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Short communication

Blockade of morphine-induced place preference by diazepam in mice

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Abstract

The effects of diazepam on morphine-induced place preference were examined in mice. Pretreatment with diazepam (2 mg/kg i.p.) 30 min prior to morphine injection significantly abolished the morphine (5 mg/kg s.c.)-induced place preference, and this effect of diazepam was antagonized by pretreatment with flumazenil. In addition, pretreatment with diazepam prevented the morphine (5 mg/kg s.c.)-induced increase in dopamine turnover in the limbic forebrain. These results suggest that pretreatment with diazepam may suppress the rewarding effects of morphine.

Keywords: Morphine; Diazepam; Conditioned place preference; (Mouse)

1. Introduction

The rewarding properties of opioid receptor agonists have been examined using the conditioned place preference paradigm. A great deal of evidence suggests that the dopamine system is involved in the reward produced by opioids. For example, the conditioned place preference produced by morphine is abolished by pretreatment with a dopamine D_1 receptor antagonist in mice (Suzuki et al., 1993). Morphine-induced place preference is also attenuated by 6-hydroxydopamine lesion of the nucleus accumbens (Shippenberg et al., 1993). Moreover, in vivo microdialysis studies have demonstrated that μ-opioid receptor agonists increase dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988). These reports have indicated that changes in mesolimbic dopamine neuron activity may play an important role in the rewarding effect of μ-opioid receptor agonists. Electrophysiological studies have indicated that systemic administration of morphine activates dopamine neurons in the ventral tegmental area (Matthews and German, 1984). Furthermore, the μ-opioid receptor agonist [D-Ala^2, MePhe^4,Gly-ol^5]enkephalin (DAGO) inhibits only non-dopaminergic neurons (γ-aminobutyric acid (GABA) interneurons projecting to the ventral tegmental area), indicating that the activation of μ-opioid receptors may increase the activity of the mesolimbic dopamine system by inhibiting GABAergic input to dopamine cells (Johnson and North, 1992).

Recently, Invernizzi et al. (1991) reported that systemic administration of diazepam decreases dopamine release in the nucleus accumbens. Furthermore, the microinjection of flurazepam into the nucleus accumbens attenuates dopamine transmission in the nucleus accumbens (Zetterström and Fillenz, 1990). In addition, the mesolimbic dopamine system is more sensitive to the benzodiazepine-induced inhibition of dopamine transmission than the nigrostriatal dopamine system (Zetterström and Fillenz, 1990; Invernizzi et al., 1991). Therefore, it would be useful to know whether or not a benzodiazepine receptor agonist suppresses the morphine-induced place preference which can be induced by an increase in dopamine transmission in the mesolimbic dopamine system.

In the present study, we investigated the effects of pretreatment with the benzodiazepine receptor agonist diazepam on morphine-induced place preference in mice. In addition, the effects of diazepam on the morphine-induced elevation of dopamine metabolites in the limbic forebrain were also examined.

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2. Materials and methods

2.1. Animals

Male *ddY* mice (20–23 g) were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed at a room 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.

2.2. Place conditioning

Place conditioning was conducted as previously described using a minor modification of a biased procedure (Suzuki et al., 1993; Funada et al., 1993). The apparatus consisted of a shuttlebox (15 × 30 × 15 cm: w × l × h) that was divided into two compartments of equal size; one compartment was white with a textured floor and the other was black with a smooth floor.

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. 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 animal on the neutral platform and allowing it free access to both compartments. The time spent in each compartment during a 900-s session was then measured by an infrared beam sensor (KN-80, Natsume Seisakusyo, Tokyo, Japan). All sessions were conducted under conditions of dim illumination (28 lux) and white masking noise. Morphine (5 mg/kg) and saline (10 ml/kg) were injected s.c. on alternate days. In a combination study, diazepam (0.5–2.0 mg/kg) was injected i.p. 30 min before morphine treatment. In an antagonism study, the mice were injected i.p. with flumazenil (30 mg/kg) 10 min before morphine treatment.

2.3. Neurochemical analysis

Using high-performance liquid chromatography with electrochemical detection (HPLC-ECD), the concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined as described previously (Narita et al., 1992). Diazepam (2 mg/kg i.p.) or vehicle (10 ml/kg) was given to the mice 30 min prior to the s.c. injection of saline or morphine. The mice were killed 50 min after s.c. injection of saline (10 ml/kg) or morphine (5 mg/kg), at the same time of day as the earlier conditioning session. The brain was quickly removed and the limbic forebrain (containing the nucleus accumbens and olfactory tubercles) was dissected on an ice-cold glass plate. Briefly, the brain was turned to expose the dorsal surface and a vertical cut was made through the anterior commissure. The resulting frontal portion was turned to expose the ventral surface. A vertical cut was made through the rhinal fissure and the small portion that included the accessory olfactory bulb and olfactory nucleus was removed. The resulting block of tissue was turned to expose the section, and the area bordered by the caudate putamen and the nucleus accumbens was cut vertically. The block of tissue that included the nucleus accumbens and olfactory tubercle was considered to be the main portion of the limbic forebrain. The tissues were frozen to −80°C and stored until analysis. The frozen tissues were homogenized in 500 μl of 0.2 M perchloric acid containing 100 μM EDTA (2Na) and 100 ng isoproterenol, as an internal standard. The homogenates were then centrifuged at 20000 × g for 25 min at 0°C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. Samples were analyzed by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). The HPLC system consisted of a delivery system (880-PU, Jasco, Japan), an analytical column (Eicompac, MA-50DS, Eicom Co., Japan) and a guard column (Eicom Co.). The electrochemical detector (EC-100, Eicom Co.) had a graphic electrode (WE-3G, Eicom Co.) and was used at a voltage setting of +0.7 V vs. an Ag/AgCl reference electrode. The mobile phase consisted of 0.1 M sodium acetate/0.1 M citric acid 1-octasulfonate and EDTA (2Na). The flow rate was set to 1.0 ml/min with a column temperature of 25°C.

2.4. Drugs

Morphine hydrochloride (Sankyo Co., Tokyo, Japan) was dissolved in sterile 0.9% NaCl solution. Diazepam (Profarma Co., Italy) and flumazenil (Yamanouchi Pharmaceutical Co., Tokyo, Japan) were suspended in vehicle consisting of 9% Tween 80 (Kishida Chemical Co., Osaka, Japan) in saline. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Research Biochemicals, Wayland, MA, USA) were dissolved in 0.02 N acetic acid for HPLC.

2.5. Statistical 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. Dopamine turnover was expressed in terms of the dopamine ratio: dopamine ratio = [DOPAC (ng/g of tissue) + HVA (ng/g)]/dopamine (ng/g). Differences in the conditioning score and in the dopamine ratio were evaluated with Dunnett's multiple comparison test.

3. Results

The saline-control mice exhibited no preference for either place; the mean conditioning score was -17.7 ± 31.3 s ($n = 10$). Diazepam (1.0 and 2.0 mg/kg) alone induced neither significant place preference nor place aversion in mice. The mean conditioning scores were 24.6 ± 45.7 s ($n = 8$) and 14.0 ± 31.2 s ($n = 7$), respectively (data not shown). Morphine (5 mg/kg) produced a significant preference for the drug-associated place ($P < 0.05$), with a mean conditioning score of 114.5 ± 30.5 s ($n = 15$). In contrast, diazepam at doses of 0.5 and 1.0 mg/kg tended to attenuate the morphine-induced place preference ($P > 0.05$). In the diazepam (2.0 mg/kg)-pretreated group, morphine-induced place preference was significantly abolished ($P < 0.05$), with a mean conditioning score of -35.3 ± 53.6 s ($n = 11$) (Fig. 1). The suppressive effect of diazepam was significantly antagonized by pretreatment with flumazenil (30 mg/kg) (mean conditioning score of 94.2 ± 27.9 s ($P < 0.05$, n = 11)). This mean conditioning score did not significantly differ from that of the morphine-treated group, and flumazenil itself did not affect the morphine-induced place preference, with a mean conditioning score of 87.9 ± 49.8 s (Fig. 1). The administration of flumazenil alone did not produce a preference for the drug- or saline-associated place (mean conditioning score of 21.4 ± 47.6 s, $n = 8$).

As shown in Fig. 2, the s.c. administration of morphine (5 mg/kg) significantly increased the dopamine ratio in the limbic forebrain: 50 min after morphine injection, the dopamine ratio was 28% ($P < 0.01$) higher

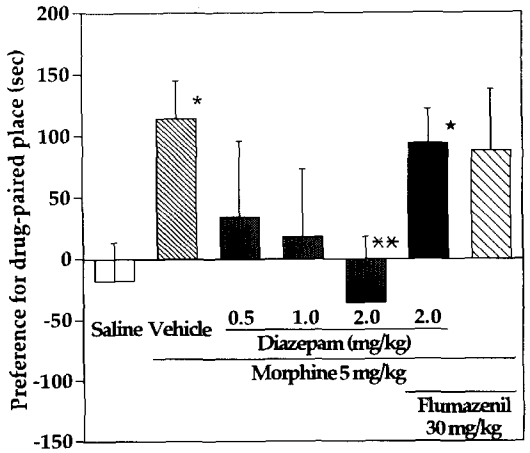

Fig. 1. Effect of diazepam on morphine-induced place preference conditioning and the antagonistic effect of flumazenil on the abolishment of morphine-induced place preference by diazepam in mice. Each column represents the mean conditioning score with S.E.M. for 8–17 mice. Mice were injected with vehicle (10 ml/kg i.p.) or diazepam (0.5–2.0 mg/kg i.p.) 30 min before treatment with morphine (5 mg/kg s.c.). Flumazenil (30 mg/kg) was injected i.p. 10 min before morphine treatment. $^{*}P < 0.05$ vs. saline control. $^{**}P < 0.05$ vs. morphine-treated group. $^{*}P < 0.05$ vs. morphine plus diazepam group.

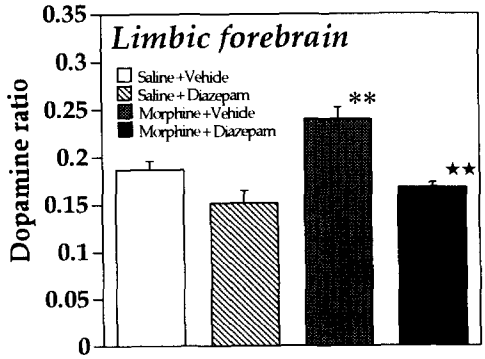

Fig. 2. Effect of pretreatment with diazepam on the morphine-induced increase in dopamine turnover in the mouse limbic forebrain. Mice were injected i.p. with diazepam (2 mg/kg) or vehicle (10 ml/kg) 30 min before morphine (5 mg/kg s.c.) treatment. Dopamine-related substances were measured by HPLC-ECD. Each column represents the mean with S.E.M. for 5–6 mice. The dopamine ratio was calculated as dopamine ratio = DOPAC + HVA/DA. $^{**}P < 0.01$ vs. saline + vehicle control. $^{**}P < 0.01$ vs. morphine + vehicle group.

than that in the saline plus vehicle-treated group. The morphine-induced increase in the dopamine ratio in the limbic forebrain was significantly suppressed by pretreatment with diazepam (2 mg/kg i.p.) (0.24 ± 0.01 to 0.17 ± 0.01; $P < 0.01$). However, diazepam (2 mg/kg i.p.) in combination with s.c. saline did not affect the dopamine ratio.

4. Discussion

The conditioned place preference paradigm is used to evaluate the relation between the rewarding stimulus properties of drugs and environmental stimuli. We previously established a method for examining morphine-induced place preference in mice (Suzuki et al., 1993; Funada et al., 1993). In the present study, we found that the benzodiazepine receptor agonist diazepam dose dependently abolishes morphine-induced place preference in mice. Furthermore, abolishment of the morphine-induced place preference by diazepam was antagonized by pretreatment with flumazenil, a benzodiazepine receptor antagonist. These results suggest that diazepam may suppress morphine-induced place preference through benzodiazepine receptors. On the other hand, diazepam alone did not induce place preference or place aversion under our conditions. This finding is inconsistent with previous findings that diazepam produces place preference (Spyraki and Fibiger, 1988). This discrepancy may be due to differences in the animal species (rat vs. mouse) and conditioning procedure (pre-conditioning procedure vs. biased procedure) used in the two studies.

Based on behavioral, neurochemical and electrophysiological studies, activation of the mesolimbic dopamine system may play an important role in mor-

phine-induced place preference (Matthews and German, 1984; Di Chiara and Imperato, 1988; Funada et al., 1993; Shippenberg et al., 1993). Recently, Johnson and North (1992) reported that the μ-opioid receptor agonist-induced excitation of dopamine cells in the ventral tegmental area may result from a reduction of the inhibitory synaptic input to the dopamine cells from GABA interneurons. This observation supports the present results that morphine-induced place preference was suppressed by diazepam, which may facilitate the inhibitory action of GABA (Twyman et al. 1989). Furthermore, we previously reported that 3 mg/kg morphine, which produces a significant conditioned place preference, also significantly elevates dopamine turnover in the limbic forebrain (Funada et al., 1993). In the present study, we further investigated whether the morphine-induced increase in dopamine turnover in the limbic forebrain was suppressed by pretreatment with diazepam. We found that although diazepam (2 mg/kg i.p.) alone did not affect dopamine turnover, it did suppress the morphine-induced increase in dopamine turnover in the limbic forebrain, which could suppress the morphine-induced place preference. Therefore, our findings suggest that the morphine-induced activation of the mesolimbic dopamine system may be suppressed by the stimulation of benzodiazepine receptors.

Under our experimental conditions, it is unclear whether the suppressive effect of diazepam on the morphine-induced activation of dopamine transmission occurs in the ventral tegmental area or in the nucleus accumbens. Many investigators have indicated that the mesolimbic dopamine neurons may be controlled through a $GABA_A$/benzodiazepine/Cl^- channel complex in the ventral tegmental area and nucleus accumbens (Zetterström and Fillenz, 1990; Invernizzi et al., 1991; Wisden et al., 1992; Chaudieu et al., 1994). However, GABA interneurons in the ventral tegmental area are innervated by descending GABA neurons from the nucleus accumbens and ventral pallidum (Kalivas, 1993). Furthermore, O'Brien and White (1987) reported that systemic administration of diazepam inhibits the activity of non-dopaminergic cells in the ventral tegmental area. Thus, the suppressive effect of diazepam on the morphine-induced increase in dopamine turnover in the limbic forebrain may occur primarily through the activation of a $GABA_A$/benzodiazepine/Cl^- channel complex located on a dopaminergic terminal in the nucleus accumbens rather than in the ventral tegmental area. Thus, the suppression of morphine-induced place preference by diazepam may be, at least in part, explained by the attenuation by diazepam of the morphine-induced increase in dopamine transmission in the limbic forebrain.

In conclusion, pretreatment with diazepam abolished the morphine-induced place preference and elevation of dopamine turnover in the limbic forebrain. These results suggest that the rewarding effect of morphine may be suppressed by activation of central benzodiazepine receptors in the nucleus accumbens.

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