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Comparative pharmacological evaluation of the cathinone derivatives, mephedrone and methedrone, in mice

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ABSTRACT

Mephedrone and methedrone are cathinone-related compounds, which act as non-selective substrates for monoamine transporters, facilitating a neurotransmitter release. We compared the acute pharmacological effects of mephedrone and methedrone, attempting to further evaluate the action mechanisms of methedrone by responsibly and ethically using mice under approved procedures. The effects of both compounds were examined from 10 to 60 min, in a series of behavioral paradigms, namely open-field, plus-maze, hot-plate and tail suspension tests, whereas neurotransmitter brain tissue levels were determined *ex vivo* by HPLC. Separate groups were pre-treated with the dopamine (DA) antagonist haloperidol, or the serotonin (5-HT) synthesis inhibitor ρCPA, to further assess the mechanisms underlying methedrone effects. The compounds caused marked hyperlocomotion, displaying dissimilar stereotyped behavior, in an open-field arena. Mephedrone caused anxiolytic-like effects, while methedrone induced anxiogenic-like actions in the elevated plus-maze. Both compounds displayed thermal antinociception, with a reduced immobility time in the tail suspension model. Mephedrone triggered a 2- and 3-fold increment of dopamine and serotonin tissue levels, respectively, in the *nucleus accumbens*, with a 1.5-fold elevation of tissue dopamine in the frontal cortex. Methedrone caused a 2-fold increment of tissue dopamine in the *nucleus accumbens* and in the striatum, and a 1.5-fold increment of serotonin tissue levels in the hippocampus and striatum. *In vivo* methedrone effects were partially inhibited by a pre-treatment with haloperidol or ρCPA. Despite similar actions on locomotion, analgesia, and depression-like behavior, the acute administration of mephedrone and methedrone elicited divergent effects on anxiety-like behavior and stereotyped movements in mice, which might be related to the distinct modulation of brain tissue neurotransmitter levels.

1. Introduction

Cathinone (Fig. S1A) is the main naturally occurring psychoactive constituent obtained from the leaves of Khat (*Catha edulis*), which has served as a basis for the development of a series of synthetic molecules used recreationally (Baumann et al., 2012). Designer cathinones easily permeate the blood–brain barrier, eliciting sympathomimetic and psychostimulant actions. Their effects in humans include paranoia, hallucinations, euphoria, aggressiveness, psychosis, and an increment of libido, in addition to the more serious occurrences of hypertension, hyperthermia, seizures, respiratory distress, together with reports of fatal outcomes (Miotto et al., 2013; Luciano and Perazella, 2014; Gregg and Rawls, 2014; Valente et al., 2014). The increased number of intoxication reports during the last 5 years has led to laws that banish the use and sale of synthetic cathinones throughout different countries, although they continue to be commercialized worldwide, especially on the Internet and in Smartshops, as components of illicit bath salts or legal highs (Wikström et al., 2010; German et al., 2014; Green et al., 2014; Valente et al., 2014).

Mephedrone (4-methylmethcathinone; Fig. S1B) and methedrone (4-methoxymethcathinone; Fig. S1C) are *para*-substituted methcathinone analogs, which primarily act as substrates for monoamine transporters, facilitating the release of neurotransmitters *via* the reversal of normal transporter fluxes (Baumann

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et al., 2012; Cameron et al., 2013; Eshleman et al., 2013; Simmler et al., 2013; Gregg and Rawls, 2014; Paillet-Loilier et al., 2014). Of note, recent reports (Simmler et al., 2014; Bonano et al., 2015; Sakloth et al., 2015) have demonstrated that both compounds display similar potencies to increase the release of monoamines *in vitro*, with a low selectivity for dopamine (DA) *versus* serotonin (5-HT) transporters (DAT and SERT, respectively). A study conducted by Marusich et al. (2012) evaluated the *in vivo* effects of a series of designer cathinones, including methedrone and mephedrone. The authors demonstrated that an acute administration of both substances caused a marked increment of locomotion activity, accompanied by a series of behavioral changes, in a functional observation battery in mice. Additional recent data (Bonano et al., 2015) has revealed that either methedrone or mephedrone induced mixed intracranial self-stimulation in rats, which is consistent with the low selectivity of these compounds for DAT *versus* SERT. In the light of literature data, the present study was designed to further compare the *in vivo* acute effects of mephedrone and methedrone, in an additional series of experimental paradigms, including stereotyped features, and depression-, painful- and anxiety-like behavior in mice. Attempts have also been made to correlate the behavioral changes elicited by both cathinone-related substances, with the variations of brain tissue monoamine levels *ex vivo*.

2. Materials and methods

2.1. Drugs

Mephedrone (2-(methylamino)-1-(4-methylphenyl)-1-propanone hydrochloride; CAS 1189726-22-4) and methedrone (1-(4-methoxyphenyl)-2-(methylamino)-1-propanone hydrochloride; CAS 879665-92-6) were obtained from the Cayman Chemical Company (Ann Arbor, Michigan, USA), as hydrochloride salts, with a purity >98%. The designer cathinones used in the present study were racemic drugs. Haloperidol, DL-ρ-chlorophenylalanine methyl ester (ρCPA), DA, 5-HT, and glutamate were obtained from the Sigma Chemical Company (St. Louis, Missouri, USA). The doses of the cathinone derivatives were selected on the basis of literature data and pilot experiments (Lisek et al., 2012; den Hollander et al., 2013). During this study, the importation of cathinone derivatives was forbidden, impeding us from performing complete dose–response studies and further biochemical analysis.

2.2. Animals

All animal care and experimental procedures were approved by the Local Animal Ethics Committee (CEUA protocol 13/00336), and were accomplished according to ARRIVE recommendations. Female C57BL-6 mice (total number of 185 animals) were used, as they are very sensitive to amphetamine-induced neuronal damage (Angoa-Pérez et al., 2012). The animals were obtained from the Central Animal House of the Universidade Federal de Pelotas (UFPEL, Brazil). The mice (17–23 g; 9–11 weeks old) were housed in groups of four per cage, and were maintained in a controlled temperature (22 ± 1 °C) and humidity (60–70%), under a 12 h light/dark cycle, with food and water *ad libitum*. The animals were acclimatized to the laboratory for at least 1 h before testing and all of the tests were performed between 8:00 AM and 3:00 PM. For the behavioral tests, the animals were visually and acoustically isolated during the experimental sessions. An observer, blind to the treatments, analyzed all of the experiments.

2.3. Protocols of treatment

The behavioral effects of a single administration (i.p.) of methedrone (15 or 30 mg/kg) were assessed during a 60 min period, in several experimental paradigms, and were compared with the effects elicited by mephedrone (30 mg/kg) (Fig. 1A) (Lisek et al., 2012; Marusich et al., 2012). Separately, the brain tissue levels of the neurotransmitters were quantified 20 min after the single (i.p.) administration of methedrone or mephedrone (both at 30 mg/kg). To evaluate some of the mechanisms underlying the methedrone effects, different groups of mice were treated with the non-selective DA receptor antagonist haloperidol (0.1 mg/kg, i.p.) given 30 min before the methedrone dosage (30 mg/kg, i.p.; Fig. 1B). Separately, the animals received the inhibitor of 5-HT synthesis ρCPA (100 mg/kg, i.p.; Fig. 1C)

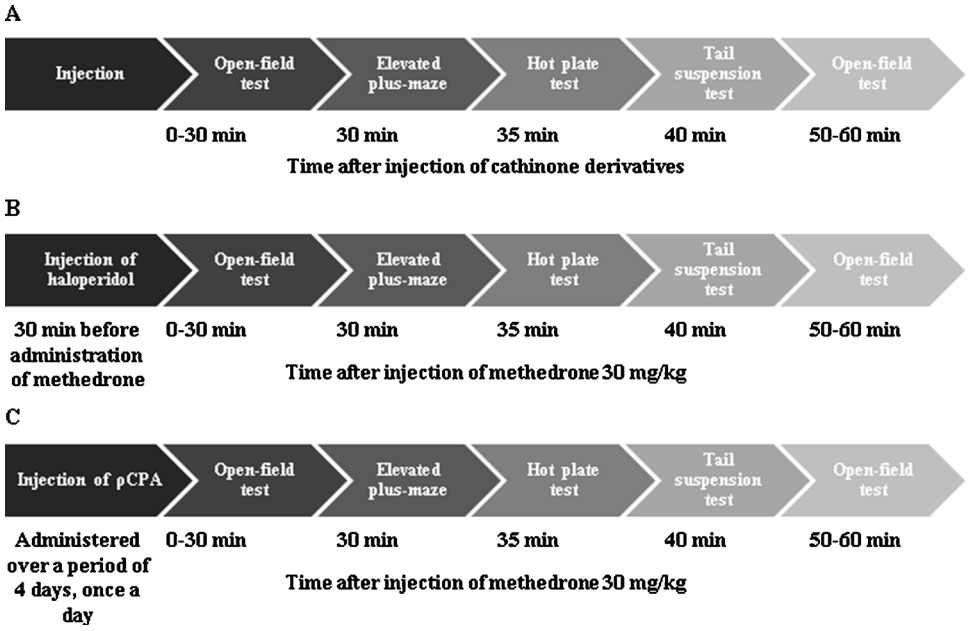

Fig. 1. General schedule of behavioral assessment used in this study. The animals were tested in a series of experimental models at different time-points after the administration of cathinone derivatives, as depicted in a schematic manner (A). The protocols of pre-treatment with haloperidol or ρCPA, before methedrone administration, are depicted in the panels B and C, respectively.