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Stereoselective action of (+)-morphine over (–)-morphine in attenuating the (–)-morphine-produced antinociception via the naloxone-sensitive sigma receptor in the mouse

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Abstract

We have previously demonstrated that (+)-morphine and (–)-morphine given spinally stereoselectively attenuate the spinally-administered (–)-morphine-produced tail-flick inhibition in the mouse. The phenomenon has been defined as antianalgesia (Wu et al., 2005). Present studies were then undertaken to determine if the systemic administration of (+)-morphine and (–)-morphine also stereoselectively attenuates the systemic (–)-morphine-produced tail-flick inhibition and the effects of (+)-morphine and (–)-morphine are mediated by the naloxone-sensitive sigma receptor activation in male CD-1 mice. Pretreatment with (+)-morphine at a dose of 0.01–10 ng/kg given subcutaneously dose-dependently attenuated the tail-flick inhibition produced by subcutaneously-administered (–)-morphine (5 mg/kg). Pretreatment with (–)-morphine (0.01–1.0 mg/kg) given subcutaneously also attenuates the (–)-morphine-produced tail-flick inhibition. The ED₅₀ values for (+)-morphine and (–)-morphine for inhibiting the (–)-morphine-produced tail-flick inhibition were estimated to be 30.6 pg/kg and 97.5 μg/kg, respectively. The attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine pretreatment was reversed by the pretreatment with (+)-naloxone or by the sigma receptor antagonist BD1047 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine dihydrobromide) given subcutaneously. Pretreatment with (+)-pentazocine, a selective sigma receptor agonist, (1–10 mg/kg) given subcutaneously also attenuates (–)-morphine-produced tail-flick inhibition, which was restored by (+)-naloxone (4 mg/kg) or BD1047 (10 mg/kg) pretreated subcutaneously. It is concluded that (+)-morphine exhibits extremely high stereoselective action over (–)-morphine given systemically in attenuating the systemic (–)-morphine-produced antinociception and the antianalgesic effect of (+)-morphine and (–)-morphine is mediated by activation of the naloxone-sensitive sigma receptor.

Keywords

sigma receptor; antianalgesia; antinociception; morphine; opioid; mice

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1. Introduction

The naturally occurring morphine alkaloid, which is isolated from the juice of the opium poppy, *papaver somniferum*, is stereochemically identified as a *levorotatory* form. (–)-Morphine interacts with μ -opioid receptors to produce potent antinociception and other pharmacological effects. On the other hand, the *dextrorotatory* (+)-morphine, which is synthesized from sinomenine (Iijima et al., 1978a), does not have any affinity for μ -opioid receptors and therefore does not produce any μ -opioid receptor mediated pharmacological effects (Jacquet et al., 1977). However, we have previously demonstrated that (+)-morphine pretreated spinally attenuates the antinociception produced by spinally-administered (–)-morphine in the mouse. Similarly, pretreatment with (–)-morphine given spinally at a dose much higher than that of (+)-morphine also attenuates the (–)-morphine-produced antinociception (Wu et al., 2004, 2005). The phenomenon has been defined as antianalgesia. The present studies were then undertaken to determine if (+)-morphine and (–)-morphine given systemically also stereoselectively attenuate the antinociception produced by systemic (–)-morphine.

The stereoselective action of (+)-morphine over (–)-morphine given spinally in attenuating the (–)-morphine-produced antinociception clearly indicates that the antianalgesic effect of (+)-morphine and (–)-morphine is not mediated by the activation of the opioid receptors. We have previously reported that the attenuation of the spinally-administered (–)-morphine-produced antinociception induced by (+)-morphine or (–)-morphine given spinally is equally reversed by (+)-naloxone and (–)-naloxone, further support that the opioid receptor mechanism is not involved in the antianalgesic effect of (+)-morphine and (–)-morphine (Wu et al., 2005).

Other (+)-opiates such as (+)-pentazocine interact with sigma receptors, which are distinct from classical opioid receptors. Sigma receptors have been reported to play an important role in the modulation of analgesia produced by μ -, δ - or κ -opioid receptor agonists (Mei and Pasternak, 2002; Marrazzo et al, 2006; Chien and Pasternak, 1994). (+)-Pentazocine antagonizes (–)-morphine-produced analgesia, which is reversed by sigma receptor antagonist haloperidol (Chien and Pasternak, 1993). We now report that (+)-morphine, (–)-morphine and (+)-pentazocine pretreated systemically all attenuated the tail-flick inhibition produced by (–)-morphine given systemically and the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine, (–)-morphine or (+)-pentazocine was reversed by (+)-naloxone and by the sigma receptor antagonist BD1047. The findings indicate that like (+)-pentazocine, (+)-morphine and (–)-morphine attenuate the (–)-morphine-produced antinociception via activation of the naloxone-sensitive sigma receptors, originally proposed by Martin et al (1976) and later by Tsao and Su (1997).

2. Materials and Methods

2.1. Animals

Male CD-1 mice weighing 25–30 g (Charles River Breeding Laboratory, Wilmington, MA) were used. Animals were housed five per cage in a room maintained at $22 \pm 0.5^\circ\text{C}$ with an alternating 12-h light-dark cycle. Food and water were available *ad libitum*. Each animal was used only once. All experiments were approved by and conformed to the guidelines of the Animal Care Committee of the Medical College of Wisconsin.

2.2. Assessment of Antinociception

The nociceptive response was measured with the tail-flick test (D'Amour and Smith, 1941). Mice were gently held with the tail placed on the apparatus to measure the latency of the tail-flick response (Model TF6, EMDIE Instrument Co., Maidens, VA). The tail-flick response was elicited by applying radiant heat to the dorsal surface of the tail. The heat stimulus was set

to provide a pre-drug tail-flick response time of 3 to 4 s. The cutoff time for the heat stimulus was set at 10 s to avoid tissue damage.

2.3. Drugs and drug administration

(-)-Morphine sulfate, (+)-morphine (base), (+)-naloxone HCl and (+)-pentazocine succinate were obtained from the National Institute on Drug Abuse (Baltimore, MD, USA). BD1047 (*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine dihydrobromide) was purchased from Tocris (Ellisville, MO). (-)-Morphine sulfate, (+)-pentazocine succinate, (+)-naloxone HCl and BD1047 were dissolved in 0.9% saline. (+)-Morphine was dissolved in 5 N hydrochloric acid and then titrated with 1 N sodium hydroxide to pH 5–6, which was then diluted to the intended concentration with 0.9 % saline for injection. Drugs were administered subcutaneously. The injection volume was 0.1 ml per 10 g mouse body weight.

2.4. Experimental Protocols

The following experiments were performed; 1) the effects of (+)-morphine, (-)-morphine and (+)-pentazocine injected subcutaneously on the tail-flick inhibition produced by (-)-morphine given subcutaneously; 2) the effect of (+)-naloxone given subcutaneously on the attenuation of the (-)-morphine-produced tail-flick inhibition induced by (+)-morphine, (-)-morphine or (+)-pentazocine given subcutaneously; 3) the effect of the sigma receptor antagonist BD1047 given subcutaneously (Romieu et al., 2000, 2002; Maurice et al., 2003) on the attenuation of the (-)-morphine-produced tail-flick inhibition induced by (+)-morphine, (-)-morphine or (+)-pentazocine given subcutaneously.

2.5. Statistical analysis

The tail-flick latencies in seconds were presented as the mean $\pm$ S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's post-test or two-way ANOVA followed by Bonferroni's post-test or unpaired Student t-test was used to test the differences between groups. Nonlinear regression model was used to fit the dose-response curve and calculate the ED₅₀ values and 95 % confidence intervals for attenuation of (-)-morphine-produced tail-flick inhibition induced by (+)-morphine and (-)-morphine. The GraphPad Prism software was used to perform the statistics (version 4.1; GraphPad Software, Inc., San Diego, CA).

3. Results

3.1. Pretreatment with (+)-morphine time- and dose-dependently attenuates the tail-flick inhibition produced by (-)-morphine

Groups of mice were pretreated subcutaneously with (+)-morphine (10 ng/kg) for various times before subcutaneous injection of (-)-morphine sulfate (5 mg/kg) and the tail-flick response was measured 30 min thereafter. Other groups of mice pretreated subcutaneously with vehicle served as controls. The subcutaneous administration of (-)-morphine sulfate (5 mg/kg) produced 8.0 to 9.0 s of tail-flick latencies in mice pretreated subcutaneously with vehicle for different times. Pretreatment with (+)-morphine (10 ng/kg) for 30 or 60 min time-dependently attenuated the tail-flick inhibition produced by (-)-morphine. However, pretreatment with (+)-morphine for 90 or 120 min did not affect the tail-flick inhibition produced by (-)-morphine (Fig. 1).

The dose-response relationship for (+)-morphine to attenuate the (-)-morphine-produced tail-flick inhibition was then studied. Groups of mice were pretreated subcutaneously with various doses (0.001 to 100 ng/kg) of (+)-morphine given subcutaneously 60 min before subcutaneous administration of (-)-morphine sulfate (5 mg/kg) and the tail-flick responses were measured 30 min thereafter. Subcutaneous injection of (-)-morphine sulfate caused an increase of the

tail-flick inhibition in mice pretreated with the vehicle. Subcutaneous pretreatment with (+)-morphine at doses from 0.01 to 10 ng/kg dose-dependently attenuated the tail-flick inhibition produced by (–)-morphine and the attenuation of the (–)-morphine-produced tail-flick inhibition reached a maximum at 10 ng/kg. A higher dose of (+)-morphine (100 ng/kg) did not further attenuate the tail-flick inhibition produced by (–)-morphine (Fig. 2A). The ED₅₀ value for (+)-morphine for attenuating the (–)-morphine-produced tail-flick inhibition was estimated to be 30.6 pg/kg.

3.2. Pretreatment with (–)-morphine dose-dependently attenuates the tail-flick inhibition produced by (–)-morphine

We have previously reported that (–)-morphine at subanalgesic doses given intrathecally also attenuates (–)-morphine-produced tail-flick inhibition (Wu et al., 2004). The dose-response relationship for (–)-morphine given systemically to attenuate the (–)-morphine-produced tail-flick inhibition was then studied. Groups of mice were pretreated subcutaneously with (–)-morphine (0.01 to 1 mg/kg) for 60 min before subcutaneous injection of (–)-morphine (5 mg/kg) and the tail-flick response was then measured 30 min thereafter. Subcutaneous pretreatment with (–)-morphine from 0.1 to 1 mg/kg dose-dependently attenuated the tail-flick inhibition produced by (–)-morphine (Fig. 2B). Subcutaneous pretreatment with 0.5 mg/kg of (–)-morphine for 60 min did not affect the baseline tail-flick latency observed at 30 min after saline vehicle injection. The ED₅₀ value for (–)-morphine for the attenuation of the (–)-morphine-produced tail-flick inhibition was estimated to be 97.3 µg/kg. Thus, (+)-morphine is approximately 3,000,000-fold more potent than (–)-morphine in attenuating the tail-flick inhibition produced by (–)-morphine.

3.3. Pretreatment with (+)-naloxone reverses the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine

The non-opioid (+)-naloxone was used as an antagonist to determine if the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine pretreatment is mediated by a non-opioid mechanism. Groups of mice were pretreated subcutaneously with (+)-naloxone (0.1 to 4 mg/kg) 20 min before subcutaneous injection of (+)-morphine (10 ng/kg) or (–)-morphine (0.5 mg/kg). (–)-Morphine (5 mg/kg) was then injected subcutaneously 60 min after (+)-morphine or (–)-morphine- morphine injection and the tail-flick response was then measured 30 min after (–)-morphine injection. Pretreatment with (+)-morphine (10 ng/kg) attenuated the tail-flick inhibition produced by (–)-morphine. The attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine pretreatment was dose-dependently reversed by the pretreatment of (+)-naloxone. The reversal almost reached its maximum at 4 mg/kg (Fig. 3A). Similarly, pretreatment with (+)-naloxone (4 mg/kg) given systemically also reversed the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (–)-morphine (Fig. 3B). (+)-Naloxone (4 mg/kg) given alone did not affect (–)-morphine-produced tail-flick inhibition and did not change the baseline of tail-flick latency (data not shown).

3.4. Pretreatment with the sigma receptor antagonist BD1047 reverses the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine

The sigma receptor antagonist BD1047 was used to determine if the sigma receptors are involved in mediating the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine. Groups of mice were pretreated subcutaneously with various doses (1 to 10 mg/kg) of BD1047 and (+)-morphine (10 ng/kg) or (–)-morphine (0.5 mg/kg) or vehicle 45 min before subcutaneous injection of (–)-morphine (5 mg/kg) and the tail-flick response was then measured 30 min after (–)-morphine injection. Pretreatment with (+)-morphine attenuated the tail-flick inhibition produced by (–)-morphine. The

attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine was dose-dependently reversed by the pretreatment of BD1047 (Fig. 4A). Pretreatment with BD1047 (10 mg/kg) given alone for 45 min did not affect the (–)-morphine-produced tail-flick inhibition and BD1047 itself alone did not affect the tail-flick latency (data not shown). Similarly, subcutaneous pretreatment with (–)-morphine (0.5 mg/kg) attenuated the (–)-morphine-produced tail-flick inhibition. The attenuation of the (–)-morphine-produced tail-flick inhibition induced by (–)-morphine was reversed by the BD1047 pretreatment (Fig. 4B).

3.5. Pretreatment with (+)-pentazocine attenuates the tail-flick inhibition produced by (–)-morphine

The results of the experiments presented above clearly indicate that the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-morphine or (–)-morphine is mediated by the activation of the naloxone-sensitive sigma receptors. (+)-Pentazocine is a selective agonist for both the naloxone-sensitive and naloxone inaccessible sigma receptors (Tsao and Su, 1997; Tam et al., 1983). Thus systemic administration of (+)-pentazocine will be expected to attenuate the (–)-morphine-produced antinociception via the stimulation of the naloxone-sensitive sigma receptors. The effect of (+)-pentazocine given systemically on the tail-flick inhibition produced by systemic (–)-morphine was then studied. Groups of mice were pretreated subcutaneously with (+)-pentazocine (1 to 10 mg/kg) for 60 min before subcutaneous injection of (–)-morphine (5 mg/kg) and the tail-flick response was then measured 30 min after (–)-morphine. Subcutaneous pretreatment with (+)-pentazocine from 1 to 10 mg/kg dose-dependently attenuated the tail-flick inhibition produced by (–)-morphine (Fig. 5).

3.6. Pretreatment with (+)-naloxone or BD1047 reverses the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-pentazocine

Groups of mice were pretreated subcutaneously with (+)-naloxone (4 mg/kg) 20 min before subcutaneous injection of (+)-pentazocine (10 mg/kg) or BD1047 (10 mg/kg) co-administered with (+)-pentazocine. (–)-Morphine (5 mg/kg) was injected subcutaneously 60 min after (+)-pentazocine injection and the tail-flick response was then measured 30 min after (–)-morphine injection. Pretreatment with (+)-pentazocine (10 mg/kg) attenuated the tail-flick inhibition produced by (–)-morphine. The attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-pentazocine pretreatment was significantly reversed by the pretreatment of (+)-naloxone (Fig. 6A) and BD1047 (Fig. 6B).

4. Discussion

4.1. Pretreatment with (+)-morphine or (–)-morphine attenuates the antinociception produced by (–)-morphine

We have previously demonstrated that pretreatment with (+)-morphine or (–)-morphine given intrathecally attenuates the tail-flick inhibition produced by (–)-morphine given intrathecally, indicating that (+)-morphine and (–)-morphine induce their antianalgesic effects against (–)-morphine-produced antinociception at the spinal cord of the mouse (Wu et al., 2005). Present study was then conducted to determine if the (+)-morphine and (–)-morphine given systemically also attenuate the antinociception produced by systemic (–)-morphine. We found that pretreatment with either (+)-morphine or (–)-morphine given systemically also attenuated the tail-flick inhibition produced by (–)-morphine given systemically. The finding suggests that (+)-morphine and (–)-morphine also elicit their antianalgesic actions at the supraspinal sites. This view is supported by our recent finding that pretreatment with (+)-morphine or (–)-morphine given into the ventral periaqueductal gray attenuates the tail-flick inhibition induced by (–)-morphine given into ventral periaqueductal gray in rats (unpublished observation).

We found that (+)-morphine at a pg/kg dose range and (–)-morphine at a higher µg/kg dose range given systemically dose-dependently attenuates the (–)-morphine-produced tail-flick inhibition. (+)-Morphine is approximately 3,000,000-fold more potent than (–)-morphine in attenuating the (–)-morphine-produced antinociception. The stereoselective action of (+)-morphine over (–)-morphine given spinally (Wu et al., 2005) or systemically in attenuating the (–)-morphine-produced antinociception clearly indicates that the antianalgesic effect of (+)-morphine and (–)-morphine is not mediated by the activation of the opioid receptors.

4.2. (+)-Naloxone and the sigma receptor antagonist BD1047 block the naloxone-sensitive sigma receptors to reverse the attenuation of the (–)-morphine-produced antinociception induced by (+)-morphine or (–)-morphine

A naloxone-sensitive, haloperidol-sensitive, [³H](+)-SKF-10047-binding protein has been purified from the rat brain membrane in an affinity chromatography originally designed to purify sigma receptors (Tsao and Su, 1997). [³H](+)-SKF-10047 binding to the protein is inhibited by (+)-pentazocine, (–)-pentazocine, (–)-morphine, (–)-naloxone, haloperidol, or (+)-SKF-10047 but not the prototypic sigma receptor ligand, such as 1.3-di-o-tolylguanidine, (+)-3-PPP, and progesterone. This receptor is therefore tentatively defined as the naloxone-sensitive sigma receptor, which resembles the opiate sigma receptor originally proposed by Martin et al. (1976). (+)-Naloxone, an enantiomer of the non-selective µ-opioid receptor antagonist naloxone, has been shown not to have any affinity for µ-opioid receptors or block µ-opioid mediated analgesia (Iijima et al., 1978b). (+)-Naloxone and the sigma receptor antagonist BD1047 were then used as the receptor antagonist to determine if the antianalgesia induced by (+)-morphine or (–)-morphine is mediated by the activation of the naloxone-sensitive sigma receptors. We found in the present study that the (+)-morphine- and (–)-morphine-induced antianalgesic effect was reversed by (+)-naloxone and by the sigma receptor antagonist BD1047. We have found in our previous studies that the antianalgesia induced by (+)-morphine or (–)-morphine is reversed by (+)-naloxone and by (–)-naloxone in the spinal cord of male CD-1 mice (Wu et al., 2004, 2005). These findings indicate that the antianalgesia induced by (+)-morphine and (–)-morphine is mediated by the activation of the naloxone-sensitive sigma receptors. Our present studies also suggest that (–)-morphine induces the acute antinociceptive tolerance to (–)-morphine via the activation of the naloxone-sensitive sigma receptors and not due to the desensitization of the µ-opioid receptors by the (–)-morphine pretreatment.

4.3. (+)-Pentazocine attenuates the (–)-morphine-produced antinociception via the activation of the naloxone-sensitive sigma receptors

(+)-Pentazocine is a selective sigma receptor agonist, which binds both the naloxone-inaccessible and naloxone-sensitive sigma receptors (Tsao and Su, 1997; Tam, 1983; Tam and Cook, 1984; Martin et al., 1984). Thus, systemic administration of (+)-pentazocine will be like (+)-morphine to attenuate the (–)-morphine-produced antinociception via the activation of the naloxone-sensitive sigma receptors. We found in the present studies that systemic administration of (+)-pentazocine attenuated the (–)-morphine-produced tail-flick inhibition. The attenuation of (–)-morphine-produced tail-flick inhibition induced by (+)-pentazocine was reversed by (+)-naloxone and by BD1047. The results clearly indicate that like (+)-morphine or (–)-morphine, (+)-pentazocine stimulates the naloxone-sensitive sigma receptor for attenuating the (–)-morphine-produced antinociception.

Mei and Pasternak (2002) have previously demonstrated that (+)-pentazocine given supraspinally attenuates the antinociception produced by (–)-morphine given supraspinally. (+)-Pentazocine given supraspinally also attenuates the antinociception produced by δ- and κ-opioid agonists as well (Mei and Pasternak, 2002). Co-administration with sigma antagonist haloperidol potentiates the (–)-morphine-produced analgesia (Chien and Pasternak, 1995).

These observations indicate that (+)-pentazocine acts as a sigma receptor agonist to attenuate the antinociception produced by (–)-morphine and other opioid agonists at the supraspinal sites.

It is concluded that pretreatment with (+)-morphine or (–)-morphine given systemically attenuates the antinociception produced by systemic (–)-morphine. The attenuation of (–)-morphine-produced antinociception induced by (+)-morphine or (–)-morphine is mediated by the activation of the naloxone-sensitive sigma receptors. Similarly, pretreatment with (+)-pentazocine systemically attenuates the systemic (–)-morphine-produced antinociception via the naloxone-sensitive sigma receptors.

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Fig. 1.

Effect of different pretreatment times with (+)-morphine on the tail-flick inhibition produced by (-)-morphine. Groups of mice were pretreated subcutaneously with (+)-morphine (10 ng/kg) or vehicle for 30, 60, 90, or 120 min. (-)-Morphine (5 mg/kg) was then injected subcutaneously and the tail-flick response was measured 30 min after (-)-morphine administration. Each column represents the mean and the vertical bar represents the S.E.M. with 7 to 8 mice in each group. Two-way ANOVA followed by Bonferroni's post-test was used to test the difference between groups. The F values of interaction, treatment and time are $F(3,53) = 14.23$, $F(1,53) = 41.12$ and $F(3,53) = 13.70$, respectively; * $P < 0.001$ when compared with vehicle pretreatment group.

Fig. 2.

Pretreatment with (+)-morphine (A) or (-)-morphine (B) attenuates the tail-flick inhibition produced by (-)-morphine. Groups of mice were injected subcutaneously with different doses of (+)-morphine (0.001 to 10 ng/kg) (A), (-)-morphine (0.01–0.5 mg/kg) (B) or vehicle 60 min before subcutaneous injection of (-)-morphine (5 mg/kg) and the tail-flick response was measured at 30 min thereafter. Vehicle pretreated group served as control. Each column represents the mean and vertical bar represent S.E.M. with 7 to 10 mice in each group. The first column from the right represented control groups, which did not include in the statistic analysis. One-way ANOVA followed by Dunnett's post-test was used to test the difference

between groups; $F(6,50) = 12.99$ (A); $F(3,32) = 17.48$ (B); * $P < 0.05$, ** $P < 0.01$ compared with the vehicle injected group (the first column from the left).

Fig. 3.

Pretreatment with (+)-naloxone reverses the attenuation of the (-)-morphine-produced tail-flick inhibition induced by (+)-morphine (A) or (-)-morphine (B). Groups of mice were pretreated subcutaneously with different doses (0.1 to 4 mg/kg) of (+)-naloxone or vehicle for 80 min and (+)-morphine (10 ng/kg) or (-)-morphine (0.5 mg/kg) for 60 min. (-)-Morphine (5 mg/kg) was then injected subcutaneously and the tail-flick response was measured 30 min after the last injection. Each column represents the mean and the vertical bar represents the S.E.M. with 8 to 12 mice in each group. The first column from the left and the first two columns from the right represented control groups, which did not include in the statistic analysis in panel A. (A) One-way ANOVA followed by Dunnett's post-test was used to test the difference

between groups, the $F(4,43) = 5.435$, * $P < 0.05$, ** $P < 0.01$ compared with the vehicle injected group (the second column from the left); (B) Student's t-test was used to test the difference between groups, ** $P < 0.01$.

Fig. 4.

Pretreatment with the sigma receptor antagonist BD1047 reverses the attenuation of the (-)-morphine-produced tail-flick inhibition induced by (+)-morphine (A) or (-)-morphine (B). Groups of mice were co-administered subcutaneously with different doses (1 to 10 mg/kg) of BD1047 and (+)-morphine (10 ng/kg) or (-)-morphine (0.5 mg/kg) 60 min before (-)-morphine (5 mg/kg) given subcutaneously. The tail-flick responses were measured 30 min after the last injection. Each column represents the mean and the vertical bar represents the S.E.M. with 8 to 10 mice in each group. The first column from the left and the first column from the right represented control groups, which did not include in the statistic analysis in panel A. (A) One-way ANOVA followed by Dunnett's post-test was used to test the difference between groups,

the $F(3,30) = 34.11$, * $P < 0.01$ compared with the vehicle injected group (the first column from the left); (B) Student's t-test was used to test the difference between groups, ** $P < 0.01$.

Fig. 5.

Pretreatment with (+)-pentazocine attenuates the tail-flick inhibition produced by (-)-morphine. Groups of mice were injected subcutaneously with different doses (1, 5, 10 mg/kg) of (+)-pentazocine or vehicle 60 min before subcutaneous injection of (-)-morphine (5 mg/kg) and the tail-flick response was measured 30 min thereafter. Vehicle pretreated group served as control. Each column represents the mean and vertical bar represent S.E.M. with 8 to 10 mice in each group. The first column from the right represented control groups, which did not include in the statistic analysis. One-way ANOVA followed by Dunnett's post-test was used to test the difference between groups; $F(3,30) = 10.40$; * $P < 0.05$, ** $P < 0.01$ compared with the vehicle injected group (the first column from the left).

Fig. 6. Pretreatment with (+)-naloxone (A) or BD1047 (B) reverses the attenuation of the (–)-morphine-produced tail-flick inhibition induced by (+)-pentazocine. Groups of mice were pretreated subcutaneously with (+)-naloxone (4 mg/kg) for 80 min, BD 1047 (10 mg/kg) for 60 min and (+)-pentazocine (10 mg/kg) for 60 min. (–)-Morphine (5 mg/kg) was then injected subcutaneously and the tail-flick response was measured 30 min after the (–)-morphine injection. Each column represents the mean and the vertical bar represents the S.E.M. with 8 to 9 mice in each group. Student's t-test was used to test the difference between groups; * $P < 0.01$ compared with the vehicle injected group.