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Involvement of AMPA/kainate and $GABA_A$ receptors in topiramate neuroprotective effects against methylphenidate abuse sequels involving oxidative stress and inflammation in rat isolated hippocampus

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ABSTRACT

Abuses of methylphenidate (MPH) as psychostimulant cause neural damage of brain cells. Neuroprotective properties of topiramate (TPM) have been indicated in several studies but its exact mechanism of action remains unclear. The current study evaluates protective role of various doses of TPM and its mechanism of action in MPH induced oxidative stress and inflammation. The neuroprotective effects of various doses of TPM against MPH induced oxidative stress and inflammation were evaluated and then the action of TPM was studied in presence of domoic acid (DOM), as AMPA/kainate receptor agonist and bicuculline (BIC) as $GABA_A$ receptor antagonist, in isolated rat hippocampus. Open Field Test (OFT) was used to investigate motor activity changes. Oxidative, antioxidant and inflammatory factors were measured in isolated hippocampus. TPM (70 and 100 mg/kg) decreased MPH induced motor activity disturbances and inhibit MPH induced oxidative stress and inflammation. On the other hand pretreatment of animals with DOM or BIC, inhibit this effect of TPM and potentiate MPH induced motor activity disturbances and increased lipid peroxidation, mitochondrial oxidized form of glutathione (GSSG) level, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in isolated hippocampal cells and decreased reduced form of glutathione (GSH) level, superoxide dismutase, glutathione peroxidase and glutathione reductase activity. It seems that TPM can protect cells of hippocampus from oxidative stress and neuroinflammation and it could be partly by activation of $GABA_A$ receptor and inhibition of AMPA/kainite receptor.

1. Introduction

Methylphenidate (MPH), a neural stimulant, used for the treatment of children with hyperactive disorders (Challman and Lipsky, 2000; Mathieu et al., 1989). MPH inhibits the dopamine and norepinephrine reuptakes into presynaptic terminals (Izenwasser et al., 1999). This agent pharmacologically acts similar to methamphetamine and cocaine and has high potential of abuse and dependency (Huss and Lehmkuhl, 2001; Schaefers et al., 2009; Tagaya, 2010). In Recent years, the frequency of its abuse was increased (Motaghinejad et al., 2015c, 2015e). The neurochemical and neurobehavioral changes induced by chronic MPH administration in adult rats remain unclear and are under investigation (Motaghinejad et al., 2015e; Réus et al., 2014; Réus et al., 2015). Chronic MPH abuse induces oxidative stress, inflammation and apoptosis in brain cells (Réus et al., 2014; Zare and Farrokhi, 2014). Recent studies indicate that MPH has neurotoxic effects in adult rat hippocampus (Motaghinejad et al., 2015e; Rozas et al., 2015; van der Marel et al., 2015).

TPM is a new generation anticonvulsant agent that inhibits AMPA/kainate receptor and activates $GABA_A$ receptors. It has indication for use in treatment of alcoholism, methamphetamine and cocaine addiction (Arnone, 2005; Garnett, 2000; Heidbreder, 2005). The neuroprotective properties of TPM have been identified in several studies but its exact mechanism of action is under investigation (Mao et al., 2012; Nazıroğlu and Yürekli, 2013; Tian et al., 2015). The previous studies suggested that TPM by inhibition of AMPA/kainate receptor or by activation of $GABA_A$ receptors can act as a protective agent against some neurodegenerative disorder (Mao et al., 2012; Raffa et al., 2010). According to these studies, TPM has antioxidant, anti-inflammatory, immunomodulatory and neuroprotective properties (Armağan et al., 2008; Cardenas-Rodriguez et al., 2013; Dudley et al., 2011). Some previous studies

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showed that TPM can act as antioxidant agent in brain of diabetic rats, these studies indicated that TPM can also manage blood glucose level and also can inhibit oxidative stress induced by high glucose in neuronal cells in animal model of diabetes (Price et al., 2011; Price et al., 2015; Shafik, 2012). TPM has antiapoptotic activity and could be a good candidate for counteracting cell damage in some neurodegenerative diseases (J. Cheng and Li, 2014; X. Cheng and Li, 2014). Recent findings also suggest that TPM can attenuate oxidative stress and mitochondrial dysfunction in neuronal cell (Tian et al., 2015). Previous studies also demonstrated that the neuroprotective agents which modulate AMPA/kainate and $GABA_A$ receptors could have potential therapeutic roles in many neurodegenerative disorders (Armağan et al., 2008; Kutluhan et al., 2009). Therefore, in present study we evaluate for the first time the role of various doses of TPM and involvement of AMPA/kainate and $GABA_A$ receptors in prevention of MPH induced oxidative stress and inflammation in adult rat hippocampus.

2. Materials and methods

2.1. Animals

Seventy male adult rats (mean weight 200 ± 8.0 g) and 8 weeks old obtained from Pasteur Institute of Iran (Tehran, Iran) and transferred to laboratory. Animals were kept for 2 weeks before start of the experiment at room temperature with free access to standard food and tap water in standard cycle of light and darkness. Our experimental protocol was approved by the Research Council of Iran University of Medical Sciences.

2.2. Drugs

TPM, MPH, DOM and BIC were purchased from Sigma-Aldrich (USA) and dissolved in normal saline for injection, freshly prepared just before use and the volumes of injections were 0.7 ml/rat. For dissolving TPM, normal saline was warmed up, to prevent use of cytotoxic solvents such as DMSO.

2.3. Experimental design

2.3.1. First part

Seventy adult male rats were divided into six groups. Group 1 (served as negative control) treated with normal saline (0.7 ml/rat) for 21 days. Group 2 (as positive control) received MPH (10 mg/kg) for 21 days. Groups 3, 4, 5, 6 and 7 were treated concurrently for 21 days by MPH (10 mg/kg, ip) and TPM with doses of 10, 30, 50, 70 and 100 mg/kg by i.p. injection respectively. After treatment of animals and based on results of behavioral changes, oxidative and inflammatory biomarkers, described below, the dominant protective dose of TPM, 100 mg/kg, was selected for second part of the experiment.

2.3.2. Second part

Forty adult male rats were divided into five groups. Group 1 (served as negative control) treated with normal saline (0.7 ml/rat) for 21 days. Group 2 received MPH (10 mg/kg) for 21 days. Group 3 received MPH (10 mg/kg) and TPM (10 mg/kg) for 21 days. Groups 4 were treated concurrently with DOM, as AMPA/kainate receptor agonist, (400 μg/kg, ip), MPH (10 mg/kg, ip) and TPM (100 mg/kg) for 21 days by i.p. injection. Groups 5 were treated concurrently with BIC, as $GABA_A$ receptor antagonist, (4 mg/kg, ip), MPH (10 mg/kg, ip) and TPM (100 mg/kg) for 21 days by i.p. injection.

On day 22, after drug administration, for all of the experimental groups, Open Field Test (OFT, a standard behavioral method used for study of hippocampus degeneration), were used to evaluate the level of motor activity. In addition, some markers of oxidative stress and inflammation were also measured in hippocampus. Also, histological studies were done by crystal violet staining for assessment of cell numbers and morphological changes in DG and CA1 areas of hippocampus in second part of experiment for further assessment of our proposal.

It should be mentioned that selection of doses of MPH and TPM were done according to previous works about the neurodegenerative doses of MPH and neurprotective doses of TPM (Clark et al., 2012; Claussen and Dafny, 2014; Cure et al., 2014; dela Pena et al., 2012; Mao et al., 2012; Motaghinejad et al., 2015e, 2015f; Réus et al., 2014, 2015; Tian et al., 2015). Also effective doses of DOM, as AMPA/kainate receptor agonist, and BIC, as $GABA_A$ receptor antagonist, were selected based on previous studies (Marriott et al., 2016; Schwarz et al., 2014; Zhu et al., 2014).

2.4. Open Field Test (OFT)

This assay was used to evaluate anxiety and locomotor activity in rodents according to references (Motaghinejad et al., 2015c, 2015d). Five 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 (Motaghinejad et al., 2015c, 2015d).

2.5. Mitochondrial preparation

By administration of 50 mg/kg of thiopental all animals were anaesthetized and hippocampal tissue was removed and isolated. The protocol was adapted from the previous studies with some modifications (Motaghinejad et al., 2015b). Hippocampus was homogenized in cold homogenization buffer. This buffer includes 25 mM 4-morpholinepropanesulfonic acid, 400 mM sucrose, 4 mM $MgCl_2$ and 0.05 mM EGTA at pH 7.3. Homogenated cells was centrifuged at 4500 *g* for 10 min, after that its supernatant was centrifuged at 12,000*g* for 10 min Final sediment was resuspended in homogenization buffer and stored on ice. Protein concentration was determined by protein Dc assay kit (Bio-Rad). The homogenized cell solutions were analyzed for measurement of factors of oxidative stress and apoptosis (Kuloglu et al., 2002; Motaghinejad et al., 2015a, 2015b).

2.6. Determination of oxidative stress factors

2.6.1. Study 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 tube, 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 x *g* for 15 min and 300 μL of *n*-Butanol was added to 300 μL of supernatant of these tubes and centrifuged for 5 min at 10,000 x *g* and absorbance of 200 μL of each tube was read at 532 nm. Results were expressed as nmol/mg of protein (Kuloglu et al., 2002; Motaghinejad et al., 2015a, 2015b).

2.6.2. GSH and GSSG levels

For measurement of GSH and GSSG levels, 25 μL of the 1X