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MEPHEDRONE ("BATH SALT") PHARMACOLOGY: INSIGHTS FROM INVERTEBRATES

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Abstract—Psychoactive bath salts (also called meph, drone, meow meow, m-CAT, bounce, bubbles, mad cow, etc.) contain a substance called mephedrone (4-methylcathinone) that may share psychostimulant properties with amphetamine and cocaine. However, there are only limited studies of the neuropharmacological profile of mephedrone. The present study used an established invertebrate (planarian) assay to test the hypothesis that acute and repeated mephedrone exposure produces psychostimulant-like behavioral effects. Acute mephedrone administration (50–1000 μM) produced stereotyped movements that were attenuated by a dopamine receptor antagonist (SCH 23390) (0.3 μM). Spontaneous discontinuation of mephedrone exposure (1, 10 μM) (60 min) resulted in an abstinence-induced withdrawal response (i.e. reduced motility). In place conditioning experiments, planarians in which mephedrone (100, 500 μM) was paired with the non-preferred environment during conditioning displayed a shift in preference upon subsequent testing. These results suggest that mephedrone produces three behavioral effects associated with psychostimulant drugs, namely dopamine-sensitive stereotyped movements, abstinence-induced withdrawal, and environmental place conditioning.

Key words: mephedrone, withdrawal, dopamine, motility, place preference, planarians.

Mephedrone [4-methylmethcathinone] is a synthetic compound of the amphetamine and cathinone classes that has emerged as a popular street drug in Europe and the USA (Winstock et al., 2010). Although the legal status of mephedrone varies between governed entities, it is available for purchase over the Internet and is often marketed as "plant feeder," "bath salts," "research chemicals," and "not for human consumption." Factors contributing to its popularity are ease of online accessibility, extensive web-based marketing, versatile administration route (intranasal, oral), high degree of purity, and high yields from simple manufacturing processes that utilize inexpensive precursors (Schifano and Corkery, 2008). Initial evidence of human mephedrone use appeared online in 2007 (Psychonaut Web Mapping Research Group, 2009). Most of what is currently known about mephedrone has been obtained from analyses of online discussions, single case reports, and small-scale focus groups (Meyer et al., 2010; Wood et al., 2010). The broadest human study to date focused on experienced drug users in the United Kingdom (Winstock et al., 2010, 2011). In that study, mephedrone was the sixth most frequently used drug (41.3% of 2295 participants) after nicotine, alcohol, cannabis, cocaine, and 3,4-methylenedioxymethamphetamine (MDMA). Evidence from human users suggests that mephedrone elicits a longer-lasting and better high than cocaine (Winstock et al., 2010). Further evidence suggests that mephedrone elicits euphoria and sociability without causing a hangover and produces a smoother high than MDMA (Newcombe, 2009). Risks of mephedrone use include toxicity (cardiac events, manganese toxicity, parkinsonian syndrome, etc.), lethality, and polydrug abuse (Al-Motarreb et al., 2010; Stepens et al., 2008).

Studies examining the pharmacological profile of mephedrone have begun to emerge. Mephedrone increases extracellular dopamine and serotonin in the mesolimbic system of rats through inhibition of dopamine and serotonin reuptake, and it is self-administered by rats (Kehr et al., 2011; Hadlock et al., 2011; Martínez-Clemente et al., 2012). In the present study, we characterized the *in vivo* behavioral effects of mephedrone in planarians, a type of aquatic flatworm that has a centralized nervous system, which some consider the simplest "brain" (Raffa and Rawls, 2008; Rawls et al., 2011). Planarians contain neurotransmitter systems, including glutamate, dopamine, serotonin, acetylcholine, and gamma-aminobutyric acid (Eriksson and Panula, 1994; Vyas et al., 2010; Nishimura et al., 2010), and to a limited extent display mammalian-equivalent behavioral responses (enhanced stereotypical activity, abstinence-related withdrawal, behavioral sensitization, cross sensitization, and conditioned place preference) following exposure to addictive substances from different drug classes (Palladini et al., 1996; Pagán et al., 2008, 2009; Rowlands and Pagán, 2008; Raffa and Rawls, 2008; Rawls et al., 2010a,b, 2011). The current experiments characterized mephedrone using three behavioral endpoints: acute stereotypical activity and its sensitivity to dopamine receptor antagonism; abstinence-induced with-

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Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; MEPH, mephedrone; VEH, vehicle.

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drawal following discontinuation of mephedrone exposure; and environmental place conditioning.

EXPERIMENTAL PROCEDURES

Subjects and Drugs

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply (Burlington, NC, USA). Upon arrival in the laboratory, planarians were maintained in the aqueous solution provided by Carolina Biological Supply, acclimated to room temperature (21 °C), and tested within 3 days of receipt. (R,S)-mephedrone was obtained from the Fox Chase Chemical Diversity Center (Doylestown, PA, USA). SCH 23390 ((R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride) was obtained from Tocris Bioscience (St. Louis, MO, USA). Stock solutions of each drug were prepared daily in a vehicle of tap water containing AmQuel® water conditioner. Treatment solutions were diluted with tap water containing AmQuel® water conditioner (1 ml Amquel per 1 gal of water).

Behavioral studies

Experiment 1: Does acute mephedrone exposure produce dopamine-sensitive stereotyped movements? Individual planarians were placed randomly into a transparent petri dish (5.5 cm diameter) containing a solution of mephedrone (0, 10, 50, 250, 100, 500, 1000 μM) for 10 min. Stereotyped movements were quantified as the number of C-like hyperkinesias during the 10-min exposure (Rawls et al., 2011; Raffa et al., 2010). Motility counts during the 10-min exposure interval were also quantified as the number of gridlines crossed, or re-crossed, by placing the petri dish over graphing paper with gridlines spaced 0.5 cm apart (Sacavage et al., 2008; Raffa and Valdez, 2001). For combined administration, stereotyped movements were quantified during a 10-min exposure to vehicle, SCH 23390 (0.3 μM), mephedrone (500 μM), or a combination of SCH 23390 (0.3 μM) (10-min pretreatment) and mephedrone (500 μM). The concentration of SCH 23390 was selected on the basis of its reported Ki values, which are 0.2 nM and 0.3 nM at D_1 and D_5 receptor subtypes, respectively (Bourne, 2001).

Experiment 2: Does the condition of mephedrone abstinence elicit a withdrawal response? A characteristic withdrawal response displayed by planarians following discontinuation of exposure to an addictive substance is reduced motility (Raffa and Valdez, 2001). Individual planarians pretreated for 60 min with mephedrone (1 μM) or vehicle were placed for 5 min into a petri dish (5.5 cm diameter) containing mephedrone (1 μM) or vehicle and motility experiments were conducted as described earlier in the text. The experiment was repeated with 10 μM mephedrone.

Experiment 3: Does mephedrone conditioning shift planarian environmental preference? Dark and "ambient" light environments were created by covering half (top and bottom) of a petri dish with black paper, and individual planarians were placed at the midline of the dish. The time spent in the non-preferred environment (light) over a 5-min interval was determined (pre-pairing response). Planarians were then conditioned with mephedrone (0, 10, 100, 500 μM) for 30 min in the non-preferred environment. Immediately following conditioning, planarians were placed back at the midline of a petri dish (half light and half dark) containing vehicle, and the time spent in the original non-preferred environment over a 5-min interval was determined (post-pairing response).

Data analysis

Comparisons of group means (±SEM) were evaluated by one-way ANOVA followed by Tukey's post hoc analysis or, for the environmental preference experiment, a two-way ANOVA (treatment, conditioning) followed by a Bonferroni test for multiple comparisons. Values of $P<0.05$ were considered statistically significant.

RESULTS

Acute mephedrone exposure elicits stereotyped movements that are attenuated by a dopamine receptor antagonist

Effects of different concentrations (0, 10, 50, 100, 250, 500, 1000 μM) of mephedrone on stereotyped movements are expressed in Fig. 1A. Planarians exposed to vehicle did not display stereotyped behaviors. Acute mephedrone exposure produced stereotyped movements that resulted in an $E_{max.}$ of 62.0±5.3 and EC_{50} of 251.1±36 (Fig. 1A). Planarian motility was significantly affected only by the two highest concentrations of mephedrone, 500 and 1000 μM, which attenuated motility by approximately 75% and 65%, respectively, relative to vehicle controls (106.1±13.6 motility counts/10 min) ($P<0.001$) (not shown).

For combination experiments with SCH 23390 (0.3 μM), a concentration of 500 μM mephedrone that produced a consistent, robust enhancement of stereotyped movements was selected (Fig. 1B). A significant main effect [$F(4,45)=21.3$, $P<0.0001$] was observed. SCH 233390 (0.3 μM) by itself did not produce stereotyped movements. Planarians exposed to mephedrone (500 μM) displayed a significant number of stereotyped movements compared with vehicle-treated planarians ($P<0.001$). Planarians pretreated for 10 min with SCH 23390 (0.3 μM) before co-exposure to a combination of SCH 23390 (0.3 μM) and mephedrone (500 μM) displayed 67% fewer stereotyped movements than planarians exposed only to mephedrone (500 μM) ($P<0.01$). The effect of SCH (0.3 μM) against a higher concentration (1000 μM) of mephedrone was less effective, with 37% fewer stereotyped movements observed in planarians treated with the drug combination ($P<0.05$, not shown). Simple co-exposure (without the pretreatment phase) of planarians to a combination of SCH 23390 (0.3 μM) and mephedrone (500 μM) resulted in 33% fewer stereotyped movements compared with mephedrone (500 μM) by itself ($P<0.05$, not shown). Mephedrone concentrations tested here did not cause lethality.

Discontinuation of mephedrone exposure elicits a withdrawal response

A significant main effect was identified for the 1 μM mephedrone data set [$F(3,36)=25.0$, $P<0.0001$]. Planarians pretreated with mephedrone (1 μM) for 60 min and then tested in vehicle (MEPH/VEH) displayed lower motility counts compared with planarians subjected to three other experimental conditions (Fig. 2): (1) mephedrone-naive planarians tested in vehicle (VEH/VEH) ($P<0.001$); (2) mephedrone-pretreated planarians tested in mephedrone (MEPH/MEPH) ($P<0.001$); and (3) vehicle-pretreated planarians tested in mephedrone (VEH/MEPH) ($P<0.001$). Mephedrone-naive planarians (VEH/VEH) displayed motility