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Cooling down the bath salts: Carvedilol attenuation of methylone and mephedrone mediated hyperthermia

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HIGHLIGHTS

- Methylone and mephedrone induce hyperthermia.
- Methylone and mephedrone increase Temperature Area Under the Curve (TAUC).
- The α_1 and β adrenergic receptor antagonist carvedilol blocks this hyperthermia.
- Carvedilol significantly reduced the TAUC associated with methylone or mephedrone.

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ABSTRACT

The use of the synthetic cathinones ("bath salts"), methylone and mephedrone, has been associated with the development of life-threatening hyperthermia. To date, no direct pharmacological intervention to mitigate the hyperthermia induced by synthetic cathinones has been identified. Here, we investigated the effects of the non-selective α_1 and β adrenergic receptor antagonist carvedilol (5 mg/kg ip) on established hyperthermia mediated by methylone and mephedrone (30 mg/kg sc) in Sprague-Dawley rats. Methylone and mephedrone induced a hyperthermic response that peaked 60 min post treatment. The administration of carvedilol 30 min after methylone or mephedrone significantly attenuated these hyperthermic responses. Analysis of the Temperature Area Under the Curve (TAUC) demonstrated carvedilol significantly reduced the TAUC associated with methylone or mephedrone alone. The present study provides the first direct pharmacological intervention for the treatment of synthetic cathinone induced hyperthermia.

1. Indroduction

Synthetic cathinone ("Bath Salts") overdose is characterized by hyperthermia, diaphoresis, tachycardia, agitation, and hypertension (Miotto et al., 2013). Synthetic cathinones are pharmacologically and structurally similar to the sympathomimetic agent 3,4-methylenedioxymethamphetamine (MDMA) (Simmler et al., 2013; Vandewater et al., 2015). Hyperthermia is linked to the cause of death resulting from synthetic cathinone overdose (Pearson et al., 2012; Kesha et al., 2013). Centrally, dopaminergic mechanisms (for a review, see Green et al., 2004) are activated to subsequently trigger peripheral noradrenergic mechanisms linked to sympathomimetic-mediated hyperthermia. Peripherally, the hyperthermic effects of MDMA have been suggested to be induced by norepinephrine (NE) mediated activation of α_1 and $\beta_{1,2,3}$ adrenergic receptors (Sprague et al., 2005). Activation of the α_1 adrenergic receptors produces vasoconstriction, preventing heat dissipation (Pedersen and Blessing, 2001). Additionally, NE binds and activates plasma membrane $\beta_{1,2,3}$ adrenergic receptors (β-AR) on brown and white adipocytes leading to the activation of lipases that catalyze the liberation of free fatty acids (FFA) from triglycerides. FFA in turn are transported to mitochondria, where they bind to uncoupling proteins (UCP1 and UCP3) and activate thermogenesis (Nakamura et al., 2001; Nedergaard et al., 2001; Sprague et al., 2007). In animal models, methylone and mephedrone have been demonstrated to induce a hyperthermic effect but

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; NE, norepinephrine; FFA, free fatty acids; CNS, central nervous system; DEA, Drug Enforcement Administration; DA, dopamine; 5HT, serotonin; NET, norepinephrine transporter; DAT, dopamine transporter; SERT, serotonin transporter; TAUC, temperature area under the curve; sc, subcutaneous; ip, intraperitoneal.

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to a lesser extent than MDMA (Baumann et al., 2012; Grecco and Sprague, 2016).

1.1. Synthetic cathinones: methylone and mephedrone

The synthetic cathinones, methylone and mephedrone, have been shown to produce psychoactivity and central nervous system (CNS) stimulation (DEA, 2013a, 2013b). Synthetic cathinones are commonly referred to by the colloquial term "bath salts" as a ruse to allow these compounds to be legally and subtly sold in retail stores (Banks et al., 2014). Structurally, methylone differs from MDMA by the addition of a ketone on the β-carbon and is occasionally referred to as bk-MDMA (Fig. 1A). Methylone is known to produce an entactogenic effect like that of MDMA, which is likely due to the close structural similarity to MDMA (Vandewater et al., 2015). Methylone emerged in the United States illegal drug market in 2009 and was made a schedule I compound by the DEA in 2011 (DEA, 2013a). Along with 3,4-methylenedioxypyrovalerone (MDPV), methylone is the most commonly abused synthetic cathinone in the U.S. (German et al., 2014).

Mephedrone (β-keto-4-methylmethamphetamine; Fig. 1B), was first reported in Europe in 2007, and the use of the drug increased rapidly from 2009 through 2010 (Dargan et al., 2011). Mephedrone could be purchased from both street-level dealers and internet sources legally in the United Kingdom until April of 2010 (Dargan et al., 2011). Pharmacologically, mephedrone resembles MDMA and cocaine by inducing euphoria, enhanced appreciation of music, general stimulation, and elevated mood (Dargan et al., 2011). Mephedrone was made a schedule I drug by the DEA in 2011 (DEA, 2013b).

Methylone and mephedrone act as nonselective releasing substrates for monoamine transporters in the CNS and significantly increase dopamine (DA), NE, and serotonin (5-HT) levels (Baumann et al., 2012). In-vitro, methylone is about half as potent as mephedrone when acting as a ligand for the NE transporter (NET), DA transporter (DAT), and 5-HT transporter (SERT) (Baumann et al., 2012). These transporters usually take a primary role of reuptake of specific neurotransmitters back into the presynaptic axon terminal (Iversen, 1971). However, when bound to the transporters methylone and mephedrone are ostensibly linked to the release of monoamines (Sitte and Freissmuth, 2010; Baumann et al., 2012). Their releasing capabilities resembled that of MDMA, but with less potency (Baumann et al., 2012).

1.2. Carvedilol and synthetic cathinones

Hyperthermia is considered one of the most severe symptoms of acute methylone and mephedrone overdose, yet treatment of this symptom remains palliative (Pearson et al., 2012; Miotto et al., 2013). In an animal model, the non-selective α_1 and $\beta_{1,2,3}$ adrenergic receptor antagonist, carvedilol has been shown to prevent or completely reverse MDMA induced hyperthermia when given 15 min before or 1 h after MDMA treatment (Sprague et al., 2005). In humans, a 50 mg oral dose of carvedilol taken one hour before a 125 mg oral dose of MDMA reduced the increases in blood pressure, heart rate, and core body temperature generated by MDMA (Hysek et al., 2012). Here, we investigate the effects of carvedilol treatment on the hyperthermia induced by methylone and mephedrone.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats were used (Harlan, Indianapolis, IN). Experimental group weight was 320.9 g ± 2.0. Animals were housed two per cage (cage size: 21.0 × 41.9 × 20.3 cm^3), maintained on a 12:12 h light/dark schedule, and provided ad libitum access to food and water. In order to maximize a thermogenic response, animals were maintained at an ambient temperature of 25 °C–27 °C, and fed a minimum 10% fat diet (Dafters, 1994; Mills et al., 2007). The optimize thermogenic conditions were maintained throughout the study. Animal maintenance and research were conducted in accordance with the eighth edition of the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health, and protocols were approved by the Bowling Green State University Animal Care and Use Committee.

2.2. Study design

Animals were randomly assigned to 4 groups (n = 5–6) with two groups administered methylone (30 mg/kg sc) and two groups administered mephedrone (30 mg/kg sc) at time zero. Thirty minutes post-treatment one methylone group (n = 5) and one mephedrone group (n = 5) received carvedilol (5 mg/kg ip). Methylone and mephedrone doses were based on previous studies investigating thermogenesis (den Hollander et al., 2013; López-Arnau et al., 2014). The carvedilol dose was determined using preceding experiments exploring the effect of carvedilol on MDMA induced hyperthermia (Sprague et al., 2005; Kiyatkin et al., 2016). Rectal temperatures were taken as a measure of core body temperature prior to synthetic cathinone administration and every 30 min post-treatment for 150 min using a Physiotemp Thermalert TH-8 thermocouple (Physitemp Instruments, Clifton, NJ) attached to a RET-2 rectal probe.

2.3. Chemicals and solutions

Methylone and mephedrone were obtained as hydrochloride salts from Cayman Chemical (Ann Arbor, MI). Carvedilol was obtained from Tocris Bioscience (Bristol, UK). Dimethyl sulfoxide (DMSO) was purchased from MP Biomedicals, LLC (Solon, OH). On the day of the experimental procedure, methylone and

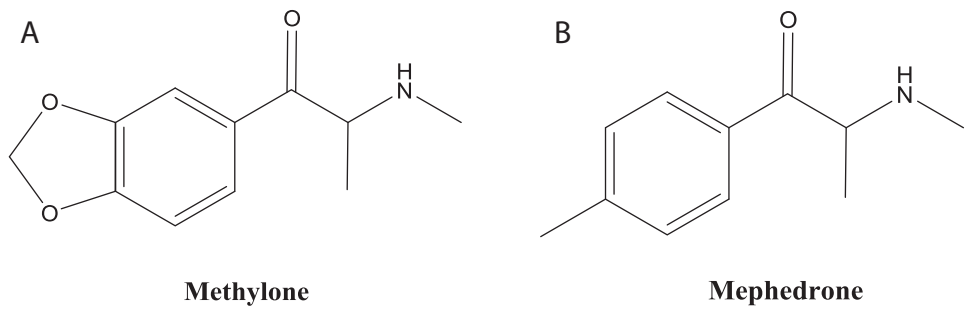

Fig. 1. Chemical structures of Methylone (A) and Mephedrone (B).