Neurochemical and Cardiovascular Effects of 4-Chloro Ring-Substituted Synthetic Cathinones in Rats

Michael R. Chojnacki, Eric B. Thorndike, John S. Partilla, Kenner C. Rice, Charles W. Schindler, and Michael H. Baumann

Designer Drug Research Unit (M.R.C., J.S.P., C.W.S., M.H.B.) and Preclinical Pharmacology Section (E.B.T., C.W.S.), National Institute on Drug Abuse Intramural Research Program, Baltimore, Maryland; and Drug Design and Synthesis Section, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism Intramural Research Programs, Rockville, Maryland (K.C.R.)

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ABSTRACT

Synthetic cathinones are a class of new psychoactive substances that display psychomotor stimulant properties, and novel cathinone analogs continue to emerge in illicit drug markets worldwide. The aim of the present study was to characterize the pharmacology of 4-chloro ring-substituted cathinones that are appearing in illicit drug markets compared with the effects of 4-methylmethcathinone (mephedrone). Synaptosomes were prepared from rat caudate for dopamine transporter (DAT) assays or from whole brain minus caudate and cerebellum for norepinephrine transporter (NET) and serotonin transporter (SERT) assays. Findings from transporter uptake inhibition and release assays showed that mephedrone and 4-chloromethcathinone (4-CMC) function as substrates at DAT, NET, and SERT, with similar potency at all three transporters. In contrast, 4-chloro-2-pyrrolidinopropiophenone (4-CzPPP) was an uptake inhibitor at DAT and NET, with similar potency at each site, but had little activity at SERT. 4-Chloroethcathinone (4-CEC) was a low-potency uptake inhibitor at DAT and NET but a substrate at SERT. In rats implanted with telemetry transmitters, mephedrone and 4-CMC increased blood pressure, heart rate, and locomotor activity to a similar extent. 4-CEC and 4-CzPPP were less potent at increasing blood pressure and had modest stimulatory effects on heart rate and activity. 4-CMC also transiently decreased temperature at the highest dose tested. All three 4-chloro ring-substituted cathinones are biologically active, but only 4-CMC has potency comparable to mephedrone. Collectively, our findings suggest that 4-CMC and other 4-chloro cathinones may have abuse potential and adverse effects in humans that are analogous to those associated with mephedrone.

SIGNIFICANCE STATEMENT

The 4-chloro ring-substituted cathinones all produced significant cardiovascular stimulation, with 4-chloromethcathinone (4-CMC) showing potency similar to mephedrone. All of the drugs are likely to be abused given their effects at the dopamine transporter, particularly 4-CMC.

Introduction

Synthetic cathinones are part of the constantly evolving group of drugs referred to as new psychoactive substances (NPSs) (Baumann et al., 2014; Rudin et al., 2021). The synthetic cathinones have pharmacological and toxicological effects that are similar to other psychomotor stimulants, and many structural analogs have been described. Synthetic cathinones represent a significant public health threat since the compounds are associated with serious intoxications and adverse effects in human users (La Maida et al., 2021; Soares et al., 2021). A small subset of these compounds includes the 4-chloro ring-substituted cathinones (see Fig. 1), where the analogs display a chloro group at the para position on the phenyl ring but show variation at the terminal amine position. These variations in structure may have profound effects on drug action at the biochemical, cellular, and organismic level (reviewed in Baumann et al., 2018). Structure activity relationship (SAR) studies for other para-substituted analogs have shown that the volume of the 4-position substituent can impact activity at serotonin transporters (SERTs) relative to dopamine transporters (DATs) (Rickli et al., 2015; Esheleman et al., 2017; Blough et al., 2019). More specifically, increasing the steric bulk at the 4-position ring-substituent increases the relative potency at SERT versus DAT, which tends to reduce abuse potential of the compounds (Bonano et al., 2015; Sakloth et al., 2015; Negus and Banks, 2017). Aside from their neurochemical and behavioral effects, psychomotor stimulants like amphetamine and cocaine are known to have cardiovascular consequences such as increases in...
heart rate (HR) and blood pressure (BP) (Havakuk et al., 2017; Schwarzbach et al., 2020). The cardiovascular effects of stimulants are due, at least in part, to their ability to elevate catecholamine levels (Egashira et al., 1991; Venton et al., 2006), which mediate changes in HR and BP via the autonomic nervous system. In particular, many stimulant drugs act as transportable substrates or uptake inhibitors at the noradrenaline transporter (NET), thereby increasing extracellular concentrations of noradrenaline from sympathetic nerve terminals (e.g., Schindler et al., 2021). Numerous synthetic cathinones are known to produce hypertension and tachycardia in both laboratory animals and humans (Spiller et al., 2011; Sutamtewagul et al., 2014; Schindler et al., 2016, 2020). For example, 4-methylmethcathinone (mephedrone), a commonly abused synthetic cathinone, increases HR and BP when administered to human subjects in a controlled laboratory setting (Papaseit et al., 2016). As far as we are aware, no studies have examined the cardiovascular effects of 4-chloro ring-substituted cathinones in laboratory animals or humans.

Given the structural similarity of synthetic cathinones to other stimulants, we wanted to investigate the neurochemical and cardiovascular effects of the 4-chloro ring-substituted cathinone analogs 4-chloromethcathinone (4-CMC), 4-chloroethcathinone (4-CEC), and 4-chloro-α-pyrrolidinopropiophenone (4-CaPPP) in comparison with the effects produced by mephedrone. Although the pharmacology of mephedrone has been studied extensively (Hadlock et al., 2011; Baumann et al., 2012; Eshleman et al., 2013; Simmler et al., 2013), less is known about 4-chloro ring-substituted cathinones. 4-CMC is known to act as a substrate (i.e., releaser) at monoamine transporters (Bonano et al., 2015; Eshleman et al., 2017; Blough et al., 2019). 4-CMC also affects intracranial self-stimulation (ICSS) in rats (Bonano et al., 2015) in a manner that suggests potential for abuse. Wojcieszak et al. (2020, 2021) reported that 4-CMC increases locomotor activity in mice but does not engender conditioned place preference (CPP), a measure of potential rewarding effects. 4-CMC is reported to have toxic effects (Luethi et al., 2019; Zhou et al., 2020) and has been detected in samples taken from human drugged driving cases and overdose autopsies (Tomeczak et al., 2018). Indeed, forensic investigations demonstrate that 4-CMC is associated with serious intoxications and even death in human users (La Maida et al., 2021). 4-CEC has been found in samples collected for the analysis of potentially abused substances (Grifell et al., 2017), and the drug increases locomotor activity in mice and generalizes to methamphetamine and cocaine in rat drug discrimination studies (Gatch et al., 2021). Xu et al. (2021) showed that rats self-administer 4-CaPPP, and the drug also generalizes to the methamphetamine stimulus cue in rat drug discrimination experiments.

In the current study, we examined the effects of 4-CMC, 4-CEC, 4-CaPPP, and mephedrone on transported-mediated uptake inhibition and release at DAT, NET, and SERT in vitro. For the in vivo physiologic studies, male rats received surgically implanted biotelemetry devices and were tested with each cathinone to determine effects on BP, HR, body temperature, and locomotor activity. Finally, antagonist studies were carried out to examine potential underlying mechanisms for the cardiovascular effects observed.

Materials and Methods

Drugs and Reagents. The synthetic cathinones 4-chloromethcathinone (4-CMC), 4-chloroethcathinone (4-CEC), 4-chloro-α-pyrrolidinopropiophenone (4-CaPPP) and 4-methylmethcathinone (mephedrone) were synthesized as HCl salts. Each compound was fully characterized by high resolution mass spectral and 400 MHz NMR analyses and gave appropriate combustion analyses for carbon, hydrogen, and nitrogen. Each compound was chromatographically homogenous by thin layer chromatography. Chemical purity for each compound was estimated to be greater than 98%. Other than the synthetic cathinones and radioligands, all chemicals and reagents were acquired from Sigma-Aldrich (prazosin HCl and R-SCH23390 HCl; St. Louis, MO) or Tocris (chlorisondamine diiodide; Minneapolis, MN). Drugs for the in vivo experiments were dissolved in saline or sterile water, and doses are expressed as the salts.

Animals. All animal procedures were approved by the National Institute on Drug Abuse (NIDA), Intramural Research Program (IRP), and Animal Care and Use Committee and followed the guidelines as outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Animals were housed in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Transporter Release and Uptake Assays in Rat Brain Synaptosomes. Twenty-four adult male Sprague-Dawley rats (Envigo, Frederick, MD) were used for the synaptosome assays (rats weighing 250–350 g). Rats were group housed in a temperature (22.2 ± 1.1 °C) and humidity (45% ± 10%)-controlled room on a 12-hour light/dark cycle (lights on at 0700) with free access to water. Rats were killed by CO2 narcosis, and synaptosomes were prepared from brains using standard procedures (Rothman et al., 2003). Transporter uptake inhibition assays were performed as described previously (Solis et al., 2017; Schindler et al., 2021). In brief, synaptosomes were prepared from caudate tissue for DAT assays or from whole brain minus caudate and cerebellum for NET and SERT assays. For release assays, 9 nM [3H]-methyl-4-phenylpyridinium (MPP+; PerkinElmer, Waltham MA) was used as the radiolabeled substrate for DAT and NET, whereas 5 nM [3H]serotonin ([3H]-5-HT; PerkinElmer) was used for SERT. All buffers used in the release assay 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]-5-HT by competing transporters. Synaptosomes were preloaded with radiolabeled substrate in Krebs-phosphate buffer for 1 hour. Release assays were initiated by incubating preloaded synaptosomes with the test drug. Release was terminated by vacuum filtration, and retained radioactivity was quantified by liquid scintillation counting. Effects of test drugs on release were expressed as percent
maximum release, with maximal release (i.e., 100% \( E_{\text{max}} \)) defined as the release produced by tyramine at doses that evoke the efflux of all 'releasable' tritium by synaptosomes (10 \( \mu \)M tyramine for DAT and NET assay conditions and 100 \( \mu \)M tyramine for SERT assay conditions). For uptake inhibition assays, 5 nM \(^{3}H\)dopamine, 10 nM \(^{3}H\)norepinephrine, and 5 nM \(^{3}H\)5-HT (all radiolabeled neurotransmitters from PerkinElmer) were used for DAT, NET, and SERT assays, respectively. To optimize uptake for a single transporter, unlabeled blockers were included that prevented uptake of \(^{3}H\)transmitter by competing transporters. Uptake inhibition was initiated by incubating synaptosomes with test compound and \(^{3}H\)transmitter in Krebs-phosphate buffer. Uptake assays were terminated by rapid vacuum filtration, and retained radioactivity was quantified with liquid scintillation counting (Baumann et al., 2013). Effects of test drugs on release and uptake inhibition were analyzed by nonlinear regression using GraphPad Prism v9.5.0 (GraphPad Scientific, San Diego, CA). Dose–response values for the uptake inhibition and release were fit to the equation \( Y(x) = Y_{\text{max}} + (Y_{\text{max}} - Y_{\text{min}}) \frac{1 + 10^{\log(P_{50} - \log(x))}}{1 + 10^{\log(P_{50} - \log(x))}} \times n \), where \( x \) is the concentration of the compound tested, \( Y(x) \) is the response measured, \( Y_{\text{max}} \) is the maximal response, \( P_{50} \) is either IC\(_{50} \) (the concentration that yields half-maximal uptake inhibition response) or EC\(_{50} \) (the concentration that yields half-maximal release), and \( n \) is the Hill slope parameter.

**In Vivo Biotelemetry.** A total of seven adult male Long-Evans rats (Charles River, Kingston, NY) were used as subjects. The rats were purchased by Data Sciences International (DSI) (St. Paul, MN) and were subsequently implanted with HD-S10 biotelemetry transmitters. The abdominal muscles and skin were then closed. Rats were treated with subcutaneous meloxicam after surgery. After recovery at 7-day quarantine, the rats were individually housed in a temperature (22.2 ± 1.1°C)- and humidity (45% ± 10%)-controlled room on a 12-hour reverse light/dark cycle (lights off at 0700) with free access to water. Food was restricted to maintain a constant or slightly elevated BP and HR when no more frequently than twice per week, typically Tuesday and Friday. Doses of the pretreatment drugs were chosen based on previous results (Schindler et al., 2016, 2019). Rats were 10 to 16 months of age over the course of the experiment.

Data from the transmitters were polled for 10 seconds every minute. The transmitters supplied readings for mean arterial pressure (BP based on the transmitter calibration supplied by the manufacturer), HR (derived from the BP signal), temperature, and activity. Activity was measured continuously by tracking the transmitter radio signal relative to two receiver antennas in perpendicular horizontal planes; therefore, the activity measures have no units. Representative time course data for the effects of 4-CMC were analyzed by two-way within-subject analysis of variance (dose × time). For the dose-effect analysis and pretreatment studies, mean data for drug effects measured over the first hour of the session were subjected to within-subject analysis of variance or mixed-effect analysis as appropriate (Prism v9.5.0). Follow up post hoc tests were carried out using Dunnett’s multiple comparison test to compare drug effects to control for the dose-effect studies or Tukey’s multiple comparison test for the pretreatment studies.

**Results**

**In Vitro Uptake Inhibition and Release.** We first tested the effects of the cathinone compounds in transporter uptake inhibition and release assays in rat brain synaptosomes. It is important to note that all drugs targeting monoamine transporters will function as efficacious inhibitors of transmitter uptake, whereas only transportable substrates (i.e., releasers) are able to evoke transporter-mediated efflux in the release assay. Thus, pure uptake inhibitors are only active in the uptake assays, whereas substrates are active in both the uptake and release assays. Figure 2 shows dose-effect functions for the compounds at transporter-mediated uptake inhibition and release at DAT, NET, and SERT. Table 1 shows IC\(_{50} \) values for uptake inhibition and EC\(_{50} \) values for release at the three transporters. As expected, mephedrone and 4-CMC functioned as fully efficacious uptake inhibitors and substrate-type releasers at all three transporters. Both compounds were nonselective transporter substrates with roughly similar potencies for inducing release at DAT, NET, and SERT. In contrast, 4-CaPPP was an uptake blocker at DAT and NET, with much weaker effects at SERT, and the drug did not function as a substrate at any transporter (i.e., see flattened release curves). 4-CEC showed low potency to block uptake at the DAT and NET but greater potency at SERT. More importantly, 4-CEC displayed no substrate activity at DAT, weak partial efficacy as a substrate at NET, and full efficacy as a substrate at SERT. The peculiar activity of 4-CEC has been previously characterized as “hybrid” transporter activity, whereby the drug produces uptake inhibition at DAT but substrate activity at SERT (Blough et al., 2014; Saha et al., 2015).

**Telemetry.** Figure 3 shows representative time course data for the telemetry experiments in those rats treated with 4-CMC or saline vehicle. After saline administration, rats showed slightly elevated BP and HR when first put into the chambers, but these effects rapidly decreased to a stable baseline after...
Effects of synthetic cathinones on inhibition of 
uptake (upper panels) and stimulation of release (lower panels) at dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters in rat brain synaptosomes. For uptake assays, synaptosomes were incubated with different concentrations of test drugs in the presence of 5 nM [3H]dopamine, 10 nM [3H]norepinephrine, or 5 nM [3H]serotonin. Data are expressed as a percentage of transmitter uptake (± S.D.) for three experiments. For release assays, synaptosomes were preloaded with 9 nM [3H]MPP⁺ (DAT and NET) or 5 nM [3H]5-HT (SERT) and then incubated with different concentrations of test drugs to evoke release via reverse transport. Data are expressed as a percentage of [3H]substrate release (± S.D.) for three experiments.

Table 1

Effects of synthetic cathinones analogs on inhibition of [3H]neurotransmitter uptake and release of [3H]MPP⁺ via DAT and NET or [3H]5-HT via SERT in rat brain synaptosomes

<table>
<thead>
<tr>
<th>Drug</th>
<th>Uptake Inhibition at DAT EC₅₀ (nM) [% Eₘₐₓ]</th>
<th>Uptake Inhibition at NET EC₅₀ (nM) [% Eₘₐₓ]</th>
<th>Uptake Inhibition at SERT EC₅₀ (nM) [% Eₘₐₓ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-CMC</td>
<td>1014 ± 78 [110]</td>
<td>542 ± 38 [109]</td>
<td>&gt;10,000 [109]</td>
</tr>
<tr>
<td>4-CEC</td>
<td>1455 ± 110 [81]</td>
<td>589 ± 44 [109]</td>
<td>&gt;10,000 [109]</td>
</tr>
<tr>
<td>4-C₇PPP</td>
<td>569 ± 57 [81]</td>
<td>764 ± 79 [107]</td>
<td>&gt;10,000 [109]</td>
</tr>
</tbody>
</table>

**Note:** Eₘₐₓ is defined as percentage of maximal releasing response as described in Materials and Methods.
4-Cz-PPP also significantly elevated BP ($F(1.659,6.635) = 6.621, P = 0.0293$) and HR ($F(2.046,8.815) = 9.197, P = 0.0079$) at 3 mg/kg when compared with saline. The increases for both BP and HR were higher at 10 mg/kg but failed to reach significance (0.08 and 0.0537, respectively). There was no significant effect on motor activity or temperature at any dose.

In the final set of experiments, we wished to examine possible mechanisms underlying the in vivo effects of 4-CMC since this specific drug had the most robust effects in the dose-response study. The effects of drug pretreatments on the responses produced by 10 mg/kg 4-CMC are depicted in Fig. 5. During the first hour after pretreatment with either water or saline vehicle, 10 mg/kg 4-CMC again increased BP (see Fig. 5: prazosin vehicle + 4-CMC, left panel $F(1.782,7.127) = 59.68, P < 0.0001$; SCH23390 vehicle + 4-CMC, middle panel $F(1.946,9.731) = 20.52, P = 0.0003$; chlorisondamine vehicle + 4-CMC, right panel $F(1.666,6.663) = 67.72, P < 0.0001$) compared with vehicle + vehicle control groups. In a similar manner, 4-CMC increased HR (prazosin vehicle + 4-CMC, $F(1.125,4.502) = 13.16, P = 0.0171$; SCH23390 vehicle + 4-CMC, $F(1.080,3.958) = 61.17, P = 0.0014$; chlorisondamine vehicle + 4-CMC, $F(1.881,7.523) = 25.51, P < 0.0001$) and locomotor activity (prazosin vehicle + 4-CMC, $F(1.783,7.132) = 38.53, P = 0.0002$; SCH23390 vehicle + 4-CMC, $F(1.093,3.959) = 61.17, P = 0.0014$; chlorisondamine vehicle + 4-CMC, $F(1.881,7.523) = 25.51, P = 0.0005$) and locomotor activity (prazosin vehicle + 4-CMC, $F(1.783,7.132) = 38.53, P = 0.0002$; SCH23390 vehicle + 4-CMC, $F(1.102,5.508) = 13.46, P = 0.0114$; chlorisondamine vehicle + 4-CMC, $F(1.212,4.847) = 7.980, P = 0.0356$). For the chlorisondamine pretreatment condition, the effect of subsequent 4-CMC administration was not significantly different from saline + saline control on BP ($P = 0.0546$). For the SCH23390 pretreatment condition, the effect of 4-CMC on activity was not significantly different ($P = 0.0838$) from water + saline control. In contrast to the dose-effect testing, there was no significant decrease in temperature for 10 mg/kg 4-CMC ($P = 0.3289$, data not shown).

Pretreatment with the alpha-adrenergic antagonist prazosin by itself caused hypotension ($P = 0.0060$), and when given as a pretreatment, prazosin reversed the increase in BP caused by 10 mg/kg 4-CMC ($P < 0.0001$). Prazosin did not affect HR or motor activity by itself, nor did it alter the changes caused by 4-CMC. Pretreatment with dopamine D1 antagonist SCH23390 did not significantly affect BP, HR, or activity but did significantly attenuate the increase in HR ($P = 0.0014$) caused by 10 mg/kg 4-CMC. For the activity measure, SCH23390 + 4-CMC was not different from water vehicle + 4-CMC, but SCH23390 + saline was significantly different from water vehicle + 4-CMC ($P = 0.0232$). The ganglionic blocker chlorisondamine had hypotensive effects ($P = 0.0014$), and this drug pretreatment reversed any increase in BP caused by 4-CMC ($P = 0.0016$). Chlorisondamine appeared to slightly decrease the 4-CMC induced tachycardia, but this...
effect was not significant. Chlorisondamine had no effect on motor activity induced by 4-CMC.

**Discussion**

As previously reported (Baumann et al., 2012; Eshleman et al., 2013; Simmler et al., 2013), we found that mephedrone is a transporter substrate that induces release at DAT, NET, and SERT. 4-CMC had nonspecific transporter releasing activity that mimicked the effects of mephedrone, congruent with findings from brain tissue (Bonano et al., 2015; Blough et al., 2019) and cells expressing human DAT, NET, and SERT (Eshleman et al., 2017; Luethi et al., 2019). The similar mechanism of action for mephedrone and 4-CMC is likely due to the similar size of the 4-methyl and 4-chloro substituents found on these compounds (Negus and Banks, 2017). Importantly, 4-CMC showed nearly identical potency to mephedrone at DAT, NET, and SERT, suggesting similar effects in vivo (Bonano et al., 2015; Negus and Banks, 2017).

Increasing the carbon chain length at the amine position of 4-CMC from a methyl to an ethyl (i.e., 4-CEC) reduced substrate activity at NET and abolished substrate activity at DAT. However, there was little difference in SERT substrate activity between 4-CMC and 4-CEC. In short, 4-CEC displays hybrid transporter activity whereby the drug acts as an uptake blocker at DAT but a substrate-type releaser at NET and SERT (Blough et al., 2014; Saha et al., 2015). The fact that 4-CEC displays substrate activity at SERT but not DAT suggests a difference in the binding pocket of SERT that can accommodate the additional steric bulk found in 4-CEC (see Saha et al., 2015). Our findings with 4-CEC are reminiscent of a prior study with 4-methylethcathinone (4-MEC) and its analogs (Saha et al., 2015). Like 4-CEC, 4-MEC displays uptake inhibition properties at DAT and transporter substrate properties at SERT. Blough et al. (2014) suggested that hybrid transporter compounds might be interesting lead compounds for medication development, warranting further investigation.

4-CzPPP showed uptake inhibition properties but was inactive in the release assays, revealing its activity as a pure uptake blocker at DAT and NET. The inability of 4-CzPPP to induce transporter-mediated release is likely due to the bulky pyrrolidine ring, which precludes its action as a transportable substrate (Kolanos et al., 2013; Saha et al., 2015). Potent uptake inhibition at DAT and NET with minimal activity at

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**Fig. 5.** Effects of 10 mg/kg 4-CMC alone or in combination with the alpha-adrenergic antagonist prazosin (Praz, 0.3 mg/kg, n = 5), the dopamine D1 antagonist SCH23390 [SCH, 0.1 mg/kg, n = 5 (except for SCH + 4CMC, n = 4)] and the ganglionic blocker chlorisondamine (chloro 1.0 mg/kg, n = 5) on BP, HR, and activity. Each bar is the mean ± S.E.M. for the first hour of the session. #Represents significant difference from vehicle (Sal or Water) + saline (Sal). *Represents significant difference from vehicle + 10 mg/kg 4-CMC.
SERT is a diagnostic feature of pyrrolidine-containing cathinones like 2-pyrroldinonovalerophenone (2PVP) (Marusich et al., 2014; Baumann et al., 2018; Schindler et al., 2020). Importantly, previous data show that increasing the z-carbon chain length of 2PPP markedly increases potency for uptake inhibition at DAT and NET (Marusich et al., 2014; Eshleman et al., 2017). The potency for uptake inhibition shown here for 4-CzPPP is similar to that reported for 2PPP in rat brain synaptosomes (Baumann et al., 2018).

As predicted from the in vitro results, 4-CMC and mephedrone displayed similar effects in vivo in male rats. At higher doses, 4-CMC and mephedrone both caused increases in BP, HR, and locomotor activity. Varner et al. (2013) demonstrated that mephedrone produces robust hypertension and tachycardia in rats. The cardiovascular effects of both mephedrone and 4-CMC are likely due to increased sympathetic tone, as increased sympathomimetic activity is a consequence of mephedrone use (James et al., 2011). Mephedrone is also known to increase locomotion (Kehr et al., 2011; Angoa-Pérez et al., 2012) and elevate extracellular concentrations of brain dopamine in rats (Kehr et al., 2011; Baumann et al., 2012), and motor stimulant effects are likely related to dopamine efflux (Heal et al., 2013). Here we show that 4-CMC has locomotor stimulant effects that are somewhat greater than those produced by mephedrone.

The only 4-chloro drug in our study that significantly affected body temperature was 4-CMC, although 4-CEC also showed nonsignificant trends for transient hyperthermia. Similar to our results, previous findings from rats show that mephedrone causes dose-dependent hyperthermia that peaks at 1 hour and slowly returns to baseline by 3 hours (Miller et al., 2013). Similarly, other investigators reported that a high dose of 10 mg/kg of mephedrone (the same dose used here) causes a transient decrease in rectal temperature, in contrast to increases in rectal temperature produced by cathinone and methcathinone (Shortall et al., 2013). Shortall et al. (2016) linked the hypothermic effect of mephedrone to activation of the serotonergic system, so it might be worth pursuing additional studies with serotonin antagonists to investigate the thermic effects of 4-CMC.

Data from our in vitro transporter studies show that 4-CEC displays weaker effects on NET and DAT when compared with effects of 4-CMC, but the 5-HT releasing capability of both drugs is similar. We observed that 4-CEC was less potent than 4-CMC in its ability to increase HR and BP, and these in vivo findings are in line with the decreased potency of 4-CEC at NET. Locomotor activity produced by 4-CEC was only noted at the highest dose, and this decreased potency compared with 4-CMC could be attributed to the lack of substrate activity at DAT. 4-CzPPP had the least potent effects on in vivo measures. It is tempting to speculate that 4-CzPPP had weak in vivo actions because it lacks substrate activity at DAT and NET, but other factors could also be involved. Previous studies show that pyrrolidine-containing cathinones like 2PPP and 2PVP have high DAT/SERT selectivity ratios, with much greater potency at DAT relative to SERT, and this ratio is correlated with reinforcing efficacy in the rat drug self-administration paradigm (Gannon et al., 2018a,b). Here we show that 4-CzPPP is also highly selective for DAT over SERT, which may explain why 4-CzPPP is readily self-administered by rats (Xu et al., 2021).

We wanted to further understand the mechanisms underlying the physiologic effects produced by 4-CMC, so we carried out experiments with various pretreatment agents. Pretreatment with the z-adrenergic antagonist prazosin caused hypotension and reversed the hypertension caused by 4-CMC, indicating that pressor effects of 4-CMC are mediated by the alpha-adrenergic system. The findings with 4-CMC are consistent with our in vitro data and replicate prior work with mephedrone (Baumann et al., 2012), indicating that activity at NET underlies elevations in BP. Prazosin pretreatment did not affect HR or motor activity by itself, nor did it alter the changes in these measures caused by 4-CMC. Prior studies show that phenolamine, a mixed z1- and z2-antagonist, reverses the hypertensive and tachycardic effects of mephedrone (Varner et al., 2013) and that prazosin is a more potent antihypertensive than phenolamine (Graham and Pettinger, 1979). However, prazosin interacts predominately with presynaptic receptors, whereas phenolamine has both pre- and postsynaptic targets (Doxyet al., 1977). Future studies could examine how phenolamine pretreatment impacts the cardiovascular effects of 4-CMC.

Pretreatment with the dopamine D1 antagonist SCH23390 did not affect the BP response but did appear to reverse locomotor activity caused by 4-CMC. The antagonistic effect of SCH23390 on motor activity, combined with in vitro substrate activity of 4-CMC at DAT, suggests that increased dopamnergic activation underlies the hyperactivity produced by 4-CMC. Our findings are consistent with prior results, which show that locomotor effects of mephedrone are blocked by SCH23390 (Lisek et al., 2012). Interestingly, SCH23390 also partially reversed the tachycardia produced by 4-CMC yet had no effect on HR alone. It seems feasible that the ability of SCH23390 to reduce HR responses to 4-CMC is linked to its antagonism of locomotor stimulation, but this hypothesis requires further investigation. The ganglionic blocker chlorisondamine had hypotensive effects on its own, as shown previously (Chadman and Woods, 2004; Schindler et al., 2019). Furthermore, chlorisondamine reversed the increases in BP caused by 4-CMC. In combination with the prazosin data, the chlorisondamine findings suggest that BP effects of 4-CMC depend upon central sympathetic outflow.

In summary, we found that 4-chloro ring-substituted cathinones are biologically active. 4-CMC exerts robust pharmacological effects that are similar to those produced by mephedrone, most likely due to the structural similarity between the 4-chloro and 4-methyl phenyl ring substituents. Negus and Banks (2017) demonstrated that increasing the size or volume of 4-position ring-substitutions on synthetic cathinones enhances the potency at SERT relative to DAT, which reduces the overall abuse liability of the compounds (Negus and Banks, 2017). However, the role of such structural changes in modulating NET potency is less well understood. Thus, future studies should investigate pharmacological effects of compounds with larger 4-position ring-substituents such as brephedrone (i.e., 4-bromo), methedrone (i.e., 4-methoxy), and 4-trifluoromethylmethcathinone to understand how varied ring-substitution affects NET function and cardiovascular effects. The hybrid transporter effects of 4-CEC were predictable given previous studies with 4-MEC (Saha et al., 2015), and the effects of 4-CzPPP align with those of other pyrrolidine-containing cathinones. Future studies should focus on the reinforcing effects of these substances, especially for 4-CMC given its similarity to mephedrone. It should be noted that all of the subjects in the current study were male rats. To further extend the generality of the findings,
future studies investigating effects of 4-chloro compounds should also include female subjects.

Authorship Contributions

Participated in research design: Chojnacki, Schindler, Baumann.
Conducted experiments: Chojnacki, Thorndike, Partilla, Schindler, Baumann.

Contributed new reagents or analytic tools: Rice.

Performed data analysis: Chojnacki, Thorndike, Schindler, Baumann.

Wrote or contributed to the writing of the manuscript: Chojnacki, Partilla, Rice, Schindler, Baumann.

References


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**Address correspondence to:** Dr. Charles W. Schindler, Designer Drug Research Unit, National Institute on Drug Abuse Intramural Research Program, National Institutes of Health, 251 Bayview Boulevard, Baltimore, MD 21224. E-mail: cschind@helix.nih.gov