Free Radical Biology and Medicine 99 (2016) 11–19

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original article

Protective effects of physical exercise on MDMA-induced cognitive and mitochondrial impairment

Ghorban Taghizadeh [a], Jalal Pourahmad [b], Hajar Mehdizadeh [a], Alireza Foroumadi [c], Anahita Torkaman-Boutorabi [a], Shokoufeh Hassani [c], Parvaneh Naserzadeh [b], Reyhaneh Shariatmadari [d], Mahdi Gholami [c], Mohammad Reza Rouini [e], Mohammad Sharifzadeh [a,d,*]

[a] Department of Neuroscience, School of Advanced Science and Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[e] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form
13 July 2016
Accepted 20 July 2016
Available online 21 July 2016

Keywords:
3, 4-methylenedioxymethamphetamine (MDMA)
Mitochondria
Physical exercise
Learning and memory
Oxidative stress
ROS
Cytochrome c

ABSTRACT

Debate continues about the effect of 3, 4-methylenedioxymethamphetamine (MDMA) on cognitive and mitochondrial function through the CNS. It has been shown that physical exercise has an important protective effect on cellular damage and death. Therefore, we investigated the effect of physical exercise on MDMA-induced impairments of spatial learning and memory as well as MDMA effects on brain mitochondrial function in rats. Male wistar rats underwent short-term (2 weeks) or long-term (4 weeks) treadmill exercise. After completion of exercise duration, acquisition and retention of spatial memory were evaluated by Morris water maze (MWM) test. Rats were intraperitoneally (I.P) injected with MDMA (5, 10, and 15 mg/kg) 30 min before the first training trial in 4 training days of MWM. Different parameters of brain mitochondrial function were measured including the level of ROS production, mitochondrial membrane potential (MMP), mitochondrial swelling, mitochondrial outermembrane damage, the amount of cytochrome c release from the mitochondria, and ADP/ATP ratio. MDMA damaged the spatial learning and memory in a dose-dependent manner. Brain mitochondria isolated from the rats treated with MDMA showed significant increase in ROS formation, collapse of MMP, mitochondrial swelling, and outer membrane damage, cytochrome c release from the mitochondria, and finally increased ADP/ATP ratio. This study also found that physical exercise significantly decreased the MDMA-induced impairments of spatial learning and memory and also mitochondrial dysfunction. The results indicated that MDMA-induced neurotoxicity leads to brain mitochondrial dysfunction and subsequent oxidative stress is followed by cognitive impairments. However, physical exercise could reduce these deleterious effects of MDMA through protective effects on brain mitochondrial function.

1. Introduction

3, 4-methylenedioxymethamphetamine (MDMA) is the most common type of amphetamine used for recreational purposes [1]. Due to widespread consumption as well as acute and chronic toxic effects, MDMA is a matter of high concern. In addition to positive effects of MDMA on mood, alertness and energy [2], MDMA-induced impairments of cognitive functions have been shown in different human and animal studies. A wide range of cognitive functions have been reported to be damaged due to MDMA consumption in humans including selective attention, spatial memory, decision making [3–5], and verbal memory [6]. However, poly drug

Abbreviations: MDMA, 3, 4-methylendioxymethamphetamine; MWM, Morris water maze test; ROS, reactive oxygen species; MMP, mitochondrial membrane potential; IP, intraperitoneally; S.C, subcutaneously; ETC, electrone transport chain; HEPES, 4-2-hydroxyethyl piperazineethanesulfonic acid; Rot, rotenone; DCFH-DA, 2′, 7′-dichlorofluorescein diacetate; Rh 123, Rhodamine 123; EGTA, Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid; ANOVA, analysis of variance

* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran.
E-mail address: msharifzadeh@sina.tums.ac.ir (M. Sharifzadeh).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.07.018
0891-5849/

use in most of MDMA users may be an important confounder of human studies which could be well controlled in animal studies in order to determine the actual MDMA-induced cognitive impairments. On the other hand, different results have been reported concerning the MDMA effects on acquisition and retention of spatial memory in rats. Sprague et al. [8] and Able et al. [7] found that MDMA partially impaired retention of spatial memory in rats while it did not affect the acquisition of spatial memory [7,8]. Nevertheless, significant MDMA-induced impairment of both acquisition and retention of spatial memory in rats has been reported in a recent study [9]. Moreover, Arias-Cavieres et al. [10] showed that non-toxic doses of MDMA resulted in spatial learning and memory impairments in rats.

MDMA-induced cognitive impairments have been attributed to the neurotoxicity caused by MDMA [11]. Oxidative stress due to production of reactive oxygene species (ROS) has been proposed as a major cause of MDMA-induced toxicity, but the source of ROS is still debated [12]. ROS are produced as a byproduct of oxidative phosphorylation during the energy production in mitochondria. Thus, mitochondrion is considered as one of the main sources of ROS production [13,14]. Increased ROS production results in mitochondrial and cellular dysfunction. A negative correlation has been found between mitochondrial dysfunction, oxidative stress and dysfunctions in behavioral tests [15]. Recent in-vitro studies have showed that MDMA and its metabolites increase ROS production in cultured cortical neurons of rats [16], mouse brain synaptosome [17], and differentiated SH-SY5Y cells [18]. In addition, Alves et al. [19] showed that MDMA administration resulted in impairment of mitochondrial electron transfer chain (ETC) subunits and subsequent decreased adenosine triphosphate (ATP) levels in the rat brain. It has been shown that mitochondrial respiration plays a pivotal role in the plasticity needed for spatial learning and memory [20]. It can be hypothesized that MDMA results in spatial learning and memory impairments through induction of mitochondrial dysfunction. However, this hypothesis needs direct confirmation because far too little attention has been paid to the mechanistic links between mitochondrial dysfunction and learning and memory impairments caused by MDMA.

Therefore, the available data about the effects of MDMA on spatial learning and memory remains inconsistent. In addition, previous data concerning the role of mitochondrial dysfunctions in MDMA-induced neurotoxicity were obtained mainly in in-vitro studies. Thus, they need confirmation by in-vivo investigations.

Physical exercise is an important non-pharmacological approach to prevent the neuronal dysfunction and death. Human studies have showed the advantageous effects of physical exercise on learning, memory and executive function as well as its protective effects in age- and disease-induced cognitive dysfuntions [21–24]. Moreover, beneficial effects of physical exercise on memory performance in different hippocampus-dependent tasks including Morris water maze test [25–27] have been reported in rodent studies. Mitochondrial-dependent mechanism is one of various mechanisms through which physical exercise exerted its neuronal protective effects. Physical exercise leads to different mitochondrial adjustments including the reinforced antioxidant networks (which results in more effective control of ROS production), decreased oxidative stress markers and improved mitochondrial ETC function [14,15,28]. So far, however, the effects of physical exercise on MDMA-induced cognitive dysfunctions have not been studied.

The present study was designed to determine the effects of MDMA on spatial learning and memory. We also investigated the possible mitochondrial mechanisms mediating the effects of MDMA on spatial learning and memory. Besides, we investigated the extent to which MDMA-induced impairments of spatial learning and memory as well as mitochondrial function was decreased by pre-exposure to physical exercise.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–250 g were purchased from Faculty of Pharmacy, Tehran University of Medical Sciences. All animals were housed in cages (five/cage), kept on a 12 h/12 h light/dark cycle with controlled temperature and humidity. Rats were handled daily and have a free access to water and food. Body temperature after MDMA injection was under control similar to Soleimani Asl et al. [9] study. All experimental procedures were approved by the Tehran University of Medical Sciences Animal Ethics Committee.

Animals were divided into the following groups (n=8 for each group):

- MDMA groups which were injected with MDMA (5, 10, and 15 mg/kg, I.P) 30 min before the first training trial in 4 training days of Morris water maze (MWM).
- Exercise groups which were further divided into short- and long-term exercise (i.e, 2 and 4 weeks treadmill exercise, respectively) and forced to run 30 min/day, 5 days/week.
- Exercise+MDMA groups which underwent the physical exercise and after completing the duration of exercise, they received MDMA (5, 10, 15 mg/kg, I.P) in training days of MWM.
- Control group which received saline instead of MDMA. They were left in the treadmill for the same time as the exercise groups without running.

2.2. Materials

MDMA was synthesized by Department of Medical Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences according to a previous method [29]. It showed an acceptable purity and its structure was fully confirmed with NMR and Mass spectrometry methodologies. 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), rotenone, 2′, 7′-dichlorofluorescein diacetate (DCFH-DA), Tris–HCl, sodium succinate, sucrose, KCl, Na_2HPO_4, $MgCl_2$, Rhodamine 123 (Rh 123), Coomassie blue, Ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), Ketamine and xylazine were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Physical exercise

Initially, for 3 days, rats were acclimated to the treadmill by placing them on a treadmill for 10 min/day at a speed of 8 m/min with no incline. Animals in exercise groups were forced to run on the treadmill at 5 days/week, 30 min/day with a speed up to 8 m/min (2 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the final 20 min) with no incline.The treadmill was equipped with the metal bar grid at the beginning of each lane through which a mild electrical shock caused tingling sensation was delivered to rats to encourage them to continue running. After a few days of treadmill running, animals were taught to continue running to avoid this shock. To insure continuous running as well as to monitor for signs of exhaustion, pain or stress, rats were observed throughout the exercise sessions.

2.4. Spatial learning and memory assessment

Spatial memory was evaluated by the MWM. Training protocol was the same as what was described previously [30]. Briefly, rats