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Research report

Mephedrone (4-methylmethcathinone) and intracranial self-stimulation in C57BL/6J mice: Comparison to cocaine

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HIGHLIGHTS

- Mephedrone and cocaine similarly affected ICSS responding in C57BL/6J mice.
- Both drugs decreased the EF50 and BSR threshold (θ_0).
- 10.0 mg/kg mephedrone (i.p.) lowered maximum operant response rate.
- Mephedrone had a slower onset of action than cocaine.

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ABSTRACT

The recreational use of cathinone-derived synthetic stimulants, also known as "bath salts", has increased during the last five years. A commonly abused drug in this class is mephedrone (4-methylmethcathinone or "meow–meow"), which alters mood and produces euphoria in humans. Intracranial self-stimulation (ICSS) measures the behavioral effects of neuroactive compounds on brain reward circuitry. We used ICSS to investigate the ability of mephedrone and cocaine to alter responding for electrical stimulation of the medial forebrain bundle in C57BL/6J mice. Adult male C57BL/6J mice ($n = 6$) implanted with unipolar stimulating electrodes at the level of the lateral hypothalamus responded for varying frequencies of brain stimulation reward (BSR). The frequency that supported half maximal responding (EF50), the BSR threshold (θ_0), and the maximum response rate were determined before and after intraperitoneal administration of saline, mephedrone (1.0, 3.0, or 10.0 mg/kg), or cocaine (1.0, 3.0, or 10.0 mg/kg). Mephedrone dose-dependently decreased EF50 (max. effect = 72.3% of baseline), θ_0 (max. effect = 59.6% of baseline), and the maximum response rate (max. effect = 67.0% of baseline) beginning 15 min after administration. Beginning immediately after administration, cocaine dose-dependently lowered EF50 (max. effect = 66.4% of baseline) and θ_0 (max. effect = 60.1% of baseline) but did not affect maximum response rate. These results suggest that mephedrone, like cocaine, potentiates BSR, which may indicate its potential for abuse. Given the public health concern of stimulant abuse, future studies will be necessary to determine the cellular and behavioral effects of acute and chronic mephedrone use.

1. Introduction

Recreational use of cathinone-derived synthetic stimulants, more commonly known as "bath salts", has increased in prevalence during the last five years. Of these, mephedrone (4-methylmethcathinone or "meow–meow") is popular among recreational users, most likely due to its availability and ability to elevate mood and produce euphoria [1]. Mephedrone use is associated with several stimulant-like drug effects, including increased concentration, talkativeness, psychomotor stimulation, reduced appetite, and insomnia [1]. Recent studies have described compulsive drug taking [2], and several deaths have been attributed to mephedrone use [3]. Not surprisingly, several countries, including the United States, have recently banned the production, possession, and sale of mephedrone and other cathinone derivatives [4].

Activation of mesocorticolimbic dopamine circuits is a common effect of drugs of abuse, and these circuits play a critical role in motivated behaviors, drug reinforcement, and drug seeking [5]. The effects of drugs of abuse on these circuits can be modeled in laboratory animals using several behavioral conditioning techniques, including intracranial self-stimulation (ICSS)

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[6,7]. ICSS measures the effects of drugs on operant responding for electrical stimulation of several brain regions, particularly the medial forebrain bundle (MFB). The MFB carries ascending dopaminergic projections from the ventral tegmental area (VTA) to targets in the nucleus accumbens (NAc) and prefrontal cortex (PFC), as well as descending glutamatergic and GABAergic fibers to the midbrain [5]. Stimulation of the MFB is potently reinforcing [8] and enhances dopamine release in terminal fields [9]. Drugs of abuse, especially psychomotor stimulants, reduce the amount of stimulation required to sustain responding, as measured by the stimulation frequency that supports half-maximal responding (EF50) or the brain stimulation reward (BSR) threshold, θ_0 [7,10,11].

In these studies, we investigated the behavioral effects of mephedrone in C57BL/6J mice using ICSS. While previous studies have examined mephedrone pharmacology and patterns of misuse in human populations (reviewed in [12]), the behavioral effects of mephedrone have been largely unexplored in rodents. BSR threshold, EF50, and maximum response rates were determined in C57BL/6J mice before and after treatment with mephedrone, cocaine, or saline vehicle to test the hypothesis that mephedrone would potentiate brain stimulation reward similarly to the psychostimulant cocaine.

2. Materials and methods

2.1. Mice

Male C57BL/6J mice ($n = 6$; Jackson Laboratories, Bar Harbor, ME) weighing at least 25 g were housed individually in polycarbonate cages (28 × 17 × 14 cm) with food and water freely available through wire lids. Cob-bedding was changed weekly, and the vivarium was 21 °C with a 12 h light cycle (lights on at 8:00 PM). Procedures, approved by the University of North Carolina Institutional Animal Care and Use Committee (IACUC), were conducted according to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 2011) between 8:30 AM and 12:30 PM.

2.2. Surgery

Under ketamine (120 mg/kg) and xylazine (18 mg/kg) (Sigma, St Louis, MO) anesthesia, mice were stereotaxically implanted with insulated monopolar stainless steel electrodes (0.28 mm diameter, Plastics One, Roanoke, VA) aimed at the right medial forebrain bundle at the level of the lateral hypothalamus (coordinates relative to bregma: AP −1.2 mm, ML −1.0 mm, DV −5.0 mm) [13], grounded to a stainless steel skull screw and secured to the skull with dental cement.

2.3. Intracranial self-stimulation

ICSS experiments were performed as previously described [14,15] in sound-attenuating chambers (16 × 14 × 13 in, MedAssociates, St Albans, VT) containing operant conditioning boxes with a grid floor (ENV-005A; MedAssociates), wheel manipulandum (ENV-113AM; MedAssociates) and house light (ENV-315W; MedAssociates). MED-PC software for Windows (v4.1; MedAssociates) controlled electrical stimulation (500 ms train of unipolar cathodal square-wave current 100 μs pulses and a trial-dependent frequency) through a stimulator (PHM-150B/2; MedAssociates) connected to a swivel commutator and insulated wire (Plastics One, Roanoke, VA) attached to the stimulating electrode. Each response (1 response = 1/4 turn of the wheel manipulandum) activated the house light and produced a stimulation. During the 500 ms stimulation period, wheel responses were recorded but did not earn additional stimulation.

Mice were initially conditioned to respond for brain stimulation reward (BSR) at a single stimulus intensity and frequency, after which stimulus intensity remained constant for each mouse. Mice were subsequently trained to respond for 15 decreasing stimulation frequencies (0.05 $\log_{10}$ steps) presented in three series. Each frequency was available for 50 s and separated by a 10 s timeout in which 5 non-contingent priming stimulations were delivered. For each response series, the maximum response rate was measured, and the frequency that maintains half-maximal responding (EF50) and sustains responding (BSR threshold or θ_0) were estimated through least squares regression. Daily baseline values were calculated from responses during the second and third series. When ICSS responding was stabilized, the mice were tested for the effects of mephedrone (1.0, 3.0, 10.0 mg/kg or saline i.p.) or cocaine (1.0, 3.0, 10.0 mg/kg or saline i.p.). After baseline responding, the mice were removed from the conditioning chambers, injected with drug, and returned immediately for four 15 min response series (i.e. 60 min of testing). Post-injection ICSS measures were expressed as a percentage of the pre-injection baseline on that day.

2.4. Histology

At the end of the experiment, 50 μm coronal brain sections were collected from each mouse following anesthesia with sodium pentobarbital (120 mg/kg i.p.) and intracardiac perfusion with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline. Sections were stained with cresyl violet for Nissl, and electrode locations were confirmed by direct microscopic visualization. One mouse died before the end of the study, and electrode placements were unavailable for this subject.

2.5. Drugs

Mephedrone (4-methylmethcathinone; generously provided by Dr. Michael Taffe, Scripps Research Institute, La Jolla, CA) and cocaine (Sigma) were dissolved in 0.9% saline and injected intraperitoneally through a 27-gauge needle in a volume of 1 ml/100 g body weight. Drug doses were given in a random order that alternated every other day with saline injections. Each drug dose was given twice and its effects on ICSS measures were averaged for each mouse. Only behavioral data from mice that received all drug doses were included in analyses of each drug effect. All experiments involving mephedrone were performed prior to scheduling by the United States Drug Enforcement Agency on October 21, 2011.

2.6. Data analysis

One-way repeated measures analysis of variance (ANOVA) determined the effects of mephedrone and cocaine on measures of ICSS. Bonferroni-corrected post hocs were performed when $p < 0.05$. Paired t-tests were used to compare repeated treatments for each drug dose and response series.

3. Results

The electrode placements are shown in Fig. 1A and B. All 6 mice that were implanted responded for electrical stimulation of the medial forebrain bundle within 2 sessions. Although electrodes were implanted at the AP (skull, relative to bregma) coordinate of −1.3 mm, tip locations varied from −1.06 to −1.58 mm. The average baseline EF50 and response threshold (θ_0) in these mice prior to all drug experiments expressed as charge delivery was -0.31 ± 0.028 μC and -0.36 ± 0.036 μC, respectively. The average baseline maximum response rate prior to all drug experiments was 162 ± 20.6 responses/50 s. The mice responded in a frequency-dependent manner, and mephedrone and cocaine produced parallel leftward shifts the rate-frequency curves, despite having different effects on maximum response rate (Fig. 1C). No significant differences were detected between drug replicates for each drug, dose, and response series.

Mephedrone dose-dependently lowered EF50 (Fig. 2) and BSR threshold (θ_0; Fig. 3) during the second ($F_{3,15} = 19.0$, $p < 0.001$; $F_{3,15} = 7.4$, $p = 0.003$, respectively), third ($F_{3,15} = 5.4$, $p = 0.01$; $F_{3,15} = 3.7$, $p = 0.04$, respectively), and fourth ($F_{3,15} = 12.6$, $p < 0.001$; $F_{3,15} = 3.7$, $p = 0.03$, respectively) 15 min post-injection response series when compared to saline vehicle. There was no significant effect on responding during the first 15 min of testing. Post hoc analysis revealed that the 3.0 mg/kg (i.p.) mephedrone dose significantly lowered EF50 during the second 15 min post-injection response series, while the 10.0 mg/kg dose (i.p.) significantly lowered EF50 during the second, third, and fourth response series. The 3.0 and 10.0 mg/kg (i.p.) mephedrone dose significantly lowered θ_0 during the second 15 min post-injection response series.

Cocaine dose-dependently lowered EF50 (Fig. 2) and BSR threshold (θ_0; Fig. 3) during the first 15 min post-injection response series when compared to saline ($F_{3,12} = 7.1$, $p = 0.005$; $F_{3,12} = 6.3$, $p = 0.008$, respectively). Cocaine lowered the EF50, but not BSR threshold, during the second ($F_{3,12} = 9.0$, $p = 0.002$) and third ($F_{3,12} = 7.4$, $p = 0.006$) 15 min post-injection response series when compared to saline. These doses did not significantly affect responding during the final 15 min of testing. Post hoc analysis revealed that the 3.0 mg/kg (i.p.) cocaine dose significantly lowered EF50 during the first and second 15 min post injection series when compared to saline vehicle. The 10.0 mg/kg (i.p.) cocaine dose significantly lowered EF50 during