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Discriminative stimulus and locomotor effects of *para*-substituted and benzofuran analogs of amphetamine

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ABSTRACT

Novel psychoactive substances have maintained a prominent role in the global drug culture, despite increased regulation by governing bodies. Novel compounds continue to become available on the market, often in "Ecstasy" or "Molly" formulations *in lieu* of MDMA, at a much faster rate than they can be properly characterized. The current study aimed to investigate the discriminative stimulus and locomotor effects of three putatively entactogenic compounds that have become increasingly prevalent on the drug market: 5-(2-aminopropyl)-benzofuran (5-APB), 6-(2-aminopropryl)-2,3-dihydrobenzofuran (6-APDB), and 4-fluoroamphetamine (4-FA). Locomotor stimulant effects were assessed in an open-field assay for locomotor activity using Swiss-Webster mice. Discriminative stimulus effects were assessed in Sprague-Dawley rats trained to discriminate either cocaine, methamphetamine, DOM, or MDMA from vehicle. The benzofuran compounds produced locomotor stimulation whereas 4-FA depressed locomotor activity. The benzofurans substituted for the discriminative stimulus effects of MDMA, but only partially or not at all for methamphetamine, cocaine, and DOM, whereas 4-FA fully substituted for MDMA, methamphetamine and cocaine, but not DOM. These results indicate an MDMA-like pattern of abuse might be expected for the benzofurans, whereas 4-FA may be substituted for psychostimulants and MDMA.

1. Introduction

Despite increased regulation, use of novel psychoactive substances (NPS) remains a prominent component of global drug culture and is especially popular among club- and rave-goers (Palamar et al., 2015, 2016a,b). In addition to voluntary use, many individuals have inadvertently taken NPS as adulterants in "Ecstasy" or "Molly" formulations (UNODC, 2014; Palamar, 2016c). Many chemical classes comprise the "Ecstasy"-like NPS, but *para*-substituted and benzofuran analogs of amphetamine are especially popular alternatives to 3,4-methylenedioxymethamphetamine (MDMA) (Palamar et al., 2016c).

Benzofuran analogs of amphetamine (benzofurans) were initially synthesized as part of investigation into structure-activity relations of ring-substituted amphetamines (Monte et al., 1993). The derivatives 5- and 6-(2-aminpropyl)-benzofuran (5- and 6-APB) and 5- and 6-(2-aminopropyl)-2,3-dihydrobenzofuran (5- and 6-APDB) were commonly used in *Benzofury* formulations, which could readily be purchased online prior to scheduling (Jebadurai et al., 2013). Chemical structures are shown in Fig. 1. User reports of the subjective experiences associated with benzofuran compounds indicate both mild hallucinogenic and entactogenic effects, described as a "more intense" version of MDMA (Erowid.org, 2015). Similarly, *para*-substituted amphetamines have been associated with entactogen-like effects and used as adulterants in "Ecstasy" formulations, with *para*-methylamphetamine, *para*-methoxyamphetamine, and 4-fluoroamphetamine (4-FA) being amongst the most prevalent compounds in this class (Elliott and Evans, 2014; Palamar et al., 2016c). 4-FA is the most commonly reported *para*-substituted analog and is sought out specifically for its effects, in addition to inadvertent, adulterated "Ecstasy" use (Linsen et al., 2015). Its chemical structure is shown in Fig. 1.

Like MDMA, benzofuran analogs of amphetamine act as substrates at the monoamine transporters with a greater affinity for the serotonin transporter (SERT) than the dopamine transporter (Rickli et al., 2015b; Baumann et al., 2011). The *para*-substituted amphetamine analogs have a greater serotonergic efficacy in terms of their capacity for promoting transmitter release than their unsubstituted amphetamine counterparts, but are still more selective for dopamine and norepinephrine transporters relative to the serotonin transporter (Nugteren-van Lonkhuyzen et al., 2015; Eshleman et al., 2016; Marona-Lewicka et al., 1995; Rickli et al., 2015a; Bauman et al., 2011). The reverse transport of dopamine by 5-APB demonstrated *in vitro* has been replicated *in vivo* using cyclic voltammetry (Dawson et al., 2014). This pharmacodynamic profile,

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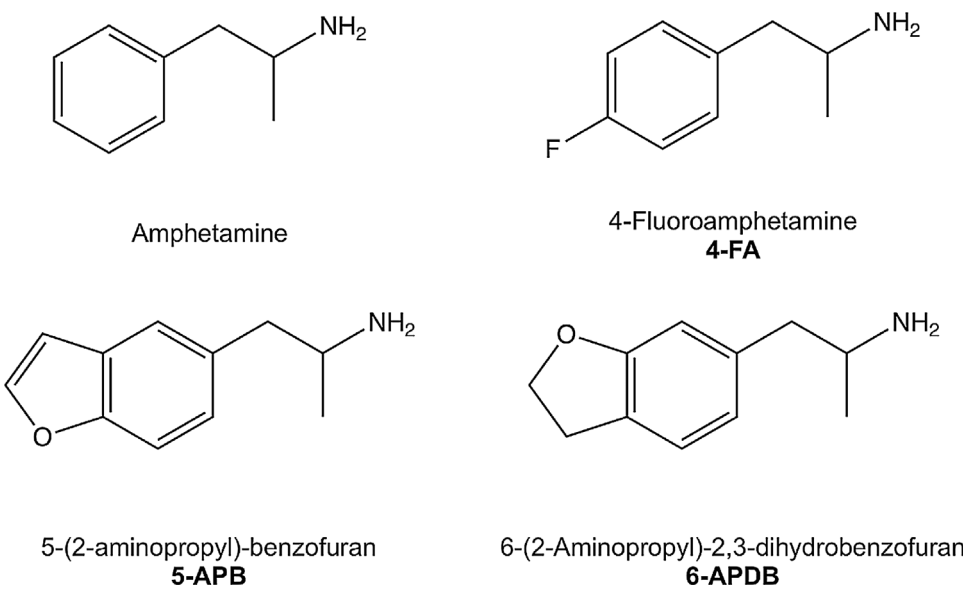

Fig. 1. Structures of amphetamine and the analogs tested in the current study.

relative to traditional, predominantly dopaminergic stimulants, is associated with unique effects and patterns of use, in that they tend to promote feelings of openness and empathy with fairly-limited or episodic consumption (Nichols, 1986; Degenhardt et al., 2010). As has been the case with many novel psychoactive substances, numerous adverse effects have been associated with benzofuran and *para*-substituted amphetamine use including hyperthermia, cardiovascular complications, psychotic episodes, and death (Chan et al., 2013; Seetohul and Pounder, 2013; Kamour et al., 2014; Hondebrink et al., 2015).

In vivo pharmacological data regarding these compounds are sparse, but some insight into their *in vivo* mechanistic and reinforcing effects has begun to emerge. 5- and 6-APDB fully substituted for the discriminative stimulus effects of the serotonin-releasing agents MBDB and MMAI (Monte et al., 1993). Assessments of 5-APB's rewarding and reinforcing effects have demonstrated robust conditioned place preference following 5-APB administration, but limited self-administration in rats (Cha et al., 2016). Conversely, 4-FA substituted fully for the discriminative stimulus effects of *d*-amphetamine, but not MBDB or MMAI (Marona-Lewicka et al., 1995). In line with its amphetamine-like discriminative stimulus, 4-FA, under the name PAL-303, was robustly self-administered by rhesus monkeys to a similar degree as cocaine (Wee et al., 2005).

These early data indicate potential mechanistic differences among these compounds that may influence differences in their subjective and reinforcing effects, with the benzofurans potentially maintaining an entactogen-like pharmacodynamic profile, whereas 4-FA may produce more stimulant-like effects. Previous studies from our laboratory have demonstrated abuse potential for the predominately serotonergic "Ecstasy" adulterant MDAI (Gatch et al., 2016), and the current study utilizes a similar approach to investigate the potential abuse liability of 5-APB, 6-APDB, and 4-FA. Assessments of locomotor activity were conducted to determine the active dose range and duration of action of these compounds. Drug discrimination experiments were performed utilizing the predominately dopaminergic training drugs methamphetamine and cocaine, as well as MDMA, which possesses a complex discriminative stimulus mediated by dopamine and 5-HT (Schechter, 1988; Goodwin et al., 2003), and DOM, a hallucinogenic phenethylamine with a 5-$HT_{2A/C}$-dependent discriminative stimulus. Analyses of the time course of locomotor effects and relative dopaminergic and serotonergic contributions to the discriminative stimuli of these drugs will provide insight into their *in vivo* pharmacology and allow for predictions regarding their patterns of use in the human population.

2. Methods

2.1. Subjects

Male Swiss–Webster mice (n = 136) were obtained from Harlan (Indianapolis, IN) at approximately 8 weeks of age and tested at approximately 10 weeks of age. Mice were group housed in cages on a 12:12-h light/dark cycle and were allowed free access to food and water. Male Sprague-Dawley rats (n = 61) were obtained from Envigo (Indianapolis, IN). All rats were housed individually and were maintained on a 12:12 light/dark cycle (lights on at 7:00 A.M.). Body weights were maintained at 320–350 g by limiting food to 15 g/day which included the food received during operant sessions. Water was readily available. All housing and procedures were in accordance with Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

2.2. Locomotor activity

The study was conducted using 40 Digiscan (model RXYZCM, Omnitech Electronics, Columbus, OH) locomotor activity testing chambers (40.5 × 40.5 × 30.5 cm) housed within sound-attenuating chambers in sets of two. A panel of infrared beams (16 beams) and corresponding photodetectors were located in the horizontal direction along the sides of each activity chamber. A 7.5-W incandescent light above each chamber provided dim illumination and fans provided an 80-dB ambient noise level within the chamber.

Separate groups of 8 mice were injected with either vehicle (0.9% saline), 5-APB (1, 2.5, 5, 10 or 25 mg/kg), 6-APDB (1, 2.5, 5, 10 or 25 mg/kg), or 4-FA (0.5, 1, 2.5 or 5 mg/kg) immediately prior to locomotor activity testing. In all studies, horizontal activity (interruption of photocell beams) was measured for 8 h within 10-min periods, beginning at 0800 h (1 h after lights on).

2.3. Discrimination procedures

Standard behavior-testing chambers (Coulbourn Instruments, Allentown, PA) were connected to IBM-PC compatible computers via LVB interfaces (Med Associates, East Fairfield, VT). The computers were programmed in Med-PC for Windows, version IV (Med Associates, East Fairfield, VT) for the operation of the chambers and collection of data.

Using a two-lever choice methodology, pools of rats previously