Toxicology and Applied Pharmacology 286 (2015) 27–35

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Neuronal changes and oxidative stress in adolescent rats after repeated exposure to mephedrone

Raúl López-Arnau [a,b], José Martínez-Clemente [a,b], Teresa Rodrigo [c], David Pubill [a,b], Jorge Camarasa [a,b,*], Elena Escubedo [a,b]

[a] Department of Pharmacology and Therapeutic Chemistry (Pharmacology Section), Faculty of Pharmacy, University of Barcelona, Spain
[b] Institute of Biomedicine (IBUB), Faculty of Pharmacy, University of Barcelona, Spain
[c] Animal Experimentation Unit of Psychology and Pharmacy, University of Barcelona, Spain

ARTICLE INFO

Article history:
Received 6 October 2014
Revised 3 February 2015
Accepted 13 March 2015
Available online 24 March 2015

Keywords:
Mephedrone
Neurotoxicity
Striatum
Frontal cortex
Hippocampus
Rat

ABSTRACT

Mephedrone is a new designer drug of abuse. We have investigated the neurochemical/enzymatic changes after mephedrone administration to adolescent rats (3×25 mg/kg, s.c. in a day, with a 2 h interval between doses, for two days) at high ambient temperature (26 ± 2 °C), a schedule that intends to model human recreational abuse. In addition, we have studied the effect of mephedrone in spatial learning and memory. The drug caused a transient decrease in weight gain. After the first dose, animals showed hypothermia but, after the subsequent doses, temperature raised over the values of saline-treated group. We observed the development of tolerance to these thermoregulatory effects of mephedrone. Mephedrone induced a reduction of the densities of dopamine (30% in the frontal cortex) and serotonin (40% in the frontal cortex and the hippocampus and 48% in the striatum) transporters without microgliosis. These deficits were also accompanied by a parallel decrease in the expression of tyrosine hydroxylase and tryptophan hydroxylase 2. These changes matched with a down-regulation of D_2 dopamine receptors in the striatum. Mephedrone also induced an oxidative stress evidenced by an increase of lipid peroxidation in the frontal cortex, and accompanied by a rise in glutathione peroxidase levels in all studied brain areas. Drug-treated animals displayed an impairment of the reference memory in the Morris water maze one week beyond the cessation of drug exposure, while the spatial learning process seems to be preserved. These findings raise concerns about the neuronal long-term effects of mephedrone.

© 2015 Elsevier Inc. All rights reserved.

Introduction

The highly restricted availability of precursors needed for the synthesis of methamphetamine and MDMA (3,4-methylenedioxymethamphetamine), and the consequent reduction in their purity (Brunt et al., 2011; Winstock et al., 2011) lead to the appearance, in the illicit market, of a new family of drugs of abuse known as “legal highs”. From their chemical structure, they are also known as “beta-ketoamphetamines”. Mephedrone (4-methyl-methcathinone) is one of these new stimulants that are mainly used by young adults and adolescents (Schifano et al., 2011) and have been readily available for legal purchase both online and in some stores. Many users consider the effects of mephedrone to be superior to those of cocaine and MDMA (Vardakou et al., 2011).

As a chemical congener of amphetamines, mephedrone could influence dopaminergic and serotonergic function. In cortical and striatal synaptosomal preparations, mephedrone was found to inhibit serotonin (5-HT) uptake with higher affinity than that of dopamine (DA) uptake, primarily acting as a substrate for plasmalemmal monoamine transporters (Baumann et al., 2012; López-Arnau et al., 2012; Martínez-Clemente et al., 2012). Our previous results suggest that the vesicular component is especially important in explaining the neurochemical effects of mephedrone (López-Arnau et al., 2012). Additionally, other authors have described that mephedrone behaves preferentially as a 5-HT and DA releaser (Hadlock et al., 2011). Administration of this drug produces dose-related increases in extracellular 5-HT and DA with the magnitude of effect on 5-HT being 2–3 times greater (Baumann et al., 2012; Kehr et al., 2011).

In rodents, mephedrone acts as a locomotor stimulant (Baumann et al., 2012; Kehr et al., 2011; López-Arnau et al., 2012; Martínez-Clemente et al., 2014) and induces self-administration. (Hadlock et al., 2011). In the basis of the 5-HT-releasing capability of mephedrone (Cameron et al., 2013), we postulated that binge administration of this

Abbreviations: 5-HT, serotonin; CAT, catalase; DA, dopamine; Gpx, glutathione peroxidase; IgG, immunoglobulin G; MDA, malondialdehyde; MDMA, 3,4-methylenedioxymethamphetamine; Meph, mephedrone; nNOS, neuronal nitric oxide synthase; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; S.E.M., standard error of the mean; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TH, tyrosine hydroxylase; TPH-2, tryptophan hydroxylase 2.

* Corresponding author at: Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain.
E-mail address: jcamarasa@ub.edu (J. Camarasa).

http://dx.doi.org/10.1016/j.taap.2015.03.015
0041-008X/© 2015 Elsevier Inc. All rights reserved.

drug could cause dysfunction of brain 5-HT or DA terminals, similarly to the effects of 5-HT transporter substrates like MDMA (Baumann et al., 2008; Commins et al., 1987; Malberg and Seiden, 1998).

Previous published results revealed that, in contrast to MDMA, methamphetamine or methcathinone (Fleckenstein et al., 2000; Haughey et al., 2000; Metzger et al., 2000), repeated mephedrone injections do not affect monoamine neurotransmitter levels (Baumann et al., 2012). On the contrary, Hadlock et al. (2011) found a persistent hippocampal serotonergic, but not striatal dopaminergic deficit after binge mephedrone exposition at high ambient temperature.

Mephedrone tends to be consumed in hot environments such as confined dancing spaces (Schifano et al., 2011) and users experience a strong desire to re-dose, leading them to ingest large amounts of the drug in binges that can last several days (Winstock et al., 2011). Accordingly, present experiments were carried out at a high ambient temperature simulating the conditions found in dance clubs. Moreover, hyperthermia by itself can induce an oxidative stress. This is one of the main factors involved in nerve terminal injury induced by amphetamines (Jayanthi et al., 1999; Yamamoto and Zhu, 1998). Therefore, in the present paper, we determined the levels of some enzymes involved in the cell redox balance and lipid peroxidation following mephedrone administration.

The aim of this paper was to investigate the effects of mephedrone in brain 5-HT or DA nerve terminals of rats, administered in a pattern used frequently to mimic psychostimulant "binge" administration. Accordingly, a multiple dose/day administration schedule was used to mimic the widespread practices of "stacking" (taking multiple doses at once) and "boosting" (taking supplemental doses over time in order to maintain the drug's effect). Additionally we investigated the consequences of a neurotoxic treatment of mephedrone in a behavioral model of spatial learning and memory.

Methods

Animals and treatment. Male Sprague–Dawley rats (115–130 g, aged 5 weeks) (Lé Genest, France) were used. The animals were housed one per cage in a regulated environment (21 ± 1 °C; 12 h light/dark cycle, lights on at 08:00 h), with free access to food and water. Experiments took place between 09:00 and 15:00 h. Experimental protocols for the use of animals in this study were approved by the Animal Ethics Committee of the University of Barcelona and following the guidelines of the European Communities Council (86/609/EEC). Animals were administered subcutaneously with three doses of saline (1 ml/kg) or mephedrone (25 mg/kg) with a 2 h interval between doses, for two consecutive days. During the treatment, the animals were maintained in an ambient temperature of 26 ± 2 °C, and were kept under this condition until 1 h after the last daily dose.

No information about the subcutaneous doses of mephedrone in humans is available. The only approach would be the doses used orally, which are comprised between 100 and 300 mg, or intranasally that can reach up to 125 mg in each insufflation (Kelly, 2011). To estimate the relevance of the doses applied in the present study to the doses taken by humans, we used a scaling method following these factors: for an animal dose of 25 mg/kg (Hadlock et al., 2011) and an allometric scaling coefficient of 0.66 (Chiou et al., 1998), the resultant human equivalent dose is 4 mg/kg. On this basis, a 25 mg/kg dose given to rats during the periadolescent period may be considered to represent a human recreational mephedrone use.

Another important issue in mephedrone studies is the frequency of dosing. Our aim was to simulate weekend mephedrone use. In this regard, we administered mephedrone in a 2 h interval since the half-life of this drug after oral administration in rats is about 30 min (Martínez-Clemente et al., 2013). The fall of mephedrone plasma levels after 2 h simulated the desire to re-dose and modeled mephedrone boosting in humans that occurs in order to extend the drug's subjective effects.

Drugs and reagents. Pure racemic mephedrone hydrochloride was synthetized and characterized by us as described (López-Arnau et al., 2012). Mephedrone solutions for injection were prepared in sterile 0.9% NaCl (saline) immediately before administration. Isoflurane was from Laboratorios Dr. Esteve (Barcelona, Spain). The other drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA). [^{3}H]ketanserin, [^{3}H]paroxetine, [^{3}H]PK11195, [^{3}H]raclopride and [^{3}H]WIN35428 were from Perkin Elmer (Boston, MA, USA). All buffer reagents were of analytical grade.

Surgical procedures and acquisition of body temperature. The animals ($n = 6$–8 per group) were allowed one week after arrival to acclimatize before surgery. Subsequently, they had implanted an electronic device (Thermo Tracker, Barcelona, Spain), which enabled continuous measurement of core body temperature. The implant was placed in the abdominal cavity, under isoflurane anesthesia. After surgery, animals received analgesic therapy for 3 days and were allowed to recover for 7 days before saline or mephedrone administration avoiding a possible influence of this type of manipulation. The devices had been programmed to record the core temperature every 5 min from 24 h prior until 24 h after drug treatment and they were removed from the rats after sacrifice. The recorded values were downloaded to a computer using a dedicated interface and processed by the software provided by the manufacturer.

Tissue sample preparation. Crude membrane preparation was prepared as described (Escubedo et al., 2005) with minor modifications. The animals were killed by decapitation under isoflurane anesthesia at 6 h (lipid peroxidation experiments; $n = 5$–7 animals/group), 1 day (lipid peroxidation experiments and antioxidant enzyme determination; $n = 6$–10 animals/group) or 7 days (radioligand binding experiments and Western blotting; $n = 5$–8 animals/group) after treatment. The brains were rapidly removed from the skull and the hippocampus, striatum and frontal cortex quickly dissected out, frozen on dry ice, and stored at −80 °C until use. When required, tissue samples were thawed and homogenized at 4 °C in 20 volumes of 5 mM Tris–HCl buffer (0.32 M sucrose) with protease inhibitors, pH 7.4. The homogenates were centrifuged at 1000 ×g for 15 min at 4 °C. Aliquots of the resulting supernatants were stored at −80 °C until use for Western blot experiments. The rest of the samples were resuspended and centrifuged at 15,000 ×g for 30 min at 4 °C. The pellets were resuspended in buffer and incubated at 37 °C for 5 min to remove endogenous neurotransmitters. The protein samples were recentrifuged. The final pellets (crude membrane preparations) were resuspended in the appropriate buffer and stored at −80 °C until use in radioligand binding experiments. Protein content was determined using the Bio-Rad Protein Reagent following the manufacturer's instructions. For measuring the malondialdehyde (MDA) production, tissue samples were homogenized on ice in 30 volumes of the MDA lysis buffer. The homogenates were centrifuged at 13,000 ×g for 10 min to remove insoluble material. Aliquots of supernatant were used for lipid peroxidation assay (see below).

DA and 5-HT transporter density and glial activation. The density of DA transporters in striatal or frontal cortex membranes was measured by [^{3}H]WIN35428 binding. Assays were performed in tubes containing 250 or 500 µl of [^{3}H]WIN35428 diluted in phosphate-buffered (final radioligand concentration, 5 nM) and 50 or 100 µg of membranes, respectively. Incubation was done for 2 h at 4 °C and non-specific binding was determined in the presence of 30 µM bupropion.

The density of 5-HT transporters in hippocampal, striatum and frontal cortex membranes was quantified by measuring the specific binding of 0.1 nM [^{3}H]paroxetine after incubation with 150 µg of protein at 25 °C for 2 h in 50 mM Tris–HCl buffer (pH 7.4), containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 ml. Clomipramine (100 µM) was used to determine non-specific binding.

Download English Version:

https://daneshyari.com/en/article/2568356

Download Persian Version:

https://daneshyari.com/article/2568356

Daneshyari.com