Pharmacology, Biochemistry and Behavior 103 (2013) 501–509

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Long-term cognitive and neurochemical effects of "bath salt" designer drugs methylone and mephedrone

Bjørnar den Hollander [a,*], Stanislav Rozov [b], Anni-Maija Linden [a], Mikko Uusi-Oukari [c], Ilkka Ojanperä [d], Esa R. Korpi [a]

[a] Institute of Biomedicine, Pharmacology, Biomedicum Helsinki, Haartmaninkatu 8, FI-00014, University of Helsinki, Finland
[b] Neuroscience Center and Institute of Biomedicine, Anatomy, Biomedicum Helsinki, Haartmaninkatu 8, FI-00014, University of Helsinki, Finland
[c] Institute of Biomedicine, Pharmacology, Drug Development and Therapeutics, FI-20014, University of Turku, Finland
[d] Hjelt Institute, Department of Forensic Medicine, Kytösuontie 11, FI-00014, University of Helsinki, Finland

ARTICLE INFO

Article history:
Received 17 August 2012
Received in revised form 12 October 2012
Accepted 15 October 2012
Available online 22 October 2012

Keywords:
Methylone
Mephedrone
Cathinone
Monoamine
Neurotoxicity
Working memory

ABSTRACT

Introduction/aims: The use of cathinone-derivative designer drugs methylone and mephedrone has increased rapidly in recent years. Our aim was to investigate the possible long-term effects of these drugs on a range of behavioral tests in mice. Further, we investigated the long-term effects of these drugs on brain neurochemistry in both rats and mice.

Methods: We treated animals with a binge-like regimen of methylone or mephedrone (30 mg/kg, twice daily for 4 days) and, starting 2 weeks later, we performed behavioral tests of memory, anxiety and depression and measured brain levels of dopamine (DA), serotonin (5-HT), their metabolites and norepinephrine (NE). 5-HT and DA transporter (5-HTT and DAT) levels were also measured in rats by [^{3}H]paroxetine and [^{3}H]mazindol binding.

Results: Mephedrone reduced working memory performance in the T-maze spontaneous alternation task but did not affect neurotransmitter levels aside from a 22% decrease in striatal homovanillic acid (HVA) levels in mice. Methylone had little effect on behavior or neurotransmitter levels in mice but produced a widespread depletion of 5-HT and 5-HTT levels in rats.

Conclusions: Both methylone and mephedrone appeared to have a long-term effect on either behavioral or biochemical gauges of neurotoxicity in rodents.

1. Introduction

Recent years have witnessed a rise in the use of a particular type of stimulant designer-drugs primarily sold to consumers via the internet as "bath salts" or "plant food" in order to circumvent laws banning their sale as products for human consumption (Vardakou et al., 2011). A large number of different substances have been identified as constituents of bath salts, but among the most prevalent drugs are mephedrone (4-methylmethcathinone) and methylone (3,4-methylenedioxymethcathinone). The surge in popularity of these drugs has led to a recent ban of these substances in both the US and EU but this has done little to reduce their availability (McElrath and O'Neill, 2011).

The effects of these substances are described by users as a mix of AMPH, MDMA and cocaine, with many users rating them as "better than cocaine" (A.R. Winstock et al., 2011). Mephedrone appears to be highly reinforcing and users experience a strong desire to redose, leading them to ingest large amounts of the drugs in binges that can last several days (A. Winstock et al., 2011). This pattern of use leads to a risk of potentially fatal overdoses, and many emergency room visits as well as some deaths have been attributed to mephedrone and methylone use (Maskell et al., 2011; Pearson et al., 2012; Wood et al., 2011). Further, the use of these substances may also be associated with more subtle long-term effects on brain chemistry and cognition. Closer examination of the effects of these drugs is needed in order to create an evidence-based public health strategy and inform users of the possible risks associated with these substances.

Methylone and mephedrone show a strong structural (see Fig. 1) and pharmacological similarity with MDMA and METH, respectively.

Abbreviations: 5-HT, serotonin; 5-HTT, serotonin transporter; AMPH, D-amphetamine; ANOVA, analysis of variance; CCD, charge coupled device; COMT, catechol-O-methyltransferase; DA, dopamine; DAT, dopamine transporter; EDTA, ethylenediaminetetraacetic acid; GFAP, glial fibrillary acidic protein; HPLC, high performance liquid chromatography; HVA, homovanillic acid; LLOQ, lower limit of quantification; MAO-A, monoamine oxidase A; MAO-B, monoamine oxidase B; MDMA, 3,4-methylenedioxymethamphetamine; METH, methamphetamine; NE, norepinephrine; NET, norepinephrine transporter; SD, standard deviation; SEM, standard error of the mean; TAAR1, trace amine associated receptor 1; TH, tyrosine hydroxylase; VMAT-2, vesicular monoamine transporter 2.

* Corresponding author at: Institute of Biomedicine, Pharmacology, Haartmaninkatu 8 (Biomedicum Helsinki), 00290 Helsinki, P.O. Box 63, 00014, University of Helsinki, Finland. Tel.: +358 9 191 25356; fax: +358 9 191 25364.
E-mail address: bjornar.denhollander@helsinki.fi (B. den Hollander).

0091-3057/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2012.10.006

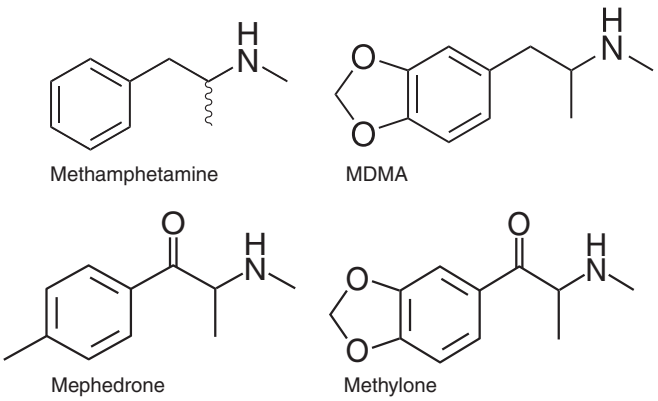

Fig. 1. Mephedrone and methylone share a strong structural similarity to METH and MDMA, respectively.

In vitro studies in synaptosomes reveal that these drugs are non-selective substrates for the monoamine transporters DAT, 5-HTT and NET, which lead to blockade of the reuptake and the enhancement of the release of monoamines by reversing the flow of the transporter, resulting in elevated synaptic neurotransmitter levels (Hadlock et al., 2011; Lopez-Arnau et al., 2012; Sogawa et al., 2011).

These findings are supported by another study which also demonstrated that a range of β-keto amphetamines, including mephedrone and methylone, inhibit the transporters and increase the release of DA, 5-HT and NE with varying potencies. Unlike the non-β-keto amphetamines, however, they exhibited an approximately 10-fold lower affinity for the TAAR1 (Simmler et al., in press). In vivo studies employing microdialysis in rats show a dose-dependent 2 to 9-fold increase in DA and 5-HT levels in the nucleus accumbens following acute challenges with methylone or mephedrone (Baumann et al., 2012; Kehr et al., 2011).

MDMA, METH and its primary active metabolite AMPH are neurotoxic and cause a long-lasting reduction in striatal and cortical DA and/or 5-HT levels in rodents (Ali et al., 1994; Battaglia et al., 1987; Friedman et al., 1998; O'Callaghan and Miller, 1994; O'Shea et al., 2001; Sabol et al., 2001), while animals as well as human users of these drugs show long-lasting and widespread decreases in 5-HTT and DAT binding (Johanson et al., 2006; McCann et al., 1998a,b; Reneman et al., 2001; Sekine et al., 2001; Semple et al., 1999). Furthermore, there is ample evidence linking the use of these substances with decreased memory function and increases in neuropsychiatric symptoms like anxiety and depression in animal models as well as humans (McCardle et al., 2004; McGregor et al., 2003; Moon et al., 2007; Parrott et al., 2000; Piper and Meyer, 2004; Sprague et al., 2003; Volkow et al., 2001). The structural and pharmacological similarities between MDMA/METH and methylone/mephedrone suggest the latter drugs may also produce similar effects on neurochemistry and neuropsychological function.

A few reports on the toxicity of these substances have already been published, but are not entirely consistent. For instance, Hadlock et al. (2011) reported decreased hippocampal 5-HT levels one week after mephedrone (4× 10 or 25 mg/kg) in rats whereas Baumann et al. (2012) found no effect of mephedrone or methylone (both 3× 3 or 10 mg/kg) on monoamine levels 2 weeks after treatment. Angoa-Perez et al. (2012) also found no effect of mephedrone (4× 20 or 40 mg/kg) on striatal DA levels or any other measure of neurotoxicity after one week in mice. These inconsistencies can be partially due to differences in the employed dosing-regimen and recovery period. However, there might also be species differences, as is the case with MDMA, which primarily affects the DA system in mice, but selectively affects the 5-HT system in rats (Logan et al., 1988). Moreover, the focus so far has been on biochemical measures of toxicity and no attention has been paid to the possible long-term cognitive and neuropsychiatric effects of methylone and mephedrone.

The present study aims to address some of these shortcomings in the literature. Specifically, our study comprised 2 experiments with the following aims: (1) to investigate the long-term effects of mephedrone and methylone on mice using behavioral models of memory, anxiety and depression; and (2) to compare the long-term effects of mephedrone, methylone and AMPH on brain monoamine levels in mice and rats. Additionally, we assessed the effect of mephedrone and methylone on 5-HTT and DAT levels in rats. We hypothesized that mice treated with methylone and mephedrone would show decreased performance on behavioral tests of memory and show anxious and depressed behavior compared to mice treated with saline and that, furthermore, methylone and mephedrone would decrease brain monoamine levels compared to saline-treated control animals and that the affected monoamine systems would differ between mice and rats.

2. Material and methods

2.1. Animals

The C57BL/J6 mice and Wistar rats used in this experiment were obtained through Nova-Scanbur (Sollentuna, Sweden). All animals were males and aged 8 weeks at arrival in the lab where they were randomized and housed in 3 per cage with food pellets (Harlan BV., Horst, Netherlands) and tap water available ad libitum at standard housing conditions (12-h light–dark cycle, lights on at 06:00 h; temperature, 20–23 °C; relative humidity, 50–60%; and aspen chip beddings). Animals were allowed 2 weeks to habituate to laboratory conditions prior to the experiments. All animal tests were approved by the Laboratory Animal Committee of the Southern Finland Provincial Government.

2.2. Drugs and dose titration

Racemic mephedrone and methylone were acquired from the Department of Forensic Medicine, Hjelt Institute, University of Helsinki, and analyzed for purity by gas chromatography and Fourier transform infrared spectroscopy which determined both compounds were pure (>95%) hydrochloride salts. D-Amphetamine sulphate was obtained from GlaxoSmithKline. The drugs were dissolved in a saline solution (0.9% NaCl) and administered intraperitoneally at a volume of 10 ml/kg for mice or 1 ml/kg for rats. Control groups were administered equal amounts of saline.

In pilot experiments in mice we tested doses of methylone and mephedrone between 15 and 60 mg/kg while observing locomotor activity and measuring body with a BAT-12 thermometer with rectal probe (Physitemp Instruments Inc., Clifton, NJ, USA). Both drugs produced hyperlocomotion and slight hyperthermia at 15 mg/kg while doses of 30 mg/kg produced a consistent 2 °C increase in body temperature and more pronounced increases in locomotor activity. Body temperatures were measured every 15 ± 5 min and reached a peak 45 ± 5 min after drug administration. Higher doses of methylone (45 and 60 mg/kg) further increased hyperthermia and caused lethality, with 100% lethality within 1 h at 60 mg/kg. Higher mephedrone doses (45 and 60 mg/kg) paradoxically caused hypothermia and produced no overt hyperlocomotion as the lower doses did. Furthermore, no lethality was observed at doses of up to 60 mg/kg of mephedrone. Due to the unpredictable and paradoxical effects of higher doses we opted to use the 30 mg/kg dose in the current experiments.

2.3. Experimental design

For the behavioral study, mice were treated with a binge-like regimen of mephedrone and methylone (30 mg/kg twice daily for 4 consecutive days) and tested on a range of behavioral tasks 2–8 weeks