The effect of 3,4- methylenedioxymethamphetamine on expression of neurotrophic factors in hippocampus of male rats

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Abstract

Background: 3,4- methylenedioxymethamphetamine (MDMA) is a chemical derivative of amphetamine that can induce learning and memory impairment. Due to the effect of neurotrophins on memory and learning, the impact of MDMA was evaluated on the brain-derived neurotrophic factor (BDNF), neurotrophin-4 (NT-4), and tropomyosin-related kinase B (Trk-β) expression in the hippocampus.

Methods: In this study, 20 adult male Wistar rats (200-250 g) received saline (1 mL) or 10 mg/kg of MDMA intraperitoneally as single or multiple injection for 2 consecutive days per week for 2 months. Expression of BDNF, Trk-β, and NT4 were assessed using Western blotting and RT PCR methods.

Results: Our results revealed that the expression of BDNF, Trk-β, and NT4 proteins and genes significantly decreased in MDMA groups compared to the sham group (p<0.05). Furthermore, the acute group showed the lowest expression of these proteins.

Conclusion: The results of the present study suggest that ecstasy administration may downregulate the expression of BDNF, Trk-β, and NT-4 in hippocampus, which is more extensive in case of acute treatment. It seems that in the chronic group, hippocampus was able to compensate the ecstasy-induced neurotoxicity.

Keywords: Ecstasy, BDNF, Trk-β, NT4

Introduction

According to the United Nation report on drug and crime, amphetamines are the second major class of illicit drugs consumed for recreational purposes worldwide (1). 3, 4-methylenedioxymethamphetamine (MDMA), known as ecstasy, is an amphetamine derivative that causes elevated mood and a heightened sense of empathy. MDMA has been shown to lead to serotonergic, dopaminergic, and noradrenergic neuronal toxicity with more affinity for serotonergic ending. This substance promotes the release and inhibits the reuptake of serotonin (5-HT) from the nerve endings (2). Previous studies have indicated cognition deficiency and memory impairment in humans following the use of MDMA; these problems could remain even after abstinence from the drug (3, 4). Several experimental studies on rodents have demonstrated that MDMA-induced neurotoxicity is characterized by cognitive impairment including deficiencies in spatial learning and memory in Morris water maze (5) and also spatial and egocentric learning in Cincinnati water maze (6), associated with serotonergic system dysfunction both in humans and animals (6, 7).

The association between serotonergic neurotoxicity with changes in the neurotrophic factors is now well-documented (8, 9). Neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin 4 are endogenous proteins that are critical for the proliferation, differentiation, and survival of central nervous system during development and neuroplasticity period throughout life (For review see von Bartheld) (10). BDNF and NT affect neuronalplastic-
Ecstasy and neurotrophins

ity and long-term potentiation through binding to the
tropomyosin receptor kinase β (TrkB) (10).

Neurotrophins may account for some of the amphet-
amines derive effects. In methamphetamine—dependent
human abusers, plasma BDNF levels remain elevated after 30
or more days of abstinence (11).

Expression of BDNF, NT3, and TrkB is also upregu-
lated in the frontal, parietal, piriform, and hippocampus
following serotonergic depleting dose of MDMA (10 mg/kg
x 4 at 2-hour intervals on a single day) 1, 7, or 24 hours
after receiving the last dose (12). However, the chronic
effects of MDMA on BDNF, NT-4, and TrkB expression
have not yet been examined. In the present study, the ef-
effect of MDMA weekend regimen (as chronic model) and
MDMA acute administration on the expression of BDNF,
NT4, and TrkB in the hippocampus were investigated.

Methods

Chemicals

MDMA was obtained by the Presidency Drug Control
Headquarters (Tehran, Iran) and dissolved in 0.9% saline.
The other chemicals, unless otherwise stated, were pur-
bred from Sigma (St. Louis, MO, USA).

Animal treatment and experimental procedures

All experimental procedures were performed in accord-
ance with the guidelines of the ethical committee of Ham-
adan University of Medical Sciences. Adult male Wistar
rats, weighing 200-250 g were included in this experi-
mental study and maintained in the colony room at a tem-
perature of 21±1°C on a 12-h light/12-h dark cycle with
access to water and food ad libitum. We checked the rectal
temperature before starting any treatment and the animals
with a body temperature higher than 37.5°C were exclud-
ed.

The rats were divided into 4 groups (n=5 per group):
Acute group received a single dose of MDMA (10
mg/kg) or saline (1mL) intraperitoneally (Shortall et al.
2013), and the chronic group received MDMA (10mg/kg)
or saline (1mL) intraperitoneally for 2 consecutive days
per week for 2 months (Shortall et al. 2013). Rats in acute
group were killed 24 hours after the treatment and those in
the chronic group were killed after one week by decapita-
tion. The hippocampi were removed, immediately frozen
in liquid N2, and maintained at -80°C for further analysis.

Western blot analysis

The right frozen hippocampi were homogenized with
200 μL lysis buffer [Ripa buffer and inhibitor cocktail (Sigma
Aldrich, St. Louis, MO, USA), 1:20] for 1 hour and
centrifuged at 12000 g for 20 minutes. Protein
concentration was determined with a Bio-Rad assay sys-
tem (Bio-Rad, San Francisco, CA, USA), and 100 mi-
crograms of total protein from each sample were dena-
tured with sample buffer (6.205 mM tris-HCl, 10% glyc-
erol, 2% SDS, 0.01% bromophenol blue and 50 mM 2-
ME) at 95°C for 5 minutes. The denatured proteins were
separated on a SDS page (10% sodium dodecyl sulphate
polyacrylamide gel) and transferred to a nitrocellulose
membrane (Amersham Pharmacia Biotech, Piscataway,
NJ, USA). Non-specific bindings were blocked with 5% nonfat dry milk, and membranes were probed with anti-
BDNF, -NT4, Trk-β (1:500; Santa Cruz, CA, USA), and
β-actin (1:1000; Sigma Aldrich, St. Louis, MO, USA)
monoclonal antibodies for 2 hours and secondary anti-
rabbit antibodies (1:5000; Santa Cruz, CA, USA) conjuga-
ted to alkaline phosphatase (for Trk-β) or horseradish
peroxidase (for BDNF and NT-4) for 1 hour. Bands were
detected using 5-bromo-4-chloro-3-indolyl phosphate in
the presence of nitroblue tetrazolium (Abcam, Cambridge,
UK) or ECL kit (Abcam, Cambridge, UK) as a chemilu-
minescent substrate. Band densities were measured by an
image analysis system (UVIdoc, Houston, TX, USA).

Reverse transcription PCR

To achieve total RNA, the left frozen hippocampi were
homogenized in 1000 μL RNA™ (Cinnagen, Tehran,
Iran). After adding ice cold chloroform, homogenates were
centrifuged (12000 g for 20 min at 4°C) and supernatant
was precipitated with isopropanol and washed with 75%
ethanol. The RNA was converted to cDNA using a cDNA
synthesis kit (Qiagen, Hilden, Germany), following the
protocol outlined by the manufacturer. RT-PCR reactions
were performed using a PCR Master Kit (Cinnagen, Teh-
ran, Iran) and the following primers:

- bdnf (forward: ggtcacacgtcggagaaag and reverse: gtc-
tatcctatgaacgcce)
- trkb (forward: aagttctaggtgctgctg and reverse: ttcttc-
tacaag acctagcttg)
- nt-4 (forward: ctcgctagggacctcttg and reverse: ccgcegcgggtgacccag)
- β-actin (forward: tgtgatggtgggaatgggtcag and reverse: ttt-
gatgacgcaagttt)

PCR reactions were performed with initial denaturation
at 95°C for 3 minutes, followed by 33 cycles at 95°C for 30
seconds, 56°C ( for bdnf and trk-β) and 61 ºC ( for nt-4) for
40 seconds and 72°C for 40 seconds, with a final elonga-
tion time at 72°C for 5 minutes.

The same annealing temperature was used for β-actin.
The products were electrophoresed in 1.5% agarose gel at
100 volts. Semi-quantitative analysis was assessed by a
digital imaging system (UVIdoc, Houston, TX, USA).

Statistical analysis

All data were presented as mean± S.E.M and analyzed
using SPSS Version 16 software. The one-way analysis of
variance (ANOVA) and Tukey multiple comparison tests
were used to analyze the significant difference between the
groups. Acceptable significance level was set at p<0.05.

Results

As there was no difference between acute and chronic
sham saline groups, herein only 1 sham group was report-
ed.

Figure 1 demonstrates the effect of MDMA on BDNF
expression both in Western blot and RT-PCR. Different
regimen of MDMA caused the downregulation of the bdnf
gene, which was statistically significant for both groups
compared with the sham group (p<0.01, Fig. 1A). Further
analysis revealed increased bdnf expression in the chronic
group compared to the acute group, but this increase was not statistically significant. Densitometry from the membrane showed a less expression of the BDNF protein in the MDMA groups compared to saline group, which was statistically significant in case of acute group (p<0.05, Fig. 1B). There was an insignificant difference between the acute and chronic groups such that the acute group expressed less BDNF. As displayed in Fig. 2A, trk-β was expressed more in the sham group, and there was a significant difference between the sham and acute groups (p<0.05). Rats that received chronic treatment showed an increase in trk-β expression, but the difference was not statistically significant. Western blot analysis revealed more expression of the Trk- β protein in the saline group compared to MDMA groups (p<0.001 and p<0.05 for acute and chronic groups, respectively, Fig. 2B). Furthermore, a significant difference was found between the acute and chronic groups, showing a lower Trk- β expression in the acute group (p<0.01).

Quantification of nt4 expression revealed a significant difference between the saline and MDMA groups (p<0.05, Fig. 3A). Moreover, MDMA administration attenuated nt4 expression related to the saline-treated group. Nt4 expression was lower in the acute group compared to the chronic group, but the difference was not significant.

In accordance with nt4 mRNA results, NT4 protein expression decreased in the MDMA-treated groups compared to saline group (p<0.05, Fig. 3B). No significant difference was obtained between the acute and chronic groups.

**Fig. 1.** Mean± S.E.M. of bdnf mRNA (A) and Protein (B) Concentrations in the Hippocampus (a p<0.05 vs. the Sham Group)

**Fig. 2.** Mean± S.E.M. of trk- β mRNA (a p<0.05 vs. the Sham Group, A) and Protein (a p<0.001 and b p<0.05 vs. the Sham Group, c p<0.01 vs. Acute Group, B) Concentrations in the Hippocampus
Discussion

The present study demonstrated neurotrophins toxicity following MDMA treatment. First, we observed that MDMA treatment caused a reduction in BDNF, Trk-β, and NT4 expression in the rat hippocampus. Secondly, the rats that received acute treatment showed more reduction compared to the rats that received chronic treatment.

Consistent with our results, Braun et al. demonstrated that administration of methamphetamine as another derivative of amphetamine affects BDNF and Trkβ expression and increases BDNF mRNA expression in the hippocampus CA1, prefrontal cortex (PFC), and locus coeruleus. Moreover, in their study, Trkβ mRNA expression was modified in the hippocampus, PFC, and striatum (13).

Several studies have demonstrated that MDMA administration can modulate BDNF, however, less is known about the changes in the NT-4 and Trk-β expression. Schaaf et al. found that BDNF expression reduces after corticosterone administration in the hippocampus (14). Neurotrophins and their receptors modulation were obtained in several brain regions after MDMA treatment. It has been reported that neurotoxic agents such as chronic stress decrease BDNF level, but they increase NT-3 levels (15, 16).

Hemmerle et al. reported that BDNF mRNA expression was elevated in both frontal and parietal cortices following MDMA treatment at 1- and 7- hour time points (Hemmerle et al. 2012). About hippocampus, the CA1 region remained unchanged for 1 and 7 hours, but was significantly elevated after 24 hours. In contrast to the increase in BDNF expression in the cortical and CA1 regions after 24 hours, MDMA treatment caused a reduction in BDNF levels in the CA3 region at 1- and 7-hour intervals (12).

These results are inconsistent with those of the present study which revealed that MDMA administration reduces neurotrophins in hippocampus. However, the difference could be due to the fact that in the current study, the examination of MDMA neurotoxicity was performed at time points different from those of Hammerle et al. study. Moreover, the results of the present study showed neurotrophins expression in the entire hippocampus, while Hammerle et al. used in situ hybridization and assessed different regions of hippocampus. Martinez- Turrillas et al. observed an increase in bdnf mRNA expression in the frontal cortex 24 and 48 hours after a single dose of MDMA and a decrease in hippocampus 24 hours, 48 hours, and 7 days following MDMA administration (17), which is in line with the results of the present study. They suggested that neurotrophins were differentially regulated in several regions of the brain. In the present study, an increased expression of BDNF, NT-4, and Trk-β was observed in the hippocampus compared to the acute group. Martinez et al. also reported that BDNF expression increases 7 days after MDMA administration compared to 24 and 48 hours after treatment (17).

The mechanism by which BDNF, NT4, and their receptor expression decrease after MDMA treatment is not yet fully understood. Several lines of evidence have shown that MDMA treatment leads to neuronal degeneration and cell death in hippocampus (18, 19). Previous studies have shown that MDMA caused an increase in dead cells but a decrease in neuronal density in the CA1 hippocampus (18). It seems that reduced neurotrophins expression in the hippocampus is the result of neuronal loss. Moreover, it is clear that serotonergic nerve cells project to the hippocampus and that MDMA administration results in serotonin depletion in this area (20). Serotonin alteration in hippocampus is likely to play a role in the changes of neurotrophin expression. Neurotrophins and serotonin are involved in dendritic growth and synaptic plasticity that improve cognition, learning, and memory (21, 22). From another aspect, there is an association between corticosterone and neurotrophin expression. It has been revealed that corti-
corticosterone regulates the expression of BDNF and Trk-β RNA in rat hippocampus and that they are downregulated following corticosterone administration (15,16,23). It seems unlikely that a decrease in BDNF, NT4, and Trk-β expression 24 hours after MDMA administration would be the result of cell loss and serotonin depletion; however, it may be tied to the increase in corticosterone following MDMA administration.

Conclusion

MDMA modulates BDNF, NT4, and Trk-β expression in hippocampus. Moreover, the rats that received acute treatment expressed lower amount of neurotrophins compared to the rats receiving chronic treatment. Therefore, it seems that the brain has no opportunity to improve the MDMA-induced toxicity in the acute administration.

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Conflict of Interests

The authors declare that they have no competing interests.

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