Brief Communication

Neuroprotective Effects of Selegiline Agent Methamphetamine-Prompted Mood-Related Behavior Disorder Mediated Via 5-HT_2 and D_2 Receptors

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Abstract

Background: Many previous studies demonstrated that methamphetamine (METH) abuses can cause mood-related behavioral changes. Previous studies indicated neuroprotective effects of Selegiline. **Methods:** Seventy male Wistar rats were randomly divided into eight groups (10 rats in each group). Group 1 and Group 2 received normal saline and methamphetamine (10 mg/kg) for 21 days, respectively. Groups 3, 4, and 5 were treated simultaneously with methamphetamine and Selegiline with doses of 10, 15, and 20 mg/kg for 21 days. Groups 6 and 7 are methamphetamine-dependent groups which received 15 mg/kg of Selegiline with haloperidol (as D_2 receptor antagonist) and trazodone (as 5-HT_2 receptor antagonist) for 21 days, respectively. In days 23 and 24, elevated plus maze (EPM) and open-field test (OFT) were conducted to assess motor activity and mood (anxiety and depression) levels. **Results:** METH as 10 mg/kg causes reduction of rearing number, ambulation distances, time spent in central square and also number of central square entries in OFT. Also METH administration causes decreases of time spent in open arm and number of open arm entries and increases of time spent in closed arm and number of closed arm entries in EPM. In contrast, Selegiline (of 10, 15, and 20 mg/kg) inhibited behavioral effects of methamphetamine in both OFT and EPM. Also administration of haloperidol and trazodone inhibited these behavioral protective effects of Selegiline and caused decrease of OFT behaviors (rearing number, ambulation distances, time spent in central square, and also number of central square entries) and also caused decreases of spend times in open arm, number of open arm entries, and also increased closed arm time spending and number of entries in closed arm in EPM. **Conclusions:** Current research showed that Selegiline via mediation of D2 and 5-HT_2 receptors inhibits METH-induced neurobehavioral changes, mood-related behavior, and motor activity disturbances.

Keywords: *D2 and 5-HT_2 receptors, methamphetamine, neurobehavioral, Selegiline*

Introduction

Methamphetamine (METH) abuses as central nervous system stimulant was increased in recent years.[1] The main mechanism and involved neurotransmitter and receptors which involved in negative neurochemical and behavioral impacts of METH were not clarified exactly.[2-4] Many previous studies demonstrated that abrupt cessation of methamphetamine can cause some mood- and motor activity-related behavior disturbances.[5,6] In recent year's attempts, the introduction of new neuroprotective and neuromodulator agent for management of METH-induced mood and motor activity disturbances was increased.[2-4,7,8] Selegiline is an irreversible selective MAO-B inhibitor, which is used for management of Parkinson's disease.[9] Studies also demonstrated that Selegiline may be a valuable anxiolytic and antidepressant agent in circumstances where mood-related behavior disorder and motor activity occur.[9,10] On the other way, it was demonstrated that many neurotransmitters, neuromodulators, and their receptors can affect mood- and motor activity-related behaviors during METH abuse syndrome.[11,12] Also it was demonstrated that mentioned receptors and neurotransmitter can modulate anxiolytic and antidepressant effects of agents such as Selegiline.[12,13] Some previous studies have shown that dopamine D2 and serotonin 5-HT2 receptor and their downstream signaling pathways can mediate mood- and motor activity-related performances.[14-17] Due to the significance of the role of dopamine D2 and serotonin 5-HT2 receptor in mediation of motor activity and mood-related performances, current research was considered to evaluate

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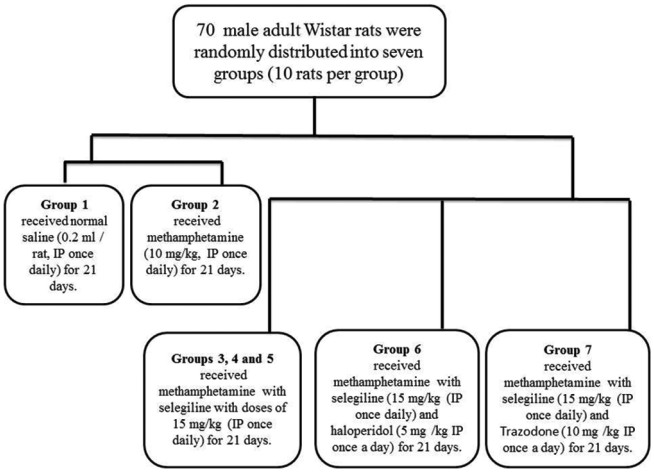
the role of these two receptors on protective effects of Selegiline in management of methamphetamine prompted mood and motor activity disorder. Results of current project can help to better understanding of the protective effects of Selegiline and METH pharmacodynamics mechanism.

Methods

Animals

Seventy male adult Wistar rats, average weighing 250 g, have located in animal house standard situation with room temperature of 22 ± 0.5°C, and light/dark cycle was 12 hr with free access to food and water. The investigational procedure of this research was approved by research committee at Qom branch of Islamic Azad University (Research Protocol and ethical code number = IR.IAU.QOM.REC.1399.027).

Experimental development



In days 23 and 24, open-field test (OFT) and EPM were conducted to assess motor activity and mood (anxiety and depression) levels. It has to be noted that doses of METH, Selegiline, trazodone, and haloperidol were selected from previous similar works.[2-4,18,19] Also administration of each METH and Selegiline (in Groups 3, 4, and 5) was with 1-hour interval, Selegiline was administered first. Also administration of each antagonist was done 1 hour before administration of Selegiline in Groups 6 and 7.

Behavioral studies

Open-field test (OFT)

The open-field device was used for evaluation of anxiety and motor activity disorder, and protocol of this test was done according to previous standard protocol studies.[20-22] According to this study, four standard behaviors were evaluated in this test:

1. Line crossing (ambulation) distance: distance of the rat passing through the grid lines.
2. Center square entries: Frequency that the rat crossed one of the red lines with all four paws in the main square.
3. Center square duration: the length of time the rat spent on the main square.
4. Rearing number: the frequency with which the rat shows a strange behavior.[20-22]

Elevated plus maze (EPM)

EPM is a commonly deployed test for the evaluation of anxiety-related behavior in rodents. Protocol of this test was done according to previous standard protocol studies. The tendency to be in the closed arms over the open arms has been representative of anxiety-like actions, while tendency to be in the open arms over the closed arms has been representative of anxiety-like actions.[23,24]

Analysis of statistics

All data were collected and analyzed by GraphPad PRISM v. 6 Software. Mean ± standard error (SEM) for each behavioral parameter was calculated. The significant differences between control and therapy groups were assessed by ANOVA and *Tukey's post-test,* and significant level ($P < 0.001$) or ($P < 0.05$) was considered for remarkable differences.

Results

Results of OFT behavior

As indicated in Table 1, administration of methamphetamine (10 mg/kg) caused decrease of rearing number, ambulation distances, central square entries, and time spent in the core area in OFT when compared to sham group ($P < 0.05$) [Table 1]. In contrast, Selegiline inhibited this effect of methamphetamine and significantly increased rearing number, ambulation distances, central square entries, and time spent in the core area when compared to METH treatment group ($P < 0.05$) [Table 1]. Pretreatment with trazodone (as 5-HT_2 receptor antagonist) and haloperidol (as D_2 receptor antagonist) inhibited mentioned protective effects of Selegiline and caused decreases of rearing number, ambulation distances, central square entries, and time spent in the core area when compared to Selegiline (15 mg/kg) in combination with methamphetamine treatment group ($P < 0.05$) [Table 1].

Results of EPM behavior

Rats in sham group spent more time in the open arms of EPM and showed increase of number of entrances to open arm of mentioned test; also sham group spent less time in the open arms of EPM and showed decreases in number of entrances in closed arm of EPM when compared to METH-treated rats ($P < 0.05$) [Table 2]. While Selegiline treatment significantly increased the existence and time spent of animals in the open arms of EPM and also significantly reduced the time spent of animals in the closed arms of EPM and caused decreases of entrances to closed arm ($P < 0.05$) [Table 2]. Pretreatment with trazodone (5-HT_2 receptor antagonist) and haloperidol

Table 1: Effects of 5-HT2 and D2 receptor selegiline protective effects on open field exploratory and anxiety like behavior in rats treated by 10mg/kg of methamphetamine

Group	Ambulation distance (cm)	Central square entries (number)	Time spent in central square (sec)	Number of rearing
Control	383±14	19±1.9	135±10	21±2
METH (10 mg/kg)	310±12[a]	6±2[a]	79±14[a]	8±2[a]
METH (10 mg/kg) + Selegiline(5 mg/kg)	362±30[b]	12±2[b]	95±14[b]	12±0.9[a]
METH (10 mg/kg) + Selegiline(10 mg/kg)	371±40[b]	15±1b	111±12b	14.9±3[a]
METH (15 mg/kg) + Selegiline(15 mg/kg)	381±26[b]	18±2	121±7[b]	17.6±4[b]
METH (10 mg/kg) + Selegiline(15 mg/kg) + Haloperidol(5 mg/kg)	319±23[c]	8±2[c]	86±11[b]	10±3[c]
METH (10 mg/kg) + Selegiline(15 mg/kg) + Trazodone (10 mg/kg)	323±29[c]	9±3[c]	95±14[c]	9±1[c]

[a]Indicates statistical significance vs. control group (P<0.05). [b]Indicates statistical significance vs. methamphetamine group (10mg/kg) (P<0.05). [c]Indicates statistical significance vs. 10mg/kg of methamphetamine in combination with 15 mg/kg of Selegiline (P<0.05). All data are expressed as Mean±SEM (n=10). METH: methamphetamine

Table 2: Effects of 5-HT2 and D2 receptor Selegiline protective effects on elevated plus maze anxiety and depression like behavior in rats treated by 10mg/kg of methamphetamine

Group	Time spent in open arms (seconds)	Time spent in closed arms (seconds)	Open arm entries (Number)	Closed arm entries (Number)
Control	183±14	135±14	21±2	7±1
METH (10 mg/kg)	118±10[a]	199±19[a]	8±1[a]	18±2[a]
METH (10 mg/kg) + Selegiline(5 mg/kg)	156±11[b]	151±16[b]	12±1[b]	14±1[a]
METH (10 mg/kg) + Selegiline(10 mg/kg)	168±12[b]	149±15[b]	14±2[b]	11±3[b]
METH (15 mg/kg) + Selegiline(15 mg/kg)	178±19[b]	120±14[b]	16±3[b]	8±2[b]
METH (10 mg/kg) + Selegiline(15 mg/kg) + Haloperidol(5 mg/kg)	129±19[c]	182±18[c]	10±3[c]	16±2[b]
METH (10 mg/kg) + Selegiline(15 mg/kg) + Trazodone (10 mg/kg)	138±29[c]	191±21[c]	11±2[c]	15±3[c]

[a]Indicates statistical significance vs. control group (P<0.05). [b]Indicates statistical significance vs. methamphetamine group (10 mg/kg) (P<0.05). [c]Indicates statistical significance vs. 10mg/kg of methamphetamine in combination with 15 mg/kg of Selegiline (P<0.05). All data are expressed as Mean±SEM (n=10). METH: methamphetamine

(as D_2 receptor antagonist) inhibited mentioned protective effects of Selegiline and caused decreases of time spent in open arm and also reduction of number of entrance in open arm and also causes increase of time spent in closed arm and increases of entrance to closed arm (P < 0.05) [Table 2].

Discussion

Present study demonstrated that that Selegiline with multiple doses can alter METH-induced neurobehavioral changes. Also according to current study, result shows that Selegiline by mediation of -HT_2 and D_2 receptor exerts its protective effects against METH-prompted neurobehavioral sequels. Results of the current work showed that METH (10 mg/kg) decreases ambulation distances, rearing number and also reduced entry and time spent in the central square of OFT. Data showed that the amount of methamphetamine used in this research could lead to disruption in motor operation. METH is a psychostimulant that its abuse was increased during recent years.[1] The exact mechanism of action this agent and also involved receptor which caused mediation of its neurobehavioral effects was not fully clarified.[25,26] Current research results have shown that methamphetamine 10 mg/kg can decrease rearing number, ambulation distances, central square entries, and time spent in the core area in OFT when compared to sham group. These data confirm previous results which indicated that METH abuses can cause mood (anxiety and depression) and motor activity disorder.[2-4,7,8,27] On the other way, Selegiline in used doses (5, 10, and 15 mg/kg) may reduce METH-induced neurobehavioral changes and increased rearing number, ambulation distances, central square entries, and time spent in the core area in OFT. Similar to previous research indicates Selegiline can change anxiety and depression in standard test such as OFT conduct in rats and modulate drug abuse-induced motor activity disturbance.[28-30] Also our data indicate that pre-treatment with trazodone (as 5-HT_2 receptor antagonist agent) and haloperidol (as D_2 receptor antagonist agent) prohibit mentioned protecting effects of Selegiline and caused decreases of rearing number, ambulation distances, central square entries, and time spent in the central core parts of OFT when compared to groups under retreatment with Selegiline (15 mg/kg) with methamphetamine treatment group. Numerous basic researches have shown that critical neurotransmitters such as serotine, norepinephrine, and dopamine play a strategic role in the expression of mood and motor exercise.[31-33] On the other way, these data can be discussed by basic concept that probably

Selegiline by modulation of 5-HT_2 and D_2 receptor exerts in neuroprotective against METH-induced behavioral disorders.[31-33] Based on this study and previous data, D_2 and 5-HT_2 receptors might be involved in brain pathways for management of depressive, anxiety, and motor activity disorder. Previous indirect evidences have shown that D_2 and 5-HT_2 receptors can modulate mood-related behavior during drug abuses, and these receptors play important roles in neuronal circuit of mood- and motor activity-related activity.[14-17]

In other parts in another part our data have shown that METH administration as 10 mg/kg can cause decrease of time of spent and number of entrances to open arm in EPM. Also METH administration increased time spent and number of entrances in closed arm in EPM. Long-term administration or abuse of METH impacts on function of amine-based neurotransmitters and by induction of dysfunction in mentioned neurotransmitter which causes express of mood-related disorders such as anxiety and depressive behavior; this concept confirms our results about role of METH-induced mood changes.[6,34] In contrast, Selegiline as 5, 10, and 15 mg/kg reduces this form of METH-induced behavioral disturbances in EPM. Selegiline possesses anti-depressant impact and can regulate depressive and anxiety by modulation of dopamine and serotonin function and probably can compensate METH-induced neurotransmitter depletion.[28,30,35] In consistent with this data, current research also indicates that pretreatment with trazodone (as 5-HT_2 receptor antagonist) and haloperidol (as D_2 receptor antagonist) can prohibit mentioned behavioral protective effects of Selegiline and causes decrease of time of spent in open arms and reduced number of entrances in open arm in EPM, METH also increased time spent and number of entrances in closed arm in EPM. In compatible with our data, many past researches have shown that Selegiline has important effects on brain neurotransmitter, and probably, its effects were modulated by multiple mentioned neurotransmitter receptors. These data can be discussed with previous concepts which confirmed the involvement of D_2 and 5-HT_2 receptors in the formation of brain pathways which occurred after uses of Selegiline especially management of mood and motor activity.[31-33]

Conclusions

According to current research results, D_2 or 5-HT_2 receptors could be involved in Selegiline behavioral protective impacts counter to METH-prompted anxiety, depression, and mood-related behavioral changes. For more approval of these effects, exact molecular, cellular, and involved signaling evaluation is needed for assessment.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Chomchai C, Chomchai S, Global patterns of methamphetamine use. Curr Opin Psychiatry 2015;28:269-74.
2. Keshavarzi S, Kermanshahi S, Karami L, Motaghinejad M, Motevalian M, Sadr S. Protective role of metformin against methamphetamine induced anxiety, depression, cognition impairment and neurodegeneration in rat: The role of CREB/BDNF and Akt/GSK3 signaling pathways. Neurotoxicology 2019;72:74-84.
3. Mozaffari S, Yasuj SR, Motaghinejad M, Motevalian M, Kheiri R. Crocin acting as a neuroprotective agent against methamphetamine-induced neurodegeneration via CREB-BDNF signaling pathway. Iran J Pharm Res 2019;18:745-58.
4. Mehrafza S, Kermanshahi S, Mostafidi S, Motaghinejad M, Motevalian M, Fatima S. Pharmacological evidence for lithium-induced neuroprotection against methamphetamine-induced neurodegeneration via Akt-1/GSK3 and CREB-BDNF signaling pathways. Iran J Basic Med Sci 2019;22:856-65.
5. Borumand MR, Motaghinejad M, Motevalian M, Gholami M. Duloxetine by modulating the Akt/GSK3 signaling pathways has neuroprotective effects against methamphetamine-induced neurodegeneration and cognition impairment in rats. Iran J Med Sci 2019;44:146-54.
6. Harro J. Neuropsychiatric adverse effects of amphetamine and methamphetamine. Int Rev Neurobiol 2015;120:179-204.
7. Alam-mehrjerdi Z, Mokri A, Dolan K. Methamphetamine use and treatment in Iran: A systematic review from the most populated Persian Gulf country. Asian J Psychiatry 2015;16:17-25.
8. Majdi F, Taheri F, Salehi P, Motaghinejad M, Safari S. Cannabinoids Δ9-tetrahydrocannabinol and cannabidiol may be effective against methamphetamine induced mitochondrial dysfunction and inflammation by modulation of Toll-like type-4 (Toll-like 4) receptors and NF-κB signaling. Med Hypotheses 2019;133:109371.
9. Tábi T, Vécsei L, Youdim MB, Riederer P, Szökő É. Selegiline: A molecule with innovative potential. J Neural Trans 2020;127:831-42.
10. Amini-Khoei H, Saghaei E, Mobini G-R, Sabzevary-Ghahfarokhi M, Ahmadi R, Bagheri N, *et al*. Possible involvement of PI3K/AKT/mTOR signaling pathway in the protective effect of selegiline (deprenyl) against memory impairment following ischemia reperfusion in rat. Neuropeptides 2019;77:101942.
11. Martinez D, Narendran R. Imaging neurotransmitter release by drugs of abuse. Curr Top Behav Neurosci 2010;3:219-45.
12. Chiu VM, Schenk JO, Mechanism of action of methamphetamine within the catecholamine and serotonin areas of the central nervous system. Curr Drug Abuse Rev 2012;5:227-42.
13. Büttner A. The neuropathology of drug abuse. Neuropathol Appl Neurobiol 2011;37:118-34.
14. Granado N, Ares-Santos S, Oliva I, O'Shea E, Martin ED, Colado MI, *et al*. Dopamine D2-receptor knockout mice are protected against dopaminergic neurotoxicity induced by

methamphetamine or MDMA. Neurobiol Dis 2011;42:391-403.
15. Hadlock GC, Chu PW, Walters ET, Hanson GR, Fleckenstein AE. Methamphetamine-induced dopamine transporter complex formation and dopaminergic deficits: The role of D2 receptor activation. J Pharmacol Exp Ther 2010;335:207-12.
16. Doyle JR, Yamamoto BK. Serotonin 2 receptor modulation of hyperthermia, corticosterone, and hippocampal serotonin depletions following serial exposure to chronic stress and methamphetamine. Psychoneuroendocrinology 2010;35:629-33.
17. van Wel JH, Kuypers KPC, Theunissen EL, Bosker WM, Bakker K, Ramaekers JG. Effects of acute MDMA intoxication on mood and impulsivity: Role of the 5-HT2 and 5-HT1 receptors. PLoS One 2012;7:e40187.
18. Wahdan SA, Tadros MG, Khalifa AE. Antioxidant and antiapoptotic actions of selegiline protect against 3-NP-induced neurotoxicity in rats. Naunyn Schmiedeberg's Arch Pharmacol 2017;390:905-17.
19. Onur MA, SEKKİN S. The neuroprotective effect of selegiline in streptozotocin induced diabetic rats. J Cell Neurosci Oxid Stress 2018;10:739-40.
20. Gould TD, Dao DT, Kovacsics CE. The Open Field test, in Mood and Anxiety Related Phenotypes in Mice. Springer; 2009. p. 1-20.
21. File SE, Lippa AS, Beer B, Lippa MT. Animal tests of anxiety. Curr Protoc Neurosci 2004; Chapter 8: Unit 8.3. doi: 10.1002/0471142301.ns0803s26.
22. Ghafarimoghadam M, Mashayekh R, Gholami M, Fereydani P, Shelley-Tremblay J, Kandezi N, *et al*. A review of behavioral methods for the evaluation of cognitive performance in animal models: Current techniques and links to human cognition. Physiol Behav 2022;244:113652.
23. Castagné V, *et al*. Rodent models of depression: Forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Pharmacol 2010; Chapter 5: Unit 5.8. doi: 10.1002/0471141755.ph 0508s49.
24. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2007;2:322.
25. Xie Z, Miller GM. A receptor mechanism for methamphetamine action in dopamine transporter regulation in brain. J Pharmacol Exp Ther 2009;330:316-25.
26. NOVÁKOVÁ, Jana. Pharmacological manipulation with 5-HT2 receptor subtype affects methamphetamine i.v. self-administration in rats. In Abstract Book of Sixth IBRO World Congress of Neuroscience. Prague (Czech Republic): Neuveden, 2003. s. 205-205. ISBN 80-239-0887-1.
27. Fonseca R, Carvalho RA, Lemos C, Sequeira AC, Pita IR, Carvalho F, *et al*. Methamphetamine induces anhedonic-like behavior and impairs frontal cortical energetics in mice. CNS Neurosci Ther 2017;23:119-26.
28. Moore JJ, Saadabadi A. Selegiline. Stat Pearls; 2020.
29. Amiri S, Amini-Khoei H, Mohammadi-Asl A, Alijanpour S, Haj-Mirzaian A, Rahimi-Balaei M, *et al*. Involvement of D1 and D2 dopamine receptors in the antidepressant-like effects of selegiline in maternal separation model of mouse. Physiol Behav 2016;163:107-14.
30. Ishikawa T, Okano M, Minami A, Tsunekawa H, Satoyoshi H, Tsukamoto Y, *et al*. Selegiline ameliorates depression-like behaviors in rodents and modulates hippocampal dopaminergic transmission and synaptic plasticity. Behav Brain Res 2019;359:353-61.
31. Ayano G. Dopamine: Receptors, functions, synthesis, pathways, locations and mental disorders: Review of literatures. J Ment Disord Treat 2016;2:2.
32. Zarrindast M-R, Khakpai F. The modulatory role of dopamine in anxiety-like behavior. Arch Iran Med 2015;18:591-603.
33. Healy D. Serotonin and depression. BMJ 2015;350:h1771.
34. Paulus MP, Stewart JL. Neurobiology, clinical presentation, and treatment of methamphetamine use disorder: A review. JAMA Psychiatry 2020;77:959-66.
35. Magyar K. The pharmacology of selegiline. Int Rev Neurobiol 2011;100:65-84.