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Research paper

Effects of chronic treatment with methylphenidate on oxidative stress and inflammation in hippocampus of adult rats

Majid Motaghinejad[a], Manijeh Motevalian[a,*], Behnaz Shabab[b]

[a] Razi Drug Research Center & Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[b] Solid Dosage Form Department, Iran Hormone Pharmaceutical Company, Tehran, Iran

HIGHLIGHTS

- Methylphenidate (MPH) abuse in adolescents and adults have markedly increased in recent years.
- Degeneration is induced by chronic administration of MPH in adult rat hippocampus.
- MPH in various doses increased the oxidative stress markers in adult rat hippocampus.
- MPH in various doses increased the markers of inflammation in adult rat hippocampus.

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ABSTRACT

Methylphenidate (MPH) is a central stimulant, prescribed for the treatment of attention deficit/hyperactivity disorder. The long-term behavioral consequences of MPH treatment are unknown. In this study, the oxidative stress and neuroinflammation induced by various doses of MPH were investigated. Forty adult male rats were divided into 5 groups; and treated with different doses of MPH for 21 days. Twenty four hours after drug treatment, Open Field Test (OFT) was performed in all animals. At the end of the study, blood cortisol level (BCL) was measured and hippocampus was isolated and oxidative stress and inflammation parameters and histological changes were analyzed. Chronic MPH at all doses decreased central square entries, number of rearing, ambulation distance and time spent in central square in OFT. BCL increased in doses 10 and 20 mg/kg of MPH. Furthermore, MPH in all doses markedly increased lipid peroxidation, mitochondrial oxidized glutathione (GSSG) level, Interleukin 1β (IL-1β) and Tumor Necrosis Factor α (TNF-α) in isolated hippocampus. MPH (10 and 20 mg/kg) treated groups had decreased mitochondrial reduced glutathione (GSH) content, and reduced superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRx) activities. 10 and 20 mg/kg of MPH change cell density and morphology of cells in Dentate Gyrus (DG) and CA1 areas of hippocampus. Chronic treatment with high doses of MPH can cause oxidative stress, neuroinflammation and neurodegeneration in hippocampus of adult rats.

1. Introduction

Methylphenidate (MPH) is an amphetamine like neural stimulant which binds to dopamine and norepinephrine transporters and inhibits their reuptake to presynaptic terminals [34]. The pharmacological properties of MPH mimic methamphetamine and cocaine but the neurochemical and neurobehavioral alterations caused by its abuse are unclear [3,17,30]. Previous studies have confirmed that chronic abuse of MPH by doses of 2, 3 and 10 mg/kg or methamphetmaine derivatives induce apoptosis, oxidative damage and DNA fragmentation to brain cells such as hippocampus in young and adult rats [2,4,30]. Furthermore, chronic use of amphetamine like agents promote neuroinflammation in glial cells as is evident by the rise in expression of inflammatory cytokines TNF-α and IL-1β levels and decrease in antioxidant enzymes in central nervous system in human and animal model [10,18,28]. All of these studies suggested that inflammatory cytokines such TNF-α and IL-1β have critical role in MPH induce neurodegeneartion. In addition, twenty days treatment with 2 mg/kg of MPH presented a rise in TBARS content, protein carbonyls and superoxide formation. Furthermore, it can cause disturbance in mitochondrial respiratory chain enzymes and decreased catalase (CAT) activity in hippocampus and cerebellum of young rats [18,26].

* Corresponding author. Fax: +98 21 88622696.
E-mail address: Motevalian.m@iums.ac.ir (M. Motevalian).

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Table 1
Effects of various doses of methylphenidate on open field exploratory and anxiety like behavior.

Group	Ambulation distance (cm)	Central square entries (number)	Time spent in central square (second)	Number of rearing
Control	400 ± 16	15 ± 1.5	115 ± 8	13 ± 2
MPH (2 mg/kg)	370 ± 19	14 ± 1	109 ± 11	12 ± 4
MPH (5 mg/kg)	365 ± 25	14 ± 1.5	104 ± 10	12 ± 0.5
MPH (10 mg/kg)	330 ± 25[a]	11 ± 3[a]	95 ± 9[a]	9 ± 1[a]
MPH (20 mg/kg	300 ± 18[a]	10 ± 1[a]	80 ± 3[a]	9 ± 2[a]

All data are given as Mean ± SEM, $N = 8$.
MPH: methylphenidate.
[a] shows significant difference from control group ($P < 0.05$).

10 mg/kg of MPH can cause increase in inflammatory markers such as IL-6 and Cycloxigenase-1 (COX-1) in mice and can induce dopaminergic neuronal cells loss by activation of microglia which suggest inflammatory pathway is important for MPH induced neurodegeneration [24]. A recent study suggests that MPH by doses of 2 and 10 mg/kg in young and adult rats, can activate apoptosis, increasing Bax and some genes involved in apoptosis and reducing Bcl-2 in some brain areas [23]. On the other hand, in recent years, chronic abuse of MPH in adults has markedly increased, but effect of MPH on adult rat brain and specially effect of chronic abuse of multiple doses from ifra to supra pharmacologic doses (2, 5, 10 and 20 mg/kg) have not been clarified specifically in rat hippocampus. Keeping in mind the role of hippocampus in cognition, depression and anxiety like behaviors, this study was designed to determine the effects of chronic administration of different doses of MPH (for 21 days) on adult rat hippocampus.

2. Materials & methods

2.1. Animals

Forty male wistar rats (8 weeks old, weighing 250 ± 2.0 g) were obtained from animal house of Iran University of Medical Sciences (Tehran, Iran) and transferred to the laboratory. For two weeks, the rats were acclimatized at controlled environmental conditions (22 ± 2 °C; 12 h light/dark cycles) and provided with a free access to commercial rat chow and tap water. All experimental protocols were in accordance with the Guide for the Care and Use of Laboratory Animals published by National Institutes of Health National Institute of Health (NIH) guidelines, Publication No.85-23, revised 1996.

2.2. Drug

Methylphenidate was purchased from Sigma–Aldrich Company (St., Louis, MO, USA).

3. Experimental design

Group 1 (negative control) received normal saline (0.6 ml/rat, ip) and groups 2, 3, 4 and 5 received 2, 5, 10, and 20 mg/kg of MPH (ip) respectively for 21 days. On day 22, Open Field Test (OFT) was performed to evaluate motor activity and depression-like behavior in animals. In addition, blood cortisol level and markers of oxidative stress and inflammation were assessed in isolated hippocampal tissues. Also histological study was done by hematoxylin and eosin (H&E) staining. The doses of MPH were selected according to our pilot study and previous studies. Multiple protocols for duration of treatment were used previously, we decided to investigate the effects of chronic doses of MPH for 21 days on adult rats based on similar studies [5,26].

4. Open field test (OFT)

This assay was used to evaluate anxiety and locomotor activity in rodents according to references [21,22]. Four typical behaviors in OFT were assessed and scored;

1. Line crossing (ambulation) distance: total distance of the grid lines crossed by each rat
2. Center square entries: number of times each rat enters the central red square lines with all four paws.
3. Center square duration: the time spent by each rat in the central square.
4. Rearing: frequency with which each rat stands on their hind legs in the maze.

5. Blood cortisol (BCL) measurement

Whole blood was collected and BCL was measured by ELISA based on references [20,27].

6. Mitochondrial preparations

All animals were anaesthetized by thiopental (50 mg/kg, ip) and hippocampus was isolated from each rat. Briefly, the isolated tissues were homogenized in cold homogenization buffer (25 mM 4-morpholinepropanesulfonic acid, 400 mM sucrose, 4 mM $MgCl_2$, 0.05 mM EGTA; pH 7.3). Homogenated tissues were centrifuged at 450 × g for 10 min, and the supernatants obtained were re-centrifuged at 12000 × g for 10 min. Finally, the sediments were re-suspended in homogenization buffer and stored at 0 °C. Total mitochondrial proteins in tissues were determined using protein Dc assay kit (Bio-Rad), briefly; Bradford reagent (1 part Bradford: 4 parts dH2O) added to serial dilution series (0.1–1.0 mg/ml) of a known protein sample concentration; e.g., BSA, dissolved in homogenization buffer. This serial dilution series were prepared for providing a standard curve. Then in separate process 10, 15, 20, 25 and 30 μl of the mentioned protein extract (homogenized cell solutions) added to multiple wells and Bradford reagent was added to each well. Density of colors of all wells were read by plate reader at 630 nm, finally by using of mentioned standard curve, protein quantity of unknown protein extracts were obtained These homogenized cell solutions were analyzed for the measurement of oxidative stress and inflammatory markers [19,20].

7. Measurement of oxidative stress parameters

7.1. Determination of lipid peroxidation

For assessment of lipid peroxidation, MDA, a natural bi-product, was measured. Briefly 100 μL of SDS lysis solution was added to both wells containing (100 μL) of sample solution or MDA standard. After shaking and incubation of these wells, 250 μL of thiobarbituric acid (TBA) reagent were added to each tube and incubated at 95 °C for 45–60 min, then tubes centrifuged at 1000 × g for 15 min and 300 μl of n-Butanol was added to 300 μL of supernatant of these