EFFECTS OF THE NON-COMPETITIVE NMDA RECEPTOR ANTAGONIST IFENPRODIL ON THE MORPHINE-INDUCED PLACE PREFERENCE IN MICE

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Abstract: The effects of ifenprodil, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, on the morphine-induced place preference were examined in mice. Morphine (1-5 mg/kg, s.c.) produced a dose-related place preference in mice. In contrast, ifenprodil alone (5-20 mg/kg, i.p.) did not produce either preference or aversion for the drug-associated place. Pretreatment with ifenprodil (5-20 mg/kg, i.p.) suppressed the place preference produced by morphine in a dose-dependent manner. These results indicate that ifenprodil suppresses the rewarding effect produced by morphine. © 1999 Elsevier Science Inc.

Key Words: morphine, NMDA, ifenprodil, conditioned place preference

Introduction

Although morphine is a potent analgesic agent that is widely used for the relief of cancer pain, it produces both psychological and physical dependence. It has been reported that the N-methyl-D-aspartate (NMDA) receptor, which is one of the principal excitatory amino acid receptors in the central nervous system (1), is involved in the development of tolerance and physical dependence caused by the chronic administration of morphine (2). As for the rewarding effect of morphine, the non-selective glutamate receptor antagonist kynurenic acid has been shown to attenuate the morphine-induced conditioned place preference (CPP) in rats (3). The CPP paradigm (4) is now commonly used to study the rewarding properties of various drugs. The morphine-induced place preference is also blocked by treatment with the non-competitive NMDA receptor antagonists dizocilpine (MK-801) and memantine (5, 6). These findings suggest that NMDA receptors may be implicated in the rewarding effect of morphine.

A variety of NMDA receptor subunits have been revealed by molecular cloning studies. There are two families of NMDA receptor subunits, NR1 and NR2 (A, B, C and D), and the...
expression of NR1 along with different NR2 subunits yields receptors with distinct pharmacological characteristics. Non-competitive NMDA receptor antagonists, such as dizocilpine and memantine, show high affinity for recombinant heteromeric NR1/NR2A and NR1/NR2B NMDA receptors (7). Recently, several lines of evidence have suggested that ifenprodil, which has been used to clinically stimulate brain circulation, selectively inhibits NR2B-containing NMDA receptors (7, 8). Thus, ifenprodil may be a useful tool for elucidating the role of NR2-containing NMDA receptors.

In the present study, to investigate the involvement of NR2B-containing NMDA receptor in rewarding effect of morphine, we examined the effect of ifenprodil on the morphine-induced place preference in mice.

Methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

Animals: Male ddY mice (20-23 g) were obtained from Tokyo Experimental Animals Inc. (Tokyo, Japan). The mice were housed at a temperature of 22 ± 1 °C with a 12-h light-dark cycle (light on 8:30 a.m. to 8:30 p.m.). Food and water were available ad libitum.

Place conditioning: Place conditioning was conducted as described previously using a minor modification of a biased procedure according to Suzuki et al (9). The apparatus consisted of a shuttlebox (15 cm x 30 cm x 15 cm; w x l x h) made of an acryl-resin board. The box was divided into two compartments of equal size by means of a sliding partition. One compartment was white with a textured floor; the other was black with a smooth floor. For conditioning, mice were immediately confined to the white compartment following drug injection and to the black compartment following vehicle injection.

Conditioning sessions (3 for drug; 3 for vehicle) were conducted once daily. Each session was 60 min in duration. On day 7, tests of conditioning were performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, and a neutral platform was inserted along the seam separating the compartments. Preference for a particular place was assessed in the drug-free state, after placing the animals on the neutral platform and allowing them free access to both compartments. The time spent in each compartment during a 900-sec session was then measured automatically in a blinded fashion by two infrared beam sensors (KN-80, Natsume Seisakusyo, Tokyo, Japan) which installed on each cover (3 cm from the center and the side) of white and black compartments. The position of the mouse was defined by the position of its whole body. All sessions were conducted under conditions of dim illumination and masking white noise.

Effect of ifenprodil on the morphine-induced place preference: Control mice were injected with vehicle (10 ml/kg) instead of drugs at each of the conditioning sessions; mice were confined to one compartment on the first day and to the other compartment on the next day after vehicle injection. This conditioning session was repeated 3 times. Morphine (1-5 mg/kg) or vehicle was injected i.p. 30 min before morphine treatment. To examine the effect of ifenprodil alone, ifenprodil or vehicle was injected i.p. on alternate days. The mice were pretreated with ifenprodil 30 min before saline injection and confined to the white compartment immediately after saline injection.

Drugs: Morphone hydrochloride was purchased from Sankyo Co., Tokyo, Japan. Ifenprodil was a generous gift from Greelan Pharmaceutical Co., Tokyo, Japan. Morphone was dissolved in saline, and ifenprodil tartrate was dissolved in DMSO (dimethyl sulfoxide; Wako Pure Chemical Ind.,
Tokyo, Japan) and diluted in 5% DMSO with 9% Tween 80/saline before use. These drugs were then injected in a volume of 10 ml/kg.

**Data analysis:** Conditioning scores represent the time spent in the drug-paired place minus the time spent in the vehicle-paired place, and are expressed as the mean ± S.E.M. Behavioral data were evaluated statistically with a one-way analysis of variance (ANOVA) followed by Dunnett’s test to determine whether individual doses produced a significant conditioning.

**Results**

**Morphine-induced place preference:** As shown in Fig. 1, the saline-control mice exhibited no preference for either drug- or saline-associated place. The mean conditioning score was -53.3 ± 34.3 sec. Morphine (1-5 mg/kg, s.c.) produced a dose-related place preference (F(3, 59) = 4.13, P<0.01). Significant conditioning was observed at doses of 1 mg/kg (95.7 ± 26.1 sec, P<0.01), 3 mg/kg (152.2 ± 24.7 sec, P<0.01) and 5 mg/kg (226.9 ± 48.4 sec, P<0.01).

**Motivational effect of ifenprodil:** Ifenprodil (5-20 mg/kg, i.p.) alone did not produce either preference or aversion for the ifenprodil-associated place (Figure 2-A). The mean conditioning...
scores associated with the vehicle, and 5, 10, or 20 mg/kg of ifenprodil were -14.8 ± 32.2 sec, 26.3 ± 52.2 sec, -1.8 ± 44.7 sec and 7.4 ± 20.0 sec, respectively.

**Effect of ifenprodil on the morphine-induced place preference:** Morphine (5 mg/kg, s.c.) produced a significant preference for the drug-associated place (Figure 2-B). The mean conditioning score was 192.8 ± 22.4 sec. However, this effect was inhibited by pretreatment with ifenprodil (5, 10 and 20 mg/kg) in a dose-dependent manner (F(3, 44) = 4.26, P<0.01). The mean conditioning scores were 89.8 ± 27.0 sec (P<0.05), 67.0 ± 35.1 sec (P<0.01) and -17.2 ± 20.0 sec (P<0.01), respectively.

![Fig. 2](image)

Effect of pretreatment with ifenprodil (5-20 mg/kg, i.p.) on the place conditioning produced by morphine (5 mg/kg, s.c.) in mice. Mice were pretreated with ifenprodil 30 min prior to saline (A) or morphine (B). Each column represents the mean with S.E.M. of 8 (A) and 16 (B) mice.

*p<0.05, **p<0.01 vs. the vehicle control (VEH).

**Discussion**

Several reports have demonstrated that some NMDA receptor antagonists, such as dizocilpine (MK-801) and phencyclidine, produce a significant place preference using the CPP method in animals (5, 10). In addition, NMDA receptor antagonists such as phencyclidine and ketamine are self-administered by monkeys (11) and are recognized as drugs of abuse in humans (12, 13). The present study showed that the NMDA receptor antagonist ifenprodil alone produced neither place preference nor place aversion. It has also been shown that ifenprodil does not lead to
abuse in clinical use. Furthermore, it has been reported that dizocilpine but not ifenprodil produces hyperlocomotion and disrupts learning (14-16). This difference in pharmacological properties may be due to a difference in affinity for NMDA receptor subunits. Ifenprodil is a non-competitive NMDA receptor antagonist which prefers NR2B-containing NMDA receptors to NR2A-containing NMDA receptors (7, 8). Other NMDA receptor antagonists (such as dizocilpine, phencyclidine and ketamine) prefer both NR2B- and NR2A-containing NMDA receptors (7, 8). Therefore, the effectiveness of ifenprodil may result from its low affinity for NR2A-containing NMDA receptors and NR2A-containing NMDA receptors may play an important role in the NMDA antagonists-induced rewarding effect. However, further investigation is necessary to clarify these mechanisms.

Earlier studies have demonstrated that the NMDA receptor antagonists dizocilpine and memantine suppress the morphine-induced place preference using the CPP method in mice and rats (5, 6). Dizocilpine and memantine have a high affinity for NR2A and NR2B-containing receptors (7), which leads to the hypothesis that NR2A and/or NR2B-containing NMDA receptors may play an important role in the rewarding effect of morphine. The present study demonstrated that the morphine-induced place preference was markedly inhibited by pretreatment with ifenprodil, which is a selective antagonist for NR2B-containing NMDA receptors. Therefore, this finding constitutes further evidence that the activation of NR2B- (rather than NR2A-) containing NMDA receptors (perhaps NR1/NR2B) may play a pivotal role in the rewarding effect of morphine.

Based on behavioral, neurochemical and electrophysiological studies, it is well known that activation of the mesolimbic dopamine (DA) system may be responsible for the morphine-induced place preference (17). Under our experimental conditions, it is unclear whether ifenprodil has a suppressive effect on the morphine-induced activation of DA transmission. Therefore, we also examined the effect of ifenprodil on the morphine-induced DA-related behavior, hyperlocomotion, in mice. The morphine-induced hyperlocomotion was dose-dependently decreased by pretreatment with ifenprodil (unpublished data), suggesting that ifenprodil may have a suppressive effect on the morphine-induced activation of DA transmission. Many investigators have indicated that mesolimbic dopamine neurons may be controlled through an NMDA receptor in the ventral tegmental area and the nucleus accumbens (18, 19). Interestingly, the density of NR2B subunit mRNA in the nucleus accumbens is higher than that of NR2A subunit mRNA (20). Therefore, these findings lead to the suggestion that ifenprodil may inhibit the activation of the mesolimbic DA system by morphine through the blockade of NR2B-containing NMDA receptors in the nucleus accumbens; as a result, ifenprodil may suppress the morphine-induced place preference. On the other hand, the NMDA receptor antagonist-induced place preference might result from activation of the mesolimbic dopamine system through NR2A-containing NMDA receptors.

The present results indicate that a selective antagonist for NR2B-containing NMDA receptors, ifenprodil, suppresses the morphine-induced place preference in mice. Unlike dizocilpine and ketamine, ifenprodil alone produced neither place preference nor place aversion. Therefore, NR2B-containing NMDA receptors may be involved in the rewarding effect of morphine, and ifenprodil may be useful for the treatment of morphine addiction.

Acknowledgments

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Reference

Ifenprodil Suppresses Morphine-induced Reward

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