

Preventive Effects of Duloxetine Against Methamphetamine Induced Neurodegeneration and Motor Activity Disorder in Rat: Possible Role of CREB/BDNF Signaling Pathway

Abstract

Background: The neuroprotective effects of duloxetine and neurodegenerative effects of methamphetamine have been shown in previous studies, but their exact mechanism remain unclear. In the current study it involved molecular mechanisms of neuroprotective effects of duloxetine against methamphetamine induced neurodegeneration were clarified. **Methods:** About 40 adult male rats randomly were divided to 5 groups. Group 1 and 2, as control and methamphetamine treated, received normal saline and methamphetamine (10 mg/kg) respectively. Groups 3, 4 and 5 concurrently treated with methamphetamine and duloxetine at doses of 10, 20 and 30 mg/kg respectively. All treatments were undertaken for 21 days. On day 22 Open Field Test (OFT) were used to examine the level of motor activity disturbance and anxiety in animals. After that hippocampus was isolated from each rat and oxidative, antioxidant, inflammatory factors and also level or expression of total and phosphorylated forms of CREB and P-CREB and BDNF proteins were measured. **Results:** Duloxetine in all mentioned doses could inhibit the effects of methamphetamine induced motor activity disturbance in MWM. Chronic abuse of methamphetamine could increase malondialdehyde (MDA), tumor necrosis factor-Alpha (TNF- α) and interleukine-1beta (IL-1 β) while caused decreases in superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities and decreased CREB (both forms) and BDNF proteins, while duloxetine could prevent these malicious effects of methamphetamine. **Conclusions:** We conclude that P-CREB/BDNF signaling pathways might have critical role in duloxetine neuroprotective effects against methamphetamine induced neurodegeneration.

Keyword: Duloxetine, methamphetamine, motor activity, neurodegeneration, P-CREB/BDNF pathway

Introduction

Duloxetine is an serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressant which is used for treatment of depression and anxiety.^[1,2] Some recent studies have indicated that this agent has anxiolytic and antidepressant effects and can modulate mood changes and neurodegenerative effects which is induced by some drug abuse.^[1,2] Duloxetine has neuroprotective, antioxidant and anti-inflammatory effects and can act against some neurodegenerative situations such as ischemia.^[3] Methamphetamine is a psychostimulant with increased rate of abuse in recent years.^[4] Previous studies have confirmed methamphetamine-induced oxidative stress, inflammation and apoptosis in brain areas such as hippocampus, but the molecular aspects and involved signaling

pathways remained unclear.^[5,6] On the other hand, previous studies have shown that cyclic AMP response element binding protein (CREB), and its production on DNA, BDNF, acts as a major transcription factor in brain development and neurogenesis.^[7,8] Based on mentioned studies it is suggested that duloxetine may protect hippocampal neurons against methamphetamine induced-neurodegeneration via regulation of CREB/BDNF pathway, but this concept was not approved definitely. Because of critical role of hippocampus in management of mood and motor activity related behavior and based on importance of P-CREB/BDNF signaling pathway in modulation of neuroprotection and motor activity, current study was designed to assess the role of these pathways

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in conferring neuroprotective effects of duloxetine against methamphetamine induced neurodegeneration in hippocampus and alterations in motor activity and anxiety disorder.

Methods

Animals

There were about 40 adult male Wistar rats, weighing between 250–300 g, were obtained from animal house of Iran University of Medical Sciences. They were kept under controlled temperature ($22 \pm 0.5^\circ\text{C}$) with 12-h light/dark cycles and had free access to food and water. Our experiments were undertaken in Iran University of Medical Sciences (IUMS, Tehran, Iran) and our experimental protocol was approved by the ethical committee of the Iran University of Medical Sciences and according to guideline of animal ethics and welfare.^[9]

Drugs

Methamphetamine and duloxetine were purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA) and freshly prepared just before use. Methamphetamine and duloxetine were dissolved in normal saline and warmed normal saline, separately. Exact doses of methamphetamine or duloxetine were calculated based on animals' weight and the amount was dissolved in 0.2 ml/rat, as volume of injection for each rat.

Experimental design

- Group 1 (as control) was administrated with normal saline (0.2 ml/rat, ip) for 21 days.
- Group 2 (as methamphetamine treated group) received methamphetamine (10 mg/kg, ip, in volume 0.2 ml/rat) for 21 days.
- Groups 3, 4 and 5 were treated concurrently with methamphetamine (10 mg/kg, ip, in volume 0.2 ml/rat) and duloxetine with doses of 10, 20 and 30 mg/kg (ip, in volume 0.2 ml/rat) respectively for 21 days. There was a 45 min time interval between administration/injection of mentioned agent (methamphetamine and duloxetine).

It should be noted that doses of methamphetamine and duloxetine for designing of current study was done according to our and previous studies.^[10-12] On the day 22, Open Field Test (OFT) were used to investigate motor activity and anxiety disorder in all animal. In order to study the effects of duloxetine against methamphetamine induced neurodegeneration and the role of P-CREB/BDNF signaling pathways in this manner, in day 22, after doing behavioral test, all animals were anesthetized by administration of 50 mg/kg of thiopental and their brain tissues were removed and hippocampus was isolated from each rat according to the guides present in previous studies.^[13,14] It should be noted that hippocampus from right hemisphere was used for evaluation of oxidative stress and inflammation biomarkers and left hemisphere's

hippocampus was used for evaluation of CREB, P-CREB and BDNF proteins' expression.

Behavioral tests

Open Field Test (OFT)

Open Field Test (OFT), as standard behavioral test for assessment of locomotors activity in rodents, was performed according to guidelines previous standard studies and protocols.^[15,16]

Molecular study

Measurement of changes in oxidative, inflammatory parameters and CREB-1 and BDNF proteins expression

Level of lipid peroxidation, malondialdehyde (MDA) production, SOD, GPx and GR activities, and also changes of level IL-1 β , TNF- α , P-CREB-1 (total and phosphorylated form), which is important in neural survival and neuroprotection, and BDNF expression were measured as described previously by standard protocols.^[17,18]

Statistical analysis

All data described as means \pm standard error of the mean (SEM), the differences between treatment groups was evaluated by one-way ANOVA with Bonferroni's post-test for group-by-group comparisons. Results were considered to be significant at $P < 0.05$ level.

Results

Effects of various doses of duloxetine on methamphetamine-induced motor activity disturbance

As shown in Table 1, our study indicates that duloxetine in a dose dependent manner inhibited methamphetamine induced decreases in central square entries, time spent in the central region, ambulation distance and rearing number in OFT, this difference was statistically significant in comparison with methamphetamine (10 mg/kg) treated groups ($P < 0.05$) [Table 1].

Effects of various doses of duloxetine on methamphetamine -induced oxidative stress and inflammation

Various doses of duloxetine (10, 20 and 30 mg/kg) reduced the methamphetamine-induced rise in MDA, IL-1 β and TNF- α level and prevented the methamphetamine induced decrease in SOD, GPx and GR activities when compared to methamphetamine treated group ($P < 0.05$) [Table 2].

Effects of various doses of duloxetine on methamphetamine-induced alterations in expressions of both forms of P-CREB and BDNF proteins

Methamphetamine (10 mg/kg) treatment noticeably reduced the relative protein expression/level of P-CREB (total and phosphorylated) and BDNF in the rats' hippocampus in comparison to the control

Table 1: Effect of various doses of duloxetine on open field exploratory and anxiety like behavior in rat under treated by 10 mg/kg of Methamphetamine

Group	Ambulation distance (cm)	Central square entries (number of entries)	Time spent in central square (sec)	Number of rearing
Control	442±12	24±1	175±12	12±2
Methamphetamine (10 mg/kg)	341±12 ^a	10±1.2 ^a	126±7 ^a	4±1 ^a
Methamphetamine (10 mg/kg) + Duloxetine (10 mg/kg)	375±16 ^b	15±1.5 ^b	145±13 ^b	4±1
Methamphetamine (10 mg/kg) + Duloxetine (20 mg/kg)	383±14 ^b	17±1.3 ^b	155±12 ^b	9±2 ^b
Methamphetamine (10 mg/kg) + Duloxetine (30 mg/kg)	394±21 ^b	22±2 ^b	169±8 ^b	10±1 ^b

^a*P*<0.05 vs control groups. ^b*P*<0.05 vs 10 mg/kg of Methamphetamine.

Table 2: The effects of various doses of duloxetine on alterations of oxidative stress and inflammatory biomarkers in mitochondria of rats treated with methamphetamine (10 mg/kg/day).

Group	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	7.1±0.8	64.2±5.1	73.3±5.1	53.1±5.1	54.4±6.2	51.2±5.3
METH (10 mg/kg)	22.4±1.6 ^a	32.3±2.1 ^a	36.2±5.6 ^a	24.1±4.1	98.5±8.4 ^a	93.6±9.1 ^a
METH (10 mg/kg) + Duloxetine (10 mg/kg)	14±1.4 ^b	46.5±6.1 ^b	51.3±6.2 ^b	36.3±5.1 ^b	90.4±5.7 ^b	88.1±10.2 ^b
METH (10 mg/kg) + Duloxetine (20 mg/kg)	12±1 ^b	53.2±3.2 ^b	61.2±6.3 ^b	44.2±6.1 ^b	66.4±6.4 ^b	66.8±3.6 ^b
METH (10 mg/kg) + Duloxetine (30 mg/kg)	11±1.8 ^b	57.1±6.5 ^b	64.3±5.2 ^b	51.3±5.1 ^b	57.2±5.3 ^b	64.9±3.1 ^b

^a*P*<0.05 vs control groups. ^b*P*<0.05 vs 10 mg/kg of Methamphetamine. METH: Methamphetamine

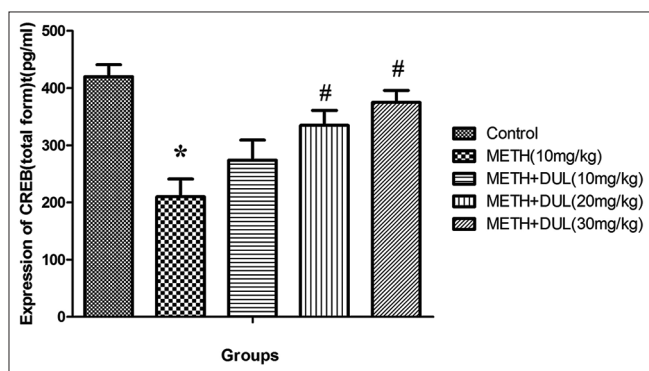


Figure 1: Shows alterations of expression/level (ELISA) of Total CREB in hippocampus in control group and group under treatment with 10 mg/kg of methamphetamine (methamphetamine treated group) and groups under treatment by methamphetamine in combination with duloxetine (10, 20, and 30 mg/kg). All data are expressed as Mean ± SEM (*n* = 8). METH: methamphetamine; DUL: duloxetine. *Shows significant level with *P* ≤ 0.001 in comparison to control group. # Shows significant level in with *P* ≤ 0.001 in comparison to methamphetamine treated group (received 10 mg/kg of methamphetamine)

group (*P* ≤ 0.001) [Figures 1-3]. Conversely, treatment with high doses of duloxetine (20 and 30 mg/kg) significantly improved the protein expression/level of CREB (total and phosphorylated) and BDNF in methamphetamine-treated animals when compared to the methamphetamine only treated group (*P* ≤ 0.001) [Figures 1-3].

Discussion

The current study demonstrated that various doses of duloxetine, possibly by modulation of P-CREB/BDNF signaling pathway, can modulate methamphetamine induced oxidative stress, inflammation and motor activity impairment. Methamphetamine is a neural stimulant which

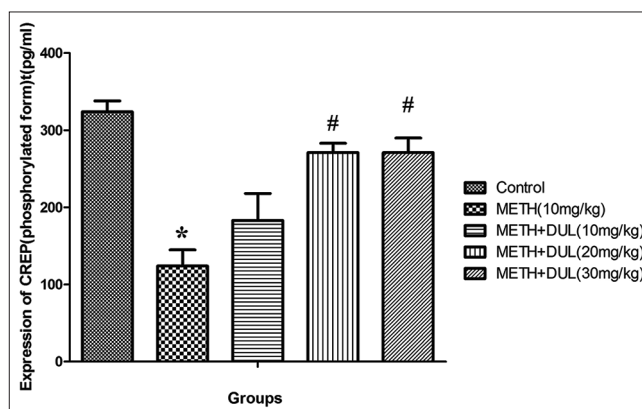


Figure 2: Shows alterations of expression/level (ELISA) of phosphorylated CREB (P-CREB) in hippocampus in control group and group under treatment with 10 mg/kg of methamphetamine (methamphetamine treated group) and groups under treatment by methamphetamine in combination with duloxetine (10, 20, and 30 mg/kg). All data are expressed as Mean ± SEM (*n* = 8). METH: methamphetamine; DUL: duloxetine. *Shows significant level with *P* ≤ 0.001 in comparison to control group. # Shows significant level in with *P* ≤ 0.001 in comparison to methamphetamine treated group (received 10 mg/kg of methamphetamine)

it's abused increased during recent year.^[19,20] Duloxetine is an antidepressant which is used primarily for the treatment of depression and anxiety disorder.^[21,22] The result of current study demonstrated that the methamphetamine with doses of 10 mg/kg causes decrease in central square entry and time spent in central square in OFT and also cause disturbance in ambulation distance and rearing while duloxetine in all mentioned doses inhibited this effects of methamphetamine. According to this data, mentioned doses of methamphetamine can activate anxiety like behavior and cause motor activity disturbance.^[19,20] Many previous clinical trials and experimental studies

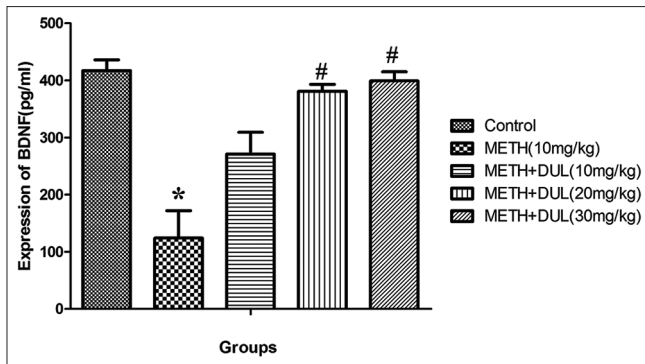


Figure 3: Shows alterations of expression/level (ELISA) of BDNF in hippocampus in control group and group under treatment with 10 mg/kg of methamphetamine (methamphetamine treated group) and groups under treatment by methamphetamine in combination with duloxetine (10, 20, and 30 mg/kg). All data are expressed as Mean \pm SEM ($n = 8$). METH: methamphetamine; DUL: duloxetine *Shows significant level with $P \leq 0.001$ in comparison to control group. # Shows significant level in with $P \leq 0.001$ in comparison to methamphetamine treated group group (received 10 mg/kg of methamphetamine)

demonstrated that duloxetine can act as antidepressant and modulate depressive and anxiety like behavior in depressed patients and subject which is induced by drug withdrawal syndrome.^[23,24] In current study, duloxetine was found to be effective in reversing methamphetamine-induced increase in MDA, TNF- α and IL-1 β levels, and reversing the reduction in SOD, GPx and GR activities in the hippocampal tissues. The previous studies indicated that chronic administration of methamphetamine caused mitochondrial dysfunction and alteration in respiratory chain enzymes in brain cells of rodents.^[25,26] These studies suggested that methamphetamine can induce oxidative stress and inflammation in the brain of rats.^[25,26] The role of antidepressant such as duloxetine in activation of antioxidant defense and its efficacy on increase of activities of antioxidant enzymes and also its anti-inflammatory properties were approved by multiple several studies.^[27-29] These reports believed that duloxetine and other similar compounds can activate or recover mitochondrial antioxidant enzymes and by this type of activation could be involved in neuroprotection against some neurotoxic agents.^[27,28] As we have noted that duloxetine can inhibit methamphetamine induced neurodegeneration in hippocampal cells, but it involved signaling pathway in this manner was not clarified, thus we evaluated the P-CREB/BDNF signaling pathway in this manner. Our data showed that duloxetine can inhibit methamphetamine induced decreases of CREB (total and phosphorylated) and BDNF proteins level/expression.^[14,18] These data are in consistency with previous works which showed that methamphetamine type stimulant can inhibit phosphorylation of P-CREB in brain cells and by this inhibition of P-CREB, production of BDNF will be inhibited.^[30,31] According to our and other previous studies duloxetine in range of 3–30 mg/kg can be effective in cognitive behavior and neurodevelopment in animal model.^[10,12] Our previous study indicated that 10 and 15 mg/kg of duloxetine, by modulation of Akt/

GSK3 signaling pathways, can inhibit methamphetamine induced neurodegeneration and cognitive behavior, but in current study duloxetine at doses of 10, 20 and 30 mg/kg can effective against methamphetamine induced changes in motor activity and neurodegeneration which confirm our previous study results,^[10] and just in high dose (20 and 30 mg/kg) could modulate CREB/BDNF signaling pathway, we can suggest that methamphetamine only in high doses can modulated critical signaling pathways. These novel results give us new insights in molecular effects of duloxetine in hippocampal cells.

Conclusions

Our data indicated that duloxetine, via modulation of production of P-CREB, BDNF, can inhibit methamphetamine induced neurodegenerative effects in adult rats. Although, these findings give us a new insight in unknown mechanisms of duloxetine neuroprotection and methamphetamine neurodegenerative effects, but further evaluation of precise molecular and cellular aspects of duloxetine protective mechanisms against methamphetamine induced neurodegeneration and neurobehavioral changes in human subject seems necessary.

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Conflicts of interest

There are no conflicts of interest.

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