European Journal of Pharmacology 745 (2014) 243–248

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural pharmacology

Epigenetic upregulation of alpha-synuclein in the rats exposed to methamphetamine

Wenda Jiang [a,*], Ji Li [a], Zhuang Zhang [a], Hongxin Wang [b], Zhejian Wang [c]

[a] Department of Neurology Fist Affiliated Hospital of Liaoning Medical University, 5-2 Renmin Street, Jinzhou, Liaoning 121000, China
[b] Institute of Medicine Liaoning Medical University, Jinzhou 121000, China
[c] Dalian Medical University, Dalian, Liaoning 116044, China

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form
23 October 2014
Accepted 23 October 2014
Available online 5 November 2014

Keywords:
α-synuclein
Methamphetamine
DNA methylation
Methyl CpG binding protein 2
DNA methyltransferase 1

ABSTRACT

Abuse of methamphetamine (METH) increases the risk of occurrence of Parkinson's disease (PD) in the individuals. Increased expression of synaptic protein α-synuclein (encoded by gene *Snca*) is remarkably associated with the neuronal loss and motor dysfunction in the patients with PD. The present study aimed to explore the epigenetic mechanism underlying the altered expression of α-synuclein in substantia nigra in the rats previously exposed to METH. Exposure to METH induced significant behavioral impairments in the rotarod test and open field test, as well as the upregulation of cytokine synthesis in the substantia nigra. Significantly increased expression of α-synuclein was also observed in the substantia nigra in the rats exposed to METH. Further chromatin immunoprecipitation and bisulfite sequencing studies revealed a significantly decreased cytosine methylation in the *Snca* promoter region in the rats exposed to METH. It was found that the occupancy of methyl CpG binding protein 2 and DNA methyltransferase 1 in *Snca* promoter region was also significantly decreased in the substantia nigra in the modeled rats. These results advanced our understanding on the mechanism of the increased incidence of PD in the individuals with history use of METH, and shed novel lights on the development of therapeutic approaches for the patients conflicted with this neurological disorder.

1. Introduction

Abuse of methamphetamine (METH) may induce substantial neurotoxicity in dopaminergic neurons in several brain regions and impair the motor and cognitive function in the individuals (Ares-Santos et al., 2013; Silverstein et al., 2011). A recent retrospective population-based large-scale cohort study found a significantly increased risk for developing Parkinson's disease in the individual with a history use of METH or amphetamine (Callaghan et al., 2012). METH is a potent inducer of dopamine release and is toxic to dopaminergic neurons. Previous studies found that exposure to METH induced significant oxidative stress resulting from the dysregulation of the dopaminergic system, hyperthermia, apoptosis, and neuroinflammation, thus leading to neurotoxicity and impairments of brain function (Silverstein et al., 2011). Although many efforts have made, currently the question remains unanswered why previous exposure to METH induces the long-lasting risk of the occurrence of Parkinson's-like behavior in the individuals.

Parkinson's disease (PD) is characterized by the selective degeneration of projecting dopaminergic neurons in the substantia nigra and the diminished dopamine levels in the striatum (Bagetta et al., 2010), and the presence of Lewy bodies and neurites containing α-synuclein aggregates in these brain regions (Dauer and Przedborski, 2003; Goedert, 2001), which leads to the impairments of motor coordination and balance. α-synuclein, encoded by *Snca*, generally binds synaptic vesicle membranes and potentially assists vesicle trafficking and the formation of soluble NSF (N-ethylmaleimide sensitive fusion protein) attachment protein receptor complex, thus contributing to the maintenance of normal synaptic function (Norris et al., 2004; Rizo and Sudhof, 2012). Meanwhile, increase of α-synuclein is substantially associated with the central neuroinflammation and neurodegeneration in the substantia nigra in the rodent model of PD (Lee et al., 2014). It was previously reported that repeated exposure to METH increased the expression of α-synuclein in the dopaminergic neurons in the substantia nigra in the rodent (Fornai et al., 2005; Mauceli et al., 2006), while the mechanism remained unclear. Currently, emerging evidences implied critical involvement of epigenetic modification of the expression of specific genes, including *Snca*, in the pathogenesis and development of several neurodegenerative diseases (Desplats et al., 2011; Tan

* Corresponding author. Tel.: +86 4162942717.
E-mail address: jiangwenda01@163.com (W. Jiang).

http://dx.doi.org/10.1016/j.ejphar.2014.10.043
0014-2999/

et al., 2014). Hence, in order to elucidate the mechanism underlying the long-lasting increased incidence of PD in the individuals with previous use of METH, the present study aimed to investigate the epigenetic mechanism underlying the alteration of α-synuclein in the substantia nigra in the rodents previously exposed to METH.

2. Materials and methods

2.1. Animals and drug administration

Adult male Wistar rats (200–220 g) were obtained from the Institutional Center of Experiment Animals, and were housed in the standard lab conditions (22 ± 2 °C and 12:12 h light cycle) with free access to food and water. All animal protocols were approved by the Institutional Animal Care and Use Committee, and were performed following the guidelines of National Institution of Health.

(+)-Methamphetamine (METH, Sigma-Aldrich) hydrochloride was dissolved in 0.9% saline. (+)–METH (20 mg/kg for 5 days) was injected intraperitoneally, and saline in the same volume was injected in the rats in control group (Ares-Santos et al., 2013). Behavioral tests were performed at 6, 10 and 14 days after the initial injection of METH to evaluate the motor function in the animals.

2.2. Motor performance testing

Motor performance was evaluated using a rotarod apparatus as previously described (Marques et al., 2014). The animals were placed in a rotarod with 60 mm diameter textured rod, 75 mm in length, rotating at a speed of 25 rpm. Each animal was tested 5 times with a 5 min interval between each trial and the maximum duration of the test was 5 min. The time spent by the animal on the rotarod was recorded as the falling latency.

2.3. Locomotor activity

Locomotor experiments were conducted as previously described (Arndt et al., 2014). The locomotor chambers were 40 × 40 × 40 cm^3 (Coulbourn Instruments) and had clear Plexiglas walls with a stainless steel floor covered with a thin layer of pine-chip bedding. Photobeams were arranged in a 16 (*x*-axis) photocell array, spaced 2.54 cm apart. During each locomotor test session (60 min), a 70-db white noise was generated to mask any possible background noise. Locomotor activity was measured by recording the moving distance in centimeters.

2.4. Enzyme-linked immunosorbent assay (ELISA)

ELISA study was performed with commercial ELISA kits to detect the content of α-synuclein (Millipore), IL-1β and TNF-α (R & D Systems) in the substantia nigra as previously reported (Ma et al., 2013). The substantia nigra tissues from the rats in all groups were collected, and processed with commercial ELISA kits following the instructions provided by the manufacturer.

2.5. Chromatin Immunoprecipitation (ChIP) and real-time PCR

The ChIP assay was carried out as described previously with minor modifications (Wang et al., 2007). The substantia nigra tissues from the rats were collected and cross-linked with 1% paraformaldehyde for 2 min. The tissue was kept in RIPA buffer (140 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 10 mM Tris–HCl, 1% Triton X-100, 0.1% Sodium dodecyl sulfate, 0.1% sodium deoxycolate) and sonicated 8 × 10 s on ice to produce DNA fragments with the size of 400–500 bp. The polyclonal antibodies against methyl CpG binding protein 2 (MeCP2) or DNA methyltransferases 1 (DNMT1) (1:100, Millipore, MA) were used to pull down the DNA fragments. Immunocomplexes were collected with the salmon sperm DNA/protein A agarose beads. After extensive wash with the buffers provided by the manufacturer, the cross-linking between histone and DNA was reversed. DNA fragments were purified, and real-time PCR was performed with the primers designed to amplify about 200- bp fragments in the promoter region of the target genes as following: for *Snca*: 5′-GGCTGTGTGAACAAAAGCAA-3′ and 5′-TGAACTTGAGCTGGCCTCTT-3′; for *Gapdh*: 5′-AGACAGCCGCATCTTCTTGT-3′ and 5′-CGTCCTCTACCATCCTCTGC-3′. The ChIP/input ratio (ChIP %) was calculated and compared among the groups.

2.6. Methylated DNA immunoprecipitation (MeDIP) assay

The MeDIP assay was carried out as previously described with minor modification (Provencal et al., 2013). Briefly, substantia nigra tissue was homogenized in the lysis buffer and genomic DNA was sonicated on ice 8 × 10 s. Sonicated samples were centrifuged at 14,000*g* for 10 min, and the supernatants were collected. The polyclonal antibody against 5-methylcytosine (1:100, Millipore) was added to each sample and incubated overnight at 4 °C with gentle mixing. The DNA–antibody complex was enriched with protein A agarose beads. DNA fragments in the input and pulled-down fractions were then purified with phenol–chloroform extraction followed by acid ethanol precipitation. Real-time PCR was performed to amplify about 250- bp segments corresponding to CpG sites within *Snca* promoter region. Primer sets were used as following: *Snca*: 5′-GGCTGTGTGAACAAAAGCAA-3′ and 5′-TGAACTTGAGCTGGCCTCTT-3′; for *Gapdh*: 5′-AGACAGCCGCATCTTCTTGT -3′ and 5′-CGTCCTCTACCATCCTCTGC-3′. Amplifications were run in triplicate, and the PCR data were analyzed as above.

2.7. Bisulfite sequencing PCR

DNA samples were prepared from the substantia nigra, purified, processed for the bisulfite modification with the EZ DNA Methylation-Gold™ kit (Zymo Research) (Liu et al., 2014). A fragment (about 250 bp) in the promoter region of *Snca* was amplified by the primers as following: (5Q.AGGTGAAATTTAGGTTATTTTTTT-3GTGAAACTCTAACTCCCTAACTCCTTCAC-3CT. The PCR product was then purified using a gel extraction kit (Qiagen) and sequenced using the reverse primer at the institutional core facility. The percentage methylation of each CpG site within the region amplified was determined by the ratio between peaks values of guanine (G) and adenine (A) (G/[G+A]), and these levels on the electropherogram were determined using Chromas software.

2.8. Retroscribed real-time PCR

Substantia nigra tissues were sampled from the rats in all group. Total RNA was prepared with Trizol reagent (Invitrogen), and reverse transcribed by using a SYBR Green reverse transcription (RT)–PCR Reagents kit (Applied Biosystems, Foster City, CA). The primers were designed to amplify about 200bP fragments within the cDNA sequence of the target genes as following: for α-synuclein: 5′-AGAAAACCAAGCAGGGTGTG-3′ and 5′-GCTCCCTCCACTGTCTTCTG-3′; and for GAPDH: 5′- AGACAGCCGCATCTTCTTGT -3′ and 5′-CTTGCCGTGGGTAGAGTCAT-3′. The threshold cycle (C_t) values for each sample were determined with the amplification plots within the logarithmic phase. Data were analyzed by using the $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical analysis

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or other commercial sources. All data were