Brain Research Bulletin 132 (2017) 82–98

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Possible involvement of CREB/BDNF signaling pathway in neuroprotective effects of topiramate against methylphenidate induced apoptosis, oxidative stress and inflammation in isolated hippocampus of rats: Molecular, biochemical and histological evidences

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ARTICLE INFO

Keyword:
Methylphenidate
Topiramate
CREB/BDNF
Neurotoxicity

ABSTRACT

Chronic abuse of methylphenidate (MPH) can cause serious neurotoxicity. The neuroprotective effects of topiramate (TPM) were approved, but its putative mechanism remains unclear. In current study the role of CREB/BDNF signaling pathway in TPM protection against methylphenidate-induced neurotoxicity in rat hippocampus was evaluated. 60 adult male rats were divided randomly into six groups. Groups received MPH (10 mg/kg) only and concurrently with TPM (50 mg/kg and 100 mg/kg) and TPM (50 and 100 mg/kg) only for 14 days. Open field test (OFT) was used to investigate motor activity. Some biomarkers of apoptotic, anti-apoptotic, oxidative, antioxidant and inflammatory factors were also measured in hippocampus. Expression of total (inactive) and phosphorylated (active) CREB and BDNF were also measured in gene and protein levels in dentate gyrus (DG) and CA1 areas of hippocampus. MPH caused significant decreases in motor activity in OFT while TPM (50 and 100 mg/kg) inhibited MPH-induced decreases in motor activity. On the other hand, MPH caused remarkable increases in Bax protein level, lipid peroxidation, catalase activity, IL-1β and TNF-α levels in hippocampal tissue. MPH also caused significant decreases of superoxide dismutase, activity and also decreased CREB, in both forms, BDNF and Bcl-2 protein levels. TPM, by the mentioned doses, attenuated these effects and increased superoxide dismutase, glutathione peroxidase and glutathione reductase activities and also increased CREB, in both forms, BDNF and Bcl-2 protein levels and inhibited MPH induced increase in Bax protein level, lipid peroxidation, catalase activity, IL-1β and TNF-α levels. TPM also inhibited MPH induced decreases in cell number and changes in cell shapes in DG and CA1 areas. TPM can probably act as a neuroprotective agent against MPH induced neurotoxicity and this might have been mediated by CREB/BDNF signaling pathway.

1. Introduction

Methylphenidate (MPH) as methamphetamine like neural stimulant commonly used for the management of children with hyperactive disorder (Challman and Lipsky, 2000; Motaghinejad et al., 2015e).This neural stimulant has high potential for abuse and is structurally and pharmacologically similar to cocaine and methamphetamines and exert its effects by inhibition of reuptake of dopamine and norepinephrine (Costa et al., 2007; Huss and Lehmkuhl, 2001; Motaghinejad et al., 2015c, 2015d; Patrick and Markowitz, 1997; Tagaya, 2010). Because of high rate of abuse of MPH in recent years, previous studies performed to clarify its harmful effects and have suggested that chronic abuse of MPH can induce oxidative stress, inflammation and apoptosis in brain cells, especially in the hippocampus, and probably by these pathways can cause neurotoxicity. But its mechanism of action and intracellular signaling pathways are under investigation (Motaghinejad et al., 2015e, 2016d; Réus et al., 2014). Also some investigations tried to introduce new protective agents for combating against this neurodegeneration (Erbaş et al., 2015; Motaghinejad et al., 2016d). One of candidates is topiramte (TPM) (Liu et al., 2009; Motaghinejad and Motevalian, 2016; Motaghinejad et al., 2016a). TPM,

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http://dx.doi.org/10.1016/j.brainresbull.2017.05.011
Received 19 September 2016; Received in revised form 13 May 2017; Accepted 19 May 2017
Available online 24 May 2017
0361-9230/

a new generation anticonvulsant, proposed for the management of migraine, obesity and other similar disorders, but its clinical outcome has not been yet approved (Arnone, 2005; Liu et al., 2009). Some ongoing reports showed that TPM affects on methamphetamine dependency but its efficacy was not approved yet(Elkashef et al., 2012; Rezaei et al., 2016), while some others showed and approved its efficacy on alcohol and nicotine dependency(Johnson et al., 2007b; Oncken et al., 2014).On the other hand, some experimental results indicated the TPM neuroprotective effects against alcohol, morphine and amphetamine induced malicious effects such as dependency and neurotoxicity (Johnson et al., 2013; Johnson et al., 2007a; Olmsted and Kockler, 2008). Some previous studies showed that the TPM has anti-apoptotic, immunosuppressive, antioxidant and neuroprotective properties (Armağan et al., 2008; Dudley et al., 2011; Mao et al., 2015). According to results of these studies TPM could act as a protective agent in therapy of some neurodegenerative disorders, but its clear mechanism of action including signaling pathways still remain unclear (Johnson et al., 2013; Motaghinejad and Motevalian, 2016; Motaghinejad et al., 2016a). On the other hand, previous studies have indicated that cyclic AMP response element binding protein (CREB) is a major transcription factor which participate in brain development, neural survival and neurogenesis and is a critical signaling pathway in neural normal activities (Blendy, 2006; Borbély et al., 2013; Lee et al., 2005; Soysal et al., 2016). Multiple enzyme family protein kinases phosphorylate this transcription factor and convert CREB to its active form phosphorylated CREB (P-CREB) (Aguiar et al., 2011; Carlezon et al., 2005; Kitagawa, 2007; Réus et al., 2011; Terranova et al., 2016). Phosphorylated CREB acts on genome of the cell and triggers production of BDNF proteins, which has a key role in neurogenesis and neuron development (Aguiar et al., 2011; Réus et al., 2011). Considering the important role of hippocampus in emotional behavior such as depression, learning, memory and anxiety-like behaviors, which would be affected by neurostimulant agents abuse, the aim of this study is to evaluate the effects of TPM against MPH-induced neurotoxicity by measurements of apoptosis, oxidative stress and biomarkers of inflammation and introduce TPM neuroprotection against MPH induced neurotoxicity and assess the possible role of the phosphorylated-CREB/BDNF in this type of neuroprotection in both DG and CA1 areas of rat hippocampus.

2. Materials & methods

2.1. Animals

Sixty adult male wistar rats (mean weight 210 ± 10 g, 10 weeks old) were obtained from experimental research center of Iran University of Medical Sciences (IUMS, Tehran, Iran). The animals transferred to the laboratory and were kept for 2 weeks before the start of experiment in a standard dark/light cycle at room temperature (22 ± 2 °C) with free access to food and tap water. All the manipulation and handeling of animals for performing the behavioral tests and killing and for isolation of brainwere done by standard protocols and expert person. Our experimental protocol was approved by the ethical committee in research deputy of IUMS.

2.2. Drug

MPH and TPM were purchased from Sigma-Aldrich Company (USA) and dissolved in normal saline for injection. All drugs freshly prepared just before use and the volume of injection was 0.7 ml/rat.

2.3. Experimental design

Sixty adult male rats were divided randomly into six groups:

- Group 1 (as negative control) treated with normal saline (0.7 ml/rat, ip) for 14 days.
- Group 2 received MPH (10 mg/kg) for 14 days.
- Groups 3 and 4 were treated concurrently with MPH (10 mg/kg, ip) and TPM at doses of 50 and 100 mg/kg (ip injection) respectively for 14 days.
- Groups 5 and 6 were treated by only TPM at doses of 50 and 100 mg/kg respectively for 14 days.

On day 15 after drug administration, Open Field Test (OFT), a standard behavioral test and as an indicator of hippocampal performance was used for evaluation of hippocampal degeneration. This test was performed for evaluation of motor activity in animals. Then, hippocampal tissues were removed and some parameters of oxidative stress, inflammation and also some proteins involved in apoptosis were measured in the hippocampus. Considering the important role of CREB and BDNF signaling pathway in neuroprotection and neural survival process, the effects of TPM on MPH induced disturbances in the CREB/BDNF signaling pathway was assessed using real-time reverse transcriptase-PCR (RT-PCR) and western blot methods at gene and protein levels respectively. In addition, changes in cell shapes and density, cell number, in dentate gyrus (DG) and Cornu Ammonis (CA1) areas of hippocampus were observed by hematoxylin and eosin (H & E) staining.

2.4. Open field test (OFT)

Open Field Test (OFT) was used as a standard behavioral test for evaluation of anxiety like behavior and locomotor activity disturbences in rodents. This is a good indicator for evaluation of hippocampal neurodegenartion. For performing this test, a box was used with the bottom divided into 16 equally spaced squares which bordered by opaque walls with 65.90 cm in length from ground. The bottom of this box was painted black, excluding the 6 mm broad white lines, which divided the ground into 16 equal squares. During the experiment, all parts of the room except for the open field were maintained at dark situation and just the apparatus was illuminated by a 100 W bulb that focused on the field from a height of about 110 cm from the ground. Before the start of experiment for each rat the open field maze was cleaned by using 75% ethyl alcohol. All behaviors were scored with Hindsight for MS-dos (version 1.5, California, USA), and each trial was recorded for latter analysis, using a video camcorder (Hitachi, VM-7500LA Tokyo, Japan,) located above the apparatus. Measures of all behaviors were obtained with an automated camera-based computer tracking system (Limelight, Actimetrics, Wilmette, Illinois, USA) on an IBM PC computer with the camera fixed to the ceiling, 2.2 m above the apparatus. In order to decrease the animals stress, all animals handled and manipulated gently by an expert person. To assess the process of habituation to the novelty of the location, rats were exposed to the maze for 5 min one day before the experiment (day 14). On the day of experiment (day 15) each rat was centrally located in the field for a maximum of 5 min and following behaviors were monitored:

- Ambulation distance: the distance with each rat crossed one of the grid lines.
- Center square entries: frequency with which the rat crossed one of the red central lines with all four paws into the central square.
- Center square duration: duration of the time rat spent in the central square
- Rearing: frequency with which the rat stood on their hind legs in the maze. This assay was used to evaluate locomotor activity and anxiety in rodents according to the references (Motaghinejad et al., 2015c; Vecsei and Widerlöv, 1988).

2.5. Mitochondrial preparation

By administration of 50 mg/kg of thiopental, all animals were anaesthetized and euthanized. Then the brain tissues were detached after the skull was dissected out for isolation of hippocampus. The hippocampal tissues were homogenized in cold homogenization buffer