J Med Sci 2008;28(6):245-253
http://jms.ndmctsgh.edu.tw/2806245.pdf

The Role of the NR2B Subunit of NMDA Receptors in Morphine Rewarding, Drug Seeking, and Behavioral Sensitization

Chung-Shiun Yang, Jen-Hsin Kao, Eagle Yi-Kung Huang, and Pao-Luh Tao*

Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, Republic of China

Background: Studies indicate that the NR2B subunits of N-methyl-D-aspartate (NMDA) receptors are involved in the rewarding effects of morphine. In the present study we further investigated the role and action sites of the NR2B subunit of NMDA receptors in morphine rewarding and other behaviors related to drug addiction, such as drug seeking and behavioral sensitization. **Methods:** A selective antagonist of the NR2B subunit of NMDA receptors, ifenprodil, was locally injected into the nucleus accumbens (NAc) or ventral tegmental area (VTA) in male Sprague Dawley (S.D.) rats to block the NMDA receptors that contain NR2B subunits. A conditioned place preference (CPP) test was used to examine the rewarding and drug-seeking effects. Locomotor activity tests were used to determine the behavioral sensitization induced by chronic morphine treatment. **Results:** Morphine-induced rewarding and drug-seeking behavior were abolished when the NR2B subunits of NMDA receptors at NAc were blocked by ifenprodil that was either coadministered with morphine during CPP conditioning or posttreated during the morphine-withdrawal period. In contrast, morphine still induced rewarding, drug-seeking behavior, and behavioral sensitization when ifenprodil was injected at the VTA. On the other hand, only when ifenprodil was coadministered with morphine did it partially inhibit the behavioral sensitization induced by morphine. **Conclusions:** These data imply that at least the NR2B subunits of NMDA receptors in the NAc, but not VTA, are involved in morphine-induced rewarding and drug-seeking effects.

Key words: morphine, NR2B, nucleus accumbens, rewarding, conditioned place preference

INTRODUCTION

Morphine is still the most effective analgesic to treat postoperative pain and cancer pain in the clinic. However, chronic use of morphine may lead to the development of physical and psychological dependence, so called addiction, which may cause social problems. Repeated administration of morphine or psychostimulants, such as cocaine or amphetamine, in animals results in increased locomotor or stereotyped behaviors[1-5]. This phenomenon can persist for a long time after drug abstinence and has been named "behavioral sensitization"[6]. Several pieces of evidence demonstrate that the mesolimbic dopaminergic system, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), is important in opioid-induced rewarding effects and behavioral sensitization[7]. Opioids activate mesolimbic dopaminergic neurons through the inhibition of GABAergic interneurons in the VTA[8] and subsequently disinhibit the dopaminergic neurons, causing an increase of dopamine transmission to the NAc[9].

Several findings have indicated that the glutamatergic system plays a role in addiction related directly or indirectly to the modification of the activity of the dopaminergic system[10-12]. Several animal studies indicated that systemic administration of glutamate receptor antagonists, in particular the N-methyl-D-aspartate (NMDA) receptor antagonists, could inhibit morphine-induced physical dependence or rewarding[13-20]. Moreover, local administration of NMDA receptor antagonists into the NAc or VTA[21-23] could also attenuate the morphine-induced rewarding effect. It has been suggested that activation of NMDA receptors in the mesolimbic pathway may play an important role in morphine addiction.

Functional NMDA receptors are heteromeric complexes with at least one NR1 subunit, one NR2 (A-D) subunit, and two other subunits[24]. NR1, NR2A, and NR2B, but not NR2C or NR2D, are distributed in reward-related brain regions[24]. Recent studies suggested that the NR2B-containing NMDA receptor may be more important in mor-

Received: August 5, 2008; Revised: August 15, 2008; Accepted: August 27, 2008
*Corresponding author: Pao-Luh Tao, Department of Pharmacology, National Defense Medical Center, P.O. Box 90048-504, Nei-Hu 114, Taipei, Taiwan, Republic of China. Tel:+886-2-87923100 ext 18639; Fax:+ 886-2-87923155; E-mail: pltao@ndmctsgh.edu.tw

phine addiction than the NR2A-containing NMDA receptors[25-27]. Systemic administration of ifenprodil, a selective antagonist of the NR2B-containing NMDA receptor, suppresses morphine-induced rewarding and drug seeking in mice[26]. Local injection of ifenprodil into the lateral ventricle also dose-dependently attenuated morphine-induced place preference in rats[27]. Intracerebroventricular injection with an antibody against NR2B subunits abolished the morphine-induced rewarding effect in mice, whereas antibodies against NR1 and NR2A subunits did not affect the rewarding effects of morphine[25].

Although the importance of the NR2B-containing NMDA receptors in morphine addiction is known, it has not yet been identified how the NR2B-containing NMDA receptors interact with addiction-related neural circuits. In the present study, we further investigated the role and sites of action of NR2B-containing NMDA receptors involved in morphine-induced rewarding, drug-seeking behavior, and behavioral sensitization in rats.

MATERIALS AND METHODS

Animals

Male Sprague Dawley (S.D.) rats, weighing 300-400 g, were used in this study (from the National Experimental Animal Centre, Taipei, Taiwan, R.O.C.). All rats were kept in an animal room with a 12 h light/dark cycle, at 25±2°C and 55% humidity. Standard diet and water were provided *ad libitum*. The animals were acclimated for at least 1 week before the experiments. The care of animals was conducted in accordance with institutional and international standards (Principles of Laboratory Animal Care, NIH) and the protocol received the approval of the Institutional Animal Care and Use Committee of the National Defense Medical Center, Taiwan, R.O.C. There were at least seven animals in each experimental group.

Intra-NAc/VTA Cannulation

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). An incision was made in the scalp, and holes were drilled in the skull for the placement of a 23 gauge stainless steel guide cannula into the NAc [anteroposterior (AP) +1.7 mm, lateral (L)± 0.7 mm, dorsoventral (DV) -7.4 mm] or VTA (AP -6.04 mm, L± 0.5 mm, DV -8.9 mm), 1.0 mm above the intended site of injection. Guide cannulae were anchored to the skull with sterile stainless steel screws and acrylic dental cement. After the surgery, a stainless steel obturator was inserted into the guide in order to prevent cannula occlusion.

Schedule of Drug Administration

A 20-day schedule was used in this study. On day -6, the cannulation surgery was performed. Conditioned place preference (CPP) testing was conducted in the morning on day 1, day 8, and day 13. Drug injections [intraperitoneally administered saline (1 ml/kg) or morphine (5 mg/kg); intra-NAc or intra-VTA administered saline (1 μl/site) or ifenprodil (1 μg/site)] and conditioning were performed from day 2 to day 7 with intraperitoneal (i.p.) saline injection in the morning and drug injection in the afternoon. For the control group, saline was injected instead of drug. Morphine administration was ceased on day 8. Ifenprodil (1 μg/site) (for the posttreated group) or saline (for the control/morphine/cotreated group) was injected twice per day (9:00 a.m. and 17:00 p.m.) for 4 days from day 9 through to day 12. Locomotor activity tests were conducted in the afternoon on day 0 (after saline injection as control), day 1 (after morphine injection as acute morphine), and day 13 (after morphine injection as chronic morphine).

There were two main groups of animals: the NAc cannulated group and the VTA cannulated group. The following four subgroups were in each of the major groups. (1) S/S (control) group: saline (i.p.) conditioning on days 2-7 and saline posttreatment at NAc or VTA (1.0 μg/site, b.i.d.) on days 9-12. (2) M/S group: morphine (5 mg/kg, i.p.) conditioning on days 2-7 and saline posttreatment at NAc or VTA (1.0 μl/site, b.i.d.) on days 9-12. (3) (M+IFEN) group: morphine (5 mg/kg, i.p.) conditioning with coadministration of ifenprodil (1.0 μg/site, NAc or VTA) on days 2-7. (4) (M-IFEN) group: morphine (5 mg/kg, i.p.) conditioning on days 2-7 with ifenprodil posttreatment at NAc or VTA (1.0 μg/site, b.i.d.) on days 9-12. There was another group (IFEN group) with NAc cannulated drug administration, with which we intended to investigate whether ifenprodil given at NAc by itself would induce rewarding or aversive effects. Rats in this group were conditioned with ifenprodil (1.0 μg/site, NAc) on days 2-7. Each subgroup contained 8-16 rats before surgery. The surviving animals of each group decreased to 7-12 after the surgery for intra-NAc or VTA cannulation and chronic treatment with drugs.

Conditioned Place Preference (CPP) Test

Drug-rewarding and drug-seeking effects were measured by the CPP test. In this study, a distinctive environment was paired repeatedly with administration of a drug and a different environment was associated with a nondrugged (saline) state. The CPP test apparatus, made from an acrylic plastic box (70 cm×25 cm×25 cm), was divided into three compartments. Two identically sized

compartments (30 cm×25 cm×25 cm) were constructed at both sides, separated by a narrower compartment (10 cm ×25 cm×25 cm). The compartments were connected by two removable doors (10 cm×10 cm) in the central unit. One of the large compartments was covered by mosaic-type paper (3 cm×3 cm black and white squares) on the three walls, as a visual cue; the other large compartment was covered by purely white paper. To provide more visual cues, a blue and a red light bulb were hung separately above the two large compartments. During the experiments, the CPP apparatus was kept in an isolated dark room, which was free from noise. After each behavioral test or place conditioning, the whole box was cleaned thoroughly to prevent interference from the smell of feces and urine. For CPP conditionings, the rats were given saline in the morning and saline (control group) or drugs in the afternoon for 6 days. A distinctive environment was paired repeatedly with administration of saline and a different environment was associated with drug injection. The animals were kept for 40 min in the corresponding compartment with the doors closed. CPP tests were performed on the day before conditioning and the days after conditioning (day 8 and day 13). We determined the place preference by placing the rats into the central compartment of the apparatus with the doors opened for 15 min. The time that the rats stayed in each compartment was recorded to determine the place preference. After repeated morphine injections in one chamber and saline in the other, rats gravitated to the drug-paired compartment in an effort to reexperience the morphine effects. Their morphine place preference indicates how intensely the drug motivates drug seeking.

Measurement of the drug-rewarding or drug-seeking effects was determined by the increase in the time that rats spent in the compartment previously paired with drug injection, compared with the time spent in the saline-paired compartment.

Locomotor Activity (for Behavioral Sensitization Test)

The experiments of locomotor activity were performed in an isolated noise-free room. The test cages were transparent standard polypropylene animal cages (38 cm×22 cm×15 cm) and were placed in a photobeam activity system (San Diego Instruments, USA). A computer control unit registered the interruption of photobeams from five individual cages. Ambulatory activity of rats was recorded after the breaking of two consecutive beams; the breaking of a single photobeam was considered as total activity. Activity was recorded in 5-min periods for 2 h immediately after drug or saline administration.

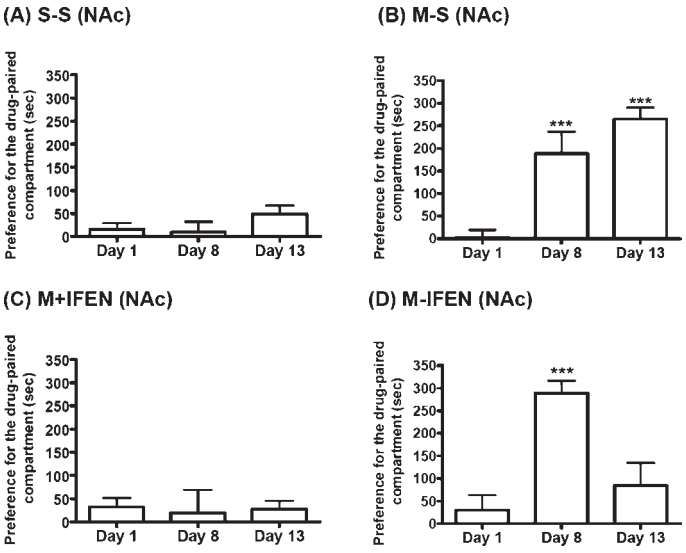

Fig. 1 Ifenprodil administratered at NAc inhibited morphine-induced rewarding and drug-seeking effects. (A) control group with saline (i.p.) conditioning on day 2-7 and saline treatment at NAc (1.0 μl/site, bid) on day 9-12 (B) chronic morphine (5 mg/kg, i.p.) conditioning on day 2-7 and saline treatment at NAc on day 9-12 (C) chronic morphine (5 mg/kg, i.p.) co-administered with ifenprodil (1.0μg/site, NAc) (day 2-7) and saline treatment at NAc on day 9-12 (D) chronic morphine (day 2-7) with ifenprodil post-treatment at NAc (day 9-12). Data are presented as mean±S.E.M. (n ≧8). One way ANOVA and Newman-Keuls test were used to analyze the data. ***$P < 0.001$ when compared to day 1.

Data Analysis

The data were expressed as the mean± SEM. Student's t test was used to analyze the differences between two groups. Analysis of variance was used to assess the statistical significance for repeated measures of the data, and the differences between the individual mean values in different groups were analyzed by ANOVA followed by the Newman—Keuls test. The differences were considered to be significant at $p<0.05$.

RESULTS

Ifenprodil Administered in the NAc Inhibited Morphine-induced Rewarding and Drug-seeking Effects

As shown in Fig. 1, no rat showed significant place preference for the drug-associated compartment before drug conditioning, which indicated that the CPP apparatus we used was of a nonbiased design[28]. In the control group (Fig. 1A), after 6 days of conditioning with saline (days 2-7, 1 ml/kg, i.p.), rats showed no place preference on day 8.

Further treatment with saline at NAc (1 μl/site) for 4 days (days 9-12) still did not induce any place preference on day 13 (Fig. 1A). In the morphine group (Fig. 1B), after 6 days of conditioning with morphine (days 2-7, 5 mg/kg, i.p.), rats showed significant place preference for the drug-paired compartment ($p<0.001$) on day 8. The rats still showed significant place preference ($p<0.001$) on day 13, even after withdrawal of morphine for 4 days and the administration of saline at NAc (1 μl/site) during those 4 days (days 9-12). These data indicate that morphine has a rewarding effect and a drug-seeking effect under our protocol. Ifenprodil (NAc, 1 μg/site) by itself did not show rewarding, aversive, or drug-seeking effects (Fig. 2). When ifenprodil (1 μg/site, NAc) was coadministered with morphine (5 mg/kg, i.p., q.d., 6 days) during conditioning, no place preference could be seen on day 8 or day 13 (Fig. 1C). Furthermore, when ifenprodil (1 μg/site, NAc, b.i.d.) was given only during days 9-12, after morphine conditioning during days 2-7, it still could abolish the drug-seeking effect on day 13, as shown in Fig. 1D.

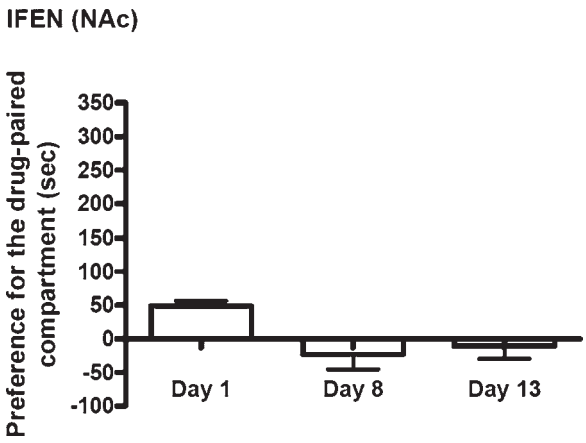

Fig. 2 Ifenprodil (NAc, 1.0 μg/site) did not induce rewarding, aversive or drug-seeking effects. Rats were given ifernprodil conditioning (1.0 μg/site, NAc) on day 2-7 and saline treatment at NAc (1.0 μl/site, bid) on day 9-12. Data are presented as mean ± S.E.M. (n=8). One way ANOVA and Newman-Keuls test were used to analyze the data.

Ifenprodil Administered in the NAc did not Inhibit Morphine-induced Behavioral Sensitization

Repeated administration of morphine results in an increase in locomotor activity that persists for a long period after drug abstinence. This is called "behavioral sensitization"[6]. In the present study, rats were treated with morphine six times in 6 days and then withdrawn from the drug for 5 days. Locomotor activity was determined on days 0, 1, and 13.

In the control group, saline (1 ml/kg, i.p.) administered acutely or chronically (1 ml/kg, i.p., days 2-7 and 1 μl/site at NAc, twice per day, days 9-12) did not affect the ambulatory activity (Fig. 3A) or total locomotor activity (Fig. 3B). Acute administration of morphine (5 mg/kg, i.p.) did not affect the ambulatory activity (Fig. 3C) or total locomotor activity (Fig. 3D) either. However, rats showed significant increases in ambulatory activity (Fig. 3C) and total locomotor activity by morphine challenge (Fig. 3D) after treatment with morphine six times in 6 days followed by withdrawal from the drug for 5 days, indicating that the animals had developed behavioral sensitization. When ifenprodil (1 μg/site, NAc) was coadministered with morphine (5 mg/kg, i.p., q.d., 6 days) during conditioning (Figs 3E & 3F) or was given only during days 9-12 after morphine conditioning (Figs 3G & 3H), rats still showed significant increases in ambulatory activity (Figs 3E & 3G) and total locomotor activity (Figs 3F & 3H) by morphine challenge. These data indicated that ifenprodil administered into the NAc did not inhibit morphine-induced behavioral sensitization.

Ifenprodil Administered in the VTA did not Inhibit Morphine-induced Rewarding and Drug-seeking Effects

In the control group (Fig. 4A), after 6 days of conditioning with saline (days 2-7, 1 ml/kg, i.p.), rats showed no place preference on day 8. Further treatment with saline in the VTA (1 μl/site) for 4 days (days 9-12) still did not induce any place preference on day 13 (Fig. 4A). In the morphine group (Fig. 4B), after 6 days of conditioning with morphine (days 2-7, 5 mg/kg, i.p.), rats showed significant place preference for the drug ($p<0.001$) on day 8 and still showed significant place preference ($p<0.001$) on day 13 even after withdrawal of morphine for 4 days, during which saline was administered in the VTA (1 μl/site, b.i.d., days 9-12). When ifenprodil (1 μg/site, VTA) was coadministered with morphine (5 mg/kg, i.p., q.d., 6 days) during conditioning (Fig. 4C), or was given only during days 9-12 (1 μg/site, VTA, b.i.d.) after morphine conditioning (Fig. 4D), rats still showed place preference on day 8 and day 13. These data indicated that ifenprodil administered in the VTA could not block the rewarding and drug-seeking effects induced by morphine.

Coadministration of Ifenprodil in the VTA with Morphine Partially Inhibits Morphine-induced Behavioral Sensitization

In the control group, saline (1 ml/kg, i.p.) administered acutely or chronically (1 ml/kg, i.p., days 2-7; and 1 μl/site at VTA, b.i.d., days 9-12) did not affect the ambulatory

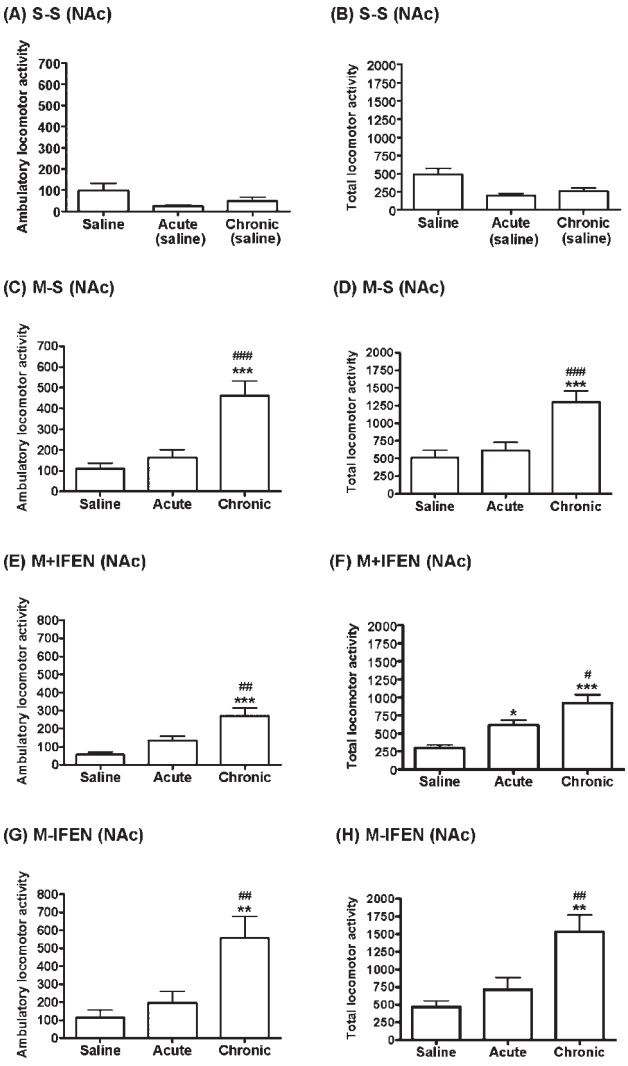

Fig. 3 Co- or post-administration of ifenprodil at NAc did not inhibit morphine-induced behavioral sensitization. Saline was injected at NAc (bid, day 9-12) after either saline (i.p.)(A, B) or morphine (5 mg/kg, i.p.)(C, D) conditioning (day 2-7). Ifenprodil (intra-NAc, 1.0 μg/site) was co-administered with morphine (day 2-7)(E, F). Ifenprodil (intra-NAc, 1.0 μ g/site) was administered after morphine conditioning (bid, day 9-12)(G, H). The ambulatory locomotor activity was shown in (A), (C), (E) and (G); the total locomotor activity was shown in (B), (D), (F) and (H). Data are presented as mean $\pm$ S.E.M. (n $\geqq$ 8). One way ANOVA and Newman-Keuls test were used to analyze the data. *P <0.05, **p < 0.01, ***p < 0.001 when compared to saline. $^{\#}P$ <0.05, $^{\#\#}p$ < 0.01 when compared to acute morphine.

activity (Fig. 5A) or total locomotor activity (Fig. 5B). Acutely administered morphine (5 mg/kg, i.p.) also did not affect the ambulatory activity (Fig. 5C) or total locomotor

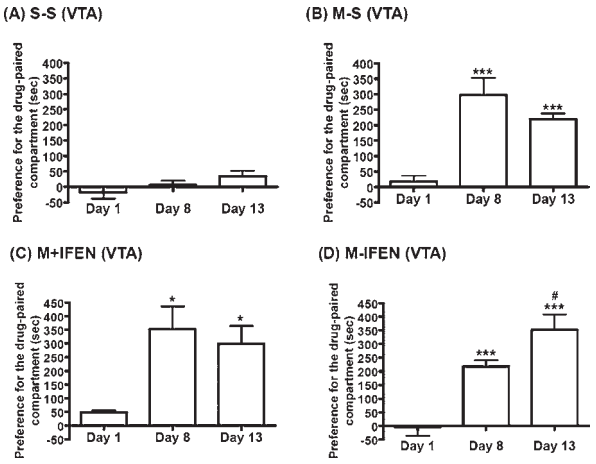

Fig. 4 Co- or post-administration of ifenprodil at VTA did not inhibit morphine-induced rewarding and drug-seeking effects. (A) control group with saline (i.p.) conditioning on day 2-7 and saline treatment at VTA on day 9-12 (B) chronic morphine (5 mg/kg, i.p.) conditioning on day 2-7 and saline treatment at VTA on day 9-12 (C) chronic morphine with ifenprodil (1.0 μ g/site) co-administered at VTA (day 2-7) (D) chronic morphine (day 2-7) with ifenprodil post-treatment at VTA (day 9-12). Data are presented as mean $\pm$ S.E.M. (n $\geqq$ 7). One way ANOVA and Newman-Keuls test were used to analyze the data. *P < 0.05, ***p < 0.001 when compared to day 1. $^{\#}P$ < 0.05 when compared to day 8.

activity (Fig. 5D). However, after treatment with morphine six times in 6 days followed by drug withdrawal and injection of saline in the VTA (1 μ l/site, bid) for 4 days, rats showed significant increases in ambulatory activity (Fig. 5C) and total locomotor activity induced by morphine challenge (Fig. 5D), indicating that the animals had developed behavioral sensitization. When ifenprodil (1 μ g/site, NAc) was coadministered with morphine (5 mg/kg, i.p., q.d., 6 days) during conditioning (Figs 5E & 5F), rats still showed significant increases in ambulatory activity (Fig. 5E) and total locomotor activity (Fig. 5F) by morphine challenge, but the increases were much less than in the morphine group (Figs 5C & 5D). When ifenprodil was given only during days 9-12 after morphine conditioning (Figs 5G & 5H), rats still showed increases in ambulatory activity (Figs 5E & 5G) and total locomotor activity (Figs 5F & 5H) induced by morphine challenge similar to the increases in the morphine group. These data indicate that coadministration of ifenprodil in the VTA with morphine partially inhibits morphine-induced behavioral sensitization. However, posttreatment with ifenprodil in

the VTA with morphine did not inhibit morphine-induced behavioral sensitization.

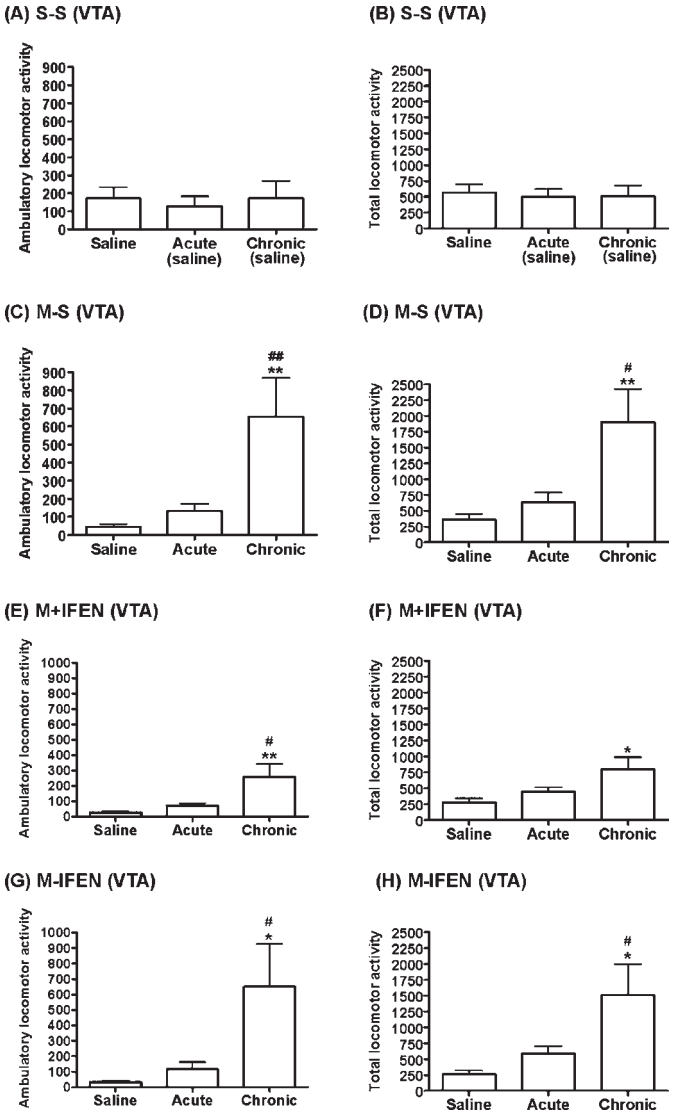

Fig. 5. The effect of co- or post-administration of ifenprodil at VTA on morphine-induced behavioral sensitization. Saline was injected at VTA (bid, day 9-12) after saline (i.p.) conditioning (day 2-7) (A, B) or morphine (5 mg/kg, i.p.) conditioning (day 2-7) (C, D). Ifenprodil (intra-VTA, 1.0 μg/site) was co-administered with morphine during day 2-7 (E, F) and was administered only after morphine conditioning (bid, day 9-12) in (G, H). The ambulatory locomotor activity was shown in (A), (C), (E) and (G); the total locomotor activity was shown in (B), (D), (F) and (H). Data are presented as mean ± S.E.M. (n ≧ 7). One way ANOVA and Newman-Keuls test were used to analyze the data. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to saline. $^{\#}P < 0.05$, $^{\#\#}p < 0.01$ when compared to acute morphine.

DISCUSSION

Several studies have demonstrated that the NR2B subunits of NMDA receptors are involved in the rewarding effects of morphine[25-27]. In the present study we further investigated the role and sites of action of the NR2B subunit of NMDA receptors in morphine rewarding, drug-seeking behavior, and behavioral sensitization. Our results indicated that the morphine-induced rewarding effect and drug-seeking behavior were abolished when ifenprodil was injected into the NAc during or after chronic morphine treatment. However, local injection of ifenprodil into the VTA did not alter the morphine-induced rewarding effect and drug-seeking behavior. On the other hand, chronic morphine-induced behavioral sensitization was not affected by the injection of ifenprodil into the NAc or VTA during or after chronic morphine treatment. These results imply that NR2B-containing NMDA receptors in the NAc, but not the VTA, play an important role in morphine-induced rewarding and drug-seeking behavior.

Activation of the NMDA receptor has been implicated in the expression of morphine tolerance and dependence. Pretreatment with competitive antagonists, LY274614, CGP39551[29,30], or a noncompetitive antagonist of the NMDA receptor, MK-801, effectively attenuates the tolerance of morphine or naloxone-precipitated withdrawal syndromes in adult rats[31-33]. In our lab we found that coadministration of a weak noncompetitive NMDA antagonist, dextromethorphan (DM), with morphine to maternal rats throughout pregnancy[34] or administration of DM to the neonatal rats after birth[35] significantly decreased naloxone-precipitated morphine-withdrawal behavior in the neonates. We also found that coadministration of DM with morphine attenuates the morphine-rewarding effect and related dopamine release at the NAc. Furthermore, DM may act at either the VTA or NAc to block the NMDA receptor activity induced by morphine withdrawal after subchronic morphine treatment[23].

Recent studies have suggested that the NR2B-containing NMDA receptor may be more important in morphine addiction than NR2A-containing NMDA receptors. Since all of these studies were investigated by either systemic[26] or intracerebroventricular injection of ifenprodil (a selective antagonist of NR2B-containing NMDA receptors)[27] or an antibody[25] against NR2B subunits, the site of action for NR2B-containing NMDA receptors is unclear. Two possible sites of action through which morphine induces place conditioning have been suggested: the NAc and VTA[36-39]. Ma and coworkers showed that injection of ifenprodil (2, 6, 20 μg/10 μl) into the right lateral ventricle of rats

could dose-dependently inhibit morphine-induced CPP when coadministered with morphine during a 4-day conditioning phase[27]. Therefore, in the present study we chose a dose of ifenprodil (1 μg/1 μl/site) and directly injected it into the NAc or VTA. This dose is at least comparable to the highest dose (20 μg) of ifenprodil that Ma et al. injected into the right lateral ventricle. We found that ifenprodil at this dose blocked morphine-induced CPP effects only when it was administered into the NAc; not when administered into the VTA. These results indicate that the NMDA receptors involved in morphine-induced rewarding and drug-seeking effects in NAc are NR2B-containing NMDA receptors. Since our preliminary data have shown that MK-801 (a selective NMDA receptor blocker) administered into the VTA (days 9-12) after morphine conditioning can block a morphine-induced drug-seeking effect (data not shown), we know that NMDA receptors in the VTA are involved in morphine-induced drug-seeking effects. However, these receptors may not be NR2B-containing NMDA receptors, and further studies are needed to elucidate this.

Behavioral sensitization is presumed to be mediated by the mesolimbic dopaminergic system[40,41], which is also thought to mediate the rewarding effect. Our previous results have shown the differential effects of DM on the rewarding and behavioral sensitization induced by subchronic morphine administration[23]. In the present study, we also found that ifenprodil administered in the NAc could block rewarding and drug-seeking effects, but not behavioral sensitization. Therefore, it seems that different neuronal systems mediate morphine-induced rewarding and behavioral sensitization.

ACKNOWLEDGMENTS

This study was supported by grants from the National Health Research Institutes (NHRI-EX96-9401NP).

REFERENCES

1. Babbini M, Davis WM. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. British journal of pharmacology. 1972;46:213-224.
2. Brady LS, Holtzman SG. Locomotor activity in morphine-dependent and post-dependent rats. Pharmacology, biochemistry, and behavior. 1981;14:361-370.
3. Kalivas PW, Duffy P. Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons. The Journal of pharmacology and experimental therapeutics. 1987;241:204-212.
4. Segal DS, Kuczenski R. Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain research. 1992;577: 351-355.
5. Segal DS, Kuczenski R. In vivo microdialysis reveals a diminished amphetamine-induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. Brain research. 1992;571:330-337.
6. Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain research. 1986;396:157-198.
7. Wise RA. Opiate reward: sites and substrates. Neuroscience and biobehavioral reviews. 1989;13:129-133.
8. Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. The Journal of physiology. 1992;450:455-468.
9. Leone P, Pocock D, Wise RA. Morphine-dopamine interaction: ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacology, biochemistry, and behavior. 1991;39:469-472.
10. Blaha CD, Yang CR, Floresco SB Barr AM, Phillips AG. Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. The European journal of neuroscience. 1997;9:902-911.
11. Floresco SB, Yang CR, Phillips AG, Blaha CD. Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. The European journal of neuroscience. 1998;10:1241-1251.
12. Tzschentke TM. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Progress in neurobiology. 2001;63:241-320.
13. Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science (New York, N.Y. 1991;251:85-87.
14. Bespalov A, Dumpis M, Piotrovsky L, Zvartau E. Excitatory amino acid receptor antagonist kynurenic acid attenuates rewarding potential of morphine. European journal of pharmacology. 1994;264:233-239.
15. Tzschentke TM, Schmidt WJ. N-methyl-D-aspartic acid-receptor antagonists block morphine-induced conditioned place preference in rats. Neuroscience letters. 1995;193:37-40.
16. Tzschentke TM, Schmidt WJ. Interactions of MK-801

and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. Behavioural brain research. 1997;84:99-107.
17. Del Pozo E, Barrios M, Baeyens JM. The NMDA receptor antagonist dizocilpine (MK-801) stereoselectively inhibits morphine-induced place preference conditioning in mice. Psychopharmacology. 1996;125: 209-213.
18. Popik P, Mamczarz J, Fraczek M, Wid?a M, Hesselink M, Danysz W. Inhibition of reinforcing effects of morphine and naloxone-precipitated opioid withdrawal by novel glycine site and uncompetitive NMDA receptor antagonists. Neuropharmacology. 1998;37:1033-1042.
19. Zhu H, Barr GA. Opiate withdrawal during development: are NMDA receptors indispensable? Trends in pharmacological sciences. 2001;22:404-408.
20. Huang EY, Liu TC, Tao PL. Co-administration of dextromethorphan with morphine attenuates morphine rewarding effect and related dopamine releases at the nucleus accumbens. Naunyn-Schmiedeberg's archives of pharmacology. 2003;368:386-392.
21. Popik P, Kolasiewicz W. Mesolimbic NMDA receptors are implicated in the expression of conditioned morphine reward. Naunyn-Schmiedeberg's archives of pharmacology. 1999;359:288-294.
22. Harris GC, Wimmer M, Byrne R, Aston-Jones G. Glutamate-associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. Neuroscience. 2004;129:841-847.
23. Lue WM, Huang EY, Yang SN, Wong CS, Tao PL. Post-treatment of dextromethorphan reverses morphine effect on conditioned place preference in rats. Synapse (New York, N.Y. 2007;61:420-428.
24. Mori H, Mishina M. Structure and function of the NMDA receptor channel. Neuropharmacology. 1995; 34:1219-1237.
25. Narita M, Aoki T, Suzuki T. Molecular evidence for the involvement of NR2B subunit containing N-methyl-D-aspartate receptors in the development of morphine-induced place preference. Neuroscience. 2000; 101:601-606.
26. Suzuki T, Kato H, Tsuda M, Suzuki H, Misawa M. Effects of the non-competitive NMDA receptor antagonist ifenprodil on the morphine-induced place preference in mice. Life sciences. 1999;64:PL151-156.
27. Ma YY, Guo CY, Yu P, Lee DY, Han JS, Cui CL. The role of NR2B containing NMDA receptor in place preference conditioned with morphine and natural reinforcers in rats. Experimental neurology. 2006;200: 343-355.
28. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Progress in neurobiology. 1998;56:613-672.
29. Tiseo PJ, Cheng J, Pasternak GW, Inturrisi CE. Modulation of morphine tolerance by the competitive N-methyl-D- aspartate receptor antagonist LY274614: assessment of opioid receptor changes. J Pharmacol Exp Ther. 1994;268:195-201.
30. Tokuyama S, Wakabayashi H, Ho IK. Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome. Eur J Pharmacol. 1996;295: 123-129.
31. Gonzalez P, Cabello P, Germany A, Norris B, Contreras E. Decrease of tolerance to, and physical dependence on morphine by, glutamate receptor antagonists. Eur J Pharmacol. 1997;332:257-262.
32. Tokuyama S, Wakabayashi H, Hoskins B, Ho IK. Naloxone-precipitated changes in biogenic amines and their metabolites in various brain regions of butorphanol-dependent rats. Pharmacol Biochem Behav. 1996;54:461-468.
33. Trujillo KA, Akil H. Opiate tolerance and dependence: recent findings and synthesis. New Biol. 1991;3:915-923.
34. Tao PL, Yeh GC, Su CH, Wu YH. Co-administration of dextromethorphan during pregnancy and throughout lactation significantly decreases the adverse effects associated with chronic morphine administration in rat offspring. Life Sci. 2001;69:2439-2450.
35. Yeh GC, Tao PL, Chen JY, Lai MC, Gao FS, Hu CL. Dextromethorphan attenuates morphine withdrawal syndrome in neonatal rats passively exposed to morphine. Eur J Pharmacol. 2002;453:197-202.
36. Phillips AG, LePiane FG. Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacol Biochem Behav. 1980;12:965-968.
37. Bozarth MA. Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. Brain Res. 1987;414:77-84.
38. Bals-Kubik R, Ableitner A, Herz A, Shippenberg TS. Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. J Pharmacol Exp Ther. 1993;264:489-495.

39. Olmstead MC, Franklin KB. The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. Behav Neurosci. 1997;111:1324-1334.
40. Joyce EM, Iversen SD. The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci Lett. 1979;14:207-212.
41. Vezina P, Stewart J. Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacol Biochem Behav. 1984; 20:925-934.