European Journal of Pharmacology 810 (2017) 63–69

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Full length article

Mephedrone exposure in adolescent rats alters the rewarding effect of morphine in adults

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ARTICLE INFO

Keywords:
Legal highs
Opioid drugs
Conditioned place preference test
Epigenetic changes
Histopathological examination

ABSTRACT

An increasing number of data show that exposure to mephedrone in adolescence can have long-lasting implication on brain activity and on peripheral organs/tissues. The aim of this study was to investigate whether adolescent exposure to mephedrone (10 mg/kg, i.p.) has influence upon the rewarding effect of morphine (5 mg/kg, i.p.) in adult rats. Thus, the adolescent rats (on the 30th PND) were treated with mephedrone for 7 consecutive days. When the animals were adult (on the 60th PND) the morphine-induced conditioned place preference (CPP) test was performed. After that, the level of DNA methylation in the striatum was investigated. DNA methylation is one of the epigenetic mechanisms which produces changes in the genome. These alterations may affect the phenotype, without effect on DNA sequences, and has influence on drug addiction. Additionally, in order to check the toxic properties of mephedrone on the peripheral organs, the histopathological examination of kidney and liver was carried out. The present experiments demonstrated that: 1) adolescent mephedrone exposure may intensify the rewarding effect of morphine in adult rats in the CPP test; 2) mephedrone may induce the alterations in DNA methylation in striatum of adult rats leading to changes in gene activity; 3) mephedrone may produce some retrogressive disturbances in kidney and liver, which confirms the toxic properties of this substance.

1. Introduction

Nowadays, there is a huge increase (especially in youths) in the use of psychoactive substances described as “legal highs”. One of them is synthetic cathinone, mephedrone (4-methyl-N-methylcathinone). Mephedrone, given at low doses, produces a psychostimulant-like effect (Gregg et al., 2013b; Opacka-Juffry et al., 2014). However, at higher doses or after chronic use, it may induce serious adverse effects such as hypertension, tachycardia, aggressive behavior and other peripheral complications (Dargan et al., 2011). The pharmacological activity of mephedrone is similar to other psychoactive drugs like amphetamine or cocaine (Kehr et al., 2011; Meng et al., 2012). Mechanism of action of mephedrone is based on modification of dopamine and serotonin release (Gołembiowska et al., 2016) and activation of dopamine and serotonin transporters in the brain (Baumann et al., 2012; Saha et al., 2015). As literature data have shown, mephedrone abuse, particularly in adolescent people, may produce long-lasting alterations in brain activity (López-Arnau et al., 2015). Because these disturbances in the mesolimbic system may be maintained for months after the final drug administration (Seiden et al., 1988; Cass and Manning, 1999), more and more data are focused on the effects of mephedrone which are formulated during adolescence. For example, Motbey et al. (2012) demonstrated the alterations in the distribution of Fos expression in adolescent rats after mephedrone administration, while Ciudad-Roberts et al. (2016) indicated that the combination of ethanol with mephedrone disturbed the oxidative stress-related enzymes - causing neurotoxicity and impairing the neurogenesis and learning in adolescent mice. The significant neuronal changes after repeated exposure to mephedrone in adolescent rats and the intensification of oxidative stress, were also confirmed by López-Arnau et al. (2015). In people mephedrone may also impair the activity of peripheral organs (Gerace et al., 2014).

Morphine is a valuable analgesic agent, but the use of it is complicated by the development of tolerance and physical dependence. Morphine induces the rewarding effect by stimulation of μ opioid receptors which are located on gamma-aminobutyric acid (GABA)

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http://dx.doi.org/10.1016/j.ejphar.2017.06.007
Received 30 November 2016; Received in revised form 25 May 2017; Accepted 7 June 2017
Available online 08 June 2017

terminals in the ventral tegmental area. The rewarding effect of morphine has been repeatedly measured in the conditioned place preference (CPP) test (Huston et al., 2013; Shippenberg et al., 2009; Listos et al., 2016). Various factors, such as the dosage, the route of administration, the individual's drug history and even diet or stress, by inducing genetic and/or epigenetic modifications, may modify the rewarding effect of morphine (Goodman, 2008; Roversi et al., 2016; Vey et al., 2016). DNA methylation, one of the epigenetic mechanisms, is an enzymatic modifications of DNA sequences by DNA methyltransferases (Suzuki and Bird, 2008), in which methyl groups are added to the DNA molecule, mainly at the 5′ position of cytosine nucleotides. It may modulate gene expression (Suzuki and Bird, 2008) and may alter the brain activity.

In the present work, we undertook to investigate whether the adolescent exposure to mephedrone has influence on the rewarding effect of morphine in adult rats. Thus, we have performed a behavioral experiment, the CPP test. After that, we analyzed the level of striatal DNA methylation. Additionally, to assess the effect of adolescent mephedrone exposure on the activity of kidney and liver in adults we performed histopathological experiments. Although many reports have described various effects of mephedrone, our study is the first which undertakes to explore the long-term effects of mephedrone on the rewarding activity of morphine in animals. Our study also aimed to evaluate the influence of adolescent exposure to mephedrone on structure of peripheral tissues.

2. Material and methods

2.1. Animals

The experiments were begun on male post-natal day 23 (PND 23) Wistar rats (80–100 g) and continued on adult rats (200–250 g). The animals were kept at room temperature of 22 ± 1 °C, on a natural day–night cycle (12 h/12 h). Standard food (Murigran pellets, Bacutil, Motycz) and tap water were freely available. All animals were housed 5–6 per cage. After one week of adaptation and handling, the animals were divided into groups (10 animals/group) and prepared for the tests. Chronic administration of mephedrone was made between 8.00 a.m. and 9.00 a.m., and the behavioral experiment (CPP protocol) was made between 9.00 a.m. and 5.00 p.m. The study was performed according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive for Care and Use of Laboratory Animals and were approved by local ethics committee (The Medical University of Lublin Committee on the Use and Care of Animals).

2.2. Drugs

In the behavioral experiments, mephedrone and morphine hydrochloride (Polfa, Kutno, Poland) were used. Both compounds were dissolved in saline and were given intraperitoneally (i.p.) in a volume of 5 ml/kg. Mephedrone was administered at the dose of 10 mg/kg (Shortall et al., 2016), i.p. and the dose of morphine was 5 mg/kg, i.p. (Listos et al., 2016). The control animals received the same volume of saline at the respective time before the test.

2.3. The procedure of mephedrone administration

Beginning at 30th PND, the rats were injected (i.p.) once daily with effective dose of mephedrone (10 mg/kg, n = 10) for a total of 7 days. Such a dose was selected on the basis of our previous unpublished study in which locomotor activity of mice was increased after injection of mephedrone at the doses of 5 and 10 mg/kg but not 2.5 mg/kg. Moreover, the literature data also confirmed the effectiveness of mephedrone at a dose of 10 mg/kg in rats (Shortall et al., 2016). Age-matched control animals (n = 10) received saline injections (i.p.) with volumes adjusted to match those of the drug-treated. Subsequently, 24 h after the mephedrone exposure (on the 37th PND), some animals (n = 10) were decapitated for the epigenetic and histopathological evaluation. Herein, the striatum was dissected, and the livers and kidneys of the animals were collected. The remaining animals (n = 40) were allowed to reach their adulthood (PND 60) so to serve as test-subjects for the behavioral, epigenetic and histopathological experiments. These adult animals were divided into several groups: the saline group with or without prior mephedrone history which did not take part in the CPP test; the saline group with or without prior mephedrone history which were taken into the CPP test; the morphine group without prior history to mephedrone; the morphine group with prior exposure to mephedrone (both of which were taken into the CPP test).

2.4. Apparatus and procedure for CPP test

On the 60th PND (3 weeks after the final mephedrone injection), the CPP test with biased protocol was performed. The applied equipment consisted of eight rectangular boxes (60 cm × 35 cm × 30 cm), each of them divided into three compartments: two of them (25 cm × 30 cm) were separated by removable guillotine doors from a small central gray area (10 cm × 10 cm). The walls and floors of the two large compartments differed in color and pattern (one had black walls and a white mesh floor, and the another had white walls with a black, solid floor. The testing boxes were kept in a soundproof room with neutral masking noise and were poorly illuminated (40 lx). During the test, the location of the subject rat was monitored through a digital video camera system positioned directly above the apparatus, and the amount of time that each rat spent in each of the two large compartments was recorded automatically using a video tracking software (Karnet, Lublin, Poland). We performed the CPP procedure based on the previous published method (Listos et al., 2016). Accordingly, we administered morphine, at a dose of 5.0 mg/kg in a 3-day schedule.

The CPP test consisted of three, typical phases: pre-conditioning, conditioning, post-conditioning.

2.4.1. Pre-conditioning

On day 1, each rat was placed separately into the central gray area for 15 min and left with free access to all the compartments. The time period, spent by each animal in the two large compartments, was measured and recorded (a baseline preference). The animals which showed similar preference for both compartments were accepted for further phases of the experiment. The neutral zone was never used during conditioning and was blocked by guillotine doors.

2.4.2. Conditioning

This phase consisted of a 3-day schedule of conditioning sessions. Each day, two sessions were performed. During the first session (the morning session), the rats received saline and were placed in the compartment with black walls and white mesh floor for 30 min. In the next session (the afternoon session), the rats received morphine (5.0 mg/kg) and were placed in the compartment with white walls and black solid floor, for the same period of time. The intervals between saline and morphine injections were at least 6 h. The procedure was repeated on the 2nd and the 3rd day of conditioning.

2.4.3. Post-conditioning

On the 5th day, similarly to the pre-conditioning phase, the animals were placed into the central gray area and the time spent in the morphine-paired compartment was recorded for each animal for 15 min. No injections were given on this day.

2.5. The locomotor activity test

During the CPP test, the locomotor activity of the individual rats was measured as the number of passes between compartments, during