1521-0103/385/3/162–170$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.122.001478
J Pharmacol Exp Ther 385:162–170, June 2023

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.)

Received August 18, 2022; accepted January 12, 2023

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-α-pyrrolidinopropiophenone (4-CαPPP) 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-CαPPP 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; Eshleman 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

This research was supported by the Intramural Research Programs of National Institutes of Health National Institute on Drug Abuse (NIDA) and National Institute on Alcohol Abuse and Alcoholism (NIAAA) [Grants Z01-DA000523 and ZA1-DA000532].

No author has an actual or perceived conflict of interest with the contents of this article.

dx.doi.org/10.1124/jpet.122.001478.

ABBREVIATIONS: 4-CEC, 4-chloroethcathinone; 4-CMC, 4-chloromethcathinone; 4-CαPPP, 4-chloro-α-pyrrolidinopropiophenone; BP, blood pressure; DAT, dopamine transporter; HR, heart rate; 4-MEC, 4-methylethcathinone; NET, norepinephrine transporter; NIDA, National Institute on Drug Abuse; SERT, serotonin transporter.

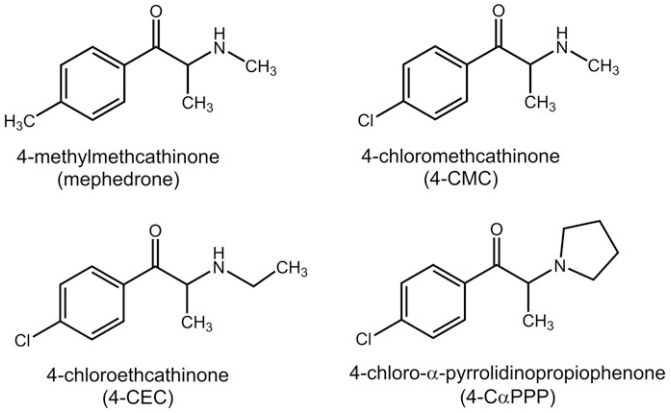

Fig. 1. Chemical structures of 4-chloromethcathinone (4-CMC), 4-chloroethcathinone (4-CEC), and 4-chloro-α-pyrrolidinopropiophenone (4-CαPPP) compared with 4-methylmethcathinone (mephedrone).

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 norepinephrine transporter (NET), thereby increasing extracellular concentrations of norepinephrine 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-CαPPP) 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 (Tomczak 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-CαPPP, 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-CαPPP, 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-chloroethcatinone (4-CEC), 4-chloro-α-pyrrolidinopropiophenone (4-CαPPP) 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 CO_2 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 [^{3}H]1-methyl-4-phenylpyridinium (MPP+; PerkinElmer, Waltham MA) was used as the radiolabeled substrate for DAT and NET, whereas 5 nM [^{3}H]serotonin ([^{3}H]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 [^{3}H]MPP+ or [^{3}H]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_{max}) defined as the release produced by tyramine at doses that evoke the efflux of all 'releasable' tritium by synaptosomes (10 μM tyramine for DAT and NET assay conditions and 100 μM 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 $Y(x) = Y_{min} + (Y_{max} - Y_{min})/(1 + 10\ \exp[(\log P_{50} - \log x)] \times n)$, where x is the concentration of the compound tested, $Y(x)$ is the response measured, Y_{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. For the surgery, the rats were anesthetized with isoflurane and the abdominal cavity was exposed. The descending aorta was isolated and the catheter from the transmitter was inserted and glued in place. The abdominal muscles and skin were then closed. Rats were treated with subcutaneous meloxicam after surgery. After recovery at DSI, the rats were shipped to NIDA in Baltimore and underwent a 7-day quarantine.

After release from 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 slowly increasing weight of approximately 400–500 g over the course of the experiment. The rats were subsequently adapted to the experimental chambers and injection procedure over a period of 3 to 4 weeks. Rats were transported to the procedure room, where the food and water were removed from the home cage and the entire home cage was placed on top of a telemetry receiver (RPC-001; DSI) inside a small acoustical chamber (BRS/LVE, Laurel, MD). Transmitters were turned on by placing a magnet near the abdomen of the rats. The chambers were then closed, and experimental parameters were monitored for 3 hours. At the end of the session, the transmitters were turned off by again placing a magnet near the abdomen of the rats, water and food were returned to the home cage, and the rats were returned to the housing room. Once experimental parameters were stable from day to day, subcutaneous injections of saline were given twice per week (typically on Tuesdays and Fridays) 5 minutes prior to the rats being placed in the experimental chamber. Once experimental parameters were stable after saline injections, experimental procedures with drug administration began. Rats in the current study were previously used to examine the cardiovascular effects of various dopamine receptor antagonists (Jordan et al., 2019). After a 2-week washout period, the current studies were begun.

Dose-effect determinations for 4-CMC, 4-CEC, 4-CαPPP, and mephedrone were determined in five to seven rats. Again, drugs were tested no more frequently than twice per week, typically Tuesday and Friday. Order for the dose-effect testing was nonsystematic, although all rats were typically tested with the same drug and dose on any given test day. All drugs were administered subcutaneously 5 minutes prior to placement of the rats into the experimental chambers, after which data collection began. Saline was tested every 2 to 3 weeks, and responses after saline were stable over the testing period. After dose-effect testing, rats were tested with various pretreatment drugs. The alpha-1 adrenergic receptor antagonist prazosin (0.3 mg/kg, sterile water vehicle), the ganglionic blocker chlorisondamine (1 mg/kg, saline vehicle), the dopamine D1 receptor antagonist SCH23390 (0.1 mg/kg, sterile water vehicle), or their vehicles were given 5 minutes (prazosin or SCH22390) or 10 minutes (chlorisondamine) prior to 4-CMC (10 mg/kg) or saline. Five min after 4-CMC or saline administration, rats in their home cages were placed in the experimental chambers, after which data collection began. 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-CαPPP 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

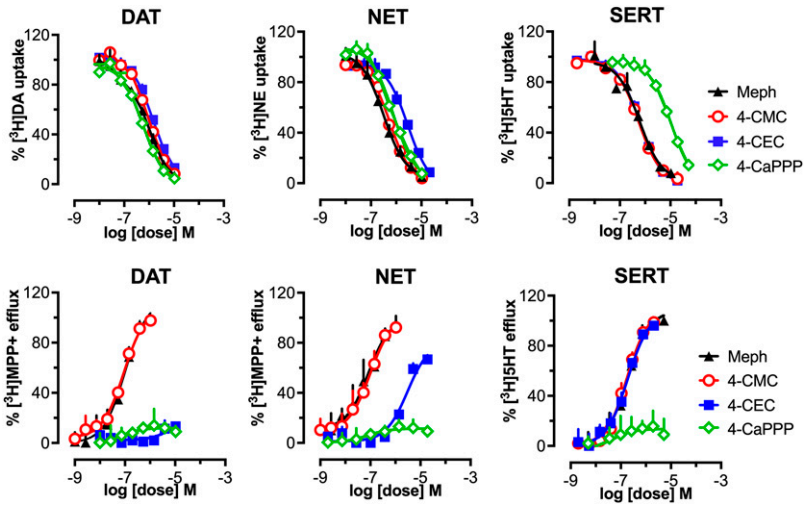

Fig. 2. Effects of synthetic cathinones on inhibition of uptake (upper panels) and stimulation of release (lower panels) at dopamine (DAT), norepinephrine (NET), and serotonin (SERT) transporters in rat brain synaptosomes. For uptake assays, synaptosomes were incubated with different concentrations of test drugs in the presence of 5 nM [^{3}H]dopamine, 10 nM [^{3}H]norepinephrine, or 5 nM [^{3}H]serotonin. Data are expressed as a percentage of transmitter uptake (± S.D.) for three experiments. For release assays, synaptosomes were preloaded with 9 nM [^{3}H]MPP$^+$ (DAT and NET) or 5 nM [^{3}H]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 [^{3}H]substrate release (± S.D.) for three experiments.

about 30 minutes. Activity was also slightly elevated right after the rats were placed in the chambers, but activity decreased to low levels by about 1 hour and remained decreased for the remainder of the 3-hour session. Temperature measures after saline administration remained fairly stable throughout the session. Across the 3-hour session, 4-CMC induced significant increases in BP ($F_{(51,404)} = 3.645$, $P < 0.0001$), HR ($F_{(51,404)} = 4.151$, $P < 0.0001$), and activity ($F_{(51,404)} = 4.975$, $P < 0.0001$) and a decrease in body temperature ($F_{(51,404)} = 13.37$, $P < 0.0001$). The lowest dose of 4-CMC showed little effect on any measure when compared with saline, whereas the two higher doses increased BP, HR, and activity in a dose-related manner. The effects of 4-CMC were primarily restricted to the first 2 hours of the session for BP, but effects on locomotor activity lasted for the full 3-hour session at the highest dose administered. The highest dose of 4-CMC produced a small decrease in temperature in the first hour of the session. Mephedrone, 4-CEC, and 4-CαPPP induced qualitatively similar time course effects on the endpoints examined (data not shown), though there were differences in potency across the drugs (see below).

Figure 4 shows the dose-effect functions for all the 4-chloro ring-substituted cathinones and mephedrone to produce physiologic effects. Because all drug effects were apparent during the first hour of the session (see Fig. 3), but some effects returned to baseline before 2 hours, we used the mean effect data from the first hour of the sessions to obtain dose-effect functions. Mephedrone produced a significant elevation in BP ($F_{(1.637,6.548)} = 35.66$, $P = 0.0004$), HR ($F_{(1.866,7.464)} = 79.95$, $P < 0.0001$), and motor activity ($F_{(1.223,4.894)} = 16.74$, $P = 0.0087$) at all three doses when compared with saline. There was a significant but small increase in temperature at the 1 mg/kg dose and a decrease at the 10 mg/kg dose.

4-CMC significantly elevated BP at 3 and 10 mg/kg ($F_{(1.350,8.097)} = 13.77$, $P = 0.0040$). 4-CMC significantly elevated HR ($F_{(1.574,9.446)} = 36.20$, $P < 0.0001$) and motor activity ($F_{(1.515,9.091)} = 19.20$, $P = 0.0009$) at both 3 mg/kg and 10 mg/kg, and the motor effects were dose dependent. 4-CMC decreased body temperature ($F_{(2.315,13.89)} = 42.10$, $P < 0.0001$) by nearly 1°C degree at 10 mg/kg ($P = 0.0006$). 4-CEC also produced a dose-dependent increase in BP ($F_{(1.537,6.147)} = 8.464$, $P = 0.0202$). 4-CEC caused modest increases in HR at all doses, whereas the increase at 10 mg/kg was slightly larger ($F_{(1.266,5.063)} = 14.56$, $P = 0.0105$). Motor activity was significantly elevated at 3 and 10 mg/kg ($F_{(1.801,7.205)} = 11.78$, $P = 0.0059$). There was a small but significant increase in temperature after 3 mg/kg 4-CEC ($F_{(1.379,5.515)} = 7.799$, $P = 0.0296$).

TABLE 1
Effects of synthetic cathinones analogs on inhibition of [^{3}H]neurotransmitter uptake and release of [^{3}H]MPP+ via DAT and NET or [^{3}H]5-HT via SERT in rat brain synaptosomes
Data are mean ± S.D. for $n = 3$ experiments performed in triplicate.

Drug	Uptake Inhibition at DAT IC_{50} (nM)	Uptake Inhibition at NET IC_{50} (nM)	Uptake Inhibition at SERT IC_{50} (nM)
Mephedrone	769 ± 106	319 ± 40	600 ± 99
4-CMC	1014 ± 78	559 ± 57	542 ± 38
4-CEC	1455 ± 110	2848 ± 292	589 ± 44
4-CαPPP	569 ± 57	764 ± 79	>10,000
	Release at DAT EC_{50} (nM) [% E_{max}][a]	Release at NET EC_{50} (nM) [% E_{max}][a]	Release at SERT EC_{50} (nM) [% E_{max}][a]
Mephedrone	103 ± 16 [114]	83 ± 20 [100]	188 ± 22 [108]
4-CMC	91 ± 11 [109]	99 ± 18 [102]	169 ± 20 [109]
4-CEC	Inactive	3058 ± 547 [81]	191 ± 26 [107]
4-CαPPP	Inactive	Inactive	Inactive

[a]% E_{max} is defined as percentage of maximal releasing response as described in Materials and Methods.

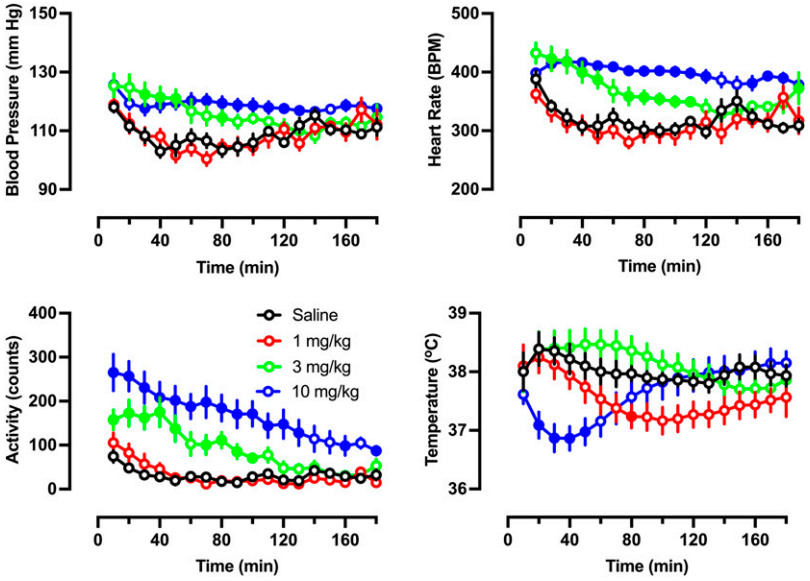

Fig. 3. Time course for the effects of various doses of 4-CMC on BP, HR, activity, and temperature over 3 hours in rats implanted with telemetry transmitters. Values represent a 10-minute mean for BP, HR, and temperature or the sum of counts over 10 minutes for activity for each rat. Solid symbols indicate significant difference from saline. Each point is the mean ± S.E.M. of seven rats.

4-Cα-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 = 0.0005) 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).

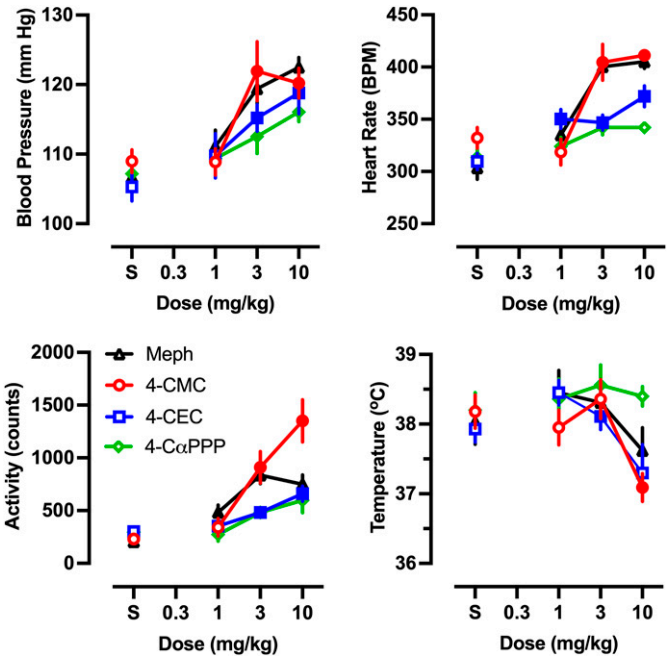

Fig. 4. Dose-effect functions for mephedrone (n = 5), 4-CMC (n = 7), 4-CEC (n = 5), and 4-CαPPP (n = 5) on BP, HR, activity, and temperature in rats implanted with telemetry transmitters. Data points are the mean ± S.E.M. over the first hour of the session. Points over S are for the saline test conducted in conjunction with each drug. Solid symbols indicate significant differences from the respective saline.

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

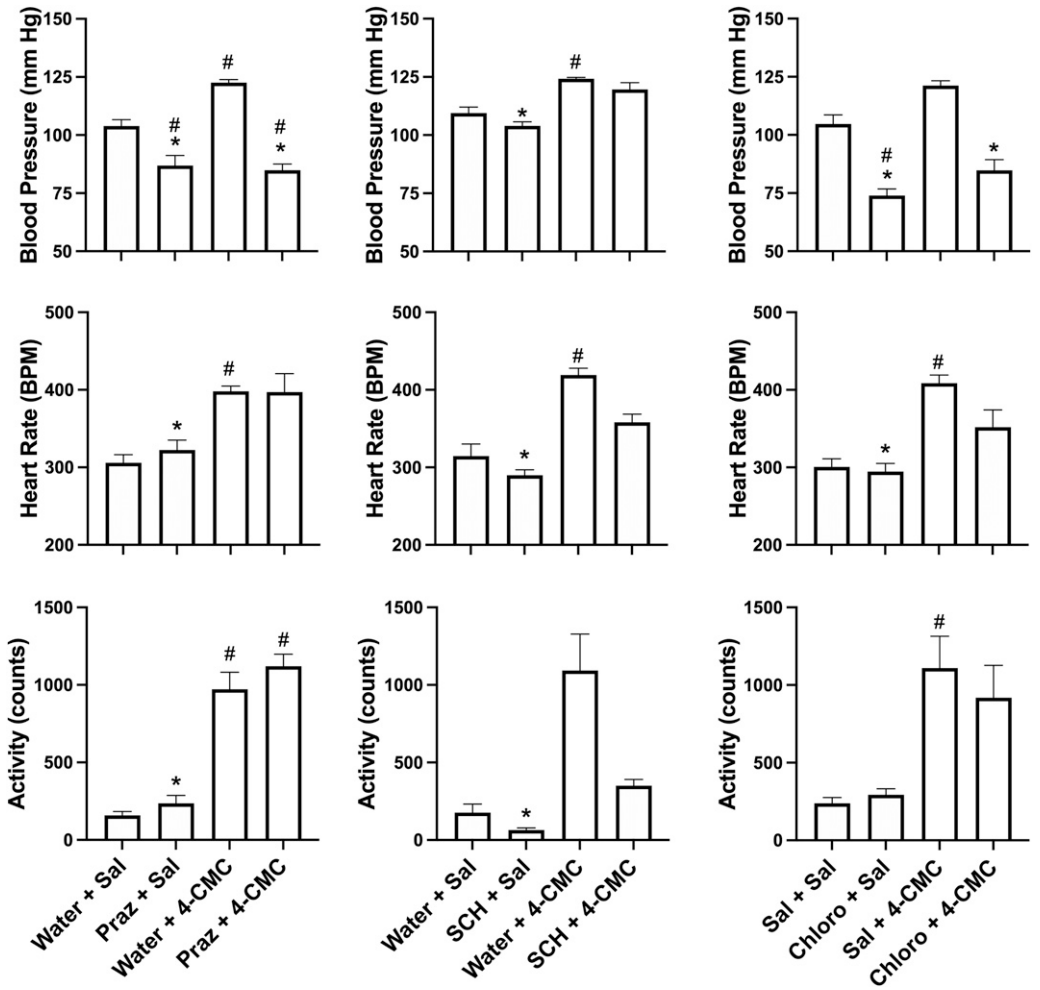

Fig. 5. Effects of 10 mg/kg 4-CMC alone or in combination with the alpha-adrenergic antagonist prazosin (Praz, 0.3 mg/kg, $n = 5$), the dopamine D1 antagonist SCH23390 [SCH, 0.1 mg/kg, $n = 5$ (except for SCH + 4CMC, $n = 4$)] and the ganglionic blocker chlorisondamine (chloro 1.0 mg/kg, $n = 5$) on 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.

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 nonselective 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-CαPPP 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-CαPPP 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

SERT is a diagnostic feature of pyrrolidine-containing cathinones like α-pyrrolidinovalerophenone (αPVP) (Marusich et al., 2014; Baumann et al., 2018; Schindler et al., 2020). Importantly, previous data show that increasing the α-carbon chain length of αPPP 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-CαPPP is similar to that reported for αPPP 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 hypothermia. Similar to our results, previous findings from rats show that mephedrone causes dose-dependent hypothermia 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-CαPPP had the least potent effects on in vivo measures. It is tempting to speculate that 4-CαPPP 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 αPPP and αPVP 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-CαPPP is also highly selective for DAT over SERT, which may explain why 4-CαPPP 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 α-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 phentolamine, a mixed α1- and α2-antagonist, reverses the hypertensive and tachycardic effects of mephedrone (Varner et al., 2013) and that prazosin is a more potent antihypertensive than phentolamine (Graham and Pettinger, 1979). However, prazosin interacts predominately with presynaptic receptors, whereas phentolamine has both pre- and postsynaptic targets (Doxey et al., 1977). Future studies could examine how phentolamine 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 dopaminergic 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-CαPPP 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

Angoa-Pérez M, Kane MJ, Francescutti DM, Sykes KE, Shah MM, Mohammed AM, Thomas DM, and Kuhn DM (2012) Mephedrone, an abused psychoactive component of 'bath salts' and methamphetamine congener, does not cause neurotoxicity to dopamine nerve endings of the striatum. *J Neurochem* **120**:1097–1107.

Baumann MH, Ayestas Jr MA, Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Ruoho AE, and Cozzi NV (2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* **37**:1192–1203.

Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, Rothman RB, Goldberg SR, Lupica CR, Sitte HH, et al. (2013) Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology* **38**:552–562.

Baumann MH, Solis Jr E, Watterson LR, Marusich JA, Fantegrossi WE, and Wiley JL (2014) Baths salts, spice, and related designer drugs: the science behind the headlines. *J Neurosci* **34**:15150–15158.

Baumann MH, Walters HM, Niello M, and Sitte HH (2018) Neuropharmacology of synthetic cathinones. *Handb Exp Pharmacol* **252**:113–142.

Blough BE, Decker AM, Landavazo A, Namjoshi OA, Partilla JS, Baumann MH, and Rothman RB (2019) The dopamine, serotonin and norepinephrine releasing activities of a series of methcathinone analogs in male rat brain synaptosomes. *Psychopharmacology (Berl)* **236**:915–924.

Blough BE, Landavazo A, Partilla JS, Baumann MH, Decker AM, Page KM, and Rothman RB (2014) Hybrid dopamine uptake blocker-serotonin releaser ligands: a new twist on transporter-focused therapeutics. *ACS Med Chem Lett* **5**:623–627.

Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, and Negus SS (2015) Quantitative structure-activity relationship analysis of the pharmacology of para-substituted methcathinone analogues. *Br J Pharmacol* **172**:2433–2444.

Chadman KK and Woods JH (2004) Cardiovascular effects of nicotine, chlorisondamine, and mecamylamine in the pigeon. *J Pharmacol Exp Ther* **308**:73–78.

Doxey JC, Smith CF, and Walker JM (1977) Selectivity of blocking agents for pre-and postsynaptic alpha-adrenoceptors. *Br J Pharmacol* **60**:91–96.

Egashira K, Morgan KG, and Morgan JP (1991) Effects of cocaine on excitation-contraction coupling of aortic smooth muscle from the ferret. *J Clin Invest* **87**:1322–1328.

Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, and Janowsky A (2013) Substituted methcathinones differ in transporter and receptor interactions. *Biochem Pharmacol* **85**:1803–1815.

Eshleman AJ, Wolfrum KM, Reed JF, Kim SO, Swanson T, Johnson RA, and Janowsky A (2017) Structure-activity relationships of substituted cathinones, with transporter binding, uptake, and release. *J Pharmacol Exp Ther* **360**:33–47.

Gannon BM, Baumann MH, Walther D, Jimenez-Morigosa C, Sulima A, Rice KC, and Collins GT (2018a) The abuse-related effects of pyrrolidine-containing cathinones are related to their potency and selectivity to inhibit the dopamine transporter. *Neuropsychopharmacology* **43**:2399–2407.

Gannon BM, Galindo KI, Mesmin MP, Sulima A, Rice KC, and Collins GT (2018b) Relative reinforcing effects of second-generation synthetic cathinones: acquisition of self-administration and fixed ratio dose-response curves in rats. *Neuropharmacology* **134**:28–35.

Gatch MB, Shetty RA, Sumien N, and Forster MJ (2021) Behavioral effects of four novel synthetic cathinone analogs in rodents. *Addict Biol* **26**:e12987.

Graham RM and Pettinger WA (1979) Effects of prazosin and phentolamine on arterial pressure, heart rate, and renin activity: evidence in the conscious rat for the functional significance of the presynaptic alpha-receptor. *J Cardiovasc Pharmacol* **1**:497–502.

Grifell M, Ventura M, Carbón X, Quintana P, Galindo L, Palma Á, Fornis I, Gil C, Farre M, and Torrens M (2017) Patterns of use and toxicity of new para-halogenated substituted cathinones: 4-CMC (clephedrone), 4-CEC (4-chloroethcatinone) and 4-BMC (brephedrone). *Hum Psychopharmacol* **32**:e2621.

Hadlock GC, Webb KM, McFadden LM, Chu PW, Ellis JD, Allen SC, Andrenyak DM, Vieira-Brock PL, German CL, Conrad KM, et al. (2011) 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse. *J Pharmacol Exp Ther* **339**:530–536.

Havakuk O, Rezkalla SH, and Kloner RA (2017) The cardiovascular effects of cocaine. *J Am Coll Cardiol* **70**:101–113.

Heal DJ, Smith SL, Gosden J, and Nutt DJ (2013) Amphetamine, past and present–a pharmacological and clinical perspective. *J Psychopharmacol* **27**:479–496.

James D, Adams RD, Spears R, Cooper G, Lupton DJ, Thompson JP, and Thomas SH; National Poisons Information Service (2011) Clinical characteristics of mephedrone toxicity reported to the U.K. National Poisons Information Service. *Emerg Med J* **28**:686–689.

Jordan CJ, Humburg BA, Thorndike EB, Shaik AB, Xi ZX, Baumann MH, Newman AH, and Schindler CW (2019) Newly developed dopamine D3 receptor antagonists, R-VK4-40 and R-VK4-116, do not potentiate cardiovascular effects of cocaine or oxycodone in rats. *J Pharmacol Exp Ther* **371**:602–614.

Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, and Yoshitake T (2011) Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. *Br J Pharmacol* **164**:1949–1958.

Kolanos R, Solis Jr E, Sakloth F, De Felice LJ, and Glennon RA (2013) "Deconstruction" of the abused synthetic cathinone methylenedioxypyrovalerone (MDPV) and an examination of effects at the human dopamine transporter. *ACS Chem Neurosci* **4**:1524–1529.

La Maida N, Di Trana A, Giorgetti R, Tagliabracci A, Busardò FP, and Huestis MA (2021) A review of synthetic cathinone-related fatalities from 2017 to 2020. *Ther Drug Monit* **43**:52–68.

Lisek R, Xu W, Yuvasheva E, Chiu Y-T, Reitz AB, Liu-Chen L-Y, and Rawls SM (2012) Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. *Drug Alcohol Depend* **126**:257–262.

Luethi D, Walter M, Zhou X, Rudin D, Krähenbühl S, and Liechti ME (2019) Parahalogenation affects monoamine transporter inhibition properties and hepatocellular toxicity of amphetamines and methcathinones. *Front Pharmacol* **10**:438.

Marusich JA, Antonazzo KR, Wiley JL, Blough BE, Partilla JS, and Baumann MH (2014) Pharmacology of novel synthetic stimulants structurally related to the "bath salts" constituent 3,4-methylenedioxypyrovalerone (MDPV). *Neuropharmacology* **87**:206–213.

Miller ML, Creehan KM, Angrish D, Barlow DJ, Houseknecht KL, Dickerson TJ, and Taffe MA (2013) Changes in ambient temperature differentially alter the thermoregulatory, cardiac and locomotor stimulant effects of 4-methylmethcathinone (mephedrone). *Drug Alcohol Depend* **127**:248–253.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). *Guide for the Care and Use of Laboratory Animals*, 8th ed, National Academies Press, Washington, DC.

Negus SS and Banks ML (2017) Decoding the structure of abuse potential for new psychoactive substances: structure-activity relationships for abuse-related effects of 4-substituted methcathinone analogs. *Curr Top Behav Neurosci* **32**:119–131.

Papaseit E, Pérez-Mañá C, Mateus JA, Pujadas M, Fonseca F, Torrens M, Olesti E, de la Torre R, and Farré M (2016) Human pharmacology of mephedrone in comparison with MDMA. *Neuropsychopharmacology* **41**:2704–2713.

Rickli A, Hoener MC, and Liechti ME (2015) Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacol* **25**:365–376.

Rothman RB, Vu N, Partilla JS, Roth BL, Hufeisen SJ, Compton-Toth BA, Birkes J, Young R, and Glennon RA (2003) In vitro characterization of ephedrine-related stereoisomers at biogenic amine transporters and the receptorome reveals selective actions as norepinephrine transporter substrates. *J Pharmacol Exp Ther* **307**:138–145.

Rudin D, Liechti ME, and Luethi D (2021) Molecular and clinical aspects of potential neurotoxicity induced by new psychoactive stimulants and psychedelics. *Exp Neurol* **343**:113778.

Saha K, Partilla JS, Lehner KR, Seddik A, Stockner T, Holy M, Sandtner W, Ecker GF, Sitte HH, and Baumann MH (2015) 'Second-generation' mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function. *Neuropsychopharmacology* **40**:1321–1331.

Sakloth F, Kolanos R, Mosier PD, Bonano JS, Banks ML, Partilla JS, Baumann MH, Negus SS, and Glennon RA (2015) Steric parameters, molecular modeling and hydropathic interaction analysis of the pharmacology of para-substituted methcathinone analogues. *Br J Pharmacol* **172**:2210–2218.

Schindler CW, Thorndike EB, Partilla JS, Rice KC, and Baumann MH (2021) Amphetamine-like neurochemical and cardiovascular effects of α-ethylphenethylamine analogs found in dietary supplements. *J Pharmacol Exp Ther* **376**:118–126.

Schindler CW, Thorndike EB, Rice KC, Partilla JS, and Baumann MH (2019) The supplement adulterant β-methylphenethylamine increases blood pressure by acting at peripheral norepinephrine transporters. *J Pharmacol Exp Ther* **369**:328–336.

Schindler CW, Thorndike EB, Suzuki M, Rice KC, and Baumann MH (2016) Pharmacological mechanisms underlying the cardiovascular effects of the "bath salt" constituent 3,4-methylenedioxypyrovalerone (MDPV). *Br J Pharmacol* **173**:3492–3501.

Schindler CW, Thorndike EB, Walters HM, Walther D, Rice KC, and Baumann MH (2020) Stereoselective neurochemical, behavioral, and cardiovascular effects of alpha-pyrrolidinovalerophenone enantiomers in male rats. *Addict Biol* **25**:e12842.

Schwarzbach V, Lenk K, and Laufs U (2020) Methamphetamine-related cardiovascular diseases. *ESC Heart Fail* **7**:407–414.

Shortall SE, Green AR, Swift KM, Fone KC, and King MV (2013) Differential effects of cathinone compounds and MDMA on body temperature in the rat, and pharmacological characterization of mephedrone-induced hypothermia. *Br J Pharmacol* **168**:966–977.

Shortall SE, Spicer CH, Ebling FJP, Green AR, Fone KCF, and King MV (2016) Contribution of serotonin and dopamine to changes in core body temperature and locomotor activity in rats following repeated administration of mephedrone. *Addict Biol* **21**:1127–1139.

Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu LH, Huwyler J, Chaboz S, Hoener MC, and Liechti ME (2013) Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* **168**:458–470.

Soares J, Costa VM, Bastos ML, Carvalho F, and Capela JP (2021) An updated review on synthetic cathinones. *Arch Toxicol* **95**:2895–2940.

Solis Jr E, Partilla JS, Sakloth F, Ruchala I, Schwienteck KL, De Felice LJ, Eltit JM, Glennon RA, Negus SS, and Baumann MH (2017) N-alkylated analogs of 4-methylamphetamine (4-MA) differentially affect monoamine transporters and abuse liability. *Neuropsychopharmacology* **42**:1950–1961.

Spiller HA, Ryan ML, Weston RG, and Jansen J (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clin Toxicol (Phila)* **49**:499–505.

Sutamtewagul G, Sood V, and Nugent K (2014) Sympathomimetic syndrome, choreoathetosis, and acute kidney injury following "bath salts" injection. *Clin Nephrol* **81**:63–66.

Tomczak E, Woźniak MK, Kata M, Wiergowski M, Szpiech B, and Biziuk M (2018) Blood concentrations of a new psychoactive substance 4-chloromethcathinone (4-CMC) determined in 15 forensic cases. *Forensic Toxicol* **36**:476–485.
Varner KJ, Daigle K, Weed PF, Lewis PB, Mahne SE, Sankaranarayanan A, and Winsauer PJ (2013) Comparison of the behavioral and cardiovascular effects of mephedrone with other drugs of abuse in rats. *Psychopharmacology (Berl)* **225**: 675–685.
Venton BJ, Seipel AT, Phillips PE, Wetsel WC, Gitler D, Greengard P, Augustine GJ, and Wightman RM (2006) Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. *J Neurosci* **26**:3206–3209.
Wojcieszak J, Kuczyńska K, and Zawilska JB (2020) Four synthetic cathinones: 3-chloromethcathinone, 4-chloromethcathinone, 4-fluoro-α-pyrrolidinopentiophenone, and 4-methoxy-α-pyrrolidinopentiophenone produce changes in the spontaneous locomotor activity and motor performance in mice with varied profiles. *Neurotox Res* **38**:536–551.
Wojcieszak J, Kuczyńska K, and Zawilska JB (2021) Behavioral effects of 4-CMC and 4-MeO-PVP in DBA/2J mice after acute and intermittent administration and following withdrawal from intermittent 14-day treatment. *Neurotox Res* **39**:575–587.
Xu P, Lai M, Fu D, Liu H, Wang Y, Shen H, and Zhou W (2021) Reinforcing and discriminative-stimulus effects of two pyrrolidine-containing synthetic cathinone derivatives in rats. *Pharmacol Biochem Behav* **203**:173128.
Zhou X, Bouitbir J, Liechti ME, Krähenbühl S, and Mancuso RV (2020) Hyperthermia increases neurotoxicity associated with novel methcathinones. *Cells* **9**:965.

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