Progress in Neuro-Psychopharmacology & Biological Psychiatry 49 (2014) 70–77

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Anxiety-like effects of meta-chlorophenylpiperazine in paradoxically sleep-deprived mice☆,☆☆

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ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 28 October 2013
Accepted 12 November 2013
Available online 19 November 2013

Keywords:
Designer drugs
Meta-chlorophenylpiperazine
Open field
Sleep deprivation
Sleep rebound

ABSTRACT

Chlorophenylpiperazines (CPP) are psychotropic drugs used in nightclub parties and are frequently used in a state of sleep deprivation, a condition which can potentiate the effects of psychoactive drugs. This study aimed to investigate the effects of sleep deprivation and sleep rebound (RB) on anxiety-like measures in mCPP-treated mice using the open field test. We first optimized our procedure by performing dose–effect curves and examining different pretreatment times in naïve male Swiss mice. Subsequently, a separate cohort of mice underwent paradoxical sleep deprivation (PSD) for 24 or 48 h. In the last experiment, immediately after the 24 h-PSD period, mice received an injection of saline or mCPP, but their general activity was quantified in the open field only after the RB period (24 or 48 h). The dose of 5 mg mL^{-1} of mCPP was the most effective at decreasing rearing behavior, with peak effects 15 min after injection. PSD decreased locomotion and rearing behaviors, thereby inhibiting a further impairment induced by mCPP. Plasma concentrations of mCPP were significantly higher in PSD 48 h animals compared to the non-PSD control group. Twenty-four hours of RB combined with mCPP administration produced a slight reduction in locomotion. Our results show that mCPP was able to significantly change the behavior of naïve, PSD, and RB mice. When combined with sleep deprivation, there was a higher availability of drug in plasma levels. Taken together, our results suggest that sleep loss can enhance the behavioral effects of the potent psychoactive drug, mCPP, even after a period of rebound sleep.

1. Introduction

Chlorophenylpiperazines (CPP) are included in the list of controlled psychotropic substances in several countries, mainly due to their toxicity and the latest findings of the isomer meta-chlorophenylpiperazine (mCPP) in ecstasy tablets (Romão et al., 2011). Previous studies have reported that mCPP promotes a feeling of wellbeing and euphoria (Tancer and Johanson, 2001, 2003). mCPP presents lower neurotoxic potential than methylenedioxymethamphetamine (MDMA), but its negative effects can be very harmful to the body (dizziness, confusion, tremors, headache and even panic attacks), similar to serotonin syndrome in psychiatric patients (Feuchtl et al., 2004; Gijsman et al., 1998; Gobbi et al., 2002). Adverse effects reported after abuse of mCPP include hallucinations, dizziness, and panic attacks, with high doses leading to respiratory depression and death (Bossong et al., 2005; EMCDDA, 2007; Gee et al., 2005).

Notably, mCPP is mostly used in nighttime environments and the users are usually in a state of sleep deprivation. Alertness and sleep disturbances are two of the problems most commonly reported by drug users (Johanson et al., 1999). In rats, sleep deprivation results in generalized symptoms leading to alterations in catecholamines, hormones,

Abbreviations: CPP, Chlorophenylpiperazines; CTRL, Control; ip, Intraperitoneal; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; mCPP, Meta-chlorophenylpiperazine; MDMA, Methylenedioxymethamphetamine; MTBE, Methyl tert-butyl ether; oCPP, Orto-chlorophenylpiperazine; OF, Open field; PSD, Paradoxical sleep deprivation; QC, Quality control; RB, Sleep rebound; SRM, Selected reaction monitoring.

☆ Note: The authors are not part of any associations or commercial relationships that might represent conflicts of interest in the writing of this study.

☆☆ Funding sources: AFIP, CAPES, CNPq and FAPESP.

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0278-5846/$ – see front matter.
http://dx.doi.org/10.1016/j.pnpbp.2013.11.007

and behavior (Andersen et al., 2005; Tufik et al., 2009). Selective sleep deprivation has been known to potentiate behavioral responses to psychostimulant drugs in rodents, particularly aggressive and stereotyped behaviors (Andersen et al., 2003, 2010; Tufik et al., 1978). Previous studies have also reported genotoxic effects in multiple organs of mice after exposure to cocaine and ecstasy associated with sleep loss (Alvarenga et al., 2011). These studies suggest that sleep deprivation may influence the effects of drugs, which is especially important when considering drug abuse by young adults.

The quantification of drugs or toxic agents in biological samples is essential for correlations with clinical signs. Studies have determined quantification of mCPP in tablets seized by police, which also identified the presence of cocaine, methamphetamine, MDMA and trifluoromethylphenylpiperazine (Siroká et al., 2013). In addition, a case report showed the mCPP levels found in fatal overdose (Kovaleva et al., 2008) and biological samples from rodents to identify the metabolic profile of mCPP (Staack and Maurer, 2003).

In order to investigate the potential interactions between mCPP and sleep in mice we examined: (i) the effects of mCPP on open-field behavior; (ii) the effects of paradoxical sleep deprivation (PSD), as well as sleep rebound (RB), on the behavioral alterations induced by mCPP, and (iii) the plasma levels of mCPP following PSD.

2. Material and methods

2.1. Animals

Male Swiss mice (3 months old), weighing 30–50 g, were obtained from CEDEME - Universidade Federal de São Paulo (UNIFESP). The mice were group-housed 10 animals per cage in polypropylene standard cages (cage size: 41 cm × 34 cm × 16.5 cm). Food (standard pellet chow feed) and drinking water were freely available except during the brief test periods. The animals were kept in artificially lighted rooms on a 12–12 h light/dark cycle (lights on at 07:00 h) and controlled temperature (22 ± 1 °C). All animal procedures followed the ethical and practical principles for research animals (Andersen and Tufik, 2010) and were approved by the Ethical Committee of the UNIFESP (#2010/1520). There was the environmental enrichment equally in all home cages with paper strips and tunnels in order to improve laboratory animals' well-being and promote the quality of animal based biomedical research (NHMRC, 2008). All possible efforts were made to reduce the number of animals used as well as discomfort to the animals.

2.2. Reagents, solutions and drug administration

All reagents were of analytical grade, solvents were of chromatographic purity, and water was purified by deionization (Milli-Q system, Millipore®, Bedford, MA, USA).

Acetonitrile and methyl tert-butyl ether (MTBE) used in the mobile phase and extraction procedure, respectively, were obtained from Tedia® (Fairfield, OH, USA). We used the analytical standard of 1-(2-chlorophenyl)piperazine (oCPP) and 1-(3-chlorophenyl)piperazine (mCPP) was obtained from Alfa Aesar® (Ward Hill, MA, USA). Stock standard solutions were prepared at 1 mg mL^{-1} in methanol. Working solutions used in the identification and MS/MS fragmentation studies were prepared in ultrapure water at 20 up to 2000 ng mL^{-1}.

mCPP was freshly diluted in saline solution (sodium chloride 0.9% w/v). The mCPP solutions were delivered intraperitoneally (ip) at a volume of 0.01 mL g^{-1} body weight. Control groups received an equivalent volume of saline solution.

2.3. Behavioral test (open field test)

The animals were individually placed in the center of the open field arena (OF) for direct quantification of general activity for 5 min. This test is used to quantitative measure of anxiety-like behavior in rodents (Fukushiro and Frussa-Filho, 2011). The OF apparatus used in the present study was a circular wooden box (40 cm in diameter and 50 cm high) with an open top and floor divided into 19 squares. Hand-operated counters were used to score total locomotion (number of any floor unit entered) and rearing (number of times the animal stood on hind legs). The observer was always unaware of the experimental design. The experimental device was cleaned with 5% alcohol solution and dried before testing each animal (Chinen et al., 2006; Frussa-Filho et al., 2004; Fukushiro et al., 2007).

2.4. Paradoxical sleep deprivation and sleep rebound procedures

The PSD procedure was conducted as described in previous studies (Silva et al., 2004; Zager et al., 2009). Briefly, PSD was induced for 24 or 48 h by placing 5 mice inside a tiled water tank (41 cm × 34 cm × 16.5 cm) containing 9 platforms, 3.5 cm in diameter, surrounded by water up to 1 cm beneath the surface. In this method, the animals are capable of moving inside the tank, jumping from one platform to the other. When the animal enters the paradoxical phase of sleep, it falls into the water, due to muscle atonia, and wakes up. Food and water were available ad libitum through a grid placed on top of the water tank. Home cage control animals were maintained in their cages in the same room. Of note, it has been previously demonstrated that this protocol suppresses 95% of paradoxical sleep in mice (Silva et al., 2004).

The RB was performed for 24 or 48 h after 24-h PSD. Thus, immediately after the 24-h PSD period, mice received the designated drug treatment and were returned to their home cages, where they could rest for 24 or 48 h before the behavioral tests. RB promotes homeostatic effects and occurs when the animal is submitted to sleep deprivation and sequentially it is placed for a period up to 48 h in a condition favorable to sleep (their original home cages) (Calzavara et al., 2008; Lima et al., 2012; Martins et al., 2008).

2.5. Experiment 1. Determination of the dose of mCPP

To determine the dose which promoted maximum behavioral effects in the OF test, mice received an injection of sterile saline NaCl 0.9% (vehicle; N = 9) or mCPP at 1.0 mg kg^{-1} (N = 10), 2.5 mg kg^{-1} (N = 10) or 5.0 mg kg^{-1} (N = 10) and were submitted to the OF test. The time between the drug administration and the exposure to the behavioral test was 30 min, based on other behavioral study that observed an induction of the depression-like behavior in rodents (Rajkumar et al., 2009). The doses of mCPP were established on concentrations injected intraperitoneally described in a previous study (1 up to 10 mg kg^{-1}, ip), which aimed to test the capacity to induce MDMA-like discriminative stimulus effects in mice (Yarosh et al., 2007).

2.6. Experiment 2. Determination of pretreatment time after mCPP administration

Parameters of open field test were evaluated after mCPP administration and the time points considered in this experiment were: 5, 15, 30, 45 and 60 min after injection. In this experiment we used the dose with desired statistical level obtained in experiment 1. After these pretreatment times, animals were submitted to the OF test. The mice were distributed into 6 groups: 5 min/mCPP (N = 9), 15 min/mCPP (N = 7), 30 min/mCPP (N = 12), 45 min/mCPP (N = 8), 60 min/mCPP (N = 9) and vehicle (N = 10).

2.7. Experiment 3. Effects PSD on the behavioral alterations induced by mCPP

We evaluated the behavioral alterations caused by the interaction between the administration of mCPP and PSD for 24 or 48 h. Given the results obtained in experiments 1 and 2, we chose the dose of 5 mg kg^{-1} and the 15-min time interval to perform this experiment.