). Initially, rats were trained to discriminate an intraperitoneal injection of mephedrone (3.2 mg/kg) from saline under a fixed-ratio 20 schedule of food presentation. Following training, cumulative doses of mephedrone and the other drugs were administered to test for substitution (80% drug-lever responding). Of the drugs tested, including those that were tested in combination with mephedrone (i.e., rimcazole, desipramine, and fluoxetine), only cocaine fully substituted for mephedrone without substantially decreasing response rate. In addition, the three drugs administered in combination with mephedrone shifted the cumulative dose-effect curves leftward (percent drug-lever responding) and down (response rate), although fluoxetine did so in a dose-dependent manner ranging from antagonism to potentiation. In summary, the discriminative stimulus effects of mephedrone were most similar to those for the central nervous system (CNS) stimulant, cocaine, and SERT and DAT activity were necessary for these effects.

1. Introduction

Mephedrone (4-methylmethcathinone) is a synthetic cathinone known for being one of the main constituents of the substances known as "bath salts", which are most often snorted, but can also be taken orally or injected (Schifano et al., 2011). Cathinone is the major psychoactive component found in the khat plant, *Catha edulis*, grown in Eastern African and the Arabian Peninsula. This plant has been referred to as a natural amphetamine due to the stimulant effects produced by chewing its leaves or bark (Kelly, 2011; Patel, 2015). Mephedrone is a substituted cathinone with structural similarity to methamphetamine and entactogenic effects similar to 3,4-mehylenedioxymethamphetamine (MDMA) as reported by its users (Carhart-Harris et al., 2011; Kapitany-Foveny et al., 2013). Although mephedrone has been reported to produce subjective effects similar to typical DAT inhibitors and substrates such as cocaine and amphetamine, respectively, it has also been reported to produce auditory and visual hallucinations that are more commonly associated with 5-HT_{2A} agonists than DAT inhibitors or substrates (Winstock et al., 2011a). With respect to its

Abbreviations: Mephedrone, (4-methylmethcathinone); PCP, (Phencyclidine); R-DOI, (2,5-Dimethoxy-4-iodoamphetamine); Δ^9-THC, (Δ^9-tetrahydrocannabinol); MDA, (3,4-Methylenedioxyamphetamine); MDPV, (4-Methylenedioxypyrovalerone); NET, (Norepinephrine transporter); SERT, (Serotonin transporter).

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mechanism of action, mephedrone binds to the three major monoamine transporters, serotonin (5-HT) type-1A and type-2A receptors, and σ type-1 receptors (Eshleman et al., 2013). Simmler et al. (2013) classified mephedrone as a "cocaine-MDMA mixed cathinone" due to its non-selective inhibition of monoamine uptake and capacity to induce the release of 5-HT.

Due to its similarity to MDMA and CNS stimulants, mephedrone has become a popular drug among recreational drug users (Winstock et al., 2011b). In 2008, mephedrone began infiltrating the Dutch ecstasy market and was found in large quantities in tablets sold as ecstasy in the Netherlands (Brunt et al., 2012). After being banned in the UK in 2010, mephedrone was found in products referred to as "legal highs" available for purchase on several UK-based websites (Brandt et al., 2010). In early 2011, there was a sharp rise in "bath salt" use reported to poison centers in the United States (Murphy et al., 2013; Yin and Ho, 2012). Mephedrone and other synthetic cathinones have been linked to a number of deaths throughout Europe and the United States and have been associated with adverse psychological, cardiovascular, and renal effects (McNeely et al., 2012; Busardo et al., 2015; Loi et al., 2015; White, 2016). Although mephedrone has been acknowledged as a drug of abuse for several years, there is still much to uncover in relation to its interoceptive effects and the receptors and transporters mediating these effects. While users of mephedrone have described its subjective effects as being most similar to MDMA, drug discrimination studies have shown asymmetrical substitution between these two drugs (Harvey and Baker, 2016; Jones et al., 2016; Papaseit et al., 2016; Varner et al., 2013).

The purpose of this study was to directly compare and contrast the discriminative stimulus effects of mephedrone with those of other abused drugs from a variety of pharmacological classes, such as mu opioid receptor agonists, DAT inhibitors and substrates, 5-HT_{2A} agonists, and NMDA receptor antagonists. Despite having different mechanisms of action, these drugs, like mephedrone, have been reported to produce a "rush" (heroin), psychomimetic effects (PCP and ketamine), hallucinations (DOI and MDA) stimulant-like effects (cocaine, MDPV, D-amphetamine, methylphenidate) or some combination of these effects (Δ^9-THC). Finally, the contribution of sigma receptors, as well as the NET and SERT, to mephedrone's discriminative stimulus effects was probed by administering cumulative doses of it after rimcazole, desipramine, and fluoxetine, respectively (Fig. 1). Rimcazole and other sigma receptor antagonists have been shown to attenuate many of the behavioral effects of DAT inhibitors and substrates such as cocaine (Hiranita et al., 2011; Katz et al., 2003; Ujike et al., 1996) and methamphetamine (Katz et al., 2003; Nguyen et al., 2005), respectively. Desipramine is a tricyclic antidepressant that binds relatively selectively to the NET and inhibits the reuptake of norepinephrine. Similar to mephedrone, desipramine can partially (Baker et al., 1993) or fully (Quinton et al., 2006) substitute for cocaine in drug-discrimination procedures, and enhance the discriminative-stimulus effects of cocaine in rats (Baker et al., 1993; Kleven and Koek, 1998) and monkeys (Spealman, 1995). Fluoxetine, on the other hand, belongs to a class of antidepressants known as selective serotonin reuptake inhibitors and has been shown to attenuate the effects of MDMA that are mediated by both the SERT and DAT (Gudelsky and Nash, 1996; Hekmatpanah and Peroutka, 1990), including its subjective and physiological effects in humans (Tancer and Johanson, 2007).

2. Materials and methods

2.1. Subjects

Twenty-five male Long-Evans hooded rats were purchased from Envigo, formally Harlan Sprague Dawley (Indianapolis, IN). Shortly after arrival, rats were individually housed in a colony room, which was maintained at a temperature of 21±2° C and 50 ± 10% relative humidity on a 14-h light/10-h dark cycle beginning at 6 a.m. Subjects were tested during the light cycle and maintained at 90% of their free-feeding weight with ad libitum access to water. Rats were trained and tested as 3 separate groups: five rats that received doses of mephedrone and rimcazole; ten rats that received doses of mephedrone, ketamine, Δ^9-THC, PCP, heroin, DOI, cocaine, D-amphetamine and desipramine; and ten rats that received doses of mephedrone, ketamine, methylphenidate, MDPV, MDA and fluoxetine. All experimental procedures were carried out in accordance with National Institutes of Health guidelines for the care and use of experimental animals and approved by the Institutional Animal Care and Use Committee at Louisiana State University Health

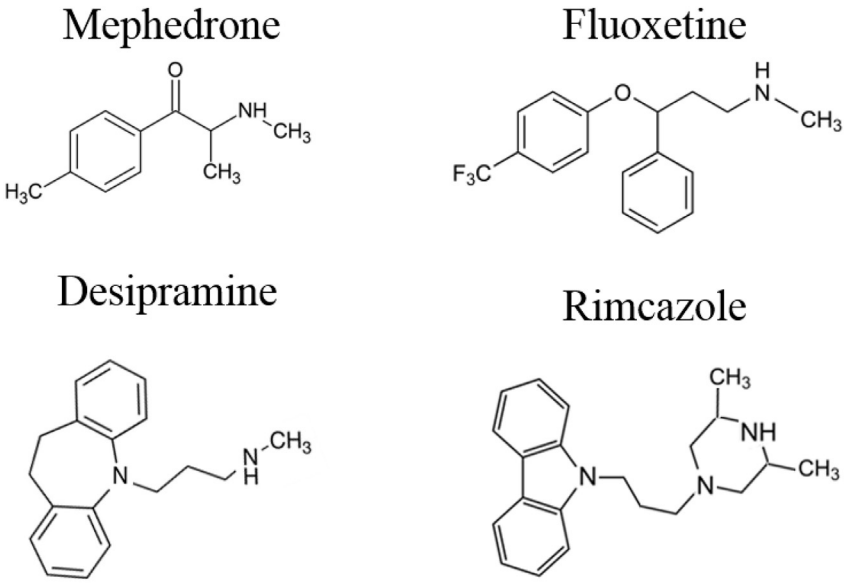

Fig. 1. Chemical structures of the cathinone mephedrone, selective serotonin reuptake inhibitor fluoxetine, tricyclic antidepressant desipramine, and sigma receptor antagonist/dopamine reuptake inhibitor rimcazole.