Biochimica et Biophysica Acta 1862 (2016) 1815–1826

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Adolescent exposure to MDMA induces dopaminergic toxicity in substantia nigra and potentiates the amyloid plaque deposition in the striatum of APPswe/PS1dE9 mice

Sonia Abad [a], Carla Ramon [a], David Pubill [a], Jorge Camarasa [a], Antonio Camins [a,b,*], Elena Escubedo [a]

[a] Department of Pharmacology, Toxicology and Therapeutic Chemistry (Pharmacology Section) and Biomedicine Institute, University of Barcelona (IBUB), Spain
[b] Networking Research Center on Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Spain

ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 24 May 2016
Accepted 21 June 2016
Available online 23 June 2016

Keywords:
3.4-Methylenedioxymethamphetamine
APPswe/PS1dE9 mice
Striatum
Dopaminergic dysfunction
Amyloid plaques

ABSTRACT

MDMA is one of the most used drugs by adolescents and its consumption has been associated with many psychobiological problems, among them psychomotor problems. Moreover, some authors described that early exposure to MDMA may render the dopaminergic neurons more vulnerable to the effects of future neurotoxic insults. Alzheimer disease (AD) is the main cause of dementia in the elderly and a percentage of the patients have predisposition to suffer nigrostriatal alterations, developing extrapyramidal signs. Nigrostriatal dysfunction in the brain of aged APPswe/PS1dE9 (APP/PS1), a mouse model of familiar AD (FAD), has also been described. The aim of the present study was to investigate the consequences of adolescent exposure to MDMA in APP/PS1 mice, on nigrostriatal function on early adulthood. We used a MDMA schedule simulating weekend binge abuse of this substance. Our MDMA schedule produced a genotype-independent decrease in dopaminergic neurons in the substantia nigra that remained at least 3 months. Shortly after the injury, wild-type animals showed a decrease in the locomotor activity and apparent DA depletion in striatum, however in the APP/PS1 mice neither the locomotor activity nor the DA levels were modified, but a reduction in dopamine transporter (DAT) expression and a higher levels of oxidative stress were observed. We found that these disturbances are age-related characteristics that this APP/PS1 mice develops spontaneously much later. Therefore, MDMA administration seems to anticipate the striatal dopaminergic dysfunction in this FAD model. The most important outcome lies in a potentiation, by MDMA, of the amyloid beta deposition in the striatum.

1. Introduction

Both the recreational use of drugs and neurodegenerative diseases still represent a big challenge for public health. Usually, drugs are consumed by adolescents and young people in a recreational context. It is also during the adolescence when the brains are suffering many transformation to achieve the necessary maturation [1], thus the impact of the drugs in this period might have negative consequences in the adulthood. 3.4-methylenedioxymethamphetamine (MDMA), also known as ecstasy, is one of the most consumed amphetamine derivative drugs due to its stimulant and mild hallucinogenic properties. Its acts mainly on the monoaminergic system in the central nervous system (CNS) by stimulating the release of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) from the mesocorticolimbic and nigrostriatal systems. This drug can also act as an agonist on central serotonin receptors and inhibits monoamine oxidase [2,3]. In mice, MDMA is considered a relatively selective dopaminergic neurotoxin, leading to a decrease in the concentration of DA and its main metabolites [4]. One of the most robust evidences by which MDMA causes long-term toxicity is through oxidative stress [5,6] mainly by DA oxidation via MAO-B [7]. It is known that oxidative stress is, in turn, a common feature of different neurodegenerative diseases [8–10].

Nowadays, Alzheimer Disease (AD) is the most common cause of dementia in the elderly. One of the neuropathological hallmarks of the AD is the presence of extracellular amyloid plaques, mostly composed of amyloid-β (Aβ) peptides, derived from sequential cleavage of amyloid-β protein precursor (APP) [11,12]. Additionally, Horvath et al., (2014) [13] reported loss of neurons in the substantia nigra (SN) and in the putamen, suggesting pre-and postsynaptic lesions of the nigrostriatal pathway in AD patients. Consequently, many patients of Alzheimer's disease develop extrapyramidal symptoms throughout the disease. This concomitant parkinsonian syndrome contributes to a worse prognosis of the disease [14,15].

Although MDMA consumption does not produce a direct injury on dopamine terminals in humans [16], it has been reported several cases of psychomotor problems in some consumers [17–19]. Flavel et al.

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

http://dx.doi.org/10.1016/j.bbadis.2016.06.016
0925-4439/

(2012) [17] have also described an abnormally large tremor during movement in abstinent ecstasy users and Parrot et al. (2002) postulate that consumers with initial psychomotor signs could have a greater risk to develop a motor disease in later stage of life [19]. In fact, a number of research efforts have been focused on demonstrating the possible relationship between MDMA consumption and dopaminergic neurodegeneration [17,20]. The effects elicited by 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP), a dopaminergic neurotoxin used as a model of Parkinson's disease, in motor brain areas (SN pars compacta (SNc) and striatum) have been shown to be more marked in adult mice treated with MDMA during adolescence [21], suggesting that early exposure to MDMA may render the dopaminergic neurons more vulnerable to the detrimental effects of further neurotoxic insults.

In the present study we used double transgenic mice APPswe/PS1dE9 (APP/PS1), which is a well-established transgenic animal model of familial Alzheimer Disease (FAD). These mice secrete elevated amounts of human Aβ peptide, inducing an age-dependent amyloid plaque deposition in the brain [22–24]. Perez et al., (2005) [25] investigated a possible dopaminergic nigrostriatal dysfunction in the brains of 3- to 17-month-old APP/PS1 mice, and reported some alterations in the nigrostriatal pathway closely related with amyloid deposition. Therefore, can be considered that adult FAD mouse model has a slight predisposition to nigrostriatal changes. No studies have been performed to now in order to investigate whether a prolonged consumption of MDMA might increase the development of dopaminergic dysfunctions in this model of FAD.

Hence, the aim of the present study was to elucidate whether prolonged and intermittent exposition to MDMA during adolescence was able to produce such dopaminergic alteration, which might trigger a nigrostriatal dysfunction in this model of FAD. To carry out the study we used a regimen of MDMA that differed from the classic short but intensely neurotoxic exposure, with the intention to model, in adolescent mice, the classical binge use of this substance during the weekends. We chose two time points to sacrifice the animals and investigate whether the treatment might accelerate or potentiate the nigrostriatal dysfunction in this model.

Furthermore, it has also been described that APP/PS1 mice display mitochondrial dysfunction in early stages of life [26], and as mitochondria is the main source of oxidative stress through the electron transporter chain, a prolonged alteration in mitochondrial function might give a rise in oxidative stress. As mentioned above, one of the mechanisms whereby MDMA produces neurotoxicity is through the production of reactive oxygen species (ROS). Thus we further investigated the role of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), since it has been reported to be a broad and powerful regulator of ROS metabolism, coordinating the expression of several genes coding for antioxidant enzymes [27]. We also determined the 4-hydroxynonenal (4-HNE), a chemotactic aldehydic end-product of lipid peroxidation playing a role in the harmful effects of oxidative stress [28].

Our findings suggest that, in APP/PS1 mice, MDMA does not produce all the typical signs of striatal dopaminergic toxicity but seems to anticipate the ulterior nigrostriatal dysfunction that these transgenic mice develop. Moreover, MDMA induced a significant neuronal loss in SN in both strains and, importantly, potentiated Aβ deposition in the striatum.

2. Materials and methods

2.1. Mice

Adolescent male APPswe/PS1dE9 and C57BL/6 mice (4–5 weeks old) were used in this study. These transgenic animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9). The animals were kept under controlled temperature, humidity and light conditions with food and water provided ad libitum. Mice were treated in accordance with the European Community Council Directive 86/609/EEC and the procedures established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Efforts were made to reduce the number of animals used. MDMA was provided by the National Health Laboratory (Barcelona, Spain).

2.2. Drug treatment

MDMA or saline were administered (5 ml/kg, s.c.) according to the treatment schedule displayed in Fig. 1. To model the pattern of weekend consumption of this substance in adolescents, we used intermittent and repeated MDMA administration, given as binges mimicking the widespread practice of "boosting". Animals received 3 doses in a day, every 3 h, once per week for 8 weeks. The doses increased along the treatment as described elsewhere [29]. Taking into account that the neurotoxic regimen in C57BL6 mice is 20 mg/kg four times in a day [30,31], the maximum doses given in our study was 10 mg/kg × 3. Drug administration was carried out at raised ambient temperature (26 ± 1 °C) until 2 h after the last dose, thus simulating the hot environments in which this substance tends to be consumed (i.e. dance clubs, raves). Afterwards, the animals were returned to normal housing conditions (22 ± 1 °C). One hour after the second daily dose of saline or MDMA, rectal temperature was measured using a lubricated, flexible rectal probe inserted into the rectum and attached to a digital thermometer (0331 Panlab SL, Barcelona, Spain). Mice were killed at 2 different points after the treatment: 2 weeks (3 months old, young adults; n(WT) = 32; n(APP) = 27) or 3 months later (6 months old, mature adults; n(WT) = 36; n(APP) = 32). The treatment groups were: Wild-type/Saline/(WT Saline), Wild-type/MDMA (WT MDMA), APPswe/PS1dE9/Saline (APP Saline), APPswe/PS1dE9/MDMA (APP MDMA).

2.3. Open field test (OF)

One week before the sacrifice, animals were monitored for 10 min in a circular arena of 40 cm of diameter. The locomotor activities were recorded and expressed as total distance traveled and average speed (Smart 3, Panlab SL, Barcelona, Spain).

2.4. Measurement of neurotransmitter levels

In the experiments aiming at analyzing neurotransmitter levels, mice were killed by cervical dislocation followed by decapitation, the brains rapidly removed and striata were dissected out on ice. Samples were prepared by sonication in 10 volumes of 0.1 M perchloric acid, centrifuged for 30 min at 12,000 ×*g* after which 40 μl of filtered supernatant (0.22 μm cellulose acetate) was injected into an HPLC system equipped with a Waters 2465 electrochemical detector set to a potential +0.70 V, column oven and a column Nova Pack C18 4 μm 3.90 × 150 mm (Waters, Milford, MA). The mobile phase consisted of purified water with 10% methanol, 1.92 mM 1-octanesulfonic acid, 0.1 mM EDTA and 10 mM phosphoric acid. Column temperature was set at 37 °C and the flow rate to 1 ml/min. The retention times for NE, 3.4-dihydroxiphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), DA, homovanillic acid (HVA) and 5-HT were 2.74, 4.46, 6.95, 8.13, 11.24 and 20.66 min, respectively.

2.5. Immunofluorescence and thioflavine S staining

Mice were anaesthetized by i.p. injection of ketamine (100 mg/kg) plus xylazine (10 mg/kg) and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, after which the brains were removed. Brains were cut into 30 μm-thick coronal sections in a cryostat and then incubated with primary antibody against tyrosine hydroxylase (TH) (1:200; BD Biosciences). Immunofluorescence procedure was carried out as described elsewhere [32]. For Thioflavine S staining, free-floating sections