Pharmacology of novel synthetic stimulants structurally related to the “bath salts” constituent 3,4-methylenedioxyamphetamine (MDPV)

Julie A. Marusich a,*, Kateland R. Antonazzo a, Jenny L. Wiley a, Bruce E. Blough a, John S. Partilla b, Michael H. Baumann b

a RTI International, 3040 Cornwallis Rd., Research Triangle Park, NC 27709, USA. Tel.: +1 919 541 6424; fax: +1 919 541 6499.

a Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA

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A B S T R A C T

There has been a dramatic rise in the abuse of synthetic cathinones known as “bath salts,” including 3,4-methylenedioxyamphetamine (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” pyrrolidinoamines has appeared on the market, with α-pyrrolidinophenone (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-pyrrolidinobutidiophenone (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.

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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-methylenedioxyamphetamine (MDPV), 3,4-methylenedioxyamphetamine (MDPV), 3,4-methylenedioxyamphetamine (MDPV).
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.

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 naive, 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 Toecris (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 μl/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 CO2 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 [3H]dopamine, 10 nM [3H]norepinephrine and 5 nM [3H]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 [3H]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 CaCl2, 0.8 mM MgCl2, 0.5 mM K2HPO4, 0.5 mM Na2SO4, 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 [3H]transmitter. Uptake inhibition assays were terminated by rapid vacuum filtration through Whatman GF/F filters, and retained radioactivity was quantified by liquid scintillation counting. For release assays, 9 nM [3H]-methyl-α-phenylglycine ([3H]MPP+) was used as the radiolabeled substrate for DAT and NET, while 5 nM [3H]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 [3H]MPP+ or [3H]serotonin by competing transporters. Synaptosomes 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 μl/kg in mice. Sterile saline was used as a comparison for all drugs for in vivo studies.
were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 h (steady state). Release assays were initiated by adding 850 μl of preloaded synaptosomes to 150 μl of test drug. Release was terminated by vacuum filtration and retained radioactivity was quantified as described for uptake inhibition.

3. Results

3.1. In vitro transporter assays

The IC50 values for inhibition of [3H]transmitter uptake at DAT, NET and SERT are summarized in Table 1; in vitro data for cocaine and amphetamine from a previously published study are included for comparison (Baumann et al., 2013b). Dose-response curves for uptake inhibition at DAT, NET, and SERT are depicted in Fig. 2. MDPV was a potent catecholamine uptake blocker with IC50 values of 4.0 ± 0.6 nM and 25.9 ± 5.6 nM at DAT and NET, respectively. By contrast, MDVP was more than 100-fold weaker at blocking uptake of serotonin, with an IC50 of 3305 ± 485 nM at SERT. Similar to MDVP, all of the other pyrrolidinophenones were catecholamine-selective uptake blockers. α-PVP and α-BPP were less potent at DAT and NET when compared to α-PVP, indicating shorter alkyl chain length produces progressively less potent effects on uptake. Nevertheless, α-BBB and α-PPP maintained selectivity for inhibition of catecholamine uptake versus SERT uptake. It should be noted that even the weakest pyrrolidinophenone compound tested here, α-BBB, is more potent than cocaine at inhibiting uptake at DAT. None of the pyrrolidinophenones displayed sufficient efficacy in the release assays to allow for the determination of EC50 values (data not shown), demonstrating that the drugs are not transporter substrates. We have previously shown that MDVP is not active in the release assay (Baumann et al., 2013b). Taken together, the in vitro data demonstrate that MDVP, α-PVP, α-BBB and α-PPP are catecholamine-selective transporter blockers, and decreasing alkyl chain length on the α-carbon reduces potency at uptake blockade.

3.2. Locomotor dose response and time course

Figs. 3 and 4 show the effects of test compounds on locomotor activity. As depicted in Fig. 3, all compounds displayed a significant main effect of dose [MDVP: F(3, 36) = 30.65, p < 0.001; α-PVP: F(3, 36) = 53.78, p < 0.001; α-BBB: F(3, 36) = 22.23, p < 0.001; α-PPP: F(3, 36) = 38.06, p < 0.001]. Post-hoc tests revealed that 1.0–3.0 mg/kg MDVP, 3.0–10.0 mg/kg α-PVP, 3.0–10.0 mg/kg α-BBB, and 10.0–30.0 mg/kg α-PPP produced significant locomotor increases compared to saline. Significant main effects for time and significant interactions were also observed for all compounds, as illustrated in Fig. 4. For the sake of comparison, the results for the saline group are shown in each panel. Over the

| Table 1 |
| --- | --- | --- |
| **Test drug** | **[3H]Dopamine uptake, IC50 (nM)** | **[3H]Noradrenaline uptake, IC50 (nM)** | **[3H]Serotonin uptake, IC50 (nM)** |
| **DAT/SERT ratio** |
| MDVP | 4.1 ± 0.6 | 25.9 ± 5.6 | 3305 ± 485 | 806 |
| α-PVP | 12.8 ± 1.2 | 142.2 ± 1.2 | >10,000 | >781 |
| α-BBB | 65.3 ± 5.7 | 91.5 ± 12.8 | >10,000 | >159 |
| α-PPP | 196.7 ± 9.9 | 447.7 ± 39.2 | >10,000 | >51 |
| Cocaine | 211 ± 19 | 292 ± 34 | 313 ± 17 | 1.5 |
| Amphetamine | 93 ± 17 | 67 ± 16 | 3418 ± 314 | 37 |

* Baumann et al. (2013b).
course of the 60 min session, habituation occurred in the saline group, resulting in attenuation of activity in later bins. For MDPV, post hoc analysis of the significant interaction \[F(15, 180) = 2.31, p < 0.01\] revealed a session-long effect, with 1.0–3.0 mg/kg producing significant increases in beam breaks compared to saline for the duration of the session. \(\alpha\)-PVP also produced a significant interaction \[F(15, 180) = 2.52, p < 0.01\]. While 3.0–10.0 mg/kg \(\alpha\)-PVP significantly increased beam breaks for the duration of the session, the 1.0 mg/kg dose significantly increased beam breaks only during the 20–50 min post-injection interval. Post-hoc analysis of the significant interaction observed with \(\alpha\)-PPP \[F(15, 180) = 6.56, p < 0.001\] revealed that 10.0 mg/kg produced significant increases in beam breaks compared to saline for all time points, whereas the 3.0 mg/kg dose significantly increased beam breaks only for the first 40 min. For \(\alpha\)-PPP, post hoc analysis of the significant interaction \[F(15, 180) = 5.02, p < 0.001\] showed that 30.0 and 10.0 mg/kg produced significant increases in beam breaks for the duration of the session and for the first 50 min of the session, respectively.

3.3. Locomotor antagonist testing

Combinations of the D1 receptor antagonist SCH23390 (0.03 mg/kg) and a single dose of each drug or saline were tested in the locomotor activity procedure. Fig. 5 shows that antagonist pretreatment attenuated the hyperactivity produced by all pyrrolidinophenones at the doses administered. The data represent total beam breaks over 60 min motor activity sessions. When mice received 1.0 mg/kg MDPV, there was a main effect of pretreatment \([F(1, 28) = 88.28, p < 0.001]\), drug \([F(1, 28) = 91.85, p < 0.001]\), and a significant interaction \([F(1, 28) = 27.39, p < 0.001]\), indicating that SCH23390 significantly decreased beam breaks for MDPV-treated mice. Administration of 3.0 mg/kg \(\alpha\)-PVP produced a main effect of pretreatment \([F(1, 28) = 45.50, p < 0.001]\), drug \([F(1, 28) = 64.05, p < 0.001]\), and a significant interaction \([F(1, 28) = 16.92, p < 0.001]\), with SCH23390 significantly decreasing beam breaks for the \(\alpha\)-PVP group. When 10.0 mg/kg of \(\alpha\)-PPP was administered there was a main effect of pretreatment \([F(1, 28) = 61.65, p < 0.001]\), drug \([F(1, 28) = 64.85, p < 0.001]\), and a significant interaction \([F(1, 28) = 31.18, p < 0.001]\), indicating that SCH23390 significantly decreased beam breaks for \(\alpha\)-PPP-treated mice. Mice that received 30.0 mg/kg \(\alpha\)-PPP showed a main effect of pretreatment \([F(1, 28) = 18.12, p < 0.001]\), drug \([F(1, 28) = 38.74, p < 0.001]\), and a significant interaction \([F(1, 28) = 5.30, p < 0.05]\), indicating that SCH23390 significantly decreased beam breaks for the \(\alpha\)-PPP group. Administration of SCH23390 plus saline did not significantly affect activity compared to saline/saline group.

3.4. Functional observational battery

Results of significant drug effects for the FOB are displayed in Table 2; behavioral data for cocaine and methamphetamine from a previously published study (Marusich et al., 2012) are included for comparison. Some drugs produced small non-significant effects on hypoaactivity, salivation, stereotyped biting, and stereotyped licking; therefore, these measures are not included in Table 2. MDPV produced significant ataxia \([H(3) = 15.94, p < 0.0033]\) at the 3.0 mg/kg dose, as compared to saline. \(\alpha\)-PPP produced significant retropulsion compared to saline at 56.0 mg/kg \([H(3) = 18.92, p < 0.0033]\). All doses of all pyrrolidinophenones significantly increased exploration compared to saline \([MDPV: \ H(3) = 18.41, p < 0.0033]; \ \alpha\)-PVP: \(H(3) = 25.98, p < 0.0033]; \ \alpha\)-PBP: \(H(3) = 25.98, p < 0.0033]; \ \alpha\)-PPP: \(H(3) = 29.32, p < 0.0033]\). MDPV produced bizarre behavior such as rearing while facing away from the wall, climbing, and jumping \([H(3) = 27.92, p < 0.0033]\), with 1.0–3.0 mg/kg producing significant differences from saline.

MDPV, \(\alpha\)-PVP, and \(\alpha\)-PPP significantly increased circular ambulations \([MDPV: \ H(3) = 24.37, p < 0.0033]; \ \alpha\)-PVP: \(H(3) = 26.01, p < 0.0033]; \ \alpha\)-PBP: \(H(3) = 19.60, p < 0.0033]\). The 3.0–10.0 mg/kg doses of MDPV, all doses of \(\alpha\)-PVP, and 10.0–30.0 mg/kg \(\alpha\)-PPP produced significant differences from saline. \(\alpha\)-PPP produced a significant effect on grooming \([H(3) = 15.19, p < 0.0033]\), with 30 mg/kg producing a significant decrease compared to saline. All doses of \(\alpha\)-PVP produced significant flattened body posture compared to saline \([H(3) = 15.55, p < 0.0033]\). All drugs produced a significant increase in hyperactivity \([MDPV: \ H(3) = 19.46, p < 0.0033]\),
Table 2
Effects of pyrrolidinophenone drugs, cocaine (COC), and methamphetamine (METH) in the FOB. Arrows denote the direction of the difference from saline with corrected alpha level (p < 0.0033). Doses (mg/kg) at which significant effects occurred are shown below arrows. For comparison purposes, doses at which locomotor activity was significantly increased during the first 10-min bin of the locomotor activity sessions are also shown.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>MDPV</th>
<th>α-PVP</th>
<th>α-PBP</th>
<th>α-PPP</th>
<th>COC</th>
<th>METH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion (first 10 min)</td>
<td>↑ 1–3</td>
<td>↑ 3–10</td>
<td>↑ 3–10</td>
<td>↑ 10–30</td>
<td>↑ 10–42</td>
<td>↑ 1–5.6</td>
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<tr>
<td>Ataxia</td>
<td>↑</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Retropulsion</td>
<td>↑</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exploration</td>
<td>↑ 1–3</td>
<td>↑ 3–10</td>
<td>↑ 3–10</td>
<td>↑ 10–30</td>
<td>↑ 10–42</td>
<td>↑ 10–56</td>
</tr>
<tr>
<td>Bizarre behavior</td>
<td>↑ 1–3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circular ambulations</td>
<td>↑ 3–10</td>
<td>↑ 3–17</td>
<td>↑ 3–30</td>
<td>↑ 10–42</td>
<td>↑ 1–10</td>
<td></td>
</tr>
<tr>
<td>Grooming</td>
<td>↑</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flattened body posture</td>
<td>↑ 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>↑ 3–10</td>
<td>↑ 3–17</td>
<td>↑ 3–30</td>
<td>↑ 10–30</td>
<td>↑ 10–42</td>
<td>↑ 1–10</td>
</tr>
<tr>
<td>Stereotyped head weaving</td>
<td>↑ 3–17</td>
<td>↑ 3–30</td>
<td>↑ 10–56</td>
<td>↑ 10 &amp; 17</td>
<td>↑ 1–10</td>
<td></td>
</tr>
<tr>
<td>Stereotyped head circling</td>
<td>↑ 10</td>
<td>↑ 3–17</td>
<td>↑ 10 &amp; 56</td>
<td>↑ 10–42</td>
<td>↑ 1–10</td>
<td></td>
</tr>
<tr>
<td>Stimulation</td>
<td>↑ 1–10</td>
<td>↑ 3–17</td>
<td>↑ 3–30</td>
<td>↑ 10–56</td>
<td>↑ 10–42</td>
<td>↑ 1–10</td>
</tr>
</tbody>
</table>

* Marusich et al. (2012).

Fig. 4. Time course effects of pyrrolidinophenone drugs on locomotor activity, plotted as a function of 10 min bins during a 60-min test session. Values represent mean ± SEM expressed as number of beam breaks for each dose (n = 8 per dose except n = 16 for saline). Asterisks (*) indicate doses and time points that showed significant increases in beam breaks compared to saline at the same time point. Panel A shows data for MDPV, panel B shows data for α-PVP, panel C shows data for α-PBP, and panel D shows data for α-PPP.

Fig. 5. Effects of vehicle or SCH23390 (0.03 mg/kg) tested in combination with vehicle or a test drug on locomotor activity during 60 min sessions. Values represent mean ± SEM expressed as total beam breaks for each dose (n = 8). Asterisks (*) indicate a significant main effect (p < 0.05) of SCH23390 (right side of panel) compared to vehicle (left side of panel).
movements [MDPV: \( H(3) = 21.14, p < 0.0033; \alpha\)-PVP: \( H(3) = 23.85, p < 0.0033; \alpha\)-PPP: \( H(3) = 23.78, p < 0.0033\)], with 10.0 mg/kg MDPV, all doses of \( \alpha\)-PVP, and 10.0 and 50.0 mg/kg \( \alpha\)-PPP producing significant differences from saline. All doses of all drugs produced a significant increase in stimulation compared to saline [MDPV: \( H(3) = 24.47, p < 0.0033; \alpha\)-PVP: \( H(3) = 29.64, p < 0.0033; \alpha\)-PBP: \( H(3) = 17.72, p < 0.0033; \alpha\)-PPP: \( H(3) = 19.54, p < 0.0033\)].

4. Discussion

Results of the present study demonstrate that MDPV and \( \alpha\)-PVP are potent uptake blockers at DAT and NET, with much weaker effects at SERT. \( \alpha\)-PBP and \( \alpha\)-PPP are also catecholamine-selective uptake blockers but display reduced potency. Our in vitro results agree with those of Melitzer et al. (2006) who demonstrated that analogs of the pyrrolidinophenone compound, pyrvalerone, are potent catecholamine uptake blockers. Importantly, none of the compounds tested here are transporter substrates. In general, drugs interacting at monoamine transporters can be classified either as blockers which inhibit transmitter uptake, or as substrates (i.e., releasers) which cause transmitter release by reversing the normal direction of transmitter flux (Baumann et al., 2013a, 2013b). Previous studies using rat brain synaptosomes or cell lines expressing human DAT, NET and SERT have shown that MDPV is a transporter blocker and not a transporter substrate (Baumann et al., 2013b; Cameron et al., 2013; Eshleman et al., 2013; Simmler et al., 2013).

The present in vitro data extend previous findings to reveal that the presence of a pyrrolidine ring in any cathinone-like compound confers potent uptake blocking properties at DAT and NET. Thus, pyrrolidinophenones are mechanistically distinct from ring-substituted cathinones, such as mephedrone and methylene, which act as non-selective substrates for monoamine transporters and trigger transmitter release (Baumann et al., 2012; Cameron et al., 2013; Simmler et al., 2013). A comparison with our previous in vitro data reveals that all the pyrrolidinophenones tested in the present study are more potent than cocaine as DAT blockers, and all except \( \alpha\)-PPP are more potent than amphetamine as DAT blockers. All pyrrolidinophenones except \( \alpha\)-PPP are more potent NET blockers than cocaine, while MDPV and \( \alpha\)-PVP are more potent NET blockers than amphetamine (Baumann et al., 2013b). The data included in Table 1 for cocaine and amphetamine were not collected at the same time as data for pyrrolidinophenones in the present study, but all of the in vitro experiments from both studies were conducted using similar assay conditions. Importantly, MDPV and \( \alpha\)-PVP are similar in potency and transporter selectivity, indicating that the presence of the 3,4-methylenedioxy substituent in MDPV does not exert much influence on the profile of transporter activity. By contrast, alkyl chain length extending from the \( \alpha\)-carbon is a critical structural feature, with shorter chain length (i.e., \( \alpha\)-PPP, methyl) yielding less potent transporter-blocking properties when compared to longer chain length (i.e., \( \alpha\)-PVP, propyl).

Consistent with their activity as catecholamine uptake blockers, all of the test drugs stimulate locomotion, and the rank order of in vivo potency parallels their potencies for blockade of DAT activity: MDPV > \( \alpha\)-PVP > \( \alpha\)-PBP > \( \alpha\)-PPP. From a comparative perspective, all of the pyrrolidinophenones tested are more potent than cocaine at blocking DAT and at stimulating locomotion (Baumann et al., 2013b; Marusich et al., 2012). The time course of locomotor effects produced by the pyrrolidinophenones is comparable to what has been seen previously for various doses of cocaine and low doses of methamphetamine, with locomotor effects dissipating over the course of 60 min. In contrast, higher doses of methamphetamine can sustain locomotor increases throughout a 90 min session (Marusich et al., 2012). The present results confirm previous reports that MDPV is a powerful locomotor stimulant (Aarde et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013; Marusich et al., 2012), and extend this observation to other pyrrolidinophenones. Additionally, the vivo potency findings indicate that DAT blockade, inhibition of dopamine uptake, and the ensuing increase in extracellular dopamine seem essential for stimulant effects. Results of the FOB show that all test drugs produce typical psychomotor stimulant actions, similar to what we have found previously for cocaine and methamphetamine (Marusich et al., 2012), though each drug produces somewhat unique effects at higher doses. At the higher doses, hyperactivity was occasionally accompanied by bizarre behaviors such as jumping or rearing while facing away from the wall, or other atypical behavior such as ataxia, flattened body posture, or retropulsion. These behaviors are similar to those observed previously with other structural classes of synthetic stimulants found in bath salts (Marusich et al., 2012). It is noteworthy that our previous study comparing the in vivo effects of synthetic stimulants to cocaine and methamphetamine (Marusich et al., 2012) was conducted under identical conditions to the experiments described here, making the results from the two studies comparable.

The antagonist experiments with SCH23390 support the role of dopamine D1 receptors in mediating locomotor effects of pyrrolidinophenones. Activation of D1 receptors expressed on medium spiny output neurons in the striatum has been implicated in psychomotor stimulant effects of cocaine, amphetamine and other stimulants, suggesting that this mechanism may also be relevant to the acute actions of pyrrolidinophenones (Lobo and Nestler, 2011; Smith et al., 2013). While few studies have examined the effects of D1 antagonists on behaviors induced by newer synthetic stimulants, previous investigations have found that SCH23390 inhibits locomotor activation produced by mephedrine in rats (Lisek et al., 2012), and produces increased self-administration of cathinone in rats (Gosnell et al., 1996). Such results suggest that the D1 receptor subtype mediates the effects of cathinone-related stimulants on various types of rodent behavior, in addition to those behaviors examined in the present study. Research with ring-substituted amphetamine analogs has shown that DAT-selective compounds produce powerful stimulant and abuse-related effects in rodents (Bauer et al., 2013; Baumann et al., 2011). Compounds with mixed DAT and SERT activity have reduced stimulant qualities, apparently due to serotonergic dampening of dopamine-mediated effects (Baumann et al., 2011; Wee et al., 2005). Therefore, it might be predicted that the catecholamine selectivity of pyrrolidinophenones may render these agents especially addictive, due to lack of inhibitory serotonergic effects. This hypothesis warrants further investigation. Additionally, given the affinity of the pyrrolidinophenones for NET, future studies should test the ability of norepinephrine receptor antagonists to influence the behavioral effects of these stimulants. It will also be crucial to examine the pharmacology of any identified brain penetrant metabolites of \( \alpha\)-PVP, \( \alpha\)-PBP, and \( \alpha\)-PPP, since bioactive metabolites may play a role in the in vivo effects of these drugs.

Human drug users prefer stimulants that possess a quick onset and short duration of action, similar to that of cocaine (Fischman, 1989), so humans are likely to prefer rapidly-acting pyrrolidinophenones over other types of stimulants. Due to the high potency of MDPV and \( \alpha\)-PVP in comparison to prototypic stimulants such as cocaine, these synthetic stimulants may have higher abuse potential. Synthetic cathinone abuse can cause severe and life-threatening side effects in humans. MDPV has been implicated as a main culprit in causing toxic effects such as tachycardia, agitation, psychosis, and violent behaviors (Murray et al., 2012; Penders et al., 2012; Spiller et al., 2011; Wyman et al., 2013). One of the more severe side effects is excited delirium, a syndrome often accompanied by hyperthermia, rhabdomyolysis and kidney failure.
present results imply that the more potent pyrrolidinophenones, along with bizarre behaviors at higher doses. Our drugs produce typical psychomotor stimulant effects at low doses, SCH23390. A functional observational battery showed that all test activation reversed by the dopamine D 1 receptor antagonist SCH23390. A functional observational battery showed that all test

5. Conclusion


