4-Methylmethcathinone (Mephedrone): Neuropharmacological Effects of a Designer Stimulant of Abuse

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ABSTRACT

The designer stimulant 4-methylmethcathinone (mephedrone) is among the most popular of the derivatives of the naturally occurring psychostimulant cathinone. Mephedrone has been readily available for legal purchase both online and in some stores and has been promoted by aggressive Web-based marketing. Its abuse in many countries, including the United States, is a serious public health concern. Owing largely to its recent emergence, there are no formal pharmacodynamic or pharmacokinetic studies of mephedrone. Accordingly, the purpose of this study was to evaluate effects of this agent in a rat model. Results revealed that, similar to methylenedioxymethamphetamine, methamphetamine, and methcathinone, repeated mephedrone injections (4×10 or 25 mg/kg s.c. per injection, 2-h intervals, administered in a pattern used frequently to mimic

psychostimulant “binge” treatment) cause a rapid decrease in striatal dopamine (DA) and hippocampal serotonin (5-hydroxytryptamine; 5HT) transporter function. Mephedrone also inhibited both synaptosomal DA and 5HT uptake. Like methylenedioxymethamphetamine, but unlike methamphetamine or methcathinone, repeated mephedrone administrations also caused persistent serotonergic, but not dopaminergic, deficits. However, mephedrone caused DA release from a striatal suspension approaching that of methamphetamine and was self-administered by rodents. A method was developed to assess mephedrone concentrations in rat brain and plasma, and mephedrone levels were determined 1 h after a binge treatment. These data demonstrate that mephedrone has a unique pharmacological profile with both abuse liability and neurotoxic potential.

Introduction

The designer drug 4-methylmethcathinone [1-(4-methylphenyl)-2-methylaminopropane-1-one; mephedrone] is among the most popular of the derivatives of the naturally occurring psychostimulant cathinone (Advisory Council on the Misuse of Drugs, 2010; Cressey, 2010; Morris, 2010). Its structure is related closely to the phenylethylamine family of illicit agents, including methamphetamine (*N*-methyl-1-phenylpropan-2-amine; METH) and methylenedioxymethamphetamine [1-(benzo[d][1,3]dioxol-5-yl)-*N*-methylpropan-2-amine; MDMA], differing by a keto group at the β carbon. Mephedrone has been readily available for purchase both online and in some stores, and its circulation has been promoted by Web-based marketing.

Its rise in popularity in the United Kingdom received international attention and led to its ban in 2010. In addition, in 2010, there were increasing reports of the abuse and seizure liability of mephedrone in regions other than Europe, including Southeast Asia, Australia, and North America. Its abuse in the United States, particularly in the form of “Ivory Wave” (e.g., its combination with the stimulant 3,4-methylenedioxypyrovalerone) has become a health concern.

Owing largely to its recent emergence, there are very few formal pharmacodynamic or pharmacokinetic studies of mephedrone. A single report by Kehr et al. (2011) indicates that mephedrone causes DA release in the nucleus accumbens. Beyond this, clinical and anecdotal reports are the primary source of information concerning mephedrone. This lack of information is problematic for public health policy makers and law enforcement organizations as they attempt to develop and implement appropriate strategies to deal with the recreational use and abuse of mephedrone and related drugs. Of further concern, it has been suggested that mephedrone may resemble dangerous drugs such as meth-

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ABBREVIATIONS: METH, *N*-methyl-1-phenylpropan-2-amine, methamphetamine; MDMA, 1-(benzo[d][1,3]dioxol-5-yl)-*N*-methylpropan-2-amine, methylenedioxymethamphetamine; DA, dopamine; 5HT, 5-hydroxytryptamine, serotonin.

cathinone, MDMA, or METH. This is problematic, because it is well established that high-dose administration of these stimulants can cause long-lasting monoaminergic deficits in humans (McCann et al., 1998; Reneman et al., 2001; Sekine et al., 2001) and nonhuman models (Hotchkiss et al., 1979; Ricaurte et al., 1980; Wagner et al., 1980; Mereu et al., 1983; Nash and Yamamoto, 1992; Sparago et al., 1996; Gygi et al., 1997; Guilarte et al., 2003). The potential clinical relevance of understanding these changes is underscored by findings that stimulant (e.g., METH) abusers often display general persistent impairment across several neurocognitive domains, including deficits in executive function and memory (Volkow et al., 2001; Scott et al., 2007).

As noted, there are currently very few published studies describing the pharmacological or toxicological impact of mephedrone. Thus, the present study addresses this issue. Results revealed that, similar to MDMA, METH, and methcathinone (Fleckenstein et al., 1999; Haughey et al., 2000; Metzger et al., 2000; Hansen et al., 2002), repeated mephedrone injections rapidly decrease dopamine (DA) and serotonin (5-hydroxytryptamine; 5HT) transporter function. Like MDMA, but unlike METH (Stone et al., 1986; Schmidt and Kehne, 1990; McCann et al., 1994; Reneman et al., 2001; Krasnova and Cadet, 2009), repeated mephedrone administrations cause persistent serotonergic, but not dopaminergic, deficits. Of note, mephedrone causes DA release from a striatal suspension approaching that of METH and is self-administered by rodents. These data demonstrate important similarities and differences among mephedrone and other related stimulants of abuse. Moreover, these data demonstrate that mephedrone has a unique pharmacological profile with both abuse liability and neurotoxic potential.

Materials and Methods

Animals. Male Sprague-Dawley rats (290–400 g; Charles River Laboratories, Inc., Wilmington, MA) were maintained under controlled lighting and temperature conditions with constant access to food and water. With the exception of rats in the self-administration experiments, which were singly housed, rats were housed three to four animals per cage during treatment. Rats were maintained in a warmer ambient environment during treatment (e.g., $\geq 27^{\circ}\text{C}$) to ensure that the mephedrone-treated rats attained hyperthermia. Temperatures were assessed at 1-h intervals beginning 30 min before the first saline or mephedrone injections. Rats were sacrificed by decapitation. All of the procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and approved by the University of Utah Institutional Animal Care and Use Committee.

Drugs and Chemicals. Mephedrone hydrochloride, METH hydrochloride, cocaine hydrochloride, and MDMA hydrochloride were supplied by the Research Triangle Institute (Research Triangle Park, NC). Fluoxetine hydrochloride, cefazolin, and heparinized saline were purchased from Sigma-Aldrich (St. Louis, MO). Mephedrone- d_3 was purchased from Cerilliant Corporation (Round Rock, TX). Mephedrone, METH, cocaine, and MDMA were dissolved in 0.9% saline vehicle before administration. Drug doses were calculated as the free base.

Synaptosomal [^3H]DA and [^3H]5HT Uptake. [^3H]DA and [^3H]5HT uptake were determined using a rat striatal (DA) or hippocampal (5HT) synaptosomal preparation as described previously (Kokoshka et al., 1998a). In brief, synaptosomes were prepared by homogenizing freshly dissected striatal or hippocampal tissue in ice-cold 0.32 M sucrose (pH 7.4) and centrifuged (800g, 12 min, 4°C).

In some experiments, small sections of the left anterior striatum and left anterior hippocampus were frozen quickly on dry ice and retained to determine DA and 5HT content. The supernatants were centrifuged (22,000g, 15 min, 4°C), and the resulting pellets were resuspended in ice-cold assay buffer [126 mM NaCl, 4.8 mM KCl, 1.3 CaCl_2 mM, 16 mM sodium phosphate, 1.4 mM MgSO_4 , 11 mM glucose, and 1 mM ascorbic acid (pH 7.4)] and 1 μM pargyline. For the IC_{50} experiments, cocaine, MDMA, or mephedrone (1 nM to 5 μM) was present in the assay tubes. Samples were incubated for 10 min at 37°C , and the assays were initiated by the addition of [^3H]DA or [^3H]5HT (0.5 or 5 nM final concentrations, respectively). After incubation for 3 min, samples were placed on ice to stop the reaction. Samples then were filtered through GF/B filters (Whatman, Clifton, NJ) soaked previously in 0.05% polyethylenimine. Filters were washed rapidly three times with 3 ml of ice-cold 0.32 M sucrose buffer using a filtering manifold (Brandel Inc., Gaithersburg, MD). For [^3H]DA uptake, nonspecific values were determined in the presence of 50 μM cocaine. For [^3H]5HT uptake, nonspecific values were determined in the presence of 10 μM fluoxetine. Radioactivity trapped in filters was counted using a liquid scintillation counter. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA).

DA and 5HT Concentrations. Striatal and hippocampal tissues were frozen quickly on dry ice and stored at -80°C until sonication (Branson Sonifier 250; Branson Ultrasonics Corporation, Danbury, CT) in 1 ml of tissue buffer [50 mM sodium phosphate dibasic and 30 mM citric acid with 10% (v/v) methanol (pH 2.5)], then centrifuged at 18,800g for 15 min at 4°C to separate the supernatant from the protein. The supernatant was centrifuged at 18,800g for 10 min at 4°C , and 25 μl was injected onto a high-performance liquid chromatography system (Dynamax AI-200 autosampler and SD-200 pump; Varian, Inc., Palo Alto, CA) coupled to an electrochemical detector ($E_{\text{ox}} = +0.70\text{ V}$; Star 9080; Varian, Inc.) to quantify the concentrations of DA and 5HT. Monoamines were separated on a Partisphere C-18 column (250 $\times$ 4.6 mm, 5 μm ; Whatman) in a mobile phase consisting of 25% (v/v) methanol, 0.04% (w/v) sodium octyl sulfate, 0.1 mM EDTA, 50 mM sodium phosphate dibasic, and 30 mM citric acid (pH 2.65) at a flow rate of 0.75 ml/min. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories).

Mephedrone Concentrations. Rat brains were homogenized separately by weighing each brain sample and homogenizing with 10 ml of Milli-Q water. For the extraction, 0.5 ml of each plasma and brain homogenate was transferred to separate glass tubes. Mephedrone- d_3 (30 mg) was added as the internal standard. A total of 0.1 ml of ammonium hydroxide and 4 ml of 1-chlorobutane/acetonitrile (4:1, v/v) were added to each tube. After mixing and centrifugation, the upper organic layers were transferred to separate culture tubes and evaporated to dryness at 40°C under air. A total of 0.1 ml of 0.2% formic acid/methanol (75:25, v/v) was added to each extract. The reconstituted extracts were transferred to separate polypropylene autosampler vials. The extracts were analyzed on an Acuity liquid chromatograph interfaced with a Quattro Premier XE tandem mass spectrometer (Waters, Milford, MA). Chromatographic conditions used a Synergi MAX-RP column (150 $\times$ 2 mm; Phenomenex, Torrance, CA). The mobile phase consisted of 0.2% formic acid/methanol (75:25, v/v) at a flow rate of 0.2 ml/min. Positive ion electrospray was used for the ionization. Selected reaction monitoring was used to monitor the peak areas for mephedrone (m/z 178 $\rightarrow$ 160) and mephedrone- d_3 (m/z 181 $\rightarrow$ 163). For both compounds, a cone voltage of 25 V and a collision energy of 15 V were used. Calibration standards (1–500 ng/ml) and quality control samples (8, 80, and 240 ng/ml) were prepared by fortification of a known concentration of drug to analyte-free matrix and were analyzed concurrently with the study samples. The study samples were diluted appropriately so that measured mephedrone concentrations were within the range of the calibration curve.

Rotating Disk Electrode Voltammetry Analysis. Rotating disk electrode voltammetry was used to measure drug-stimulated DA release using a modification of previously published procedures used to measure potassium-stimulated DA release from rat striatal suspensions (Volz et al., 2007) and METH-induced vesicular DA efflux (Volz et al., 2006). Striatal suspensions were placed in the glass chamber at 37°C, and a detection current baseline was established as described previously (Volz et al., 2007). The striatal suspensions then were preloaded with 10.2 μ l of 20 μ M DA solution (resulting in a concentration of 400 nM DA inside the chamber), and within 3 min, the detection current returned to the original baseline. After the DA was preloaded onto the striatal suspensions, a small quantity of assay buffer [126 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl₂, 16 mM sodium phosphate, 1.4 mM MgSO₄, and 11 mM glucose (pH 7.4)] containing 0.25 mM mephedrone, MDMA, or METH (resulting in 5 μ M concentrations inside the glass chamber) was added to the glass chamber to stimulate DA release. The resulting current outputs caused by DA release were recorded and converted to extravesicular DA concentration versus time profiles by calibrating with known DA concentrations as described previously (Volz et al., 2006). The initial velocities of mephedrone-stimulated DA release were calculated from the first 3 s of release and normalized to striatal wet weight as described previously (McElvain and Schenk, 1992; Volz et al., 2007).

Food Training. Rats were restricted to approximately 90% of their free-feeding food quantity and then placed in operant chambers connected to a personal computer running Graphic State software (Coulbourn Instruments, Allentown, PA). Each chamber was equipped with two retractable levers, one of which was the “active” lever resulting in the delivery of a food pellet, whereas the other lever had no programmed consequences. Training consisted of an overnight 14-h schedule of food reinforcement (45 mg of rodent grain food pellets; Bio-Serv, Frenchtown, NJ) at FR1, with only the stimulus-appropriate (drug) lever eliciting the reward.

Catheter Implantation. After food training, rats were anesthetized with 90 mg/kg ketamine i.p. and 7 mg/kg xylazine i.p., and indwelling catheters consisting of a threaded connector, Silastic tubing (10 cm, 0.51 mm o.d.; Dow Corning, Midland, MI), ProLite polypropylene surgical mesh (Atrium Medical Corporation, Hudson, NH), and dental cement were implanted subcutaneously proximal to the scapula. The distal end of the catheter tubing was inserted into the right jugular vein and was secured to the surrounding tissue with sutures. To maintain catheter patency, animals were infused daily with 0.1 ml of antibiotic solution containing cefazolin (10.0 mg/ml) dissolved in heparinized saline (70 U/ml; Sigma-Aldrich), followed by an infusion of 0.05 ml of heparin and 0.05 ml of heparinized glycol to lock the catheter.

Self-Administration. After 3 days of recovery, animals were assigned randomly to self-administer either mephedrone (0.24 mg per 10- μ l infusion), METH (0.24 mg per 10- μ l infusion), or saline (10- μ l infusion) for 7 or 8 days (4 h/day, room temperature 29°C). For each active lever press, an infusion pump (Coulbourn Instruments) connected to a liquid swivel (Coulbourn Instruments) suspended

outside the operant chamber delivered a 10- μ l infusion over a 5-s duration through polyethylene tubing located within a spring leash (Coulbourn Instruments) tethered to the rat. During this period, both levers were retracted. After the infusion, the levers remained retracted for an additional 20 s. The active lever was counterbalanced within each group. Pressing the inactive lever resulted in no programmed consequences, although it was recorded. Rectal temperatures were measured using a digital thermometer (Physitemp Instruments, Inc., Clifton, NJ) approximately 30 min after the end of each session.

Data Analysis. Statistical analyses between two groups were performed using a two-tailed Student's *t* test. Statistical analyses among multigroup data were conducted using one-way analysis of variance, followed by a Newman-Keuls post hoc test. Differences among groups were considered significant if the probability of error was $\leq 5\%$. IC₅₀ values were determined using a least-squares, non-linear regression fit with a minimum of eight data points (determined in triplicate) per curve and competing drug concentrations ranging from 1 nM to 5 μ M. Lever pressing and mephedrone intake during self-administration were analyzed using a two-way repeated-measures analysis of variance with Bonferroni multiple comparisons post hoc analysis. Rotating disk electrode DA release velocities during the first 3 s were calculated using linear regression. IC₅₀ values were determined, and all of the statistical analyses were performed using Prism 5 (GraphPad Software, Inc., San Diego, CA).

Results

Results presented in Fig. 1 reveal that repeated mephedrone injections (4 $\times$ 10 or 25 mg/kg s.c. per injection, 2-h intervals, administered in a pattern used frequently in rodent models to mimic psychostimulant “binge” treatment) cause a rapid (within 1 h) decrease in DA and 5HT transporter function, as assessed in striatal and hippocampal synaptosomes, respectively. This decrease was not likely due to residual drug introduced by the original subcutaneous injections, because other studies have demonstrated that the preparation of synaptosomes “washes” the drug from the preparation (Fleckenstein et al., 1997; Kokoshka et al., 1998b). Of note, the IC₅₀ value of mephedrone to inhibit DA uptake was similar to that of METH, whereas the IC₅₀ value for 5HT uptake was similar to that of MDMA (Table 1). Furthermore, mephedrone increased core body temperatures throughout the course of treatment from an average of 37.8 $\pm$ 0.1°C* for saline-treated rats to averages of 39.5 $\pm$ 0.1* and 40.0 $\pm$ 0.1°C* for rats treated with 10 and 25 mg/kg mephedrone, respectively (*, $p \leq 0.05$, significant difference from all of the other groups). Administration of 4 $\times$ 1 or 3 mg/kg s.c. per injection, 2-h intervals, was without effect on dopamine transporter or 5HT transporter function, as assessed 1 h

Fig. 1. Repeated mephedrone injections rapidly (within 1 h) decrease hippocampal synaptosomal 5HT uptake (A) and striatal synaptosomal DA uptake (B). Rats received mephedrone (4 $\times$ 10 or 25 mg/kg s.c. per injection, 2-h intervals) or saline (1 ml/kg s.c. per injection, 2-h intervals) and were sacrificed 1 h after the final injection. Columns represent means, and vertical lines represent 1 S.E.M. determinations in 6–10 rats. *, $p \leq 0.05$, significant difference from saline controls.

TABLE 1

IC₅₀ values for striatal DA uptake and hippocampal 5HT uptake in synaptosomes

IC₅₀ values represent the means of at least three independent experiments and were obtained as described under *Materials and Methods*.

Drug	DA Uptake IC ₅₀	5HT Uptake IC ₅₀
	<i>nM</i>	<i>nM</i>
Mephedrone	467 ± 17	558 ± 48
MDMA	1216 ± 263	291 ± 36
Cocaine	1032 ± 96	1036 ± 137
METH	291 ± 4*	NA

* Values were reported previously (Fleckenstein et al., 1997).

NA, not assessed.

after treatment. Specifically, striatal [³H]DA uptake levels were 1.7 ± 0.2, 1.8 ± 0.1, and 1.4 ± 0.1 fmol/μg protein for saline-, 4 × 1 mg/kg mephedrone-, and 4 × 3 mg/kg mephedrone-treated rats, respectively (*n* = 7–8; *p* > 0.05). Hippocampal [³H]5HT uptake levels were 0.8 ± 0.1, 0.7 ± 0.1, and 0.9 ± 0.1 fmol/μg protein for saline-, 4 × 1 mg/kg mephedrone-, and 4 × 3 mg/kg mephedrone-treated rats, respectively (*n* = 7–8; *p* > 0.05). Both doses of mephedrone increased core body temperatures throughout the course of treatment from an average of 37.4 ± 0.1°C* for saline-treated rats to averages of 38.3 ± 0.1°C* and 39.0 ± 0.1°C* for rats treated with 4 × 1 and 3 mg/kg mephedrone, respectively (*, *p* ≤ 0.05, significant difference from all of the other groups).

Data presented in Fig. 2 demonstrate that repeated mephedrone administrations (4 × 10 or 25 mg/kg s.c. per injection, 2-h intervals) also caused persistent decreases in hippocampal 5HT transporter function (Fig. 2A) and 5HT levels (Fig. 2B), as assessed 7 days after treatment. The 4 × 25 mg/kg mephedrone treatment decreased striatal 5HT levels as well 4.0 ± 0.6, 3.5 ± 0.5, and 2.1 ± 0.2* pg/μg protein

for saline, 4 × 10 mg/kg, and 4 × 25 mg/kg, respectively (*n* = 6–10; *, *p* ≤ 0.05, significant difference from saline controls). In contrast, mephedrone treatment was without effect on striatal DA transporter function (Fig. 2C), dopamine transporter immunoreactivity (data not shown), or DA concentrations (Fig. 2D) as assessed 7 days after treatment. Mephedrone increased core body temperatures throughout the course of treatment from an average of 37.7 ± 0.1°C* for saline-treated rats to averages of 39.2 ± 0.2* and 39.7 ± 0.1°C* for rats treated with 10 and 25 mg/kg mephedrone, respectively (*, *p* ≤ 0.05, significant difference from all of the other groups). Administration of 4 × 1 or 3 mg/kg mephedrone s.c. per injection, 2-h intervals, did not decrease DA or 5HT levels, as assessed 7 days after treatment. Specifically, striatal DA content was 110.9 ± 6.2, 119.8 ± 4.5, and 131.3 ± 7.3 pg/μg protein for saline-, 4 × 1 mg/kg per injection mephedrone-, and 4 × 3 mg/kg per injection mephedrone-treated rats, respectively (*n* = 8; *p* = 0.08), with DA content being slightly increased after 4 × 3 mg/kg mephedrone per injection. Hippocampal 5HT content was 5.7 ± 0.6, 6.7 ± 1.5, and 6.6 ± 0.8 pg/μg protein in saline-, 4 × 1 mg/kg per injection mephedrone-, and 4 × 3 mg/kg per injection mephedrone-treated rats, respectively (*n* = 7–8; *p* > 0.05). Both doses of mephedrone significantly increased core body temperatures throughout the course of treatment from an average of 37.4 ± 0.1°C* for saline-treated rats to averages of 38.8 ± 0.2* and 39.0 ± 0.1°C* for rats treated with 1 and 3 mg/kg mephedrone, respectively (*, *p* ≤ 0.05, significant difference from the saline-treated rats).

To determine brain and plasma mephedrone levels, a method was developed using liquid chromatography-mass spectrometry. Results from this assay revealed plasma levels

Fig. 2. Repeated mephedrone injections cause persistent decreases in hippocampal synaptosomal 5HT uptake (A) and hippocampal 5HT content (B), but not in striatal synaptosomal DA uptake (C) or striatal DA content (D) as assessed 7 days after treatment. Rats received mephedrone (4 × 10 or 25 mg/kg s.c. per injection, 2-h intervals) or saline vehicle (1 ml/kg s.c. per injection, 2-h intervals) and were sacrificed 7 days later. Tissues were assayed as described in "Synaptosomal [³H]DA and [³H]5HT Uptake" and "DA and 5HT Concentrations" (under *Materials and Methods*). Columns represent the means, and vertical lines represent 1 S.E.M. determinations in 6 to 10 rats. *, *p* ≤ 0.05, significant difference from all of the other groups.

Fig. 3. Mephedrone causes DA release from a striatal suspension. A 5.0 μ M concentration of METH, mephedrone, or MDMA was applied to a striatal suspension that was preloaded with DA (see *Materials and Methods*; $n = 7$ –11). The initial velocities of DA release (determined over the first 3 s) for METH, mephedrone, and MDMA were $0.29 \pm 0.01^*$, $0.25 \pm 0.01^*$, and $0.16 \pm 0.01^*$ nmol/(s $\cdot$ g tissue wet weight), respectively [$F_{(2,4071)} = 85.2509$; $^*p \leq 0.05$, significant difference from all of the other groups]. The maximal DA release for METH, mephedrone, and MDMA were $3.8 \pm 0.6^*$, $2.7 \pm 0.2^*$, and $1.7 \pm 0.2^*$ nmol/g tissue weight, respectively ($^*p \leq 0.05$, significant difference from all of the other groups).

of 384.2 ± 62.2 and 1294.3 ± 145.5 ng mephedrone/ml plasma as assessed 1 h after 4×10 or 25 mg/kg s.c. per injection, 2-h intervals, respectively. Whole-brain levels of 2.1 ± 0.2 and 7.8 ± 0.9 ng mephedrone/mg tissue were found 1 h after 4×10 and 25 mg/kg s.c. per injection, 2-h intervals, respectively.

DA release was assessed after application of 5.0 μ M METH, mephedrone, or MDMA onto striatal suspensions that were preloaded with DA (Fig. 3). Results revealed that the initial velocities (determined over the first 3 s) were $0.29 \pm 0.01^*$, $0.25 \pm 0.01^*$, and $0.16 \pm 0.01^*$ nmol DA/(s $\cdot$ g tissue wet weight) for METH, mephedrone, and MDMA, respectively [$F_{(2,4071)} = 85.2509$; $^*p \leq 0.05$, significant difference from all of the other groups]. The maximal values for DA release for METH, mephedrone, and MDMA were $3.8 \pm 0.6^*$, $2.7 \pm 0.2^*$ and $1.7 \pm 0.2^*$ nmol DA/g tissue weight, respectively ($^*p \leq 0.05$, significant difference from all of the other groups).

Figure 4A demonstrates that rodents self-administer mephedrone. Whereas saline self-administering animals ($n = 10$) decreased pressing from day 1 of self-administration

to day 8, mephedrone self-administering rats ($n = 13$) increased pressing [drug $\times$ day interaction, $F_{(7,147)} = 24.88$, $p \leq 0.05$; Fig. 4A]. Mephedrone self-administering animals increased daily drug intake from 1.77 ± 0.15 mg on day 1 to 6.78 ± 1.00 mg on day 8 [$F_{(7,84)} = 17.59$; $p \leq 0.05$]. Discrimination of the reinforced lever from the inactive lever increased from a ratio of 2.65:1 reinforced presses per inactive press on day 1 to 10.71:1 reinforced presses per inactive press on day 8 in mephedrone self-administering rats. Approximately 85% of mephedrone self-administering rats increased drug intake on 3 or more consecutive days. Mephedrone self-administration also increased core body temperature (assessed 30 min after the end of each daily session) from an average of $37.3 \pm 0.1^\circ\text{C}$ for saline-controls to an average of $38.0 \pm 0.1^\circ\text{C}$ for mephedrone self-administering rats ($^*p \leq 0.05$, significant difference from saline controls).

For comparison with data presented in Fig. 4A, animals were allowed to self-administer METH under identical conditions (e.g., same dosing, duration of sessions, etc.) as were used to study mephedrone self-administration (Fig. 4B). Again, saline self-administering animals ($n = 8$) decreased pressing from day 1 of self-administration to day 7, whereas METH self-administering rats ($n = 8$) rapidly acquired stable lever-pressing behavior [$F_{(6,84)} = 23.63$; $p \leq 0.05$]. Daily drug intake averaged 2.55 ± 0.06 mg of METH per session across the 7 days of treatment. Discrimination of the reinforced lever from the inactive lever averaged a ratio of 10.1:1 reinforced presses per inactive press in the METH self-administering animals. METH self-administration also increased core body temperature (assessed 30 min after the end of each daily session) from an average of $37.6 \pm 0.1^\circ\text{C}$ for saline controls to an average of $38.2 \pm 0.2^\circ\text{C}$ for METH self-administering rats ($^*p \leq 0.05$, significant difference from saline controls).

Discussion

The stimulant/hallucinogen mephedrone has received recent international attention. Most abusers report that, in terms of its subjective effects, the agent most resembles MDMA (Carhart-Harris et al., 2011). However, some abusers also liken its subjective effects to those of cocaine (Carhart-Harris et al., 2011). Of further interest are reports that, unlike MDMA (First and Tasman, 2010), some mephedrone abusers tend to binge on mephedrone (Schifano et al., 2010; but also see Carhart-Harris et al., 2011).

The present study demonstrates that mephedrone has sev-

Fig. 4. Mephedrone (A) and METH (B) are self-administered by rats. Rats were food-trained as described under *Materials and Methods*. After catheter implantation, rats were given access (4-h sessions, 7–8 days) to saline (10 μ l per infusion), mephedrone (0.24 mg per 10- μ l infusion), or METH (0.24 mg per 10- μ l infusion) according to an FR1 schedule of reinforcement as described under *Materials and Methods*. $^*p \leq 0.05$, significant difference from saline controls.

eral pharmacological characteristics in common with other well characterized psychostimulants such as MDMA and METH. First, the IC_{50} value for inhibition of striatal synaptosomal DA uptake resembles that of METH, whereas the IC_{50} value for inhibition of hippocampal synaptosomal 5HT uptake resembles that of MDMA. Second, such as METH and MDMA (Fleckenstein et al., 1999; Haughey et al., 2000; Metzger et al., 2000; Hansen et al., 2002), repeated high-dose injections of mephedrone, administered in a regimen designed to mimic binge use in humans, causes rapid decreases in DA and 5HT transporter function. Third, each of these agents promotes stimulant-induced hyperthermia.

Although METH and MDMA share many characteristics, one important factor that distinguishes METH and MDMA is that the latter causes persistent serotonergic deficits in rat and human models but largely spares dopaminergic neurons (Stone et al., 1986; Schmidt and Kehne, 1990; McCann et al., 1994; Reneman et al., 2001). In this respect, mephedrone more closely resembles MDMA. This is of interest, because most individuals who abuse mephedrone report subjective effects reminiscent of MDMA (Schifano et al., 2010; Carhart-Harris et al., 2011), suggesting similarities in the underlying mechanisms of action of these agents.

Despite the similarities noted above with the effects of MDMA, mephedrone causes greater DA release as assessed in a striatal suspension preloaded with equimolar concentrations of DA. In fact, in response to application of a 5 μ M concentration of the drug [a concentration selected based, in part, upon studies by Clausung et al. (1995) wherein extracellular brain levels in the μ M range were demonstrated after amphetamine administration], the in vitro DA release capacity of mephedrone approaches that of METH. Although this study is limited in that only a single drug concentration was employed, its results are consistent with recent microdialysis findings by Kehr et al. (2011) that mephedrone caused DA release (albeit the present study examined DA release from a striatal suspension) and mephedrone caused greater DA release than MDMA. These data are also consistent with reports by some users that the subjective effects of mephedrone resemble those of METH or a combination of MDMA and cocaine (Carhart-Harris et al., 2011). Finally, these data are consistent with our finding that mephedrone is readily self-administered by rats (Fig. 4). Of note, METH is a potent DA-releasing agent (Bowyer et al., 1993; Kuczenski et al., 1995; Tata and Yamamoto, 2007), and its high-dose administration causes persistent dopaminergic deficits (for review, see Hanson et al., 2004; Yamamoto and Bankson, 2005; Tata and Yamamoto, 2007, and references therein). Because mephedrone has DA-releasing capability resembling METH and yet does not cause dopaminergic deficits, it is of significant interest in terms of studying the differential mechanisms underlying the long-term damage caused by these stimulants.

Of note, mephedrone concentrations were evaluated and detected in both rat plasma and brain samples after controlled administration of mephedrone. Mean whole-brain levels of 2.1 ± 0.2 ng mephedrone/mg tissue were found 1 h after 4×10 mg/kg s.c. per injection, 2-h intervals. This value compares with mean brain levels of 4.3 ± 0.5 ng/mg tissue as reported 1 h after 4×5 mg/kg METH s.c. per injection, 2-h intervals (Truong et al., 2005). However, any comparison between these METH and mephedrone data must be made

very cautiously, because studies designed specifically to compare pharmacokinetics are necessary to address differences and similarities between the drugs.

Given the DA-releasing capacity of mephedrone, the finding that mephedrone readily penetrates the blood-brain barrier, that mephedrone is readily self-administered by rats, and that the reinforcing effects of psychostimulants are associated with increases in brain DA levels (Volkow et al., 1999), it is reasonable to speculate that mephedrone may have significant abuse liability. Indeed, results presented in Fig. 4A demonstrate that mephedrone is readily self-administered, as assessed over 8 days of exposure to 4-h sessions (0.24 mg/kg per infusion). For comparison, the ability of METH to elicit self-administration under identical experimental conditions was assessed. Results confirmed numerous reports that, like mephedrone, METH is readily self-administered. However, in contrast to effects of mephedrone, lever-pressing behavior did not increase over the 8-day duration of the experiment, possibly due to the increased stereotypy associated with this high infusion dose. Several factors probably account for this differential response, including differences in pharmacokinetics, differences in DA-releasing capabilities, and potential long-term consequences of repeated exposures (e.g., repeated high-dose METH administrations cause persistent dopaminergic damage, whereas data presented in Fig. 2, C and D, reveal that repeated high-dose mephedrone administrations do not cause such deficits).

In summary, mephedrone is a unique psychostimulant of abuse that shares pharmacological properties similar to, and yet distinct from, both METH and MDMA. Its ability to cause subjective effects resembling MDMA reportedly likely contributes to its abuse. However, its ability to cause DA release greater than MDMA may be particularly problematic in that, in comparison to MDMA, this drug may have enhanced abuse liability more resembling that of DA-releasing agents such as METH. Before this report, clinical and anecdotal reports have been the primary source of information concerning the stimulant. As noted above, this lack of reliable information is particularly problematic for public health policy makers and law enforcement organizations as they attempt to develop and implement appropriate strategies for dealing with the escalating recreational use of this substance and products that contain mephedrone and related drugs. In fact, the U.S. Drug Enforcement Administration recently appealed for information concerning mephedrone and its analogs. Thus, additional studies are needed to further investigate the impact of mephedrone and also the various synthetic analogs that are an important public health concern.

Authorship Contributions

Participated in research design: Hadlock, Webb, McFadden, Chu, Andrenyak, Gibb, Wilkins, Hanson, and Fleckenstein.

Conducted experiments: Hadlock, Webb, McFadden, Chu, Ellis, Allen, Andrenyak, Vieira-Brock, German, Conrad, and Hoonakker.

Contributed new reagents or analytic tools: Andrenyak and Wilkins.

Performed data analysis: Hadlock, Webb, McFadden, Chu, Andrenyak, Vieira-Brock, German, Wilkins, and Fleckenstein.

Wrote or contributed to the writing of the manuscript: Hadlock, Webb, Gibb, Hanson, and Fleckenstein.

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