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Pharmacology of novel synthetic stimulants structurally related to the "bath salts" constituent 3,4-methylenedioxypyrovalerone (MDPV)

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ABSTRACT

There has been a dramatic rise in the abuse of synthetic cathinones known as "bath salts," including 3,4-methylenedioxypyrovalerone (MDPV), an analog linked to many adverse events. MDPV differs from other synthetic cathinones because it contains a pyrrolidine ring which gives the drug potent actions as an uptake blocker at dopamine and norepinephrine transporters. While MDPV is now illegal, a wave of "second generation" pyrrolidinophenones has appeared on the market, with α-pyrrolidinovalerophenone (α-PVP) being most popular. Here, we sought to compare the in vitro and in vivo pharmacological effects of MDPV and its congeners: α-PVP, α-pyrrolidinobutiophenone (α-PBP), and α-pyrrolidinopropiophenone (α-PPP). We examined effects of test drugs in transporter uptake and release assays using rat brain synaptosomes, then assessed behavioral stimulant effects in mice. We found that α-PVP is a potent uptake blocker at dopamine and norepinephrine transporters, similar to MDPV. α-PBP and α-PPP are also catecholamine transporter blockers but display reduced potency. All of the test drugs are locomotor stimulants, and the rank order of in vivo potency parallels dopamine transporter activity, with MDPV > α-PVP > α-PBP > α-PPP. Motor activation produced by all drugs is reversed by the dopamine receptor antagonist SCH23390. Furthermore, results of a functional observational battery show that all test drugs produce typical stimulant effects at lower doses and some drugs produce bizarre behaviors at higher doses. Taken together, our findings represent the first evidence that second generation analogs of MDPV are catecholamine-selective uptake blockers which may pose risk for addiction and adverse effects in human users.

This article is part of a Special Issue entitled 'CNS Stimulants'.

1. Introduction

In the past few years, products containing synthetic stimulants have flooded the recreational drug marketplace in the United States (U.S.) and elsewhere (Baumann et al., 2013a; Psychonaut, 2009; U.S. Drug Enforcement Administration, 2013a). These products, often sold under the guise of "bath salts," "plant food," or "research chemicals," contain psychoactive cathinone derivatives and are purchased online, at gas stations, or at head shops as "legal" alternatives to illicit drugs (Karila and Reynaud, 2011; Schifano et al., 2011; Winstock and Ramsey, 2010; Winstock et al., 2011). From 2010 to 2011, the number of calls to U.S. poison control centers reporting exposure to synthetic cathinones increased from 303 to 6138, and patients with acute toxicity began presenting to emergency departments (American Association of Poison Control Centers (2012)). The abuse of synthetic stimulants can result in severe side effects including tachycardia, hyperthermia, agitation, delusions, and violent behaviors leading to suicide or homicide (EMCDDA, 2010; Kelly, 2011; Ross et al., 2011; Spiller et al., 2011). In response to the heightened public health threat, federal legislation was enacted in 2012 and 2013 to permanently ban the three most common constituents in these products: 3,4-methylenedioxypyrovalerone (MDPV), 3,4-methylene

Abbreviations: methylone, 3,4-methylenedioxymethcathinone; MDPV, 3,4-methylenedioxypyrovalerone; mephedrone, 4-methylmethcathinone; α-PBP, α-pyrrolidinobutiophenone; α-PPP, α-pyrrolidinopropiophenone; α-PVP, α-pyrrolidinovalerophenone; DAT, dopamine transporter; FOB, functional observational battery; NET, norepinephrine transporter; SERT, serotonin transporter.

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dioxymethcathinone (methylone) and 4-methylmethcathinone (mephedrone).

Initial pharmacological investigations showed that synthetic cathinones exert their effects by interacting with monoamine transporters for dopamine (DAT), norepinephrine (NET) and serotonin (SERT) (Cozzi et al., 1999; Hadlock et al., 2011; López-Arnau et al., 2012; Martínez-Clemente et al., 2012). More recent data reveal that ring-substituted cathinones, like mephedrone and methylone are transporter substrates which cause the release of dopamine, norepinephrine and serotonin by reversing the normal direction of transporter flux (Baumann et al., 2012; Cameron et al., 2013; Simmler et al., 2013) in a manner similar to amphetamine (Baumann et al., 2013b; Fleckenstein et al., 2000). MDPV is structurally distinct from other synthetic cathinones due to the presence of a pyrrolidine ring, which gives the drug potent actions as a transporter blocker at DAT and NET (Baumann et al., 2013b; Eshleman et al., 2013; Simmler et al., 2013). Thus, MDPV displays a molecular mechanism of action that is similar to cocaine rather than amphetamine (Baumann et al., 2013b; Fleckenstein et al., 2000). Systemic administration of MDPV or mephedrone to rats increases extracellular concentrations of dopamine in mesolimbic reward circuits (Baumann et al., 2012, 2013b; Kehr et al., 2011; Wright et al., 2012). Consistent with dopaminergic activation, synthetic cathinones increase locomotor activity in rodents, similar to the effects of classical stimulants like amphetamine and cocaine (Fantegrossi et al., 2013; Lisek et al., 2012; López-Arnau et al., 2012; Marusich et al., 2012). A functional observational battery (FOB) revealed that MDPV, mephedrone, and methylone produce typical stimulant effects including hyperactivity and stereotyped behavior, comparable to that found for cocaine, amphetamine and methamphetamine (Gauvin and Baird, 2008; Marusich et al., 2012). Perhaps more importantly, MDPV and mephedrone are readily self-administered by rats, indicating a propensity for abuse and addiction (Aarde et al., 2013; Hadlock et al., 2011; Motbey et al., 2013; Watterson et al., 2012a, 2012b).

Since the three most common synthetic stimulants were banned in the U.S., manufacturers have introduced novel replacement cathinones as a means to skirt regulatory control, a trend which is expected to continue (Brandt et al., 2010; Shanks et al., 2012). For the purposes of the present paper, synthetic stimulants which were legal prior to legislation enacted in 2012 (U.S. Congress, 2012) are referred to as “first generation” drugs, while newer stimulants are referred to as “second generation” drugs. Despite the fact that a host of cathinone compounds may be present in synthetic stimulant products (Shanks et al., 2012; Spiller et al., 2011), MDPV is the chief compound found in blood and urine from patients admitted to emergency departments for treatment of acute toxicity due to synthetic stimulant exposure (Murray et al., 2012; Penders et al., 2012; Spiller et al., 2011; Wyman et al., 2013). Such data point to MDPV as a principal culprit in mediating medically-relevant adverse effects. Recently, a number of second generation MDPV analogs have appeared in the marketplace, with α-pyrrolidinovalerophenone (α-PVP) being the most popular and widespread (Marinetti and Antonides, 2013; Shanks et al., 2012; U.S. Drug Enforcement Administration, 2013b). As shown in Fig. 1, pyrrolidinophenones like α-PVP, α-pyrrolidinobutiophenone (α-PBP) and α-pyrrolidinopropiophenone (α-PPP) are structurally similar to MDPV (Meltzer et al., 2006), yet little is known about their mechanism of action or behavioral effects.

The purpose of the present study was to evaluate in vitro and in vivo effects of second generation stimulants that are structurally-related to MDPV. To this end, in vitro transporter activity at DAT, NET and SERT was assessed for MDPV, α-PVP, α-PBP, and α-PPP. In vivo pharmacology of these compounds was assessed by measuring locomotor activity and effects in an FOB. Our findings provide the first evidence that second generation pyrrolidinophenones like α-PVP are potent catecholamine-selective transporter blockers which can elicit psychomotor stimulant effects via a dopaminergic mechanism. As such, these agents would be expected to pose substantial risks for abuse and addiction.

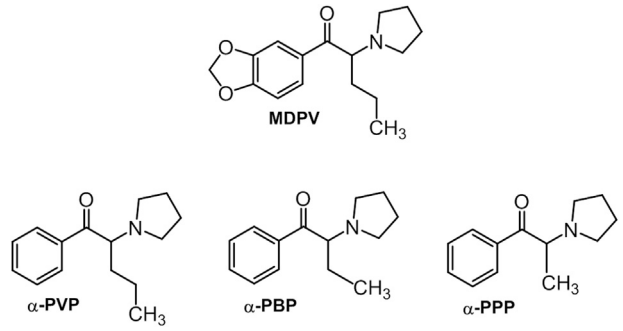

Fig. 1. Chemical structures of pyrrolidinophenone compounds in comparison to MDPV.

2. Methods and materials

2.1. Subjects

Adult male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) weighing 300–400 g (total $n = 36$) were housed three per cage. Adult male ICR mice (Harlan, Frederick, MD, USA) weighing 30–55 g (total $n = 112$) were housed individually. Animals were housed in polycarbonate cages with hardwood bedding. All animals were drug and test naïve, and were housed in temperature-controlled conditions (20–24 °C) with a 12 h standard light–dark cycle. Animals had *ad libitum* access to food and water in their home cages at all times. Rat experiments were approved by the Institutional Animal Care and Use Committee at NIDA IRP, while mouse experiments were approved by the Institutional Animal Care and Use Committee at RTI International. All research was conducted as humanely as possible, and followed the principles of laboratory animal care (National Research Council, 2011). The authors consulted the ARRIVE guidelines for reporting experiments involving animals, and all efforts were made to minimize animal suffering, reduce the number of animals used, and utilize alternatives to in vivo techniques, if available.

2.2. Drugs

α-PVP, α-PBP, and α-PPP were purchased from Cayman Chemical (Ann Arbor, MI, USA). SCH23390 was purchased from Tocris (Minneapolis, MN, USA). MDPV was synthesized in house at RTI using standard synthetic procedures. MDPV was formulated as a recrystallized HCl salt and was >97% pure. The purity was assessed by several analytical techniques including carbon, hydrogen, nitrogen (CHN) combustion analysis and proton nuclear magnetic resonance spectroscopy. All drugs were dissolved in sterile saline (Butler Schein, Dublin, OH, USA). Doses are expressed as mg/kg of the salt, and were administered at a volume of 10 ml/kg in mice. Sterile saline was used as a comparison for all drugs for in vivo studies.

2.3. In vitro uptake and release assays

Rats were euthanized by CO_2 narcosis, and brains were processed to yield synaptosomes as previously described (Baumann et al., 2013b; Rothman et al., 2003). Synaptosomes were prepared from rat striatum for the DAT assays, whereas synaptosomes were prepared from whole brain minus striatum and cerebellum for the NET and SERT assays. For uptake inhibition assays, 5 nM [^{3}H]dopamine, 10 nM [^{3}H]norepinephrine and 5 nM [^{3}H]serotonin were used to assess transport activity at DAT, NET and SERT, respectively. The selectivity of uptake assays was optimized for a single transporter by including unlabeled blockers to prevent uptake of [^{3}H]transmitter by competing transporters. Uptake inhibition assays were initiated by adding 100 μl of tissue suspension to 900 μl Krebs-phosphate buffer (126 mM NaCl, 2.4 mM KCl, 0.83 mM $CaCl_2$, 0.8 mM $MgCl_2$, 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4, 11.1 mM glucose, 0.05 mM pargyline, 1 mg/mL bovine serum albumin, and 1 mg/mL ascorbic acid, pH 7.4) containing test drug and [^{3}H]transmitter. Uptake inhibition assays were terminated by rapid vacuum filtration through Whatman GF/B filters, and retained radioactivity was quantified by liquid scintillation counting. For release assays, 9 nM [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP+) was used as the radiolabeled substrate for DAT and NET, while 5 nM [^{3}H]serotonin was used as a substrate for SERT. All buffers used in the release assay methods contained 1 μM reserpine to block vesicular uptake of substrates. The selectivity of release assays was optimized for a single transporter by including unlabeled blockers to prevent the uptake of [^{3}H]MPP+ or [^{3}H]serotonin by competing transporters. Synaptosomes

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