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Original Article

Asymmetric Involvement of Central and the Peripheral NMDA Glutamate Receptors in the Expression of Withdrawal Syndrome in Morphine-Dependent Mice

Mahboubeh Kamali[1], Hedayat Sahraei[2], Maryam Khosravi[1], Shahin Hassanpour[3], Habib Yaribeygi[2]*

1. Department of Biology, Islamic Azad University, North Tehran Branch, Tehran, Iran
2. Neurosciences Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Section of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract

Introduction: Morphine withdrawal syndrome is mediated via several central and peripheral neurological pathways. In the present study we investigated the role of N-methyl-D aspartic acid (NMDA) glutamate receptor on naloxone-induced withdrawal syndrome in morphine-conditioned mice.

Materials and Methods: We designed two separate experiments. In experiment one, 30 male NMRI mice were divided into 5 groups, pretreated with memantine (0.1, 1 and 5 mg/kg; I.P.) followed by morphine-dependence period for 3 days. In the other experiment, 48 male NMRI mice distributed into 8 groups, pretreated with intra-accumbens (IAc) memantine (1 and 5 µg/animal) within the right, left and both side of nucleus accumbens (RNAcc, LNAcc and BNAcc) followed by I.P. morphine-dependence (3 days). On day 4, in both experiments, morphine was injected into mice, followed by naloxone. Then naloxone-induced total jumping count, jump height and defecation in morphine-conditioned mice were recorded for 30 min.

Results: Pre-treatment by I.P. injection of memantine significantly attenuated naloxone precipitated jumping count/30 min, jumping height (mm) and fecal material output in morphine dependent mice ($P<0.05$). Also, IAC pretreatment with memantine in LNAcc, RNAcc and BNAcc significantly declined the effect of I.P. injection of naloxone on total jumping count and jumping height ($P<0.05$), pretreatment within memantine in LNAcc, RNAcc and BNAcc had no effect on defecation ($P>0.05$).

Conclusion: These findings indicated asymmetric involvement of central and peripheral NMDA glutamate receptors in withdrawal syndrome development in morphine-dependent mice.

Keywords:
Memantine;
NMDA glutamate receptors;
Morphine withdrawal syndrome;
Mice

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***Correspondence to:**
H. Yaribeygi

Tel: +9821 26127286

Email:
habib.yari@yahoo.com

Introduction

Opioids are predominantly used for pain relief and are commonly utilized as psychoactive agent (Rehni et al., 2012). Chronic administration of opiates such as morphine can lead to physical and psychological dependence such as withdrawal syndrome which occurs after cessation of the drug therapyor when an opioid antagonist is administered (Fichna et al., 2007). Sometimes withdrawal syndrome becomes a

life-threatening clinical condition (Sekiya et al., 2004). Animal model studies indicated that daily injection of morphine decreases locomotor activity (Dehghani et al., 2013). Opioid withdrawal syndrome has severe side effects such as abnormal posture, anxiety, diarrhea and hypothermia (Fichna et al., 2007) and no effective drug has been developed to this day to relieve opioid withdrawal syndrome treatment (Kotlińska, 2001).

It is well-documented that mu, kappa and delta opioid receptors are responsible for development of morphine addiction (Karimi et al., 2011). However, investigations suggest that several other neurotransmitters, e.g. dopamine, glutamate and Gama-amino-Butyric-acid (GABA) play central roles in withdrawal syndrome in morphine-dependent subjects as well. N-methyl-D aspartic acid (NMDA) glutamate receptor has been indicated to attenuate morphine withdrawal signs (Sekiya et al., 2004; Benturquia et al., 2007). NMDA glutamate receptors has been identified in peripheral as well as and central nervous system (PNS and CNS) including in ventral tegmental area (VTA), prefrontal cortex (PFC) and nucleus accumbens (NAc) (McRoberts et al., 2001). Moreover, morphine elicits its reinforcing properties at the level of VTA and NAc through projections originating from the PFC (Kotlińska, 2001); Do Couto et al., 2004). This wide neural network within the CNS suggests that the glutamatergic system might play an important role in opiate-induced withdrawal syndrome (Maldonado et al., 2003).

It has been reported that there is an asymmetry between right and left sides of the NAc in response to neurotransmitter/neuropeptide systems. For instance, IAC injection of cholecystokinin-8 (CCK-8) into the right NAc increased dopaminergic system activity compared to injection into the left NAc (Esmaeili et al., 2012). Chronic administration of morphine can lead to withdrawal syndrome; however, neurochemical, behavioral and clinical effects of NMDA receptors induced in RNAc and LNAc are not completely elucidated. The hypothesis of current study was to elucidate the role of central and peripheral NMDA glutamate receptors on naloxone-induced withdrawal syndrome.

Therefore. we designed this study to introduce a new pharmacological agent for managing withdrawal syndrome as a highly prevalent behavior among addicts. In this study we determined the effects of intraperitoneal (I.P.) and IAC injections of memantine (a non-competitive NMDA receptor antagonist) on naloxone-induced behaviors in morphine- dependent male mice. Moreover, to investigation the possible asymmetry between NMDA glutamate receptors in LNAc and RNAc, effects of IAC injection of memantine in both of LNAc and RNAc were evaluated.

Materials and methods

Animals

Male NMRI mice (Weight: 30±2 g) were purchased from Razi Vaccine and Serum Research Institute, Alborz, Karaj, Iran. The animals were housed in groups of six per cage under an ambient temperature of 22±1 ˚C and 12/12 h light/dark cycle with *ad libitum* access to food and water. The animals were randomly allocated to different experimental groups and used once in each experiment. Animals were housed under standard laboratory conditions in accordance with Baqiyatallah University of Medical Sciences, Community for Laboratory Animal Care and Use.

Experimental Drugs

Experimental drugs included Morphine Sulphate (narcotic opioid analgesic; TEMAD Co., Temad, Iran), Naloxone hydrochloride (opioid receptor antagonist; Sigma®, USA) and Memantine (a non-competitive NMDA receptor antagonist; Sigma®, USA) Morphine and naloxone hydrochloride were dissolved in sterile pyrogen-free 0.9% NaCl solution (saline). Also to induce anesthesia; ketamine chloride and diazepam chloride were both applied. The selection of morphine, memantine and naloxone doses were determined based on the pilot and previous studies (Popik et al., 1998; Do Couto et al., 2004; Wang et al., 2005; Esmaeili et al., 2012).

Morphine addiction procedure

Two experiments designed to investigate the effects of central and peripheral memantine on naloxone-induced withdrawal syndrome in morphine-dependent mice. Experiment 1 was designed to investigate the role of peripheral NMDA glutamate receptors on naloxone-precipitated withdrawal syndrome in

morphine-conditioned mice whereas in experiment 2, the effects of central NMDA glutamate receptors (within the NAcc) were examined. In this study, a 3 day morphine addiction period was applied to all mice using the method previously described by Marshall and Graham-Smith (MARSHALL and Grahame-Smith, 1971). Before to the study, animals were kept for 3 days in the laboratory to adapt to the new environment. Then all animals were weighted and pretreated with memantine followed by morphine (except saline treated groups) during the course of the 3 days. On Day 1, animals were pre-treated with different doses of memantine based on the group type (0.1, 1 and 5 mg/kg for I.P. injected groups or 1 and 5 µg/kg for IAC injection followed by morphine (SC; 50, 50 and 75 mg/kg) at 8 A.M, 12 A.M and 16 P.M, respectively. The following day Mice were pretreated with memantine followed by SC morphine injection at doses of 75, 75 and 100 mg/kg at 8 A.M, 12 and 16 P.M, respectively. On day 3, animals received SC injections of morphine (100, 100 and 125 mg/kg) following memantine administration at 8 A.M, 12 and 16 P.M, respectively.

The experimental procedure

In experiment 1, thirty male NMRI mice randomly allocated into 5 experimental groups (n=6). Group 1 served as control and received I.P. injection of 0.1 mg/kg saline three times daily at 8 A.M, 12 and 16 P.M (2 injections at the time with 30 min interval). Group 2, I.P. injection of saline (0.1 mg/kg) followed by S.C. injection of morphine at 8 A.M, 12 and 16 P.M. The interval between saline and morphine injection was 30 min. Groups 3, 4 and 5 followed the similar procedure except those mice received 0.1, 1 and 5 mg/kg memantine (I.P.) instead of saline. All groups received injections for a period of 3 continuous days. Then, Naloxone (2 mg/kg, I.P.) was injected in the morning of day 4, 120 min after administering morphine (50 mg/kg, SC). Afterwards, naloxone-induced withdrawal syndrome which included total jumping count, jump height (mm) and fecal materials (mg) in morphine-dependent mice were monitored for 30 min. To condition mice to the injection process and minor palpations a5-day recovery period was considered during which mice were moved and injected daily.

Surgical procedure

In experiment two, for implantation of the IAC cannula, the animals were anesthetized by ketamine hydrochloride (50 mg/kg) and diazepam hydrochloride (5 mg/kg) and a stainless steel (23-gauge thin-walled) guide cannula (Razipakhsh, Iran) was stereotaxically implanted into the right, left or both sides of NAcc. The coordinates for stereotaxic surgery was selected according to Paxinos and Watson atlas (AP= 0 mm, ML= 1.2 mm, DV= 4.5 mm) (Paxinos, 2007). Experimental injections were applied by a thin-walled (30-gauge) stainless steel injecting cannula, (Razipakhsh co., Iran) which extend 1.0 mm beyond the guide cannula. A 60 cm long polyethylene-20 tubing (Parsian tube, Iran) attached the injecting cannula to a 10-µl Hamilton syringe. The solutions mentioned above prior were injected over a period of 60 sec. Furthermore, the same time interval (60 sec) was allowed to diffuse the solution from the tip of the cannula into the nucleus. Total volume of injected drug into the nucleuses were 0.5 µl/mice. After surgery, animals were transferred to individual corresponding cages. To adapt mice to injection process and lower surgical stress, 7-day recovery period was given.

In experiment two, 48 male NMRI mice randomly allocated into 8 experimental groups (n=6). Similar to experiment one, morphine addiction period (50-125 mg/kg for 3 days) was applied to the mice in experiment 2. All injections were similar to the experiment 1 except that animals received IAC injections of memantine instead of I.P. injections. Group 1, served as control and received I.P. injection of saline (0.1 mg/kg) at 8 A.M, 12 and 16 P.M, respectively. In third injection mice received IAC injection of 1 µL/kg saline 30 min prior to I.P. administration of saline. Group 2, was intraperitoneally treated with saline (0.1 mg/kg) followed by SC injection of morphine at 8 A.M and 12 P.M. In the final injection (at 16 P.M), mice were IAC injected with 1 µL/kg saline followed by SC injection of morphine. Group 3, received morphine in first two injections (at 8 A.M and 12 P.M). Then, in third injection mice IAC injected with memantine (1 µg/mouse in 2.5 µL) within the right NAcc (RNAcc) followed by SC injection of morphine. In group 4, mice were SC injected with morphine in 2 first injections at 8 A.M and 12 P.M while in the last injection at 16 P.M, IAC injected with memantine. (1

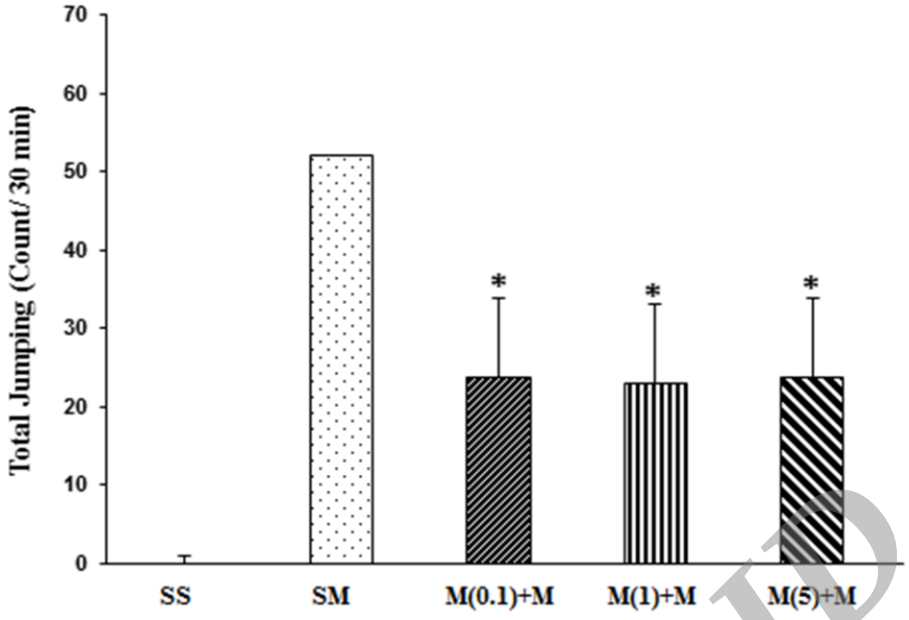

Fig.1. Effect of intraperitoneal (I.P.) injection of Memantine (0.1, 1 and 5 mg/kg) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced total jumping (count per 30 min) in morphine-dependent mice. n=6 per group. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group (P<0.05). (*Groups*: SS=saline+saline, SM= saline+morphine, M(0.1)+M= memantine (0.1 mg/kg)+morphine, M(1)+M= memantine (1mg/kg)+morphine, M(5)+M= memantine (5mg/kg)+morphine).

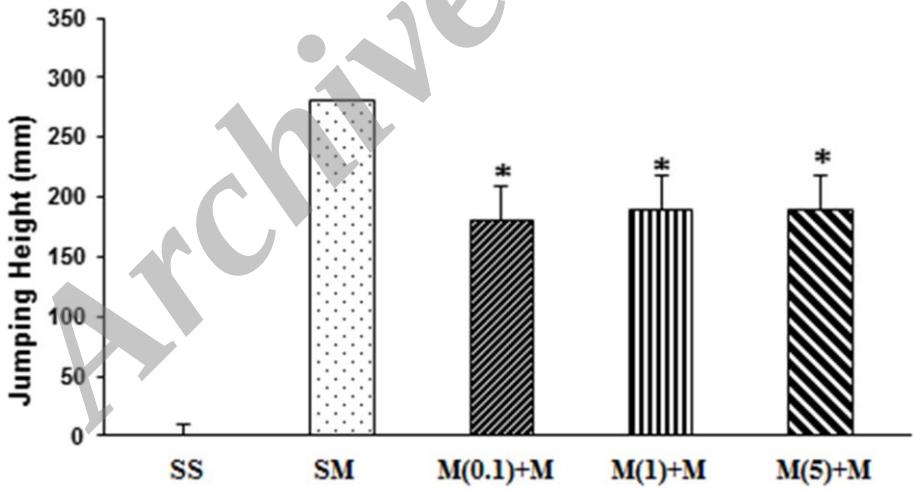

Fig.2. Effect of intraperitoneal (I.P.) injection of Memantine (0.1, 1 and 5 mg/kg) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced jump height (mm) in morphine-dependent mice. n=6 per group. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group (P<0.05). (SS=saline+saline, SM=saline+morphine, M (0.1) + M=memantine (0.1 mg/kg)+ morphine, M(1)+M= memantine(1mg/kg)+morphine, M(5)+M= memantine (5mg/kg)+morphine).

µg/mouse in 2.5 µL) within the left NAcc (LNAcc) followed by morphine injection (SC). In group 5, animals treated using SC injection of morphine at 8 A.M and 12 P.M. Then, in the third injection (at 16 P.M) mice were IAC injected using 1 µg/mouse of memantine in 2.5 µL within the both side of NAcc (BNAcc) prior to SC injection of morphine. In all groups, in the third injection, the interval between IAC and SC injections was 30 min. Mice in groups 6, 7 and 8 followed the procedure similar to the 3, 4 and 5 except those mice received 5 µg/mouse of memantine in 2.5 µL instead of 1 µg/mouse. All

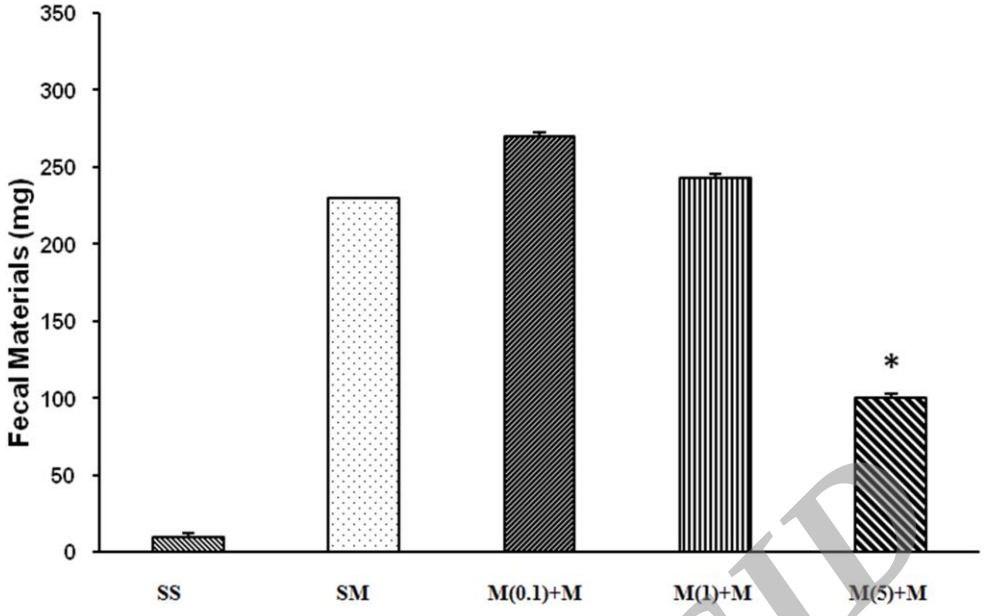

Fig.3. Effect of intraperitoneal (I.P.) injection of Memantine (0.1, 1 and 5 mg/kg) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced fecal materials (mg) in morphine-dependent mice. n=6 per group. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group (P<0.05). (SS= saline+saline, SM= saline+morphine, M(0.1)+M=memantine (0.1mg/kg)+morphine, M(1)+M= memantine(1mg/kg)+morphine, M(5)+M= memantine(5mg/kg)+morphine).

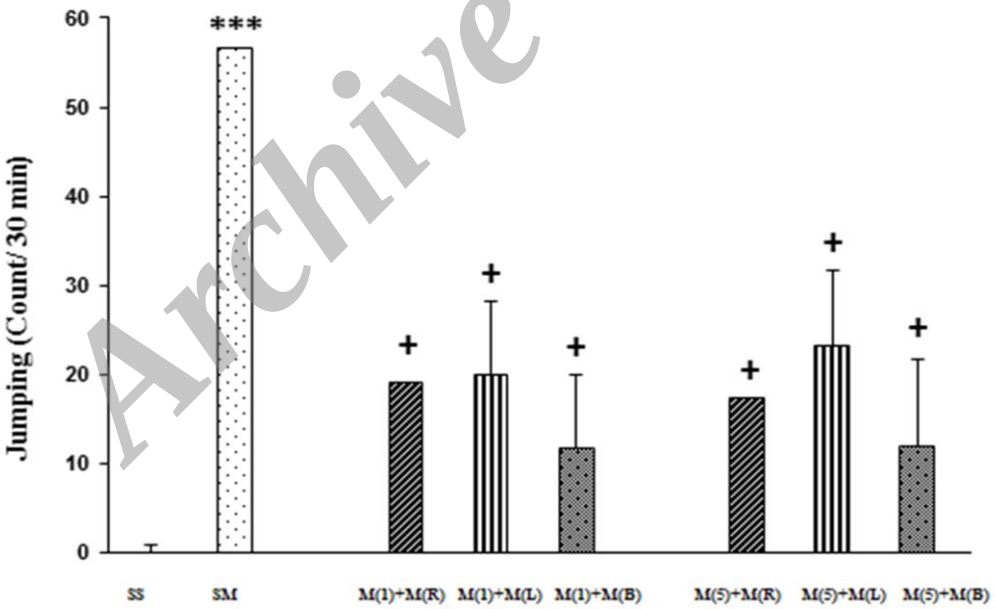

Fig.4. Effect of intra-accumbens (IAC) injection of Memantine (1 and 5 µg/mouse) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced total jumping (count per 30 min) in morphine-dependent mice. n=6 per group. RNAcc: right nucleus accumbens, LNAcc: left nucleus accumbens, BNAcc: both side of nucleus accumbens. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group and pluses indicate on significance to morphine (SM) group (P<0.05). (groups: SS= saline+saline, SM=saline+morphine, M(1)+M(R)= memantine (1µg/mice)+morphine(RNA cc), M(1)+M(L)= memantine (1µg/mice)+morphine(LNA cc), M(1)+M(B)= memantine (1µg/mice)+morphine(BNA cc), M(5)+M(R)= memantine (5µg/mice)+morphine(RNA cc), M(5)+M(L)= memantine (5µg/mice)+morphine (LNA cc), M(5)+M(B)= memantine (5µg/mice)+morphine(BNA cc)).

animals treated with this timetable for 3 consecutive days. On day 4, all mice subcutaneously injected with 50 mg/kg of morphine followed by I.P. administration of 2 mg/kg naloxone. The interval between morphine and naloxone injection was 120 min. Then, total jumping count, jump height (mm) and fecal materials (mg) in morphine-dependent mice monitored for 30 min.

During the experiments, each mouse was used once. Mice were sacrificed painlessly afterwards in accordance to mentioned guidelines. The direct placement of the guide cannula into the NAcc was confirmed via the injection of methylene blue onto of cerebrospinal fluid (CSF) and IAC injection of methylene blue followed by slicing the frozen brain tissue at the end of each experiment.

Statistical analysis

Data was analyzed by using one way analysis of variances (ANOVA) followed by Tukey-Kramer multiple comparison test using SPSS 16.0 for Windows and is presented as mean ± SEM. $P<0.05$ was considered as significant

Results

Effect of I.P. injection of memantine on naloxone-precipitated total jumping count in morphine-dependent mice is presented in table 1. According to the data, injection of naloxone (opioid receptor antagonist) on day 4, significantly increased total jumping count per 30 min in. the morphine treated group (group 2) compared with control ($P<0.05$). Additionally, pre-treatment by I.P. injection of memantine significantly diminished naloxone induced jumping behavior in morphine-conditioned mice ($P<0.05$). Also, no significant difference was observed after I.P. administration of different levels of memantine (0.1, 1 and 5 mg/kg) on total jumping count in mice ($P>0.05$) (figure 1).

As seen in the figure 2, I.P. injection of naloxone on day 4, significantly amplified jumping height (mm) in morphine treated mice ($P<0.05$). Furthermore, effect of naloxone on jumping height in morphine-addicted mice was significantly attenuated in memantine-treated animals compared to control group ($P<0.05$). Likewise, there was no significant difference on jumping height using various levels of memantine (0.1, 1 and 5 mg/kg) ($P>0.05$) (figure 2).

Effect of I.P. injection of memantine on naloxone-induced fecal excretion (mg) in morphine-dependent mice is presented in figure 3. Based on data, I.P. injection of naloxone on day 4, significantly increased defecation (mg) in morphine addicted mice ($P<0.05$). Also, there was no significant effect on naloxone-precipitated fecal material (mg) in animals pre-treated with 0.1 or 1 mg/kg memantine ($P>0.05$) whereas I.P. injection of 5 mg/kg memantine significantly diminished fecal material output compared to control animals ($P<0.05$) (figure 3).

In The next step in this study, we investigated the role of central NMDA glutamate receptors on naloxone-induced withdrawal syndrome in morphine-dependent mice. As seen in figure 4, injection of naloxone on day 4, significantly raised total jumping (count per 30 min) in morphine-conditioned mice. Also, pre-treatment with IAC injection of memantine (1µg/animal) in LNAcc and RNAcc significantly weakened the effect of I.P. injection of naloxone on total jumping count compared to the control group ($P<0.05$). Moreover, IAC administration of memantine (1µg/animal) in BNAcc significantly blocked naloxone-induced total jumping count in comparison to control group ($P<0.05$), nevertheless, the results was not significant between LNAcc, RNAcc and BNAcc ($P>0.05$). Similarly, pre-treatment with memantine (5µg/animal) in LNAcc and RNAcc significantly declined the effect of I.P. injection of naloxone on total jumping count compared to the control group ($P<0.05$) however, pre-treatment in RNAcc had better effect but not more significant than LNAcc ($P>0.05$). Moreover, IAC administration of 5µg/mouse memantine in BNAcc expressed a significant effect on reducing naloxone-precipitated total jumping count compared to control group ($P<0.05$). However, no significant difference was observed between IAC injection in LNAcc, RNAcc and BNAcc ($P>0.05$) (figure 4).

We determined effects of IAC injection of memantine followed by SC injection of morphine on naloxone-induced jump height (mm) in morphine-dependent mice. According to our results, injection of naloxone on day 4, significantly increased jump height in morphine-conditioned mice ($P<0.05$). Also, pre-treatment with IAC injection of memantine (1µg/animal) in LNAcc, RNAcc and BNAcc (for 3 continuous days) significantly decreased effects of naloxone administration (on day 4) in morphine-

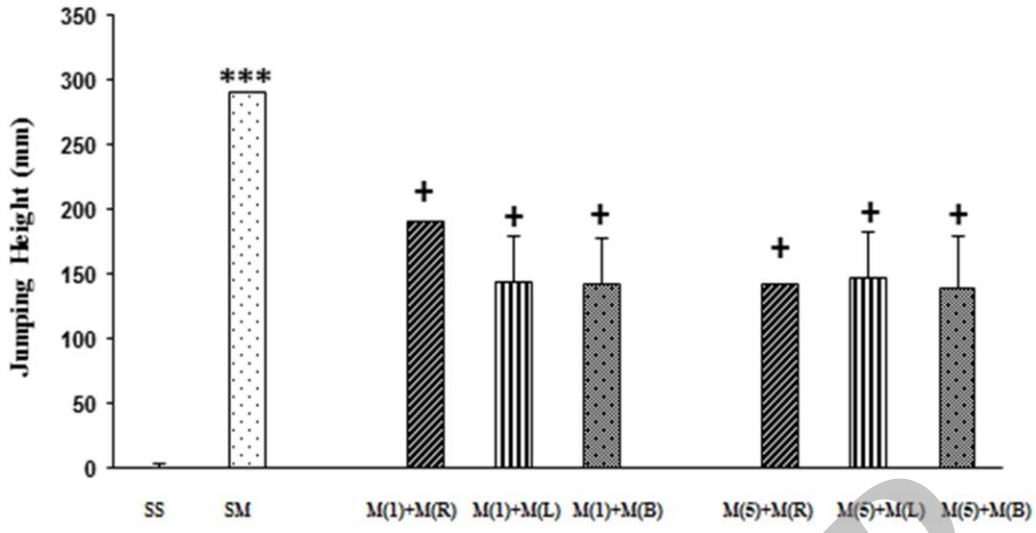

Fig.5. Effect of intra-accumbens (IAC) injection of Memantine (1 and 5 µg/mouse) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced jump height (mm) in morphine-dependent mice. n=6 per group. RNAcc: right nucleus accumbens, LNAcc: left nucleus accumbens, BNAcc: both side of nucleus accumbens. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group and pluses indicate on significance to morphine (SM) group ($P<0.05$). (Groups: SS= saline+saline, SM=saline+morphine, M(1)+M(R)= memantine (1µg/mice)+morphine(RNA cc), M(1)+M(L)= memantine (1µg/mice)+morphine(LNA cc), M(1)+M(B)= memantine (1µg/mice)+morphine(BNA cc), M(5)+M(R)= memantine (5µg/mice)+morphine(RNA cc), M(5)+M(L)= memantine (5µg/mice)+morphine(LNA cc), M(5)+M(B)= memantine (5µg/mice)+morphine(BNA cc)).

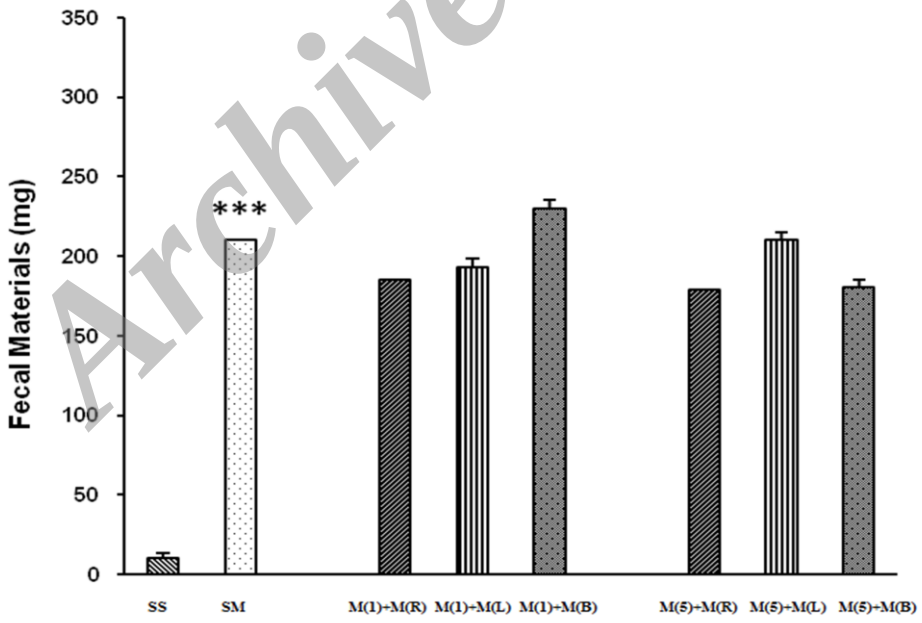

Fig.6. Effect of intra-accumbens (IAC) injection of Memantine (1 and 5 µg/mouse) followed by subcutaneous (S.C.) injection of Morphine (50-125 mg/kg in a 3-day schedule) on naloxone-induced fecal materials (mg) in morphine-dependent mice. n=6 per group. RNAcc: right nucleus accumbens, LNAcc: left nucleus accumbens, BNAcc: both side of nucleus accumbens. Data are presented as the mean ± SEM. Asterisks indicate significant difference in each group compared with control (SS) group ($P<0.05$).(Groups: SS= saline+saline, SM=saline+morphine, M(1)+M(R)= memantine (1µg/mice)+morphine(RNA cc), M(1)+M(L)= memantine (1µg/mice)+morphine(LNA cc), M(1)+M(B)= memantine (1µg/mice)+morphine(BNA cc), M(5)+M(R)= memantine (5µg/mice)+morphine(RNA cc), M(5)+M(L)= memantine (5µg/mice)+morphine(LNA cc), M(5)+M(B)= memantine (5µg/mice)+morphine(BNA cc)).

dependent mice (P<0.05). IAC injection of memantine in RNAcc had more but not significant effect on blocking naloxone induced jumping height (P>0.05). Then we measured effects of pre-treatment with 5µg/mouse memantine on jump height. As observed in the data, pre-treatment with IAC injection of memantine (5µg/mouse) in LNAcc, RNAcc and BNAcc significantly weakened the effect of peripheral administration of naloxone-induced jump height in morphine-conditioned mice (P<0.05) but there was no significant difference between IAC injections in LNAcc, RNAcc and BNAcc (P>0.05) (figure 5).

Effect of IAC injection of memantine on naloxone-induced fecal excretion (mg) in morphine-addicted mice is presented in figure 6. Based on the data, administration of naloxone on day 4, significantly increased fecal excretion (mg) in morphine addicted animals compared to the control group (P<0.05). In this study, IAC injection of animals with memantine (1µg/mouse) in RNAcc, LNAcc and BNAcc had no significant effect on total defecation amount in comparison to control group (P>0.05). Interestingly, pre-treatment with IAC injection of memantine (5µg/mouse) in RNAcc, LNAcc and BNAcc, was not able to g/lessen the effect of I.P. injection of naloxone in morphine-dependent mice (P>0.05) (figure 6).

Discussion

The withdrawal syndrome after the cessation of opioid therapy remains a challenge in the clinical treatment of pain. In agreement with previous studies, our results indicated that blocking of opioid receptors by naloxone, worsened withdrawal symptom in morphine-depended mice (Tzschentke, 2007; Karimi et al., 2011). Based on the protocol we described above, administration of memantine during the morphine treatment protocol attenuated the exacerbation of naloxone-precipitated opioid withdrawal syndrome. These results indicate that both central and peripheral administration of memantine attenuates behavioral manifestations of morphine withdrawal symptoms in mice. Previously it has been reported that Caroverine and Ketamine inhibit NMDA receptors associated with experience of morphine withdrawal symptom in rats (Kratzer and Schmidt, 2003; Jovaiša et al., 2006). In addition, Dextromethorphan (a non-competitive NMDA receptors antagonist) attenuates higher vulnerability to thermal hyperalgesia caused by prenatal morphine exposure in rat offspring (Tao et al., 2011). Molecular studies revealed that abnormal expression of synaptic $NR2_A$ and $NR2_B$ subunits of NMDA receptors can lead to decreased postsynaptic density-93 proteins and impairs morphine dependence in mice (Liaw et al., 2008). Also, Kulkarni et al., (Kulkarni et al., 2008) reported that effects of Ascorbic Acid on blocking opiate's withdrawal syndrome might be mediate dvia glutamatergic system. Recently it was discovered that peripheral NMDA receptors are essential in visceral pain transmission which provides a novel neurological mechanism for visceral hyperalgesia. Anatomical studies revealed that in addition to expression of NMDA receptors in dorsal root ganglia (DRG), peripheral afferent nerves innervating somatic tissues also express NMDA receptors (McRoberts et al., 2001). We found that I.P. injection of memantine inhibited behavioral responses to naloxone-precipitated jumping in opioid withdrawal syndrome in mice. It is reported that intravenous (I.V.) injection of NMDA antagonist inhibits voltage-dependent channels and decreases permeability in neurons innervating visceral tissues (McRoberts et al., 2001).

Our results also revealed that IAC injection of memantine (within the NAc) prior to I.P. morphine administration significantly diminished total jump count and jump height in naloxone-induced opioid withdrawal syndrome. It is well-documented that administration of morphine downregulates NMDA glutamate receptors in NAc (Martin et al., 2004). In this study we used IAC injection of memantine before I.P. administration of morphine. So to our knowledge, morphine was not able to down-regulate NMDA glutamate receptors in NAc in mice. NMDA receptors are ionotropic glutamate receptors characterized by slow deactivation and high Ca^{2+} permeability (McRoberts et al., 2001). Opioid receptors signaling are related through intracellular transducer G proteins from different subfamilies (Mu, Delta and Kappa receptors). Opioid receptors predominantly couple to pertussis toxin sensitive Gαi and $G\alpha_o$ classes which are further responsible for the inhibition of adenylyl cyclase (AC) / cyclic adenosine mono-phosphate (cAMP) pathway (Rehni et al., 2012). The direct interaction between Mu and Delta opioid receptors with NMDA glutamate receptors is not fully identified but it seems that they might interact via intercellular

protein kinase (PK) (Maldonado et al., 1992). It seems that morphine (via Mu and Delta opioid receptors) inhibits calcium influx into the cells and decrease phosphatidylinositol (PI) and cAMP second messenger system. This phenomenon reduces protein kinase C (PKC) and protein kinase A (PKA) activity. On the other hand, memantine or Caroverine blocks the effect of morphine on NMDA glutamate receptors and calcium channels which were formerly associated with the experience of morphine withdrawal (Fundytus and Coderre, 1994; Kratzer and Schmidt, 2003)

Morphine dependence is one of the side effects of acute drug administration. Acute morphine uptake leads to cellular second messenger activities and influence on membrane potential by lowering calcium influx and then impairing neurotransmitter release (Bodnar, 2008). Long term exposure of neural system to morphine leads to adapting to opioids and up-regulation cAMP and PK activities (Rehni et al., 2012; Bodnar, 2013). In withdrawal syndrome, on one hand, morphine addiction increased cAMP and PK activities. On the other hand, in absence of morphine, normal cellular physiological functions reinforces cAMP and PK production. So high levels of second messengers and PK activity leads to developing somatic signs such as jumping behavior, piloerection, diarrhea and etc. (Fundytus and Coderre, 1994).

It was reported that morphine withdrawal syndrome develops in specific limbic areas such as central nucleus of the amygdala, raphe nuclei and NAc (Sekiya et al., 2004). Morphine withdrawal induces glutamate release in the ventral tegmental area (VTA) and NAc (Wang et al., 2005). It is reported that microinjection of NMDA receptor antagonist into the central nucleus of the amygdala decreases naloxone-induced morphine withdrawal symptom (Sekiya et al., 2004). In accordance with previous studies, obtained results in this study revealed that IAC injection of memantine into NAc significantly diminished morphine withdrawal somatic signs (jumping count and height). These findings suggest that activation of the glutamatergic system in specific areas such as NAc during morphine withdrawal might contribute to the negative effects. Molecular research revealed that opiate withdrawal-associated induction of ΔFosB in the NAc is regulated by glutamate activity in the VTA. Hence, ΔFosB isoforms in the shell of NAc increase following naloxone injection in morphine-treated rats. It seems that NMDA receptor antagonists block c-fos expression during morphine withdrawal period (Wang et al., 2005). Based on these results; no significant difference observed on effects of IAC injection of memantine in LNAc, RNAc and BNAc. Our study has shown that morphine affects both sides of NAc and induced dependence syndrome mediated via both sides of NAc.

Defecation and diarrhea is common withdrawal sign of opioid-dependence (Mori et al., 2014). In this study I.P. administration of naloxone increased fecal excretion in morphine dependent mice. It is reported that blockade of peripheral opioid receptors leads to decline in fluid absorption from the jejunum and colon in morphine-dependent rats .while IAC injection of naloxone methiodide did not induce diarrhea in morphine-dependent mice (Mori et al., 2014). Based on current study, presumably peripheral opioid receptors play an important role in withdrawal-induced diarrhea in opioid-dependent patients. In this study, peripheral memantine (I.P.) decreased defecation whereas IAC injection of memantine in RNAc, LNAc and BNAc was not able to block this effect. NMDA receptors and messenger RNA have been identified in both myenteric and submucosa of intestine. It is reported that NMDA receptor antagonist, memantine can decline colon and rectum stimulation. For instance, I.V injection of memantine attenuated the viscera-motor response to noxious colorectal distention (McRoberts et al., 2001). Our observations identify a role for peripheral NMDA receptors in naloxone-induced defecation in morphine-conditioned mice. We think central administration of memantine was able to inhibit some of the somatic signs of morphine withdrawal (Sekiya et al., 2004). So perhaps effect of memantine on fecal excretion is more related to peripheral (in the gastrointestinal tract) but not central NMDA receptors.

Conclusion

In conclusion, according to these results; central and peripheral injection of NMDA receptor antagonists decreased naloxone-induced withdrawal symptom in morphine-depended mice. It seems that distribution of glutamatergic projections and receptor subtypes within the shell and core of the NAc are different (Wang et al., 2005). So we believe that more studies

need to investigate the role of NMDA receptors in shell and core of the NAc as well as other brain regions in opiates withdrawal syndrome. Also, further studies are needed to determine the role of other glutamatergic receptors for withdrawal syndrome control in patients. Additionally, the exact contribution of peripheral and central NMDA receptors in withdrawal behavior remains to be elucidated.

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Conflict of Interest

The authors declare that have no conflict of interests in this study.

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