Neuropharmacology xxx (2017) 1–11

Contents lists available at ScienceDirect

Neuropharmacology

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

Assessing the role of dopamine in the differential neurotoxicity patterns of methamphetamine, mephedrone, methcathinone and 4-methylmethamphetamine

John H. Anneken [a, b], Mariana Angoa-Perez [a, b], Girish C. Sati [c], David Crich [c], Donald M. Kuhn [a, b, *]

[a] Research & Development Service, John D. Dingell VA Medical Center, Detroit, MI, USA
[b] Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA
[c] Department of Chemistry, Wayne State University, Detroit, MI, USA

ARTICLE INFO

Article history:
Received 6 June 2017
Received in revised form
17 August 2017
Accepted 25 August 2017
Available online xxx

Keywords:
Methamphetamine
Mephedrone
4-Methylmethamphetamine
Methcathinone
Dopamine
Neurotoxicity

ABSTRACT

Methamphetamine and mephedrone are designer drugs with high abuse liability and they share extensive similarities in their chemical structures and neuropharmacological effects. However, these drugs differ in one significant regard: methamphetamine elicits dopamine neurotoxicity and mephedrone does not. From a structural perspective, mephedrone has a β-keto group and a 4-methyl ring addition, both of which are lacking in methamphetamine. Our previous studies found that methcathinone, which contains only the β-keto substituent, is neurotoxic, while 4-methylmethamphetamine, which contains only the 4-methyl ring substituent, elicits minimal neurotoxicity. In the present study, it was hypothesized that the varying neurotoxic potential associated with these compounds is mediated by the drug-releasable pool of dopamine, which may be accessed by methamphetamine more readily than mephedrone, methcathinone, and 4-methylmethamphetamine. To test this hypothesis, L-DOPA and pargyline, compounds known to increase both the releasable pool of dopamine and methamphetamine neurotoxicity, were combined with mephedrone, 4-methylmethamphetamine and methcathinone. Methamphetamine was also tested because of its ability to increase releasable dopamine. All three regimens significantly enhanced striatal neurotoxicity and glial reactivity for 4-methylmethamphetamine. Methcathinone neurotoxicity and glial reactivity were enhanced only by L-DOPA. Mephedrone remained non-neurotoxic when combined with either L-DOPA or pargyline. Body temperature effects of each designer drug were not altered by the combined treatments. These results support the conclusion that the neurotoxicity of 4-methylmethamphetamine, methcathinone and methamphetamine may be differentially regulated by the drug-releasable pool of dopamine due to β-keto and 4-methyl substituents, but that mephedrone remains non-neurotoxic despite large increases in this pool of dopamine.

Published by Elsevier Ltd.

1. Introduction

The β-ketoamphetamine mephedrone (MEPH)[1] is a common constituent of bath salts drug cocktails, and despite being a controlled substance of abuse, is still being used frequently (Hockenhull et al., 2016; Papaseit et al., 2016). Users of the drug report subjective effects including feelings of euphoria, well-being, and altered sensory perceptions, but acute toxicity and occasional deaths have also been reported (Papaseit et al., 2016). MEPH has a markedly similar neurotransmitter releasing effect on dopamine (DA) and serotonin (5-HT), mediated by their respective reuptake

* Corresponding author. R & D Service (11R), John D. Dingell VA Medical Center, 4646 John R, Detroit, MI, 48201, USA.
E-mail address: donald.kuhn@wayne.edu (D.M. Kuhn).

[1] *Abbreviations*: 4 MM, 4-methylmethamphetamine; 5-HT, serotonin; ANOVA, analysis of variance; DA, dopamine; DAT, dopamine transporter; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acid protein; HPLC, high performance liquid chromatography; Iba-1, ionized calcium-binding adapter molecule 1; MeCa, methcathinone; MEPH, mephedrone; METH, methamphetamine; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter-2.

http://dx.doi.org/10.1016/j.neuropharm.2017.08.033
0028-3908/Published by Elsevier Ltd.

transporters, in comparison with methamphetamine (METH), its non-β-keto analog (Baumann et al., 2012; Cameron et al., 2013; Eshleman et al., 2013; Golembiowska et al., 2016; Lopez-Arnau et al., 2012; Simmler et al., 2013; Suyama et al., 2016). Additionally, MEPH shares similarities with METH in its acute effects on thermoregulation (Baumann et al., 2012; Martinez-Clemente et al., 2014; Shortall et al., 2016), locomotor stimulation (Baumann et al., 2012; Lopez-Arnau et al., 2012; Marusich et al., 2012; Motbey et al., 2012; Nguyen et al., 2016; Wright et al., 2012), and indicators of addictive liability (Creehan et al., 2015; Hadlock et al., 2011; Karlsson et al., 2014; Lisek et al., 2012). Despite these similarities, these two compounds differ in their ability to evoke long-term toxicity to DA nerve endings.

It has been well established in rodent models that the classic amphetamines, including METH, elicit long-lasting damage to DA nerve terminals. For METH, this is typically manifested as reductions in markers of presynaptic dopaminergic integrity such as DA, the dopamine transporter (DAT), and the synthetic enzyme tyrosine hydroxylase (TH) (Moratalla et al., 2015). This toxicity is thought to be mediated via pathways involving neuroinflammation and oxidative stress (Halpin et al., 2014a; Yamamoto and Raudensky, 2008). It has been proposed that the excessive DA release evoked by METH is a primary factor, as the metabolism and auto-oxidation of DA is known to generate reactive species that can contribute to neuronal damage (Halpin et al., 2014a). MEPH has generally not been found to evoke this long-lasting dopaminergic toxicity. Most rodent studies under standard conditions known to evoke neurotoxicity with METH have not reported similar neurochemical or inflammatory changes in MEPH-treated animals (Angoa-Perez et al., 2012, 2013; Anneken et al., 2015; Anneken et al., 2017; Baumann et al., 2012; den Hollander et al., 2013; Motbey et al., 2013), except under harsher environmental conditions (den Hollander et al., 2014; Martinez-Clemente et al., 2014).

MEPH differs from METH by 2 substituents: a β-keto group, and a 4-methyl group on the phenyl ring. A recent study in this lab investigated the toxicity of two intermediate compounds, methcathinone (β-keto; MeCa) and 4-methylmethamphetamine (4-methyl; 4 MM) (Anneken et al., 2017) (see Fig. 1). MeCa, although less potent, elicited dopaminergic toxicity resembling METH, while 4 MM resembled MEPH in that it had greatly diminished dopaminergic toxicity compared to METH. The mechanism of this differential toxicity remains to be elucidated. Multiple studies have shown that increases in the releasable pool of DA augment METH toxicity (Guillot et al., 2008; Kita et al., 1995; Kuhn et al., 2008; Thomas et al., 2008, 2009). MEPH, which is non-toxic, enhances METH toxicity as well and may do so via interactions with the releasable pool of DA (Angoa-Perez et al., 2013), as it has been reported that METH and MEPH both release DA via reverse transport through the DAT in vitro (Simmler et al., 2013). However, Eshleman et al. (2013) observed that MEPH is much less effective at releasing vesicular norepinephrine via the vesicular monoamine transporter ($VMAT_2$) in vitro than METH, and also less effective in the amount of release it evokes via DAT reverse transport, releasing half the amount of DA observed with METH. The inability of MEPH alone to increase the cytosolic, drug-releasable pool of DA in a $VMAT_2$-dependent manner could explain its low neurotoxic potential by comparison to METH, which releases DA from vesicles into the cytosol, and then through the DAT into the synapse. MeCa, which is neurotoxic, also released a greater amount of DA via the DAT when compared to non-toxic MEPH (Eshleman et al., 2013). In the same study, while MeCa had a lower binding affinity and release profile at $VMAT_2$ compared to METH, it was found to release norepinephrine from $VMAT_2$ in slightly higher amounts than MEPH (42% compared with 33%).

To test whether these variations in dopamine release could account for the differential toxic potential among these structural analogs, we hypothesized that increasing the drug-releasable pool of DA, by administration of either the DA precursor L-DOPA, the monoamine-oxidase (MAO) B inhibitor pargyline, or a mild dose of METH, which can release vesicular DA to the cytosol, would impart toxicity to MEPH, as well as enhance the dopaminergic toxicity of the two closely related compounds, 4 MM and MeCa.

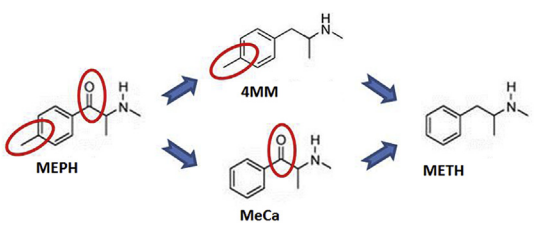

Fig. 1. Comparative structures of MEPH, METH, and intermediate structures. Diagram depicts the structures of the related compounds methamphetamine (no structural substituents), 4-methylmethamphetamine (4-methyl), methcathinone (β-keto), and mephedrone (4-methyl and β-keto). Reprinted from Anneken et al. (2017) with permission from the American Society for Pharmacology and Experimental Therapeutics.

2. Materials and methods

2.1. Drugs and reagents

(R,S)-N-Methcathinone HCl and (R,S)-mephedrone HCl were provided by the NIDA Research Resources Drug Supply Program. Racemic 4-methlymethamphetamine HCl was synthesized as described by Davis et al. (2012) from methylamine HCl and 4-methylphenylacetone purchased from Alfa Aesar (Ward Hill, MA, USA). (+)- Methamphetamine HCl, pargyline HCl, L-3,4-dihydroxyphenylalanine (L-DOPA), S-(-)-carbidopa, DA, polyclonal antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and all buffers and HPLC reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bicinchoninic acid protein assay kits for Western blot analysis were obtained from Pierce (Rockford, IL, USA). Polyclonal antibodies against rat TH were produced as previously described (Kuhn and Billingsley, 1987). Monoclonal antibodies against rat DAT were generously provided by Dr. Roxanne Vaughan (University of North Dakota, Grand Forks, ND, USA). IRDye secondary antibodies for Odyssey Imaging Systems were purchased from LiCor Biosciences (Lincoln, NE, USA).

2.2. Animals

Female C57BL/6 mice (Harlan, Indianapolis, IN, USA) weighing 18–25 g at the time of experimentation were housed 5–7 per cage in large shoe-box cages in a light- (12 h light/dark) and temperature-controlled room. Female mice were used as they have been shown to be impacted by the neurotoxicity induced by amphetamines and to maintain consistency with our previous studies of METH and β-ketoamphetamine interactions (Angoa-Perez et al., 2012, 2013; Anneken et al., 2015; Anneken et al., 2017). Mice had free access to food and water. The mice used were randomly divided into treatment groups. The Institutional Care and Use Committee of Wayne State University approved the animal care and experimental procedures. All procedures were also in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* and were conducted in compliance with ARRIVE guidelines and under IACUC-approved protocols.

Please cite this article in press as: Anneken, J.H., et al., Assessing the role of dopamine in the differential neurotoxicity patterns of methamphetamine, mephedrone, methcathinone and 4-methylmethamphetamine, Neuropharmacology (2017), http://dx.doi.org/10.1016/j.neuropharm.2017.08.033