Original Article

Neuroprotective Effects of Forced Exercise and Bupropion on Chronic Methamphetamine-induced Cognitive Impairment via Modulation of cAMP Response Element-binding Protein/Brain-derived Neurotrophic Factor Signaling Pathway, Oxidative Stress, and Inflammatory Biomarkers in Rats

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Abstract

Background: Forced exercise can act as non-pharmacologic neuroprotective agent. In current study, we tried the involved molecular mechanisms of protective effects of forced exercise against methamphetamine induced neurodegeneration. **Materials and Methods:** Forty adult male rats were divided to Group 1 and 2 which received normal saline and methamphetamine (10 mg/kg) respectively for 30 days. Groups 3, 4 and 5 were treated with methamphetamine for first 15 days and then were treated by forced exercise, bupropion (20 mg/kg/day) or combination of them for the following 15 days. Between 26th and 30th days, Morris Water Maze (MWM) was used to evaluate the cognition. On day 31, hippocampus was isolated from each rat and oxidative, antioxidant and inflammatory factors also the level of total and phosphorylated forms of cAMP response element-binding protein (CREB) and brain derived neurotrophic factor (BDNF) proteins were also evaluated. **Results:** Chronic abuse of methamphetamine could decreases cognition and increase malondialdehyde (MDA), Tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), while caused decreases in superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities all these changes was significant ($P < 0.001$) in compared to control group while treatment with bupropion, forced exercise and bupropion in combination with forced exercise could prevent all these malicious effects of methamphetamine ($P < 0.001$). Bupropion, forced exercise and bupropion in combination with forced exercise could activate CREB (both forms) and activates BDNF proteins' expression with $P < 0.001$ in methamphetamine treated rats. **Conclusions:** P-CREB/BDNF signaling pathways might have critical role in forced exercise protective effects against methamphetamine induced neurodegeneration.

Keywords: *Cognition impairment, forced exercise, methamphetamine, neurodegeneration, P-CREB/BDNF pathway*

Introduction

Brain-derived neurotrophic factor (BDNF) is a major neurotrophin in the brain which is produced by the effect of phosphorylated cAMP response element-binding protein (P-CREB) on DNA, and the abnormal regulation of BDNF may contribute to the pathophysiology of mood disorders such as depression.[1,2] Glycogen synthase kinase-3-beta (GSK3β) is an important intracellular regulatory protein that is subject to phosphorylation by growth factor-stimulated signaling pathways (such as BDNF).[3,4] GSK3β is inhibited upon serine 9-phosphorylation by growth factor receptor-induced activation of Akt.[5] GSK3β is a protein kinase that has important regulatory effects in neural plasticity and survival.[5,6] For example, increased GSK3β activity impairs the activation of CREB[7] and promotes cell death.[8,9] Foulstone *et al*. reported that BDNF, like insulin and epidermal growth factor, can inhibit GSK3β through increased serine-phosphorylation in cerebellar granule cells.[10] In addition, previous works showed the role of P-CREB/BDNF signaling pathway on cognitive activity.[11] Suppression of P-CREB/BDNF signaling

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pathway can induce cognitive impairment and cause increase of oxidative stress and inflammatory biomarkers.[4] Methamphetamine is a neurostimulant with an increased rate of abuse in recent years.[12] The mechanism of methamphetamine action is to increase the release of dopamine, norepinephrine, and to a lesser extent serotonin into synaptic terminals and cause hyperstimulation of receptors in acute phase and downregulation of receptors in chronic phase.[13-16] Chronic abuse of methamphetamine and its withdrawal can induce behavioral changes such as cognitive (learning and memory) impairment as evidenced in human and experimental models.[13-16] Experimental studies have confirmed the potential effect of methamphetamine in inducing neurodegeneration in some areas of the brain such as hippocampus which is responsible for cognitive impairment.[14] Previous studies have confirmed methamphetamine-induced oxidative stress, inflammation, and apoptosis in brain areas such as hippocampus.[17,18] Previous studies indicate that methamphetamine and its derivate can cause neurodegeneration, and probably suppression of some signaling pathways such as CREB/BDNF can cause this type of methamphetamine-induced neurodegeneration.[19] According to previous studies, some neuroprotective medications were used for the treatment of methamphetamine and other psychostimulant-induced behavioral, cognitive, molecular, and cellular disorders.[20,21] One of the first drugs to be approved for use in this treatment is bupropion.[20,21] It is an antidepressant used for the management of methamphetamine and nicotine cessation syndrome, but its molecular and signaling mechanisms of action remain unclear.[20,21] In addition to medicines, physical activity can inhibit oxidative stress and inflammation in brain cells and can protect them from neurodegenerative effects of drug abuse.[22-25] Chronic exercise in mice may induce behavioral changes involving BDNF pathways.[26] Because of the importance of P-CREB/BDNF signaling pathway in the modulation of neuroprotection and enhancement of cognitive performance, this study was designed to assess the role of these pathways in conferring the neuroprotective effects of forced exercise and its combination with bupropion (as standard medication) against methamphetamine-induced neurodegeneration and alterations in cognitive activities. Furthermore, this study intends to evaluate the role of forced exercise as an adjunct therapy of bupropion for methamphetamine-induced behavioral and molecular malicious effects. This can also make a better understanding of the mechanisms involved in amphetamine toxicity, forced exercise, and bupropion neuroprotection.

Materials and Methods

Animals

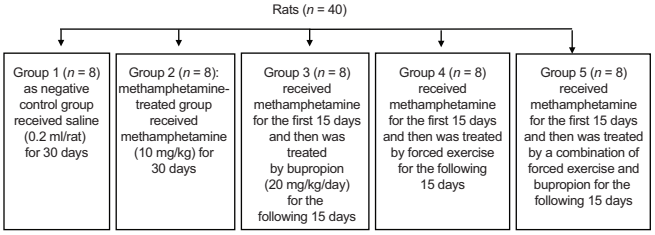
Forty adult male Wistar rats, weighing between 250 and 300 g, were obtained from the animal house of Iran University of Medical Sciences (IUMS), Tehran, Iran. They were kept under controlled temperature (22°C ± 0.5°C) with 12-h light/dark cycles and had free access to food and water. Our experiments were undertaken at IUMS in 2017, and our experimental protocol was approved by the Ethical Committee of the IUMS and according to the Guidelines of Animal Ethics and Welfare.[27]

Drugs

Methamphetamine (a psychostimulant agent) and bupropion (as an antidepressant and psychostimulant agent cessation medication) were purchased from Sigma-Aldrich Co., St. Louis, Missouri, USA, and freshly prepared just before use. Methamphetamine was dissolved in normal saline. Bupropion was dissolved in warmed normal saline. Exact doses of methamphetamine or bupropion were calculated based on animals' weight, and this amount was dissolved and made up to 0.2 ml/rat as the volume of injection for each rat. All administrations were done by intraperitoneal injection.

Experimental design

As it shown in the below chart forty adult rats were randomly divided to Various groups of experimental animals treated by drugs and exercise.



The dose of methamphetamine and bupropion was administered according to previous studies.[21] Also, the use of forced exercise protocol has been described below.

Between 26th and 30th days, Morris water maze (MWM) test was used to evaluate learning and spatial memory in all the treated animals. In order to study the effects of forced exercise against methamphetamine-induced neurodegeneration and the role of P-CREB/BDNF signaling pathways, in day 31, all animals were anesthetized by the administration of 50 mg/kg of thiopental and their brain tissues were removed and hippocampus was isolated from each rat according to the guidelines present in previous studies.[28,29] It should be noted that hippocampus from the right hemisphere was used for the evaluation of oxidative stress and inflammatory biomarkers, and left hemisphere's hippocampus was used for the evaluation of P-CREB and BDNF proteins' expression.

Treadmill forced exercise protocol

All animals were made to run on a motor-driven treadmill (ModelT408E, Diagnostic and Research Instruments Co., Taoyuan, Taiwan). Exercise training

program for rats in Groups 3 and 5 consisted of uninterrupted running on treadmill, 6 times a week for 45 min/day. For the 1st week, the running speed was set at 10 m/min and from the 2nd week till the end of experiment, it was maintained at 15 m/min. The slope and intensity of exercise was set as 0° for the first 10 min, 5° for the next 10 min, and 15° for the last 25 min.[24,30]

Behavioral method

Morris water maze task

MWM apparatus includes a black-colored circular tank, filled with water, 160 cm in diameter and 90 cm in height, which was fixed at the center of the experimental lab. This equipment was divided into four quadrants (north, east, west, and south) and filled with water to the height of 50 cm. The operator stays in the northeast part of the room. A disk on the platform with 15 cm diameter, which was hidden, was located 1 cm beneath the surface of the water. In the first 4 days of the experiment, which is called training procedure, the mentioned platform was randomly inserted persistently in one of the quarters. An automated infrared tracking system (CCTV B/W camera, SBC-300 [P], Samsung Electronics Co, Ltd., Korea) recorded the position of the experimental animals in the tank. The camera was mounted 2.4 m above the surface of the water.[31,32]

Handling

On the 1st day before the start of the experiment, all rats, one by one, were positioned on the tank that was filled with 40°C water, at room temperature (25°C ± 2°C), and the experimenter guided the rats for swimming and to reach the quarter where the platform was placed. In our experiment, the platform was situated on the southeast quarter of a tank.[31,32]

Training procedure

Some discriminate landmarks (such as a distinguish picture, window, and door) were placed in the extra maze in the room for spatial cues for animal learning about the platform's position. As mentioned above, the position of the platform was set up in the southeast quarter of the MWM tank with 25-cm distance from the edge of the tank and 1 cm beneath the surface of the water. For the evaluation of learning procedure, each rat was experimented for four trials in a day for 4 days. Each animal was randomly located in four quarters (north, east, west, and south). During the learning procedure, if the rats were found in the platform within 60 s, the trial was automatically closed by a computer. However, if they could not, the trial was stopped automatically by the computer. In the learning experiment, the following two parameters were evaluated:

1. The time of escape latency which was characterized by the time to find the hidden platform
2. Traveled distance which was confirmed by the distance each animal spent to reach and find the hidden platform.

In memory assessment procedure, on the 5th day (probe day), the platform was removed, and the animals were randomly terrified of the water from one of the above-mentioned directions (almost east), and the percentage of animal presence in target quarter (southeast quarter) was recorded and calculated.[32-34]

Mitochondrial preparations

Animals were anesthetized using sodium thiopental (50 mg/kg, i.p.), and the hippocampus was isolated from each rat. The isolated tissues were homogenized in cold homogenization buffer (25 mM 4-morpholinepropanesulfonic acid, 400-mM sucrose, 4-mM magnesium chloride, 0.05-mM ethylene glycol tetraacetic acid, pH 7.3), and the homogenized tissues were centrifuged at 450 ×*g* for 10 min. The supernatants obtained were re-centrifuged at 12,000 ×*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 a Dc protein assay kit (Bio-Rad, California, USA). Briefly, Bradford reagent (1 part of Bradford reagent: 4 parts of distilled water) was added to serial dilution series (0.1–1.0 mg/ml) of a known protein sample concentration, for example, bovine serum albumin, dissolved in homogenization buffer. These serial dilution series were prepared and used for providing a standard curve. On the other hand, 10, 15, 20, 25, and 30 μl of the protein extract (homogenized cell solutions) were added to multiple wells. Bradford reagent was also added to each well. The density of colors of all wells was read by the plate reader at 630 nm. Finally, by using the standard curve, protein quantity in the extracts was obtained. These homogenized cell solutions, containing mitochondria of hippocampal cells, were analyzed for the measurement of oxidative stress and inflammatory markers.[28,35-37]

Measurement of oxidative stress parameters

Determination of lipid peroxidation

For the assessment of lipid peroxidation, malondialdehyde (MDA) – a natural by-product – was assessed. Briefly, 100 μL of SDS lysis solution was added to wells containing 100 μL of sample solution or MDA standard. After shaking and incubation of these wells, 250 μL of thiobarbituric acid (TBA) reagent was added to each well and incubated at 95°C for 45–60 min. Next, the tubes were centrifuged at 1000 ×*g* for 15 min, and 300 μl of n-Butanol was added to 300 μL of the supernatant. Then, the tubes were centrifuged for 5 min at 10,000 ×*g*. Finally, the absorbance was read at 532 nm, and the results obtained were expressed as nmol/mg of protein.[3,28,29,35-38]

Study of manganese superoxide dismutase activity

In order to determine the superoxide dismutase (SOD) activity, 20 μL of unknown sample solution was added to each sample and 2nd blank wells. 20 μL of double-distilled

water was added to the 1st and 3rd blank wells. Next, 200 μL of WST working solution (1 ml of water-soluble tetrazolium salt; WST dissolved in 19 ml of buffer solution) was added to each well and mixed. 20 μL of dilution buffer was added to the 2nd and 3rd blank wells. Furthermore, 20 μL of enzyme working solution was added to each sample and the 1st blank well. After mixing thoroughly, the plates were incubated at 37°C for 20 min, and absorbance was read at 450 nm using a microplate reader. As recommended by the manufacturer, SOD activity was calculated using the following equation: SOD activity = ([(A_{blank} 1 − A_{blank} 3) − (A_{sample} − A_{blank} 2)]/[A_{blank} 1 − A_{blank} 3]) ×100. Data were reported as U/ml/mg protein.[1,29,38]

Determination of glutathione peroxidase activity

For the assessment of glutathione peroxidase (GPx) activity, 20 μL of the sample (which was diluted previously by assay buffer) or assay buffer alone was added to sample and its corresponding well. Following this, 200 μL of reaction solution was added to each well. After setting up the microtiter plate reader at 340 nm over a time period of 8 min at 25°C, 20 μL of peroxide substrate solution was added to each well and absorbance was measured. For data evaluation, delta optical density (OD) between 2 and 8 min was used. As recommended by the manufacturer, change in absorbance (ΔA_{340}/min) was calculated by the following equation:

ΔA_{340}/min = A_{340}nm (start) − A_{340}nm (stop)/reaction time (min), any change in absorbance is directly proportional to GPx activity.[3,38]

GPx activity: ΔA_{340}/min × reaction volume (ml) × dilution factor of the original sample/extinction coefficient for NADPH at 340 nm × volume of the tested sample. Results were expressed as mU/mg protein.[3,38]

Determination of glutathione reductase activity

To assess glutathione reductase (GR) activity, 25 μl of the sample (which was diluted beforehand with the assay buffer) or assay buffer alone was added to the sample and its corresponding well and then, 250 μl of the special reaction solution was added to each well according to manufacturer's instructions. After that, the micro plates were read at 340 nm. The OD of sample wells was inserted in the standard curve which was drawn previously by manufacturer's kits, and the activity of GR in unknown sample solution was measured by the insertion of OD and calculation of enzyme activity. Results were expressed as mU/mg protein.[3,37-40]

Determination of protein expression alteration

Concentrations (expression of protein) of BDNF, CREB (total and phosphorylated), tumor necrosis factor-alpha (TNF-α), and interleukin (IL) in cell lysate of hippocampal tissue were measured by using a commercially available ELISA kit (Genzyme Diagnostics, Cambridge, USA). Briefly, wells containing sheep anti-rat BDNF, CREB (total and phosphorylated), IL-1β, and TNF-α polyclonal antibody (Sigma Chemical Co., Poole, Dorset, UK) were washed three times with washing buffer (0.5 M of sodium chloride [NaCl], 2.5 mM of sodium dihydrogen phosphate [NaH_2PO_4], 7.5 mM of Na_2HPO_4, and 0.1% tween 20, pH 7.2). Then, 100 ml of 1% (w/v) ovalbumin (Sigma Chemical Co., Poole, Dorset, UK) solution was added to each well and incubated at 37°C for 1 h. Following three washes, 100 ml of samples and standards were added to each well and incubated at 48°C for 20 h. After three washes, 100 ml of the biotinylated sheep anti-rat BDNF, CREB (total and phosphorylated), IL-1β, or TNF-α antibody (1:1000 dilutions in washing buffer containing 1% sheep serum, Sigma Chemical Co., Poole, Dorset, UK) was added to each well. Next, after 1-h incubation and three washes, 100 ml avidin-HRP (Dako Ltd, UK) (1:5000 dilution in wash buffer) was added to each well, and the plate was incubated for 15 min. After washing three times, 100 ml of tetramethylbenzidine substrate solution (Dako Ltd., UK) was added to each well and then incubated for 10 min at room temperature. Then, 100 ml of 1 M H_2SO_4 was added, and absorbance was read at 450 nm. Results were expressed as ng for IL-1β/ml or TNF-α/ml of suspension in hippocampus tissues. BDNF and CREB (total and phosphorylated) were reported as pg/ml of suspension in hippocampal tissues.[1,41-44]

Statistical analysis

The data were analyzed by GraphPad PRISM v.6 software (2016) (Graph Pad Company, San Diego, USA). First, the normality of continuous variables (behavioral and molecular parameters) was assessed using Kolmogorov–Smirnov test. Based on this test, all variables were normally distributed. All data were described as means ± standard error of the mean. The difference between treatment groups was evaluated by one-way ANOVA with Bonferroni's *post hoc*-test for group-by-group comparisons. Results were considered to be statistically significant at $P < 0.001$ level.

Results

Forced exercise improved the spatial memory in methamphetamine-induced cognitive impairment

Parameter of escaped latency and traveled distance during 4 days training in the MWM for group under treatment by methamphetamine (10 mg/kg) was increased, and it was statistically significant compared to that of negative control group ($P < 0.001$) [Figure 1a and b]. The swimming speed was not altered during training trials in control and methamphetamine-treated groups ($P < 0.001$) [Figure 1c]. In addition, the presence (%) of animals in target quarter with methamphetamine (10 mg/kg) was decreased ($P < 0.001$) [Figure 1d]. Treatment with bupropion (20 mg/kg/day), forced exercise, and

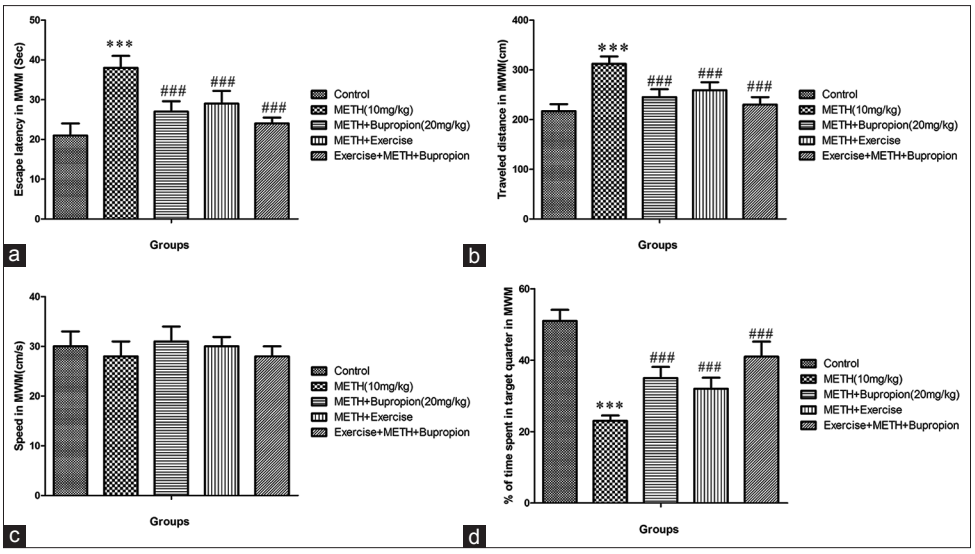


Figure 1: An average of escape latency (a), traveled distance (b), swimming speed, (c) and percentages of time spent in target quarter in probe trial (d) in negative control group and group under treatment with 10 mg/kg of methamphetamine (positive control) and groups under treatment by methamphetamine in combination with bupropion (20 mg/kg), forced exercise, or under bupropion in combination with forced exercise across all training days by using Morris water maze in rats. All data are expressed as mean ± standard error of the mean (*n* = 8). METH: Methamphetamine, MWM: Morris water maze. ***Significant level with *P* < 0.001 in comparison to negative control group, ###Significant level with *P* < 0.001 in comparison to methamphetamine-treated group (received 10 mg/kg of methamphetamine)

bupropion in combination with forced exercise in methamphetamine-dependent animals reduced escape latency time and traveled distance during training period in the MWM in comparison to those of methamphetamine-dependent group ($P < 0.001$) [Figure 1a and b]. Furthermore, this treatment increased the time spent by animals in target quarter in comparison to those of methamphetamine-dependent group ($P < 0.001$) [Figure 1d].

Forced exercise prevented the methamphetamine-induced oxidative stress

Methamphetamine administration significantly increased the lipid peroxidation as indicated by elevated mitochondrial MDA level and also decreased the SOD, GPx, and GR activities when compared to the negative control ($P < 0.001$) [Table 1]. Conversely, treatment with bupropion (20 mg/kg/day), forced exercise, by mentioned protocol, and bupropion in combination with forced exercise reduced the methamphetamine-induced rise in MDA level and inhibited the methamphetamine-induced decrease in SOD, GPx, and GR activities when compared to the methamphetamine-treated alone group (with $P < 0.001$) [Table 1].

Forced exercise prevented the methamphetamine-induced rise in inflammatory biomarkers

The animals in methamphetamine-treated groups demonstrated significant elevations in IL-1β and TNF-α levels as compared to the negative control group ($P < 0.001$) [Table 1]. Conversely, treatment with bupropion (20 mg/kg/day), forced exercise, by mentioned protocol, and bupropion in combination with forced exercise prevented the methamphetamine-induced rise in pro-inflammatory biomarkers when compared to methamphetamine-treated alone group ($P < 0.001$) [Table 1].

Forced exercise inhibited the methamphetamine-induced alterations in expressions of both forms of phosphorylated forms of cAMP response element-binding protein and brain-derived neurotrophic factor proteins

Methamphetamine (10 mg/kg) treatment markedly reduced the relative protein expression/level of P-CREB (total and phosphorylated) and BDNF in the rats' hippocampus in comparison to the negative control group ($P < 0.001$) [Figure 2a-c]. Conversely, treatment with bupropion (20 mg/kg/day), forced exercise, by mentioned protocol, and bupropion in combination with forced exercise significantly improved the protein expression/level of CREB (total and phosphorylated) and BDNF (total and phosphorylated) in methamphetamine-treated animals when compared to the methamphetamine only-treated group ($P < 0.001$) [Figure 2a-c].

Discussion

Methamphetamine is a neural stimulant, with high potential for abuse, which causes increase in the release of dopamine and norepinephrine into synaptic cleft.[45,46] Methamphetamine-like compounds cause increased dopamine, serotonin, and noradrenaline release in brain, and this leads to the downregulation of the mentioned neurotransmitter receptors.[18,46] The consequence of this phenomenon is cognitive impairment and neurodegenerative events (due to lack of the mentioned neurotransmitter receptor) during chronic abuse and withdrawal syndrome

Table 1: The effects of forced exercise training on alterations of oxidative stress and inflammatory biomarkers in mitochondria of rats treated with methamphetamine (10 mg/kg/day)

Groups	MDA (nmol/ mg of protein)	SOD (U/ml/ mg protein)	GPx (U/ml/ mg protein)	GR (U/ml/ mg protein)	TNF-α (ng/ml)	IL-1β (ng/ml)
Control	7.3±0.8	64.1±3.1	73.2±4.2	55.8±5.3	58.6±7.1	55.3±2.2
METH (10 mg/kg)	23.2±1.5[a]	28.3±5.1[a]	32.2±5.1[a]	20.2±3.9	108.5±9.8[a]	105.5±7.2[a]
METH (10 mg/kg) + bupropion (20 mg/kg)	12±1.2[b]	49.2±4.1[b]	45.3±6.1[b]	35.2±6.1[b]	90.5±2.5[a]	85.1±6.1[a]
METH (10 mg/kg) + forced exercise	13±1.5[b]	45.2±3.2[b]	41.1±6.1[b]	37.1±6.6[b]	91.6±3.5[b]	88.6±3.9[b]
METH (10 mg/kg) + bupropion (20 mg/kg) + forced exercise	10±1.1[b]	55.1±4.2[b]	68.2±8.1[b]	49.6±5.2[b]	66.4±4.1[b]	62.7±2.1[b]

[a]P<0.001 versus control groups, [b]P<0.001 versus 10 mg/kg of METH. METH: Methamphetamine, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GR: Glutathione reductase, TNF-α: Tumor necrosis factor-alpha, IL-1β: Interleukin-1β

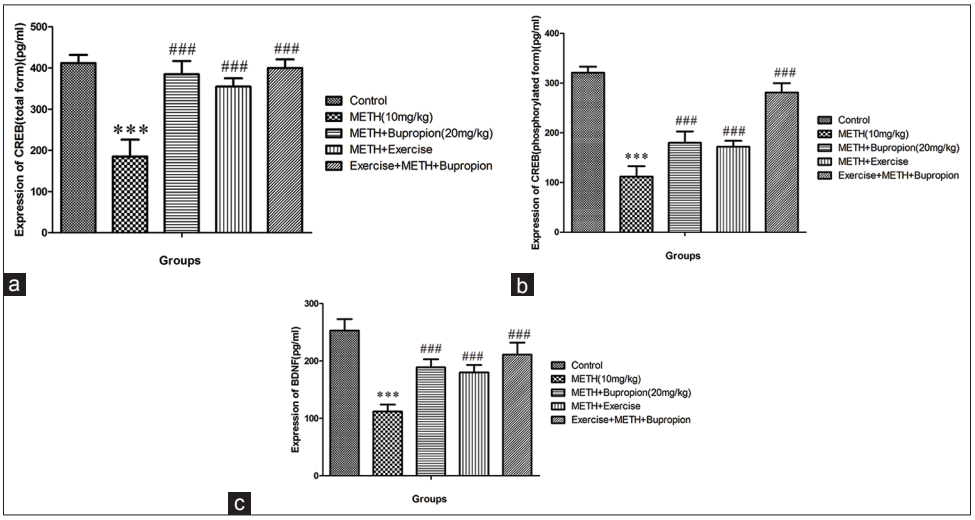


Figure 2: Alterations of expression/level (ELISA) of cAMP response element-binding protein (total form) (a), phosphorylated forms of cAMP response element-binding protein, (b) and brain-derived neurotrophic factor (c) in hippocampus in negative control group and group under treatment with 10 mg/kg of methamphetamine (positive control) and groups under treatment by methamphetamine in combination with bupropion (20 mg/kg), forced exercise, or under bupropion in combination with forced exercise. All data are expressed as mean ± standard error of the mean (n = 8). METH: Methamphetamine, P-CREB: Phosphorylated forms of cAMP response element-binding protein. ***Significant level with P < 0.001 in comparison to negative control group, ###Significant level with P < 0.001 in comparison to methamphetamine-treated group (received 10 mg/kg of methamphetamine)

of this agent.[47,48] Previous studies indicated that chronic administration of methamphetamine caused mitochondrial dysfunction and alteration in respiratory chain enzymes in brain cells of rodents; these studies suggested that methamphetamine can induce oxidative stress and inflammation in the brain of rats.[49-51] According to these studies, some parts of methamphetamine-induced neurodegeneration were mediated by the induction of lipid peroxidation, inhibition of antioxidant enzyme activity, or activation of neuro-inflammation.[50,51]

Our study suggested that chronic administration of methamphetamine at a dose of 10 mg/kg can increase escape latency and traveled distance in learning time and also cause decrease of percentage presence in target quarter in probe trial; these data are inconsistent with those of previous studies.[47,52] From our molecular findings, it can be suggested that methamphetamine (10 mg/kg) can alter oxidative stress situation and neuro-inflammation. The present study indicated that methamphetamine can decrease SOD, GPx, and GR activities while increasing MDA level, as a marker of lipid peroxidation, TNF-α, and IL-1β in rat hippocampus.

Regarding neuroprotective medicines and activities, previous studies reported the role of forced exercise or bupropion in the enhancement of cognitive impairment;[20,21,24] however, their combination and their effects in methamphetamine-induced cognitive impairment have not been reported previously. Forced exercise, which is used in the activation of neural performance, can be effective in the treatment of neurobehavioral and neurochemical disorders related to various drug abuse and their withdrawal syndromes.[22-25] The role of bupropion and its neuroprotective effects in the activation of antioxidant defense and decrease in neuro-inflammation were reported in some previous studies.[20,53] Many previous studies have reported the role of forced exercise in the activation of antioxidant enzyme and inhibition of lipid peroxidation and neuro-inflammation,[54,55] but its effect on the management of methamphetamine-induced oxidative stress and neuro-inflammation has not been reported until now.[54,55]

The current study demonstrated that forced exercise alone or its combination with bupropion (as the standard treatment of methamphetamine cessation syndrome) can modify methamphetamine-induced oxidative stress, inflammation, and cognitive (learning and memory) impairment. According to our study, some parts of its effects on the management of oxidative stress and inflammation are clarified. Our study showed that forced exercise, bupropion, and bupropion in combination with forced exercise could alter methamphetamine-induced cognitive impairment. Furthermore, our data revealed that treatment by forced exercise, bupropion, and bupropion in combination with forced exercise is effective in reversing methamphetamine-induced decrease in SOD, GPx, and GR activities and increase in MDA, TNF-α, and IL-1β levels in the hippocampal tissues. We showed that forced exercise in combination with bupropion can activate mitochondrial antioxidant enzymes (by increasing SOD, GPx, and GR activities) and inhibit lipid peroxidation and neuro-inflammation occurrence (by decrease of TNF-α and IL-1β) in methamphetamine-treated rats, and these effects could be involved in neuroprotection against some amphetamine-type stimulants.[32,54,55] These parts of data suggested that forced exercise can act as an adjunct therapy for bupropion in the management of methamphetamine-induced neurodegeneration and can also potentiate bupropion neuroprotective effects in this manner. Furthermore, according to our findings, this protocol probably by the modulation of P-CREB/BDNF signaling pathway, an important pathway on neuroprotection and neural repairing, could inhibit methamphetamine-induced behavioral and molecular changes. For clarification of the mechanism, we have tried to evaluate possible signaling pathways involved in cognitive activity and neurodevelopment. Thus, we evaluated the P-CREB/BDNF signaling pathway. According to the obtained data, methamphetamine can decrease CREB (in total and phosphorylated forms) and BDNF protein level/expression, while forced exercise, bupropion, and bupropion in combination with forced exercise can inhibit methamphetamine-induced decline of CREB (both forms) and BDNF protein level/expression in methamphetamine-treated rats. According to the present data, methamphetamine by inhibition of CREB and its product, BDNF, can cause some of the mentioned neurodegenerative events (oxidative stress and inflammation) in hippocampal cells and also cause some of the mentioned neurobehavioral disorders such as cognitive impairment. These parts of data are in consistent with those of previous works which showed that methamphetamine-type stimulant probably can inhibit P-CREB/BDNF in brain cells,[19,56,57] but its relation with the activation of neurobehavioral and neurodegenerative disorders (oxidative stress and inflammation) has not been reported yet. According to the current study, we can suggest the role of P-CREB/BDNF in methamphetamine-induced outcomes. Furthermore, it has been shown somehow by many previous studies that forced exercise or bupropion beneficial effects against neurodegenerative events were mediated probably by the modulation of P-CREB/BDNF and other similar signaling pathways, but definite role of this signaling pathway has not been approved yet.[58-61] Moreover, according to the present findings, the beneficial neurobehavioral and neurochemical effects of bupropion and forced exercise or their combination might act through P-CREB/BDNF pathways and rescue cells from methamphetamine-induced damage and trigger neuroprotection.

Conclusions

From the data obtained, we can conclude that forced exercise can be suggested as an adjunct therapy for the management of methamphetamine-induced cognitive impairment and neurodegeneration and, also, for the first time, the current study showed that P-CREB/BDNF signaling pathway might be involved in forced exercise or bupropion protective effects against methamphetamine-induced neurobehavioral and biochemical malicious effects. Certainly, further study is needed in this manner.

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Conflicts of interest

There are no conflicts of interest.

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