Journal of Chemical Neuroanatomy 83–84 (2017) 69–74

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

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

Atypical dopamine efflux caused by 3,4-methylenedioxypyrovalerone (MDPV) *via* the human dopamine transporter

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ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form 20 January 2017
Accepted 24 January 2017
Available online 3 February 2017

Keywords:
3,4-methylenedioxypyrovalerone
Dopamine transporter
Amphetamine
Drosophila

ABSTRACT

Synthetic cathinones are similar in chemical structure to amphetamines, and their behavioral effects are associated with enhanced dopaminergic signaling. The past ten years of research on the common constituent of bath salts, MDPV (the synthetic cathinone 3,4-methylenedioxypyrovalerone), has aided the understanding of how synthetic cathinones act at the dopamine (DA) transporter (DAT). Several groups have described the ability of MDPV to block the DAT with high-affinity. In this study, we demonstrate for the first time a new mode of action of MDPV, namely its ability to promote DAT-mediated DA efflux. Using single cell amperometric assays, we determined that low concentrations of MDPV (1 nM) can cause reverse transport of DA *via* DAT. Notably, administration of MDPV leads to hyperlocomotion in *Drosophila melanogaster*. These data describe further how MDPV acts at the DAT, possibly paving the way for novel treatment strategies for individuals who abuse bath salts.

1. Introduction

The neurotransmitter dopamine (DA) mediates behaviors relating to reward, motivation, attention, and cognition (Iversen and Iversen, 2007; Bjorklund and Dunnett, 2007). Important to dopamine neurotransmission is the dopamine transporter (DAT). DAT is a presynaptic membrane protein responsible for the reuptake and recycling of DA following vesicular release (Giros and Caron, 1993). Dysfunctions in DAT can lead to dopamine-associated neuropsychiatric disorders including ADHD, autism spectrum disorders, schizophrenia, and bipolar disorder (Gowrishankar et al., 2014). DAT is also the target of commonly abused psychostimulants and controlled substances, namely cocaine and amphetamine (AMPH). Cocaine acts as a high-affinity antagonist of the transporter and blocks DA uptake, whereas AMPH acts as a substrate of the transporter and, through a series of intracellular mechanisms, causes DAT to reverse transport or "efflux" DA into the extracellular space (Schmitt and Reith, 2010). The actions of cocaine and AMPH on the DAT are well-known to play a role in their rewarding properties and abuse potential. Thus, determining the effects of psychostimulants on DAT function is important for understanding the neural and molecular mechanisms underlying psychostimulant drug action.

In recent years, the abuse of synthetic cathinones or "bath salts" has become a major world-wide health concern (German et al., 2014). These substances are synthetic derivatives of the naturally-occurring stimulant, cathinone, found in the flowering plant *Catha*

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http://dx.doi.org/10.1016/j.jchemneu.2017.01.004
0891-0618/

edulis (Brenneisen et al., 1990). The psychoactive effects of synthetic cathinones vary from the cocaine-like stimulant effects seen with 3,4-methylenedioxypyrovalerone (MDPV) (Baumann et al., 2013) to the MDMA-like empathogenic effects of methylone (3,4-methylenedioxymethcathinone) (Liechti, 2015). Among a number of identified biological sites, cathinones are known to target proteins that modulate dopamine neurotransmission, increasing dopaminergic signaling and associated behaviors (Glennon et al., 1987; Nguyen et al., 2016; King et al., 2015; Novellas et al., 2015; Kehr et al., 2011; Bonano et al., 2014), including drug-seeking (Watterson et al., 2013; Lisek et al., 2012). When consumed in small doses, cathinones can lead to euphoria, alertness, increased libido, and elevated blood pressure. When consumed at higher doses, tremors, seizures, paranoia, violent behavior, psychoses, tachycardia (Borek and Holstege, 2012), delusions/hallucinations (Penders and Gestring, 2011), and death (Wyman et al., 2013) can occur. A recent report released by the Substance Abuse and Mental Health Services Administration (SAMHSA) showed that nearly 23,000 emergency room visits in 2011 were a result of cathinone abuse (SAMHSA 2013 Bath Salts Report https://www.dea.gov/druginfo/drug_data_sheets/Bath_-Salts.pdf). Due to the high risk associated with the use and the abuse potential of these compounds, the Drug Enforcement Administration (DEA) designated mephedrone (4-methylmethcathinone), methylone and MDPV as Schedule 1 substances under the Controlled Substances Act http://www.who.int/medicines/areas/quality_safety/4_13_Review.pdf (DEA Drug Fact Sheet on Synthetic Cathinones). Nonetheless, illegal manufacturers continue to circumvent this ban by synthesizing "designer" substances with novel chemical structures but which produce similar psychostimulant effects (Marusich et al., 2014). These compounds are readily available and sold with fraudulent labels such as "plant food", "research chemicals", or "bath salts" at gas stations, tobacco stores, and over the Internet with a warning that the contents are not intended for human consumption. Their continued production and availability make it nearly impossible to control the exponentially rising sales and consumption of synthetic cathinones.

Despite increased data regarding the use and abuse of cathinones (Zawilska and Wojcieszak, 2013), little is known about their mechanism of action. To address this issue, several research groups have begun to study the chemistry, pharmacology, and behavioral effects of various synthetic cathinones. Of these, MDPV is most commonly implicated in high-risk use (Borek and Holstege, 2012; Wyman et al., 2013; Marusich et al., 2014; Coppola and Mondola, 2012a; Ross et al., 2012; Wright et al., 2013; Murray et al., 2012; Kriikku et al., 2011). First synthesized in 1969, MDPV gained popularity much later in 2010 (2014 World Health Organization Critical Review Report on MDPV). As a highly lipophilic analogue of the synthetic cathinone pyrovalerone (Coppola and Mondola, 2012b), MDPV readily crosses the blood-brain barrier. Importantly, MDPV, when administered to animals exhibits striatal distribution, a brain region enriched in DA projections (Novellas et al., 2015). MDPV also shows high abuse potential in animal behavioral tasks (King et al., 2015; Novellas et al., 2015; Kehr et al., 2011; Watterson et al., 2014).

Early research on MDPV demonstrated that this drug acts similarly to cocaine (a known DAT blocker), but with a 10- to 50-fold higher potency (Baumann et al., 2013; Cameron et al., 2013). However, increasing data suggests that there may be more to the action of MDPV. Work from Baumann et al. showed that after intravenous administration of MDPV, DA levels remain elevated for far longer than after cocaine administration (Baumann et al., 2013). In addition, MDPV administration results in long lasting cross-sensitization in mice, similar to the effects of methamphetamine (Watterson et al., 2013). These results suggest that MDPV, in addition to acting as a DAT blocker, may also display other modes of action. To examine further the molecular actions of MDPV on the DAT, we performed amperometric studies. Specifically, to obtain greater temporal resolution, we studied MDPV action on human DAT (hDAT) by employing single cell amperometry. This assay has been previously used to discriminate AMPH versus cocaine actions in a single cell and these results have been reproduced in different model systems (Cartier et al., 2015). Further, we assessed MDPV-induced behaviors in *Drosophila melanogaster*, specifically focusing on known DAT-associated behaviors. *Drosophila* is a powerful genetic model for studying behaviors that are associated with DA as well as promoted by psychostimulants (Cartier et al., 2015; Hamilton et al., 2014; Hamilton et al., 2013), as several genes that regulate DA transport, synthesis, and signaling are conserved between flies and humans (Yamamoto and Seto, 2014).

2. Methods

2.1. Drugs

(±)-3,4-Methylenedioxypyrovalerone HCl (MDPV), was synthesized in racemic form in our laboratories. Chemical and structural analysis included nuclear magnetic resonance spectroscopy, gas- and liquid- chromatography/mass spectrometry, thin layer chromatography, and melting point determination. All data were consistent with the expected structures. All other drugs used in this study including their salt and enantiomeric forms were as follows and purchased from Sigma-Aldrich (St. Louis, MO): Dopamine,D-amphetamine hemisulphate salt and cocaine hydrochloride.

2.2. Amperometry

Chinese hamster ovary (CHO) cells stably expressing hDAT (here defined as hDAT cells) were plated at a density of ~20,000 per 35-mm culture dish. To preload cells with DA, dishes were washed with KRH assay buffer (130 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 25 mM HEPES, 1.1 mM $MgSO_4$ 2.2 mM $CaCl_2$, pH 7.4) supplemented with 10 mM dextrose, 100 μM pargyline, 1 mM tropolone, and 100 μM ascorbic acid, and incubated with 1 μM DA in KRH assay buffer for 20 min at 37 °C. To record DA efflux, a carbon fiber electrode (ProCFE; fiber diameter of 5 μm; obtained from Dagan Corporation) juxtaposed to the plasma membrane and held at +700 mV (a potential greater than the oxidation potential of DA) was used to measure DA flux through oxidation reactions. Amperometric currents in response to the addition of 1 nM MDPV were recorded using an Axopatch 200B amplifier (Molecular Devices, Union City, CA) with a low-pass Bessel filter set at 1 kHz; traces were digitally filtered offline at 1 Hz using Clampex9 software (Molecular Devices, Union City, CA). DA efflux was quantified as the peak value of the amperometric current.

2.3. Drosophila melanogaster behavior

To measure the locomotor response to MDPV we used the TriKinetics *Drosophila* Activity Monitoring (DAM) system as described in earlier studies (Pfeiffenberger et al., 2010; Andretic et al., 2005). Wild-type Oregon-R male flies were entrained for seven days in 12:12 h light:dark (LD) cycles at 25 °C on standard cornmeal-molasses medium. On day two, flies were transferred individually to activity tubes and acclimated for a period of five days. On day seven, flies were transferred into identical activity tubes containing 20 μM MDPV or vehicle (water) in standard medium. Flies were continuously monitored for movement using activity monitors (DAM5, Trikinetics). Activity was measured as the number of times a fly crossed the infrared beam (beam crosses)