Original Article

Possible Role of Cyclic AMP Response Element Binding/ Brain-Derived Neurotrophic Factor Signaling Pathway in Mediating the Pharmacological Effects of Duloxetine against Methamphetamine Use-Induced Cognitive Impairment and Withdrawal-Induced Anxiety and Depression in Rats

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Abstract

Background: Duloxetine is used for treating depression and anxiety. The current study evaluated the effects of duloxetine against methamphetamine withdrawal-induced anxiety, depression, and motor disturbances and methamphetamine use-induced cognitive impairments. **Materials and Methods:** Ninety-six adult male rats were used for two independent experiments. Each experiment consisted of Groups 1 and 2 which received normal saline (0.2 ml/rat) and methamphetamine (10 mg/kg) respectively, Groups 3, 4, and 5 received both methamphetamine and duloxetine at doses of 5, 10, and 15 mg/kg, respectively. Groups 6, 7, and 8 received 5, 10, and 15 mg/kg of duloxetine, respectively. All administrations were performed for 21 days. In experiment 1, elevated plus maze (EPM), open-field test (OFT), forced swim test (FST), and tail suspension test (TST) were used to examine anxiety and depression in animals during withdrawal period. In experiment 2, Morris water maze (MWM) test was used to assess the effect of methamphetamine use followed by duloxetine treatment, on learning and memory. In the experiments, the expression of cyclic AMP response element binding (CREB) and brain-derived neurotrophic factor (BDNF) proteins were evaluated using enzyme-linked immunosorbent assay. **Results:** In the first experiment, duloxetine at all doses attenuated methamphetamine withdrawal induced-depression, anxiety, and motor disturbances in FST, OFT, EPM, and TST. In the second experiment, duloxetine at all doses attenuated methamphetamine use-induced cognitive impairment in MWM. In both experiments, duloxetine activated cAMP, CREB, and BDNF proteins' expression in methamphetamine-treated rats. **Conclusions:** Duloxetine can protect the brain against methamphetamine withdrawal-induced mood and motor disturbances and can also inhibit methamphetamine-induced cognitive impairment, possibly via cAMP/CREB/BDNF signaling pathway.

Keywords: *Anxiety, cognition impairment, depression, duloxetine, methamphetamine*

Introduction

Methamphetamine is a neurostimulant carrying a high potential for abuse. To date, the biochemical and behavioral consequences associated with chronic use of methamphetamine remain unclear.[1,2] Methamphetamine increases the release of dopamine, norepinephrine, and, to a lesser extent, serotonin into synaptic terminals, and this increase causes the hyperstimulation of receptors in acute phase and downregulation of receptors in chronic phase.[3-6] Chronic abuse of methamphetamine has been shown to induce withdrawal syndrome associated with behavioral changes such as anxiety and depression-like behavior and also motor disturbances in rodent experimental model.[7,8] Moreover, methamphetamine abuse causes cognitive (learning and memory) impairment.[2] Experimental studies have confirmed the potential effect of methamphetamine in altering the expression of proteins associated with mood and cognitive behavior in some areas of brain such as hippocampus.[9,10]

Duloxetine is a serotonin-norepinephrine reuptake inhibitor (SNRI) which is used for the treatment of depression, anxiety,

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and cognition deficits.[11,12] Some recent studies have revealed that duloxetine, due to its effect on both serotonin and norepinephrine receptors, appears to be an effective sedative, anxiolytic, and antidepressant agent.[11,12] Hence, duloxetine can be used to subside devastating signs of drug abuse-induced cessation syndrome, such as anxiety, depression, and motor activity disorder.[12] Some similar studies demonstrated that duloxetine can improve the cognitive deficits induced by neurotoxic substances.[11,13] It can be effective in managing drug abuse disorders, such as alcohol dependency and its withdrawal.[14] On the other hand, cyclic AMP response element binding protein (CREB) is an important transcription factor involved in the regulation of genes such as brain-derived neurotrophic factor (BDNF) which is profoundly associated with development and neurogenesis.[15,16] According to previous studies, cAMP/CREB/BDNF signaling pathway plays critical role in the inhibition of expression of anxiety and depression and is also associated with enhancement of cognition.[17-19] Keeping in view the important role of duloxetine as a SNRI in the management of mood disorder and enhancement of cognition, the current study was aimed to evaluate the effect of duloxetine against methamphetamine withdrawal-induced stress, anxiety, depression, and motor activity disturbances and methamphetamine use-induced cognitive impairment. Moreover, we also studied the role of cAMP/CREB/BDNF signaling pathway in the modulation of neurobehavioral changes during methamphetamine use and withdrawal and with duloxetine treatment.

Materials and Methods

Animals

Ninety-six adult male Wistar rats, weighing between 250 and 300 g, were purchased from the Animal House of Iran University of Medical Sciences. They were kept under controlled temperature room (22°C ± 0.5°C) with 12-h light/dark cycle and had free access to food and water. The experimental protocol was approved by the Ethical Research Committee of the Iran University of Medical Sciences.

Drugs

Methamphetamine and duloxetine were purchased from Sigma_Aldrich Co (St. Louis, Missouri, United States), and freshly prepared just before use.

Experimental design

Experiment 1

In the first experiment, the study aimed to evaluate the effect of duloxetine against methamphetamine-induced anxiety, depression, and motor activity disturbances during withdrawal period. Forty-eight animals were randomly divided into eight groups as follows:

- Group 1 (control): Animals received normal saline (0.2 ml/rat, i.p.) for 21 days
- Group 2 (methamphetamine treated): Animals received methamphetamine (10 mg/kg, i.p.) for 21 days
- Groups 3, 4, and 5: Animals were treated concurrently with methamphetamine (10 mg/kg) and duloxetine at the doses of 5, 10, and 15 mg/kg, respectively, for 21 days
- Groups 6, 7, and 8: Animals received duloxetine at the doses of 5, 10, and 15 mg/kg, respectively, for 21 days.

The doses for methamphetamine and duloxetine were selected from previously published literature.[20-22]

From the 22nd to 28th days, some behavioral tests, such as open-field test (OFT), elevated plus maze (EPM) test, forced swim test (FST), and tail suspension test (TST) were performed to investigate the anxiety and depression levels of the experimental animals. These tests were performed with an interval of 1 day such that the results of one test cannot affect the other.[23-26] As we aimed to study the effect of duloxetine against methamphetamine-induced withdrawal syndrome, the behavioral tests were performed following the cessation of methamphetamine. Following behavioral examination, animals were sacrificed, and the hippocampus was used to evaluate the protein expression of cAMP, CREB, and BDNF. Moreover, we studied the relationship between these proteins and cognition.

Experiment 2

In the second experiment, we studied the effect of duloxetine against methamphetamine-induced cognitive impairment. Forty-eight animals were randomly divided into eight groups as follows:

- Group 1 (control): Animals received normal saline (0.2 ml/rat, i.p.) for 21 days
- Group 2 (methamphetamine treated): Animals were treated with methamphetamine (10 mg/kg, i.p.) for 21 days
- Groups 3, 4, and 5: Animals were treated concurrently with methamphetamine (10 mg/kg) and duloxetine at the doses of 5, 10, and 15 mg/kg, respectively, for 21 days
- Groups 6, 7, and 8: Animals received duloxetine at the doses of 5, 10, and 15 mg/kg, respectively, for 21 days
- Keeping in view the goal of this experiment, the cognitive functions were assessed using Morris water maze (MWM) task between the 17th and 21st days of the experiment. On the 28th day, animals were sacrificed and the hippocampal tissue was used to study the protein expression of cAMP, CREB, and BDNF and the relationship between these and cognition-related behaviors.

Behavioral tests

Open-field test

OFT was used to assess locomotor activity in animals.[23,24] The animals were evaluated based on the following five behaviors:

1. Ambulation distance: Distance crossed by the rat across the grid lines
2. Central square entries: The number of times the rat entered the central square with all the four paws
3. Central square duration: The time spent by the rats in the central square
4. Rearing: Frequency with which the rats stood on the hind legs in the maze.[23,24]

Forced swim test

FST is frequently used to evaluate depression-like behavior. The time of swimming, as a marker of nondepressive behavior, was recorded according to the previous studies.[23-25]

Elevated plus maze test

EPM is a test used to assess anxiety in experimental animals. The time spent by the animal in the open arms was recorded according to previous studies.[23,24,26]

Tail suspension test

TST is a test to confirm depression. The immobility time during the 5 min was recorded which was suggestive of depression-like behavior.[23,24]

Morris water maze task

MWM task, a standard behavioral test for the evaluation of cognition, was performed based on a previous study.[23,24] In this test, time of escape latency which was characterized by the time spent to find the hidden platform, distance traveled by the animal to find the hidden platform, and the percentage of time spent by the animal in the target quadrant were measured.

Measurement of protein expression

Animals were anesthetized using sodium thiopental (50 mg/kg, i.p.), and the hippocampus was isolated from each rat.[27] We studied the immunoreactivity of cAMP, CREB (total and phosphorylated), and BDNF (total) in homogenized hippocampal tissue by enzyme-linked immunosorbent assay commercial kits (MyBioSource, San Diego, USA).[19,27,28]

Statistical analysis

The data were analyzed using GraphPad PRISM software v. 6 and expressed as mean ± standard error of the mean. The differences between control and treatment groups were evaluated using one-way ANOVA followed by *post hoc* Tukey's test. $P < 0.05$ was considered statistically significant.

Results

Experiment 1

Assessment of open-field test

As shown in Table 1, the group treated with methamphetamine had fewer central square entries and spent less time in the central region of the OFT in comparison with the control groups ($P < 0.05$) [Table 1]. This group also showed more rearing and had longer ambulation distance in OFT ($P < 0.05$) [Table 1]. We found that duloxetine in a dose-dependent manner inhibited this effect of methamphetamine and increased the frequency of central square entries, time spent in the central region, rearing number, and ambulation distance in OFT ($P < 0.05$) [Table 1]. On the other hand, duloxetine alone at all doses increased the frequency of central square entries, time spent in the central region, rearing number, and ambulation distance in OFT when compared to methamphetamine in combination with duloxetine-treated groups ($P < 0.05$) [Table 1]. Moreover, these effects were significant for central square entries and time spent in the central region which confirmed the anxiolytic effect of duloxetine in methamphetamine-treated group ($P < 0.05$) [Table 1]. In addition, duloxetine treatment alone did not affect locomotor activity which was confirmed by no significant difference in rearing and ambulation distance in OFT [Table 1].

Table 1: Effect of various doses of duloxetine on open-field exploratory and anxiety-like behavior methamphetamine-treated rats

Group	Ambulation distance	Central square entries	Time spent in central square (s)	Frequency of rearing
Control	442±12	24±1	175±12	12±2
METH (10 mg/kg)	341±12[a]	10±1.2[a]	126±7[a]	4±1[a]
METH (10 mg/kg) + DUL (5 mg/kg)	375±16[b]	15±1.5[b]	145±13[b]	4±1
METH (10 mg/kg) + DUL (10 mg/kg)	383±14[b]	17±1.3[b]	155±12[b]	9±2[b]
METH (10 mg/kg) + DUL (15 mg/kg)	394±21[b]	22±2[b]	169±8[b]	10±1[b]
DUL (5 mg/kg)	430±15[c]	28±2[a,c]	189±8[a,c]	14±6[c]
DUL (10 mg/kg)	434±13[c]	29±3[a,c]	195±7[a,c]	14±5[c]
DUL (15 mg/kg)	448±14[c]	31±3[a,c]	199±5[a,c]	15±3[c]

[a]$P<0.05$ versus control groups, [b]$P<0.05$ versus 10 mg/kg of methamphetamine, [c]$P<0.05$ versus 10 mg/kg of methamphetamine in combination with duloxetine with doses of 5, 10, and 15 mg/kg. METH: Methamphetamine, DUL: Duloxetine

Assessment of forced swim test

The swimming time was less for the animals treated with methamphetamine as compared to the control group ($P < 0.05$) [Figure 1a]. In contrast, duloxetine (10 and 15 mg/kg) significantly improved the swimming time in methamphetamine-treated animals ($P < 0.001$) [Figure 1a]. Furthermore, duloxetine treatment alone at all doses increased swimming time in FST when compared with methamphetamine in combination with duloxetine-treated groups ($P < 0.05$). However, this effect was not significant in comparison to the control group [Figure 1a].

Assessment of elevated plus maze

Animals that received normal saline spent more time in the open arms of EPM in comparison with methamphetamine-treated group ($P < 0.05$) [Figure 1b]. Our data showed that animals treated with duloxetine at doses 5, 10, and 15 mg/kg spent considerably more time in the open arms of EPM as compared to the methamphetamine-treated group ($P < 0.05$) [Figure 1b]. Furthermore, duloxetine treatment alone at all doses increased the time spent by the animals in open arms when compared to methamphetamine in combination with duloxetine-treated groups ($P < 0.05$) [Figure 1b]. However, this effect was not significant in comparison to the control group [Figure 1b].

Assessment of tail suspension test

Immobility time in methamphetamine groups was considerably more in comparison to the control group in TST ($P < 0.05$) [Figure 1c]. Duloxetine (10 and 15 mg/kg) reduced the immobility time in comparison with the methamphetamine group ($P < 0.05$) [Figure 1c]. Moreover, duloxetine treatment alone at all doses reduced the immobility time when compared with methamphetamine in combination with duloxetine-treated groups ($P < 0.05$). However, this effect was not significant in comparison to the control group [Figure 1c].

Duloxetine inhibited methamphetamine-induced alterations in the expressions of cyclic AMP, cyclic AMP response element binding, and brain-derived neurotrophic factor proteins

Methamphetamine administration markedly reduced the relative protein expression/levels of cAMP, CREB (total and phosphorylated), and BDNF in the hippocampal tissue when compared to the control group ($P < 0.05$) [Figure 2a-d]. Conversely, high doses of duloxetine (10 and 15 mg/kg) significantly improved the protein levels of cAMP, CREB (total and phosphorylated), and BDNF in methamphetamine-treated animals when compared to the methamphetamine-treated group ($P < 0.05$) [Figure 2a-d]. In addition, duloxetine treatment alone at all doses increased the levels of cAMP, CREB (total and phosphorylated), and BDNF as compared to methamphetamine in combination with duloxetine-treated groups ($P < 0.05$) [Figure 2a-d]. For doses 10 and 15 mg/kg, duloxetine treatment alone increased the levels of these proteins as compared to the control group ($P < 0.05$) [Figure 2a-d].

Experiment 2

Evaluation of escape latency and distance traveled during the training days in Morris water maze

The parameters such as escape latency and distance traveled during the 4-day training for methamphetamine group were significantly higher than that of the control animals ($P < 0.05$) [Figure 3a and b]. In contrast, duloxetine treatment at all doses inhibited this methamphetamine-induced increase when compared to methamphetamine-treated

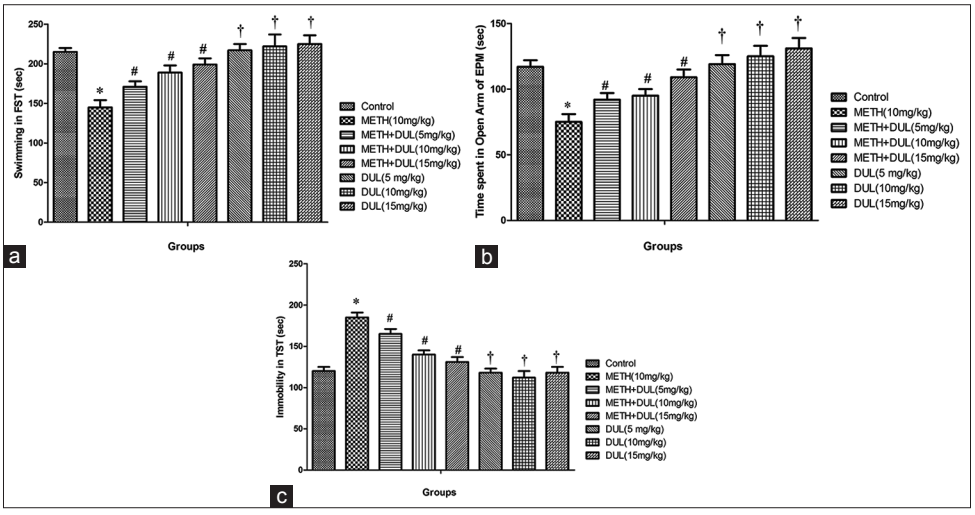

Figure 1: Assessment of anxiety and depression-like behavior in control group, and groups under treated by duloxetine with doses of 5, 10, and 15 mg/kg alone or in combination with 10 mg/kg of methamphetamine in experiment 1. (a) swimming time (seconds) in forced swim test, (b) Time spent in open arms (seconds) in elevated plus maze, (c) immobility (second) in tail suspension test. All data are expressed as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ versus control group. #$P < 0.05$ versus 10 mg/kg of methamphetamine. †$P < 0.05$ versus 10 mg/kg of methamphetamine in combination with duloxetine with doses of 5, 10, and 15 mg/kg. METH: Methamphetamine. DUL: Duloxetine

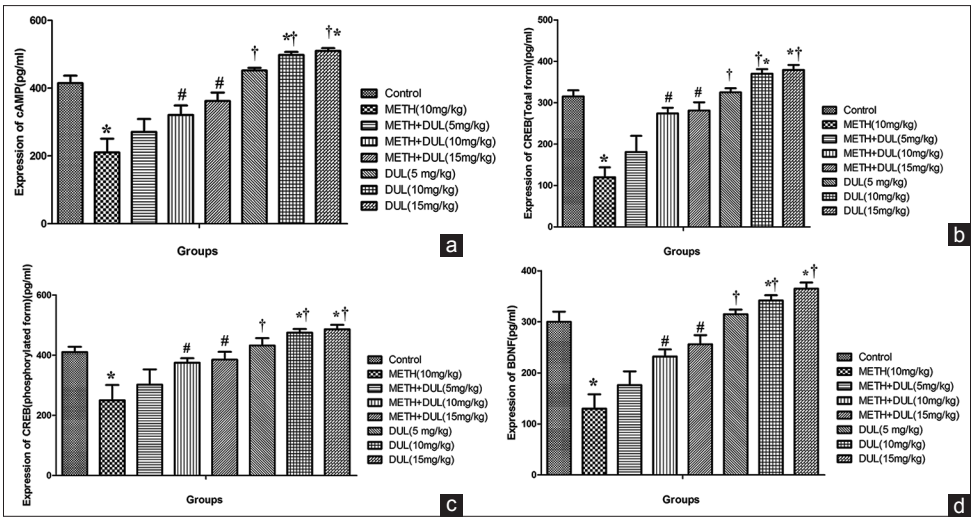

Figure 2: Hippocampal protein levels in control group, and groups treated by duloxetine with doses of 5, 10, and 15 mg/kg alone or in combination with 10 mg/kg of methamphetamine in experiment 1. (a) cyclic AMP, (b and c) total and phosphorylated cyclic AMP response element binding, (d) brain-derived neurotrophic factor. All data are expressed as mean ± standard error of the mean (*n* = 8). **P* < 0.05 versus control group. #*P* < 0.05 versus 10 mg/kg of methamphetamine. †*P* < 0.05 versus 10 mg/kg of methamphetamine in combination with duloxetine with doses of 5, 10, and 15 mg/kg. METH: Methamphetamine. DUL: Duloxetine

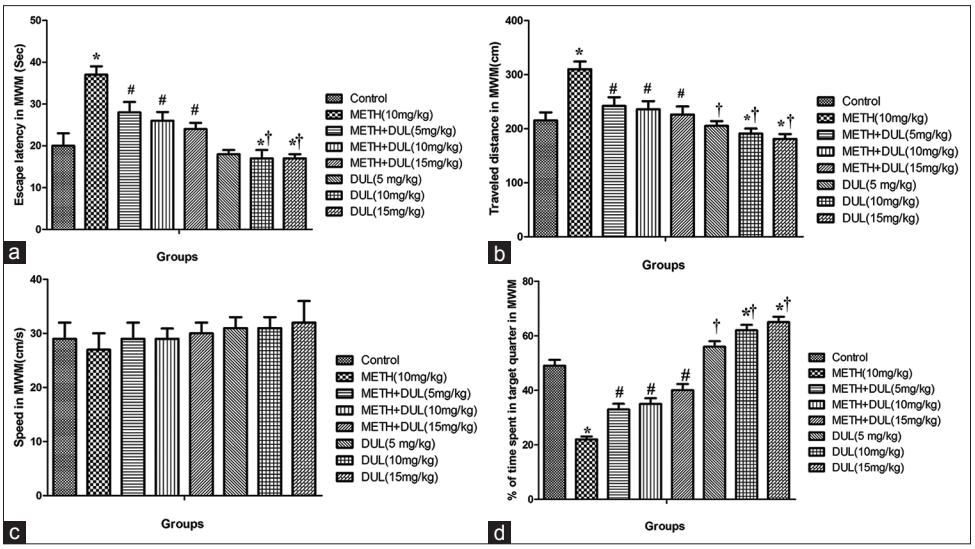

Figure 3: Assessment of learning and memory-related behavior in control group, and groups treated by duloxetine with doses of 5, 10, and 15 mg/kg alone or in combination with 10 mg/kg of methamphetamine in experiment 2. (a) average of escape latency, (b) average of traveled distance, (c) average of swimming speed, and (d) percentages of time spent in target quarter in probe trial across all training days using Morris water maze. All data are expressed as mean ± standard error of the mean (*n* = 8). **P* < 0.05 versus control group. #*P* < 0.05 versus 10 mg/kg of methamphetamine. †*P* < 0.05 versus 10 mg/kg of methamphetamine in combination with duloxetine with doses of 5, 10, and 15 mg/kg. METH: Methamphetamine. DUL: Duloxetine

animals [Figure 3a and b]. Moreover, duloxetine alone at 5, 10, and 15 mg/kg doses markedly reduced escape latency and distance traveled during the training days in comparison with methamphetamine in combination with duloxetine-treated groups ($P < 0.05$) [Figure 3a and b], whereas duloxetine at doses 10 and 15 mg/kg significantly decreased escape latency and distance traveled when compared to the control group ($P < 0.05$) [Figure 3a and b].

Evaluation of swimming speed during training days

The swimming speed was comparable during training trials between the experimental groups, suggesting that exposure to methamphetamine (10 mg/kg) alone or in combination with duloxetine (5, 10, and 15 mg/kg) did not cause any motor disturbances [Figure 3c].

Evaluation of time spent in the target quadrant in probe trial

Methamphetamine group spent less time (expressed as percentage) in the target quadrant as compared to the control group ($P < 0.05$) [Figure 3d]. In contrast, duloxetine at all doses diminished this effect of methamphetamine in comparison with the methamphetamine-treated group ($P < 0.05$) [Figure 3d]. Duloxetine treatment at all

doses considerably increased the percentage of time spent in the target quadrant when compared with the animals that had received methamphetamine in combination with duloxetine ($P < 0.05$) [Figure 3d], whereas duloxetine treatment alone at doses 10 and 15 mg/kg significantly improved the percentage of the time spent in target quadrant when compared to the control group ($P < 0.05$) [Figure 3d].

Duloxetine inhibited the methamphetamine-induced alterations in the expressions of cyclic AMP, cyclic AMP response element binding, and brain-derived neurotrophic factor proteins

Methamphetamine treatment significantly reduced the protein expression/levels of cAMP, CREB (total and phosphorylated), and BDNF in the hippocampal tissue as compared to the control group ($P < 0.05$) [Figure 4a-d]. Conversely, high doses of duloxetine (10 and 15 mg/kg) treatment remarkably improved the protein levels of cAMP, CREB (total and phosphorylated), and BDNF in methamphetamine-treated animals when compared to the animals that had received methamphetamine ($P < 0.05$) [Figure 4a-d]. Furthermore, duloxetine treatment alone at all doses improved cAMP, CREB (total and phosphorylated), and BDNF levels in comparison with the methamphetamine in combination with duloxetine-treated groups ($P < 0.05$) [Figure 4a-d], whereas duloxetine treatment alone at doses 10 and 15 mg/kg increased the expression of these proteins compared to the control group ($P < 0.05$) [Figure 4a-d].

Discussion

The current study demonstrated that duloxetine at multiple doses can reduce methamphetamine withdrawal-induced anxiety (in EPM), depression (in FST, TST, and OFT), and motor activity (in OFT). In addition, we also showed that duloxetine administration can profoundly improve cognitive impairment (in MWM) caused by methamphetamine abuse. These beneficial effects of duloxetine appeared to be mediated by cAMP/CREB/BDNF signaling pathway.

Methamphetamine is a neural stimulant which causes increased release of dopamine and norepinephrine into the synaptic cleft.[29] However, the long-term neurobehavioral and neuromolecular consequences of its abuse and withdrawal remain unclear.[3-6] Duloxetine is an antidepressant of the SNRI class.[30] It is used primarily for the treatment of depression, general anxiety disorder, social phobia, and panic disorder. It can also act as a cognitive enhancer.[30] According to a previous study, duloxetine and other similar compounds can be effective for the management of severe behavioral disorders which may occur during the abuse or withdrawal period of drugs.[31-33]

Our first experiment for OFT demonstrated that methamphetamine withdrawal increases depression, as observed by reduced frequency of central square entries, time spent in central square, and rearing. Methamphetamine withdrawal also caused a considerable decrease in the ambulation distance, suggestive of motor disturbance.[29] In agreement with the previous report, all doses of duloxetine evaluated in this study reduced this depression-like behavior and motor activity disturbance in OFT.[33,34] Moreover, duloxetine treatment alone at all doses increased central square entries, time spent in central square, rearing, and ambulation distance in OFT. These data are inconsistent with the previous studies which have demonstrated that duloxetine can attenuate depression-like behavior and also can increase motor activity disturbance induced by some drug abuse.[11,12] Our findings suggest that the antidepressant

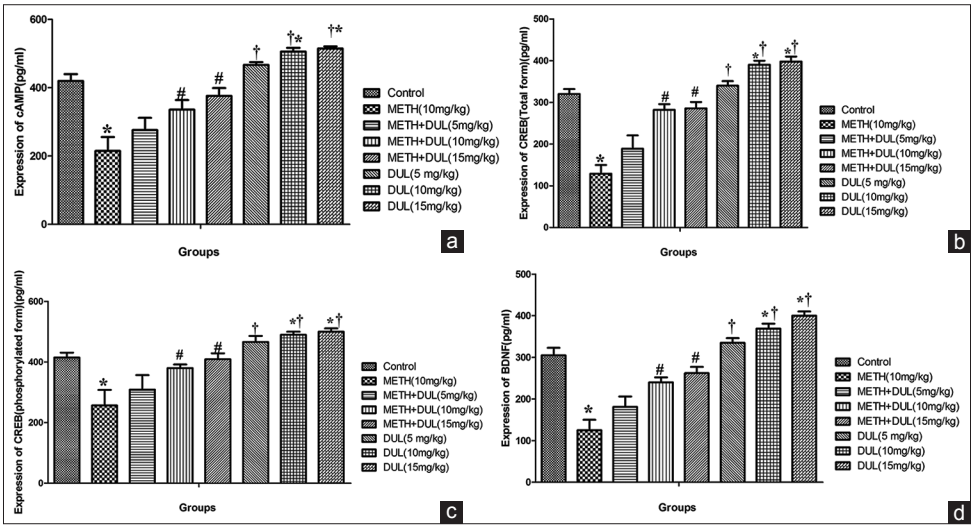

Figure 4: Hippocampal protein levels in control group, and groups treated by duloxetine with doses of 5, 10, and 15 mg/kg alone or in combination with 10 mg/kg of methamphetamine in experiment 2. (a) cyclic AMP, (b and c) total and phosphorylated cyclic AMP response element binding, (d) brain-derived neurotrophic factor. All data are expressed as mean ± standard error of the mean ($n = 8$). *$P < 0.05$ versus control group. #$P < 0.05$ versus 10 mg/kg of methamphetamine. †$P < 0.05$ versus 10 mg/kg of methamphetamine in combination with duloxetine with doses of 5, 10, and 15 mg/kg. METH: Methamphetamine. DUL: Duloxetine

action of duloxetine against methamphetamine withdrawal-induced depression-like behavior is unlikely to be due to reduction in motor activity as the animals receiving duloxetine alone demonstrated attenuated depression-like behavior accompanied by increase in motor activity. This notion has been confirmed by many experimental studies which have shown that duloxetine can act as an antidepressant and motor activity enhancer in depressed individuals during withdrawal syndrome.[11,12] In addition, duloxetine has been shown to modulate the cortical excitability and improve motor activity and reaction speed in depressed people.[13,35] Furthermore, according to our study, there is no significant effects in duloxetine alone-treated groups when compared to control group in OFT behaviors, which shows that duloxetine alone has no significant effects on locomotor activity and its effects are probably caused by antianxiety effects. According to the present study, methamphetamine withdrawal decreased the swimming time in FST and increased immobility time in TST. Treatment with duloxetine, in the presence or absence of methamphetamine, significantly improved the swimming time in FST and reduced immobility time in TST. Depression is one of the major behaviors observed during the withdrawal of abuse of methamphetamine family-like compound.[3,36] Duloxetine, due to its antidepressant effect, can increase serotonin and norepinephrine in brain synapse and by this mechanism, it can modulate depressive-like behavior during withdrawal syndrome of drug abuses.[35,37]

In the second experiment, methamphetamine alone or its combination with duloxetine did not alter the speed of rats in MWM, whereas in FST, methamphetamine caused a marked decrease in swimming time. Treatment by duloxetine, with and without methamphetamine, increased the swimming time in FST. It should be mentioned that, in MWM, speed was not changed in all treatment groups and this parameter (speed in MWM) indicates speed, which means that the movement changes over time. While in FST, we just reported and discussed swimming time and evaluated only the time of swimming activity, and we did not have the need to know the changes of speed of swimming. On the other hand, in OFT, we evaluated ambulation distance which indicates changes of distance traveled by the animal.[23-26] The speed in MWM, swimming time in FST, and ambulation distance in OFT are three parameters assessed routinely in behavioral tests. Furthermore, swimming time, ambulation distance, and speed are representative of antidepressive behavior, motor activity, and coordination, respectively.[23-26] In the current study, withdrawal of methamphetamine reduced the duration of time spent in open arms in EPM. In addition, duloxetine, with and without methamphetamine, increased the duration of time spent in open arms in EPM during withdrawal period. Studies have shown that many antidepressant and anxiolytic agents can alleviate anxiety- and depression-like behavior in drug abusers during withdrawal period.[35,38,39] In our study, duloxetine as a potent SNRI alleviated methamphetamine cessation-induced anxiety in EPM. This finding is quite intriguing as methamphetamine abuse is known to cause depletion of dopamine and norepinephrine which may augment anxiety- and depression-like behavior after its cessation. Duloxetine, due to its antidepressant and anxiolytic effects, can reduce this methamphetamine-induced anxiety and depression.[39,40]

The result of the second experiment demonstrated that chronic administration of 10 mg/kg methamphetamine can increase escape latency and distance traveled in learning time in MWM and decrease the time spent in the target quadrant in MWM. However, the swimming speed in MWM remained unaffected. In accordance to previous findings, our results indicated methamphetamine-induced reduced learning and memory function.[41-43] Methamphetamine-like compounds cause release of dopamine, serotonin, and adrenaline in brain, which in turn leads to the downregulation of the respective receptors and may induce cognitive impairment.[44] According to our results, treatment with duloxetine, with and without methamphetamine, improved learning and memory in MWM. Previous studies have also confirmed the protective effect of duloxetine and similar antidepressant effect on learning and memory.[45,46]

To study the mechanism involved in mediating the protective effects of duloxetine against methamphetamine cessation-induced anxiety, depression, and motor activity disturbances, and also the protective effect of duloxetine against methamphetamine-induced cognition impairment, we have evaluated the cAMP/CREB/BDNF signaling pathway. In both experiments, methamphetamine reduced both the total and phosphorylated forms of cAMP and CREB protein level/expression, and also caused marked reduction in BDNF levels. These data are consistent with previous findings which have shown that methamphetamine can inhibit phosphorylation and activation of cAMP and CREB in brain cells and ultimately reduce BDNF production.[19,27,47] The role of cAMP/CREB/BDNF signaling pathway in the modulation and suppression of anxiety, depression, and motor activity disturbances is well evident in literature.[19,27] Studies have also demonstrated the prominent role of cAMP/CREB/BDNF in the enhancement of cognition.[19] In line with these findings, methamphetamine abuse and its withdrawal may have caused mood disturbances and cognitive impairment at least partly by inhibiting cAMP/CREB/BDNF signaling. Furthermore, our data showed that duloxetine, with and without methamphetamine, can improve cAMP and CREB protein levels, thereby increasing BDNF production in treated rats; these data are in consistent with previous results.[18,27,48] Numerous neuroprotective agents induce antidepressant, antianxiety, and cognition enhancement effects against neurodegenerative and neurobehavioral disorders through the modulation of cAMP/CREB/BDNF and other similar signaling pathways.[18,49,50] Here,

duloxetine may have reduced anxiety- and depression-like behavior in methamphetamine-dependent rats, possibly through the modulation of cAMP/CREB/BDNF pathway. These novel results give us new insights regarding the molecular action of duloxetine and methamphetamine in hippocampal cells. Further studies are warranted to confirm these findings and to explore other signaling pathways that may be modulated during methamphetamine abuse and withdrawal.

Conclusion

Duloxetine can be effective against methamphetamine cessation-induced anxiety, depression, and motor activity disturbances and can inhibit methamphetamine-induced cognitive impairment. In this regard, cAMP/CREB/BDNF signaling pathway is of particular importance.

Suggestion and limitation

Pharmacological blockade of the signaling pathway can help in further clarification of the mechanisms of action of these agents.

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Conflicts of interest

There are no conflicts of interest.

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