Behavioural Brain Research 323 (2017) 133–140

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

The neuroprotective effect of memantine on methamphetamine-induced cognitive deficits

Jian-Dong Long[a], Yao Liu[b], Dong-Liang Jiao[b], Yu-Jun Wang[a], Gui-Ying Zan[a], Yun-Yue Ju[b], Min Zhao[b,*], Jing-Gen Liu[a]

[a] Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica and Collaborative Innovation Center for Brain Science, Chinese Academy of Sciences, Shanghai 201203, China
[b] Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China

HIGHLIGHTS

- METH impaired long term memory retention.
- METH changed expression levels of Bcl-2 and cleaved caspase-3 in the prefrontal cortex.
- MEM pretreatment improved METH-induced cognitive function.
- MEM pretreatment reversed METH-induced changes of protein levels of apoptotic-related gene.

ARTICLE INFO

Article history:
Received 25 October 2016
Received in revised form
12 December 2016
Accepted 25 January 2017
Available online 29 January 2017

Keywords:
Methamphetamine
Memantine
Cognitive deficits
Apoptosis
Prefrontal cortex

ABSTRACT

Repeated exposure to methamphetamine (METH) can cause severe neurotoxicity to the cortical neurons. In the present study, we investigated the effect of METH on cognitive function deficits, and determined the neuroprotective effects of memantine (MEM) on memory impairment induced by METH. The protein levels of Bcl-2 and cleaved caspase-3 in prefrontal cortex (PFC) were further examined to exploring the underlying mechanism. We found that repeated METH administration impaired long term (24 h) memory retention without affecting short term (5 min) memory retention. Co-administration of MEM with METH before training session significantly improved METH-induced cognitive function. METH significantly decreased expression level of Bcl-2 and increased expression level of cleaved caspase-3 in the PFC. The changes can be prevented by MEM pretreatment. Thus, these results demonstrated that MEM pretreatment reversed METH-induced changes of protein levels of apoptotic-related gene, and produced protective effects against METH-induced cognitive deficits, suggesting the effectiveness of MEM may be due to its anti-apoptotic activity.

1. Introduction

Methamphetamine (METH) is the most commonly used amphetamine-type stimulant that has already been listed as the second most abused illicit substance in the world [1]. Acute administration of METH brings about euphoria and hyperactivity due to increased release of monoamine neurotransmitters [2,3]. Repeated exposure to METH causes not only dependence and withdrawal syndrome [4,5] but also a series of neurotoxic side effects [6,7], including dopaminergic nerve terminal toxicity [8], degeneration of non-monoaminergic cells in the somatosensory cortex, and increased risk of developing Parkinson's disease [9]. Recent studies have suggested that METH-induced abnormal changes of neurotransmitter activities can cause learning, memory, cognitive or executive function deficits [10,11]. Studies from structural and functional imageology in humans demonstrated that abuse of METH resulted in the shrink of the prefrontal cortex [12], loss of dopamine transporters in the striatum [13], or neurodegenerative changes in the cerebrum [14]. A better understanding of the neurobiological mechanism of METH-induced cognitive deficits will lead to effective therapeutic strategies for preventing METH abuse. Accumulative studies suggested that METH produced persistent effect in the dopaminergic system with decreasing dopamine terminal integrity, including dopamine content [15], metabolites [16] and transporters [17]. Hsieh and colleagues proposed that METH abuse caused an overflow of dopamine in the striatum, which led

* Corresponding author.
E-mail address: drminzhao@gmail.com (M. Zhao).

http://dx.doi.org/10.1016/j.bbr.2017.01.042
0166-4328/

to excessive glutamate release into the prefrontal cortex (PFC), and induced damage to cortical neurons [18]. There was also evidence showing that METH resulted in neuronal injuries [19,20]. A recent study demonstrated METH-induced memory impairment was related with reduction of *n*-methyl-D-aspartate (NMDA) receptor ligand binding in the prefrontal cortex and hippocampus [21].

Apoptosis or programmed cell death is an important process in healthy and diseased states of the brain [22]. It has been well defined that the death signal increases the translocation of Bcl-2 family members including pro-apoptotic (e.g. Bax, Bad, Bid) and anti-apoptotic (e.g. Bcl-2, Bcl-XL) to the mitochondrial membrane and releases of cytochrome c into the cytosol, resulting in activation of caspase 9. Active caspase 9 activates the effector caspase-3 and begins the degradation phase of apoptosis [23]. Krasnova et al. [24] has demonstrated that amphetamine causes apoptosis in the striatum through a mitochondria-dependent mechanism.

Memantine (MEM) is a non-competitive antagonist of the NMDA receptor and effective in treating moderate to severe, but not mild, Alzheimer's disease [25]. MEM is also effective in treating vascular or other kinds of dementia [26] and preventing pathological neuronal damages [27,28]. Recent studies demonstrate that MEM can prevent METH-induced cell death [24,29] and cognitive deficits in rodents [30], however, it is unclear what mechanism is related to the protective effects of MEM. Here, we first utilized mice novel task recognition task to verify whether repeated METH treatment induced recognition memory impairment, and the different retention session was applied. We then investigated the effects of MEM on METH-induced cognitive deficits under different pretreatment conditions. Finally, the change of protein levels of apoptosis-relevant gene in PFC was examined in order to understanding potential mechanism.

2. Material and methods

2.1. Animals

Male Kunming mice weighting 20–25 g (8–10 weeks)were obtained from the Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China). Mice were housed five per cage and maintained on a 12 h light/dark cycle with access to food and water *ad libitum*. All experimental procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and antibodies

METH was obtained from China academy of military medical science, and was dissolved in saline to a final concentration of 5 mg/kg before intraperitoneal (i.p.) injections. Memantine Hydrochloride Tablets was produced by Rottendorf Pharma GmbH (Germany), and was grinded and dispersed in saline before intragastric administration. The antibodies of Bcl-2, Bax and cleaved caspase-3 were purchased from Cell Signaling and diluted 1:500 for western blotting analysis.

2.3. Novel object recognition task (NORT)

The NORT was carried out as preciously described [31,32]. The experimental apparatus consisted of a Plexiglas open field box ($30 \times 30 \times 40$ cm high) with a textured floor. The apparatus was located in a sound-attenuated room and was illuminated with an approximately 20 lm light. The NORT procedure consisted of three sessions: habituation, training, and testing. The animals were videotaped in both training and testing session. Each mouse was individually habituated to the box for 10 min exploration in the absence of objects for three consecutive days (habituation session, day 1–3). During the training session, two identical objects were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore in the box for 5 min (day 4). An animal was considered to be exploring the object when its head was facing the object (the distance between the head and object is an approximately 1 cm or less) or it was touching or sniffing the object. The time spent exploring each object was recorded by an experimenter blinded to the identity of the treatments, using stopwatches. After training, mice were immediately returned to their home cages. During the testing sessions, the animal was placed back into the same box 5 min or 24 h after training, with one of the familiar objects replaced by a novel object. The animals were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded as described above. Throughout the experiments, the objects were used in a counter balanced manner in terms of their physical complexity and emotional neutrality. Thus, we biased preference to any one of these objects observed in the training session. An exploratory ratio of the amount of time spent exploring the identical or novel object over the total time spent exploring both objects was used to measure cognitive function. The discrimination index was calculated as a ratio of the time spent exploring the novel object minus the familiar object over the total exploring time in the testing session.

2.4. Experimental schedule

In typical experimental conditions, mice were given saline (i.p.) or METH (5 mg/kg, i.p.) four doses (separated by 2 h) two days before the habituation session (day −1) (Fig. 1A). To study the therapeutic effects of co-treatment of MEM (5 mg/kg, or 10 mg/kg, p.o.), MEM was administrated 1 h each time before METH injection. The following 6 groups were prepared: saline/saline, MEM (5 mg/kg)/saline, MEM (10 mg/kg)/saline, saline/METH, MEM (5 mg/kg)/METH, and MEM (10 mg/kg)/METH (Fig. 1B). To test the effects of post-administration of MEM, MEM (5 mg/kg, or 10 mg/kg, p.o.) was administrated immediately after training (Fig. 1C). To study weather neuron apoptosis happens in the PFC, and testify the protective effect of MEM, mice were sacrificed at certain time points after METH treatment or MEM co-treatment for western blot analysis.

2.5. Subcellular fractionation

Immediately after sacrifice the brains were rapidly removed from the skull. Coronal brain sections (1 mm thick) were obtained using a mice brain slicer (Braintree Scientific Inc., Braintree, MA). Both sides of the medial prefrontal cortex were punched from brain slices using a blunt-end, 16-gauge syringe needle. In all subsequent procedures, the tissues were maintained at 4 °C. The tissue was homogenized with 30 strokes with a Teflon pestle in 10 vol of PIRA lysis buffer (Beyotime) with protease inhibitor cocktail (Roche). The homogenate was centrifuged at $1000 \times g$ for 10 min, and the pellet was discarded. The supernatant was collected for western blotting analysis.

2.6. Immunoblotting of Bax, Bcl-2, and cleaved caspase-3

Equal amounts of protein were electrophoresed on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Bax, Bcl-2, and cleaved caspase-3 were detected using a polyclonal rabbit anti-Bax, anti-Bcl-2, and anti-cleaved caspase-3 serum (Cell signaling), respectively at a 1:500 dilution in Tris-Buffered Saline (TBS) containing 5% non fat dry milk and 0.05% Tween-20. After incubation with Horseradish Peroxidase (HRP)-conjugated goat anti-rabbit IgG (Santa Cruz) at a 1:2000 dilution, bands were developed with a chemiluminescent substrate (RPN2132, Amer-