Original Article

Repeated Pretreatment of Morphine Prevents Morphine-induced Amnesia: A Possible Involvement for Dorsal Hippocampal NMDA Receptors

Mohammad-Reza Zarrindast PhD•[1,2,3,4], Fereshteh Asadi MSc[5], Ameneh Rezayof PhD[6]

Abstract

Background: Learning and memory processes can be affected by morphine administration. It has been previously demonstrated that the effects of morphine depend on the timing of drug administration. In the present study, the effects of microinjections of a NMDA receptor agonist and antagonist into the CA1 regions of the dorsal hippocampi (intra-CA1) on repeated pretreatment of morphine-induced prevention of morphine-induced amnesia have been investigated.

Methods: Step-through inhibitory avoidance task of memory has been used to examine retrieval of memory formation, 24 h after training in male Wistar rats.

Results: The results indicate that post-training administration of morphine (7.5 mg/kg) impaired memory retrieval, but not in the animals, which received previous repeated morphine (7.5 and 10 mg/kg) injections followed by morphine withdrawal. Repeated co-administration of NMDA (7.5 and 10 ng/rat, intra-CA1) with an ineffective dose of morphine (5 mg/kg), once daily for three days reversed morphine-induced amnesia. Repeated bilateral intra-CA1 microinjections of NMDA, once daily for three days followed by a five-day washout had no effect on the expression of amnesia produced by post-training morphine. Three-day administration of the NMDA receptor antagonist, D-AP5 (0.5 – 2 μg/rat, intra-CA1) followed by a five-day washout had no effect on morphine-induced amnesia. On the other hand, intra-CA1 microinjections of the same doses of D-AP5 prior to injection of 7.5 mg/kg of morphine (per day×3 days) decreased the reversal of morphine-induced amnesia.

Conclusion: These data imply that the dorsal hippocampal NMDA receptor mechanism(s) may modulate the effect-induced by repeated morphine administration on a challenge dose of morphine-induced amnesia.

Keywords: D-AP5, memory, morphine, NMDA, rat(s)

Introduction

Administration of morphine[1–4] and psychostimulants such as amphetamine or cocaine[5,6] increases locomotor activity in rodents, which becomes progressively stronger with repeated intermittent injections. This phenomenon is named drug-induced sensitization. Investigations aimed at testing the mechanisms involved have indicated that different neurotransmitter systems in various brain sites may play a role in this behavioral sensitization, such as the ventral pallidal dopaminergic system,[7] the central cholinergic system,[8] or opioidergic system in the ventral tegmental area.[9]

The most excitatory synaptic neurotransmitter, glutamate,[10] is known to play a major role in excitatory and synaptic transmission and sensitization.[11] Glutamate acts through two types of receptors, ionotropic glutamate (iGluRs) and metabotropic glutamate (mGluRs), which mediate the excitatory signal between neurons.[12] iGluRs are ligand-gated ion channels that mediate rapid changes in the permeability of cations such as sodium, calcium, and potassium,[10] while mGluRs belong to the family of G-protein coupled receptors that control intracellular signaling cascades.[12]

The involvement of glutamate and dopamine neurotransmission in the ventral tegmental area, nucleus accumbens, prefrontal cortex, and amygdala in behavioral sensitization is well established.[13,14] Glutamatergic receptors are also involved in the behavioral sensitization to cocaine[15] and nicotine.[11] Moreover, it has been shown that the NMDA receptor mechanism may have a role in ethanol sensitization.[16] NMDA receptors are also involved in opiate-induced antinociceptive tolerance and locomotor sensitization in rats, thus NMDA receptor antagonists inhibit antinociceptive tolerance and locomotor sensitization induced by morphine.[17] On the other hand, repeated administration of morphine followed by withdrawal after five days, has been

Authors affiliations: [1]Department of Pharmacology, School of Medicine and Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, [2]School of Cognitive Science, Institute for Research in Fundamental Sciences (IPM), Tehran, [3]Institute for Cognitive Science Studies, Tehran, [5]School of Biology, Azad University of Damghan, Damghan, [6]Department of Animal Biology, School of Biology, College of Science, University of Tehran, Tehran, [4]Department of Neuroscience, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran

•**Corresponding author and reprints:** Mohammad-Reza Zarrindast PhD, Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P. O. Box 13145-784, Tehran, Iran.
Fax: +98-216-640-2569, Tel: +98-216-640-2569,
E-mail: zarinmr@ams.ac.ir; zrrndst@yahoo.ca
Accepted for publication: 18 August 2010

previously shown to induce locomotor sensitization in rats[18] and inhibit morphine-induced amnesia.[19]

Therefore, the aim of the present study determined whether the NMDA receptor mechanism in dorsal hippocampal CA1 is involved in the inhibition of morphine-induced amnesia, with repeated intermittent injections of morphine. A step-through type of passive avoidance task, which is a widely used method to test long-term memory in rodents was used. It has been suggested that the task may rely on hippocampal functions.[20]

Materials and Methods

Animals

Male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 200 – 250 g at the time of surgery were used as subjects in this study. Animals were housed five per cage with a 12-h light/12-h dark cycle and controlled temperature (22±2 °C). They had *ad libitum* access to food and water. All animals were allowed to adapt to the laboratory conditions for at least one week before surgery and were handled for five min/day during this adaptation period. Each animal was used once only. Eight animals were used in each experimental group.

Surgical procedures and intra-CA1 microinjections

Rats were placed in a stereotaxic apparatus under anesthesia with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg) plus xylazine (4 mg/kg). The skin was incised and the skull cleaned. Two stainless-steel, 22-gauge guide cannulas were placed (bilaterally) 1 mm above the intended injection site according to the atlas of Paxinos and Watson.[21] Stereotaxic coordinates for the CA1 regions of the dorsal hippocampi were incisor bar (-3.3 mm), -3 to -3.5 mm (depending on body weight) posterior to bregma, ±1.8 to 2 mm lateral to the sagittal suture and -2.8 to -3 mm ventral of the dorsal surface of the skull. Cannulas were secured to anchor jewelers' screws with dental acrylic. Stainless steel stylets (27-gauge insect pins) were inserted into the guide cannulas to keep them free of debris. All animals, after a brief anesthetic clearing period, were allowed to recover from surgery for one week. For drug microinjection, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannula). The injection solutions were administered manually in a total volume of 1 μL/rat (0.5 μL/side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs. Animals were immediately placed in the home cage after injection.

Drugs

The drugs used in the present study were morphine sulphate (Temad Co., Tehran, Iran), NMDA (N-methyl-D-aspartate acid) and D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5, Tocris Cookson Ltd., UK). All drugs were dissolved in sterile saline. Doses of morphine expressed as salt were injected subcutaneously (s.c.) at a volume of 1 mL/kg. NMDA and D-AP5 were injected bilaterally into the dorsal hippocampal CA1 regions (intra-CA1). Control animals received 0.9% physiological saline. The drugs' doses were chosen based on our previous experiments.[9,22,23]

Step-through inhibitory avoidance apparatus

A learning box consisted of two compartments, one light (white opaque resin, 20 cm×20 cm×30 cm) and the other dark (black opaque resin, 20 cm×20 cm×30 cm). A guillotine door opening (7 cm×9 cm) was constructed on the floor in the center of the partition between the two compartments. Stainless steel grids (2.5 mm in diameter) were placed at 1 cm intervals (distance between the centers of grids) on the floor of the dark compartment to produce a foot shock. Intermittent electric shocks (50 Hz, 3 s, and 1 mA intensity) were delivered to the grid floor of the dark compartment by an insulated stimulator.

Behavioral procedures

Training

Training was based on our previous studies.[24] All animals were allowed to habituate in the experimental room for at least 30 min prior to the experiments. Then, each animal was gently placed in the light compartment of the apparatus; after 5 sec the guillotine door was opened and the animal was allowed to enter the dark compartment. The latency with which the animal crossed into the dark compartment was recorded. Animals that waited more than 100 sec to cross to the dark compartment were eliminated from the experiments. Once the animal crossed with all four paws to the next compartment, the guillotine door was closed and after 10 sec, the rat was taken into its home cage (habituation trial). The acquisition trial was carried out 30 min after the habituation trial. The animal was placed in the light compartment and 5 sec later the guillotine door was opened, and as soon as the animal crossed to the dark (shock) compartment the door was closed and a foot shock (50 Hz, 1mA and 3 sec) was immediately delivered to the grid floor of the dark room. After 20 sec, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the procedure was repeated. The rat received a foot-shock each time it re-entered the dark compartment and had placed all four paws in the compartment. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds.

Retrieval test

Twenty-four hours after training, a retrieval test was performed to determine long-term memory. Each animal was placed in the light compartment for 20 sec; the door was

opened and the step-through latency was measured for entering into the dark compartment. The test session ended when the animal entered the dark compartment, but a cut-off time of 300 sec was applied for those animals, which remained in the light compartment. During these sessions, no electric shock was applied.

Experimental design

Effect of morphine on memory retrieval

In this experiment, the effect of post-training administration of morphine on memory retrieval was examined. Four groups of animals received subcutaneous injections of saline (1 mL/kg) or three different doses of morphine (2.5, 5, and 7.5 mg/kg) immediately after training.

Effects of three-day repeated pretreatment of morphine on acute test dose of morphine-induced amnesia

In this experiment, the effect of previous repeated injections of morphine on amnesia induced by an acute test dose of morphine was investigated in seven groups of animals. All animals received subcutaneously (s.c.) different doses of morphine (0, 5, 7.5, and 10 mg/kg), once daily for three days, followed by five days washout. Three groups of the animals received post-training injections of saline (1 mL/kg) and the other four groups received a test dose of morphine (7.5 mg/kg) immediately after training. Memory retrieval of the animals was tested 24 h later. The particular schedule of repeated administration was based on our previous studies where morphine sensitization was significantly produced in the rats.[9]

Effects of intra-CA1 microinjection of NMDA on the response to three-day repeated pretreatment with morphine

In this experiment, three-day repeated bilateral intra-CA1 microinjections of different doses of NMDA in the presence or absence of three-day repeated pretreatment of morphine on amnesia-induced post-training morphine was examined in eight groups of animals. The first four groups of animals received bilateral intra-CA1 microinjections of saline (1 μL/rat) or different doses of NMDA (5, 7.5, and 10 ng/rat) and 5 min later they were given saline (1 mL/kg) once daily for three days. After a five-day washout, the animals received a post-training test dose of morphine (7.5 mg/kg) and memory was tested 24 h later. The next four groups of animals received bilateral intra-CA1 microinjections of saline (1 μL/rat) or different doses of NMDA (5, 7.5, and 10 ng/rat) and 5 min later they received morphine (5 mg/kg) once daily for three days. After a five-day washout, the animals received a post-training test dose of morphine (7.5 mg/kg) and memory was tested 24 h later.

Effects of intra-CA1 microinjection of D-AP5 on the response to three-day repeated pretreatment with morphine

In this experiment, three-day repeated bilateral intra-CA1 injections of different doses of D-AP5 with or without morphine on amnesia induced by post-training morphine was examined. Eight groups of animals received bilateral intra-CA1 injections of saline (1 μL/rat) or different doses of D-AP5 (0.5, 1, and 2 μg/rat) and 5 min later the animals received either saline (1 mL/kg) or morphine (7.5 mg/kg) once daily for three days. After a five-day washout, the animals received a post-training test dose of morphine (7.5 mg/kg) and memory retrieval was tested 24 h later.

Data analysis

Since data displayed normality of distribution and homogeneity of variance, the results were statistically evaluated by analysis of variance one- and two-way (ANOVA), in which mean±SEM of step-through latencies of the experimental groups on the test day were compared. Further analyses for individual between-group comparisons were carried out with post-*hoc* Tukey's test. In all comparisons, $P<0.05$ was considered to indicate statistical significance.

Histology

After the testing sessions each rat was deeply anesthetized and 1 μL of a 1% methylene-blue solution was bilaterally infused into the CA1 (0.5 μL/side), as described in the drug section. The animal was subsequently decapitated, its brain removed and placed in formaldehyde (10%). After several days, the brains were sliced and the sites of injections were verified according to the atlas of Paxinos and Watson.[21] Data from the animals with injection sites located outside the CA1 regions were not used in the analysis.

Results

Effects of morphine on memory formation

Figure 1 shows the effects of post-training of morphine injections (2.5, 5, and 7.5 mg/kg, s.c.) on step-through latency. One-way ANOVA revealed that post-training s.c. administration of morphine (2.5, 5, and 7.5 μg/rat) altered the step-through latency in the inhibitory avoidance task [$F(3, 28)=8.9$, $P<0.001$]. Post-*hoc* analysis indicated that the higher dose of morphine (7.5 mg/kg) impaired memory.

Effects of three-day repeated pretreatment of morphine on acute test dose of morphine-induced amnesia

As shown in Figure 2, repeated administration of different doses of morphine (5, 7.5, and 10 mg/kg, s.c.) once daily for three days followed by a five-day drug-free period altered memory impairment induced by a challenge dose of morphine (7.5 mg/kg) [One-way ANOVA, $F(4, 35)=15.3$, $P<0.001$]. Post-*hoc* analysis indicated that repeated pre-exposure to morphine (7.5 and 10 mg/kg) prevented the expression of amnesia produced by post-training morphine. On the other hand, one-way ANOVA revealed that repeated pre-exposure to higher doses of morphine (7.5 and

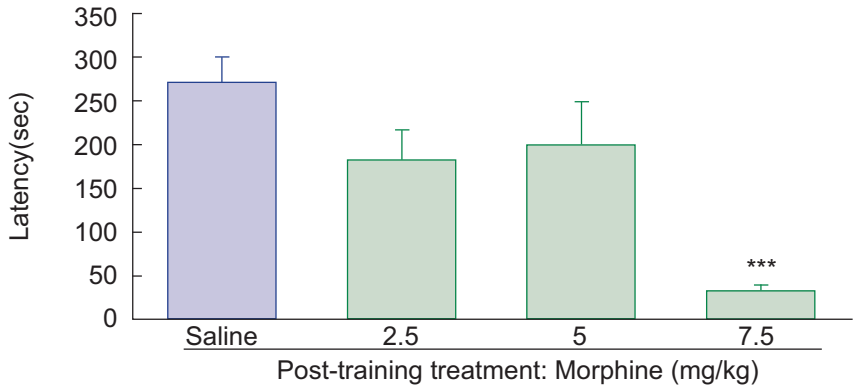

Figure 1. Effects of post-training administration of morphine on memory formation. Animals received s.c. injections of saline (1 mL/kg) or different doses of morphine (2.5, 5 and 7.5 mg/rat) immediately after training. The test was achieved 24 h after training. Data are expressed as mean±SEM of eight animals in each group. ***P<0.001 different from the saline group.

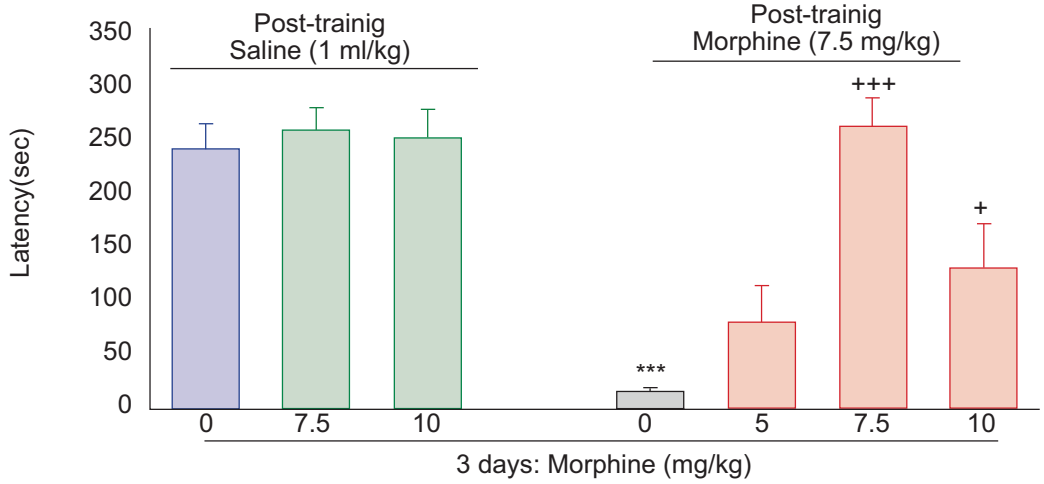

Figure 2. Effects of three-day repeated pretreatment of morphine on acute test dose of morphine-induced amnesia. Animals received repeated administrations of saline (1 mL/kg) or different doses of morphine (5, 7.5 and 10 mg/kg, s.c.) once daily for three days and after a five-day drug free period, saline (1 mL/kg) or a challenge dose of morphine (7.5 mg/kg) were administered immediately after training. The test was achieved 24 h after training. Data are expressed as mean±SEM of eight animals in each group. ***P<0.001 different from three-day saline/post-training saline group. +P<0.05, +++P<0.001 different from three-day saline/post-training morphine group.

10 mg/kg) had no effect on memory retrieval in animals, which received post-training administration of saline [F(2, 21)=0.14, P>0.05].

Effects of intra-CA1 injections of NMDA on the response to repeated pretreatment with morphine

Figure 3 shows the effect of repeated pre-treatment administration of NMDA (5, 7.5, and 10 ng/rat, intra-CA1) with or without morphine (5 mg/kg, s.c.) followed by five days free of the drugs on morphine-induced amnesia. Two-way ANOVA indicated a significant difference between the effects of NMDA alone and NMDA plus morphine (5 mg/kg) on memory retrieval [For treatment, F (1, 56)=22.27, P<0.001; dose, F(3, 56)=17.47, P<0.001; and treatment×dose interaction, F(3, 56)=3.20, P<0.05]. Post hoc analysis also revealed that NMDA administration increased the response of repeated morphine (5 mg/kg, s.c.) treatment [F(3,28)=21.5, P<0.001]. Furthermore repeated injection of the same doses of NMDA (10 ng/rat) by itself had no effect on morphine-induced amnesia [F(3,28)=1.85, P>0.05].

Effects of intra-CA1 injection of D-AP5 on the response to repeated pretreatment with morphine

Figure 4 shows the effect of repeated pre-treatment administration of D-AP5 (0.5, 1, and 2 µg/rat, intra-CA1) with or without morphine (7.5 mg/kg, s.c.) followed by five days free of the drugs on morphine-induced amnesia. Two-way ANOVA indicated a significant difference between the effects

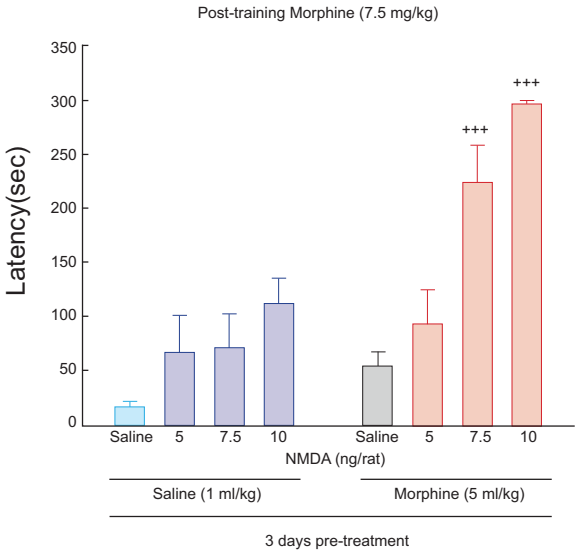

Figure 3. Effects of repeated pretreatment of NMDA with or without morphine on acute test dose of morphine-induced amnesia. Animals received three-day bilateral intra-CA1 microinjections of saline (1 μL/rat) or different doses of NMDA (5, 7.5 and 10 ng/rat), 5 min prior to the three-day repeated injections of saline (1 mL/kg) or morphine (5 mg/kg). After a five-day drug-free period, all animals received a challenge dose of morphine (7.5 mg/kg) immediately after training. The test was performed 24 h after training. Data are expressed as mean±SEM of eight animals in each group. +++P<0.001 different from the saline/morphine (5 mg/kg) group.

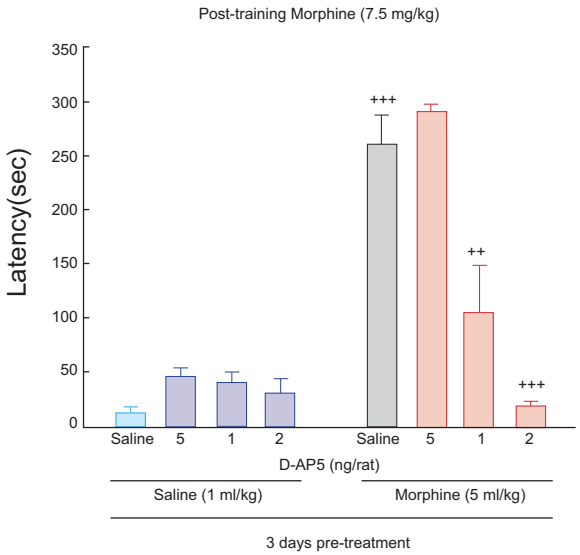

Figure 4. Effects of repeated pretreatment of D-AP5 with or without morphine on acute test dose of morphine-induced amnesia. Animals received three-day bilateral intra-CA1 microinjections of saline (1 μL/rat) or different doses of NMDA (0.5, 1 and 2 μg/rat), 5 min prior to the three-day repeated injection of saline (1 mL/kg) or morphine (7.5 mg/kg). After a five-day drug-free period, all animals received a challenge dose of morphine (7.5 mg/kg) immediately after training. The test was achieved 24 h after training. Data are expressed as mean ± SEM of eight animals in each group. ***P<0.001 different from the saline control group. ++P<0.01, +++P<0.001 different from the saline/morphine (7.5 mg/kg) group.

of D-AP5 alone and D-AP5 plus morphine (7.5 mg/kg) on memory retrieval [For treatment, $F(1, 56) = 95.73$, $P<0.001$; dose, $F(3, 56)= 21.83$, $P<0.001$; and treatment×dose interaction, $F(3, 56) = 22.43$, $P<0.001$]. Post hoc analysis also revealed that repeated administration of D-AP5 decreased the response of repeated injections of morphine (7.5 mg/kg) and showed amnesia [$F(3,28)=24.9$, $P<0.001$]. Furthermore repeated injections of the same doses of D-AP5 alone elicited no response [$F(3,28)=2.33$, $P>0.05$].

Discussion

The present results revealed that a post-training subcutaneous morphine injection induced memory impairment. The data agreed with those presented previously in our laboratory[24,25] and by other investigators.[26,27] It has been shown that morphine which was administered post-training, pre-training or pre-test showed an amnesic effect,[28–30] which appeared to be mediated via the μ-opioid receptors.[30,31] The memory impairment observed in the present study by an acute dose of morphine was attenuated in animals which received three-day previous repeated administrations of different doses of morphine (7.5 and 10 mg/kg) followed by a five-day washout. It is well known that repeated administration of morphine elicits behavioral sensitization. It should be considered that the schedule of morphine sensitization was significantly produced in the rats.[9]

Our previous studies have also shown that the GABAergic receptors of the dorsal hippocampus, dopaminergic receptors of the ventral tegmental area and central cholinergic system are involved in the inhibition of morphine-induced amnesia in morphine-sensitized mice[8,32] and rats.[9] Generally, behavioral sensitization to morphine may be due to changes in opioidergic, dopaminergic and/or GABAergic neurotransmission.[18,33–35] Furthermore, it has been suggested that the increase in NMDA receptor expression is associated with morphine-induced behavioral sensitization.[36] Considering that glutamate neurotransmission[37,38] is also implicated in behavior and NMDA receptors have an important role in the opiate antinociceptive tolerance and locomotor sensitization in rats,[17] we aimed to test the involvement of NMDA receptors of the CA1 regions on the inhibition of morphine-induced amnesia in morphine-sensitized rats.

In the present study, three-day previous repeated intra-CA1 microinjections of NMDA followed by a five-day washout had no effect on post-training morphine-induced memory impairment. Repeated intra-CA1 microinjections of NMDA with repeated subcutaneous administration of an ineffective dose of morphine also reversed morphine

amnesic response with a potentiation. These experiments may show that the dorsal hippocampal NMDA receptor mechanism(s) is involved in the response elicited by the repeated morphine administration. This may be in agreement with data reported by others which have indicated that repeated administration and withdrawal of drugs alter the expression of various glutamate receptors in different sites of the brain.[39,40] On the other hand, acute administration of morphine has also been demonstrated to enhance glutamate neurotransmission in the nucleus accumbens via an increase in mRNA levels of NMDA receptor subunits.[41] Furthermore, involvement of NMDA receptors in the behavioral sensitization to nicotine,[11] cocaine,[15] amphetamine,[42] ethanol[16] and apomorphine[43] has been shown. Considering that several evidences indicate the involvement of NMDA receptors in the behavioral sensitization to drug abuse, it may be proposed that the dorsal hippocampal NMDA receptors are involved in the inhibition of morphine-induced amnesia in morphine-sensitized mice.

To further support NMDA involvement in the response induced by three-day morphine pretreatment, the NMDA receptor antagonist D-AP5 was used in the present experiments. The present data indicated that repeated intra-CA1 administration of D-AP5 by itself had no significant response on memory retrieval. On the other hand, the inhibition of acute morphine-induced amnesia by repeated administration of morphine was significantly reduced in the animals that had previously received intra-CA1 administration of D-AP5 for three days. In agreement with our results, it has been shown that the blockade of NMDA receptors inhibits the development of morphine tolerance and physical dependence.[17,44] Therefore, it appears that repeated intermittent morphine administration reverses impairment induced by an acute challenge dose of morphine through a glutamatergic mechanism in the dorsal hippocampus. It seems that the increase in NMDA receptor expression is associated with the inhibition of morphine-induced amnesia in morphine-sensitized mice. This hypothesis can be supported by several investigations that show which the glutamatergic system is a potential target in morphine-induced behavioral sensitization.[3,36] Furthermore, our previous results have indicated that the central dopaminergic[8,19] and dorsal hippocampal GABAergic system[32] are involved in the effects of morphine sensitization on learning and memory processes. Considering that the glutamatergic, dopaminergic and GABAergic systems have functional interactions in behavioral sensitization,[3,14,35] involvement of an indirect pathway in this process seems likely.

In conclusion, morphine induces amnesia in a passive avoidance task in rats which can be reversed by repeated pretreatment of the opioid followed by wash out. The dorsal hippocampal NMDA receptor mechanism is involved in the response-induced by repeated administration of morphine.

Acknowledgment

The authors wish to thank Dr. Touraj Nayernouri for his assistance in preparing this manuscript. This project was supported by the Institute for Cognitive Science Studies.

References

1. Kuribara H. Modification of morphine sensitization by opioid and dopamine receptor antagonists: evaluation by studying ambulation in mice. *Eur J Pharmacol.* 1995; **275:** 251 – 258.
2. Shippenberg TS, Heidbreder C, Lefevour A. Sensitization to the conditioned rewarding effects of morphine: pharmacology and temporal characteristics. *Eur J Pharmacol*. 1996; **299:** 33 – 39.
3. Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci*. 2002; **12:** 610 – 615.
4. Li KW, Jimenez CR, van der Schors RC, Hornshaw MP, Schoffelmeer AN, Smit AB. Intermittent administration of morphine alters protein expression in rat nucleus accumbens. *Proteomics.* 2006; **6:** 2003 – 2008.
5. Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev*. 1991; **16:** 223 – 244.
6. Kalivas PW, Alesdatter JE. Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther.* 1993; **267:** 486 – 495.
7. Zarrindast MR, Ebrahimi-Ghiri M, Rostami P, Rezayof A. Repeated pre-exposure to morphine into the ventral pallidum enhances morphine-induced place preference: involvement of dopaminergic and opioidergic mechanisms. *Behav Brain Res*. 2007a; **181:** 35 – 41.
8. Zarrindast MR, Farahmandfar M, Rostami P, Rezayof A. The influence of central administration of dopaminergic and cholinergic agents on morphine-induced amnesia in morphine-sensitized mice. *J Psychopharmacol.* 2006; **20:** 59 – 66.
9. Zarrindast MR, Farajzadeh Z, Rostami P, Rezayof A, Nourjah P. Involvement of the ventral tegmental area (VTA) in morphine-induced memory retention in morphine-sensitized rats. *Behav Brain Res.* 2005; **163:** 100 – 106.
10. Chaffey H, Chazot PL. NMDA receptor subtypes: structure, function, and therapeutics. *Curr Anaesth Crit Care.* 2008; **19:** 183 – 201.
11. Jain R, Mukherjee K, Balhara YP. The role of NMDA receptor antagonists in nicotine tolerance, sensitization, and physical dependence: a preclinical review. *Yonsei Med J.* 2008; **49:** 175 – 188.
12. Shin SS, Martino JJ, Chen S. Metabotropic glutamate receptors (mGlus) and cellular transformation. *Neuropharmacology.* 2008; **55:** 396 – 402.
13. Cadoni C, Di Chiara G. Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. *Neuroscience*. 1999; **90:** 447 – 455.
14. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology*. 2000; **151:** 99 – 120.
15. Ghasemzadeh MB, Mueller C, Vasudevan P. Behavioral sensitization to cocaine is associated with increased glutamate receptor trafficking to the postsynaptic density after extended withdrawal period. *Neuroscience.* 2009; **159:** 414 – 426.
16. Meyer PJ, Phillips TJ. Behavioral sensitization to ethanol does not result in cross-sensitization to NMDA receptor antagonists.

Psychopharmacology (Berl). 2007; **195:** 103 – 115.
17. Mendez IA, Trujillo KA. NMDA receptor antagonists inhibit opiate antinociceptive tolerance and locomotor sensitization in rats. *Psychopharmacology (Berl)*. 2008; **196:** 497 – 509.
18. Zarrindast MR, Heidari-Darvishiani A, Rezayof A, Fathi-Azarbaijani F, Jafari-Sabet M, Hajizadeh-Moghaddam A. Morphine-induced sensitization in mice: changes in locomotor activity by prior scheduled exposure to GABAA receptor agents. *Behav Pharmacol*. 2007b; **18:** 303 – 310.
19. Zarrindast MR, Rezayof A. Morphine state-dependent learning: sensitization and interactions with dopamine receptors. *Eur J Pharmacol.* 2004; **497:** 197 – 204.
20. Izquierdo I, da Cunha C, Rosat R, Jerusalinsky D, Ferreira MB, Medina JH. Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behav Neural Biol.* 1992; **58:** 16 – 26.
21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 6th ed. San Diego, CA: Academic Press; 2007.
22. Jafari-Sabet M, Zarrindast MR, Rezayat M, Rezayof A, Djahanguiri B. The influence of NMDA receptor agonist and antagonist on morphine state-dependent memory of passive avoidance in mice. *Life Sci.* 2005; **78:** 157 – 163.
23. Rezayof A, Sharifi K, Zarrindast MR, Rassouli Y. Modulation of ethanol state-dependent learning by dorsal hippocampal NMDA receptors in mice. *Alcohol.* 2008; **42:** 667 – 674.
24. Ahmadi SH, Zarrindast MR, Haeri-Rohani A, Rezayof A, Nouri M. Nicotine improves morphine-induced impairment of memory: possible involvement of N-Methyl-D-Aspartate receptors in the nucleus accumbens. *Development Neurobiol.* 2007; **67:** 1118 – 1127.
25. Homayoun H, Khavandgar S, Zarrindast MR. Morphine state-dependent learning: interactions with alpha2-adrenoceptors and acute stress. *Behav Pharmacol.* 2003; **14:** 41 – 48.
26. Castellano C. Effects of morphine and heroin on discrimination learning and consolidation in mice. *Psychopharmacologia*. 1975; **42:** 235 – 242.
27. Nishimura M, Shiigi Y, Kaneto H. State dependent and/or direct memory retrieval by morphine in mice. *Psychopharmacology (Berl)*. 1990; **100:** 27 – 30.
28. Izquierdo I. Effect of naloxone and morphine on various forms of memory in the rat: possible role of endogenous opiate mechanisms in memory consolidation. *Psychopharmacology (Berl)*. 1979; **66:** 199 – 203.
29. Bruins Slot LA, Colpaert FC. Opiate states of memory: receptor mechanisms. *J Neurosci.* 1999a; **19:** 10520 – 10529.
30. Bruins Slot LA, Colpaert FC. Recall rendered dependent on an opiate state. *Behav Neurosci.* 1999b; **113:** 337 – 344.
31. Saha N, Datta H, Sharma PL. Effects of morphine on memory: interactions with naloxone, propranolol, and haloperidol. *Pharmacology.* 1991; **42:** 10 – 14.
32. Zarrindast MR, Hoghooghi V, Rezayof A. Inhibition of morphine-induced amnesia in morphine-sensitized mice: involvement of dorsal hippocampal GABAergic receptors. *Neuropharmacology.* 2008; **54:** 569 – 576.
33. Spanagel R, Shippenberg TS. Modulation of morphine-induced sensitization by endogenous kappa opioid systems in the rat. *Neurosci Lett.* 1993; **153:** 232 – 236.
34. Kuribara H. Modification of morphine sensitization by opioid and dopamine receptor antagonists: evaluation by studying ambulation in mice. *Eur J Pharmacol*. 1995; **275:** 251 – 258.
35. Leite-Morris KA, Fukudome EY, Shoeb MH, Kaplan GB. GABA(B) receptor activation in the ventral tegmental area inhibits the acquisition and expression of opiate-induced motor sensitization. *J Pharmacol Exp Ther.* 2004; **308:** 667 – 678.
36. Sepehrizadeh Z, Sahebgharani M, Ahmadi S, Shapourabadi MB, Bozchlou SH, Zarrindast MR. Morphine-induced behavioral sensitization increased the mRNA expression of NMDA receptor subunits in the rat amygdala. *Pharmacology*. 2008; **81:** 333 – 343.
37. Wolf ME, Jeziorski M. Co-administration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. *Brain Res.* 1993; **613:** 291 – 294.
38. Tzschentke TM, Schmidt WJ. Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. *Behav Brain Res.* 1997; **84:** 99 – 107.
39. Carlezon WA Jr, Todtenkopf MS, McPhie DL, Pimentel P, Pliakas AM, Stellar JR, et al. Repeated exposure to rewarding brain stimulation downregulates GluR1 expression in the ventral tegmental area. *Neuropsychopharmacology.* 2001; **25:** 234 – 241.
40. Lu W, Monteggia LM, Wolf ME. Withdrawal from repeated amphetamine administration reduces NMDAR1 expression in the rat substantia nigra, nucleus accumbens and medial prefrontal cortex. *Eur J Neurosci*. 1999; **11:** 3167 – 3177.
41. Jacobs EH, Wardeh G, Smith AB, Schoffelmeer AN. Morphine causes a delayed increase in glutamate receptor functioning in the nucleus accumbens core. *Eur J Pharmacol.* 2005; **511:** 27 – 30.
42. Prieto-Gómez B, Vázquez-Alvarez AM, Martínez-Peña JL, Reyes-Vázquez C, Yang PB, Dafny N. Methylphenidate and amphetamine modulate differently the NMDA and AMPA glutamatergic transmission of dopaminergic neurons in the ventral tegmental area. *Life Sci.* 2005; **77:** 635 – 649.
43. Acerbo MJ, Lee JM, Delius JD. Sensitization to apomorphine, effects of dizocilpine NMDA receptor blockades. *Behav Brain Res*. 2004; **151:** 201 – 208.
44. Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*. 1991; **251:** 85 – 87.