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Intraplantar injection of bergamot essential oil induces peripheral antinociception mediated by opioid mechanism

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ABSTRACT

This study investigated the effect of bergamot essential oil (BEO) containing linalool and linalyl acetate as major volatile components in the capsaicin test. The intraplantar injection of capsaicin (1.6 μg) produced a short-lived licking/biting response toward the injected paw. The nociceptive behavioral response evoked by capsaicin was inhibited dose-dependently by intraplantar injection of BEO. Both linalool and linalyl acetate, injected into the hindpaw, showed a significant reduction of nociceptive response, which was much more potent than BEO. Intraperitoneal (i.p.) and intraplantar pretreatment with naloxone hydrochloride, an opioid receptor antagonist, significantly reversed BEO- and linalool-induced antinociception. Pretreatment with naloxone methiodide, a peripherally acting μ-opioid receptor preferring antagonist, resulted in a significant antagonizing effect on antinociception induced by BEO and linalool. Antinociception induced by i.p. or intrathecal morphine was enhanced by the combined injection of BEO or linalool. The enhanced effect of combination of BEO or linalool with morphine was antagonized by pretreatment with naloxone hydrochloride. Our results provide evidence for the involvement of peripheral opioids, in the antinociception induced by BEO and linalool. Combined administration of BEO or linalool acting at the peripheral site, and morphine may be a promising approach in the treatment of clinical pain.

1. Introduction

The essential oil of bergamot (BEO; Citrus bergamia, Risso) is one of the most common essential oil and most familiar to the general public. BEO is obtained by cold pressing of the epicarp and in part of the mesocarp of the fresh fruit of bergamot. BEO consists of a volatile fraction (93–96%) and a non volatile fraction (4–7%); the former fraction contains monoterpene and sesquiterpene hydrocarbons and oxygenated derivatives such as linalool and linalyl acetate, and the latter fraction contains waxes, polymethoxylated flavones, coumarins and psoralens such as bergamottin and bergapten (Mondello et al., 1993; Dugo et al., 2000). Recently, BEO has been shown to reduce neuronal damage caused by excitotoxic stimuli (Corasaniti et al., 2007). (−) Linalool is the natural occurring enantiomer of the monoterpene compound found in the essential oil extracted from aromatic plants such as sauge, lavender, rose wood, thyme and bergamot. As previously reported (Peana et al., 2002, 2003, 2004), (−) linalool administration produced anti-inflammatory and antinociceptive activities in several behavioral assays. However, the antinociceptive efficacy of intraplantar BEO and linalool on capsaicin-induced nociceptive response is unknown.

The capsaicin (8-methly-*N*-vanillyl-6-noneamide) test is widely used as a model of pain in mice (Sakurada et al., 1992), rats (Pelissier et al., 2002) and humans (Hughes et al., 2002). We previously reported that subcutaneous (s.c.) injection of capsaicin into the hindpaw produced a short-lasting paw-licking/biting response, which was dose-dependently inhibited by intrathecally (i.t.) administered morphine (Sakurada et al., 1994). Activation of primary afferent nociceptors by capsaicin causes the release of nociceptive transmitters, substance P and glutamate from the dorsal spinal cord *in vivo* and *in vitro* (Gamse et al., 1979; Ueda et al., 1993; Sorkin and Mcadoo, 1993). In addition, it has been shown that capsaicin excites the C-fiber population of nociceptive afferents through transient receptor potential vanilloid type-1 (TRPV-1) receptors located in C-fiber type nociceptors (Di Marzo et al., 2002; Szallasi et al., 2007).

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In this study, the effects of BEO on capsaicin-evoked acute pain were investigated in comparison with linalool. In addition, here intraplantar injection BEO and linalool were tested for (1) production of antinociception in a capsaicin pain model, (2) assessment of the involvement of peripheral opioid system, and (3) modulation of the antinociceptive effect of morphine.

2. Materials and methods

2.1. Animals

Male ddY (SD) mice (Shizuoka Laboratory Center, Japan) weighing 22–26 g, at the time of testing, were used in these experiments. The mice were individually housed in a colony maintained in a controlled environment (12 h light/dark cycle, room temperature 23 °C, 50–60% relative humidity).

The animals had unlimited access to food pellets and water. All behavioral experiments took place during the light period between 10:00 and 17:00 h in a quiet room. The animals belonging to the various treatment groups ($n=10$ each group) were tested in randomized order. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the Committees of Animal Care and Use of Daiichi College of Pharmaceutical Sciences and Tohoku Pharmaceutical University.

2.2. Capsaicin test

Antinociception was assessed using the capsaicin test (Sakurada et al., 1992). To reduce variability, each mouse was acclimatized to an acrylic observation chamber (22.0×15.0×12.5 cm) for approximately 1 h before the injection of capsaicin. The mouse was injected 20 µl of a solution of capsaicin (1.6 µg/paw) beneath the skin of the plantar surface of the right hindpaw using a 50 µl Hamilton microsyringe with a 26-gauge needle as quickly as possible, with only minimal animal restraint. Following capsaicin injection, the animals were immediately placed in the test box for a 5-min observation period. Licking/biting behavior induced by intraplantar injection of capsaicin was observed as an indicator of nociceptive response. The accumulated response time in second spent in licking/biting the capsaicin-injected paw was measured for a period of 5 min immediately after subcutaneous (s.c.) injection of capsaicin.

2.3. Experimental protocol

BEO, (±) linalool, linalyl acetate, lidocaine and morphine were injected s.c. into the plantar surface of the right hindpaw 10 min before local injection of capsaicin. Morphine was also injected i.t. into the subarachnoid space according to the method of Hylden and Wilcox (1980). Briefly, the lumbar puncture was performed using a 28 gauge needle attached to a 5 µl Hamilton microsyringe. The needle was inserted between L5 and L6, and drugs were delivered in a volume of 5 µl in conscious mice. The mice were not anesthetized during these procedures. Puncture of the dura was behaviorally indicated by a slight flick of the tail. The opioid receptor antagonists, naloxone and its quarternary form, naloxone methiodide, were pre-injected i.p. or into the hindpaw 10 min before intraplantar injection of BEO, linalool, lidocaine or morphine. The effect of BEO and linalool, administered s.c. into the plantar surface of the contralateral (left hindpaw) or ipsilateral paws, was also studied. In all experiments the observer was unaware of the treatment.

2.4. Statistical analysis

Nociceptive behavior for each treatment group was expressed as mean ± S.E.M. The ID_{50} values with 95% confidence limits were calculated for reduction in the capsaicin-induced nociceptive response with a computer-associated curve-fitting program (GraphPad Prism; GraphPad Software, Inc., San Diego, CA). Statistical differences between groups were established using Dunnett's test for multiple comparisons after analysis of variance (ANOVA). The 5% ($P<0.05$) level of statistical significance was set in all experiments.

2.5. Materials

BEO was kindly provided by the company "Simone Gatto" (San Pier Niceto, Messina, Italy) together with the certificate of analysis carried out by the "Stazione Sperimentale per le Industrie delle Essenze e dei Derivati dagli Agrumi" (SSEA, Reggio Calabria, Italy). The composition of the essential oil of bergamot used here has been previously reported by Corasaniti et al. (2007). Briefly, BEO contained 0.38% of D-limonene, 70.26% of linalyl acetate, 18.95% of linalool, 0.62% of γ-terpinene, and 0.03% of β-pinene. The following drugs and chemicals were used: (±) linalool, linalyl acetate (Nacalai tesque, Kyoto, Japan) and morphine hydrochloride (Sankyo, Tokyo, Japan), naloxone hydrochloride, naloxone methiodide, lidocaine hydrochloride monohydrate and capsaicin (Sigma Chemical Co., St. Louis, MO). BEO, linalool and linalyl acetate were diluted in jojoba wax (Simmondsia chinensis) (K.S.A. International, Co. Ltd., Kanagawa, Japan) to reach total amount of 1.25–20 µg. Jojoba wax alone gave no effect on capsaicin-induced nociception. Morphine hydrochloride, lidocaine hydrochloride, naloxone hydrochloride and naloxone methiodide injected peripherally were dissolved in physiological saline (0.9% wt/vol). For i.t. injection, morphine was dissolved in sterile artificial cerebrospinal fluid (CSF) containing 126.6 mM NaCl, 10.0 mM $NaHCO_3$, 2.5 mM KCl, 2.0 mM $MgCl_2$, 1.3 mM $CaCl_2$ and 1.0 mM glucose. Capsaicin was dissolved in 100% dimethylsulfoxide (DMSO). Concentrated stock solutions of capsaicin were diluted with physiological saline (0.9% wt/vol).

3. Results

3.1. Effects of BEO, linalool and linalyl acetate injected into the hindpaw

The effects of BEO, linalool and linalyl acetate were examined when injected into the hindpaw plantar surface in mice. Licking/biting behavior after intraplantar injection of BEO (5 and 10 µg/paw), linalool (2.5, 5.0 and 10 µg/paw) linalyl acetate (10 and 20 µg/paw) and jojoba wax was not statistically different when compared with saline-treated group (Table 1). Only the maximum concentration of BEO (20 µg/paw) produced a significant nociceptive response, which peaked at 5–10 min and had almost disappeared by 10–15 min after intraplantar injection.

Table 1
Effects of BEO, linalool, linalyl acetate and jojoba wax injected into the hindpaw in mice.

Treatments	Concentrations (µg/paw)	Licking/biting responses (s)
Saline	20 µl	16.2 ± 4.9
Jojoba wax	20 µl	16.4 ± 3.0
BEO	5	16.9 ± 3.5
	10	23.4 ± 6.8
	20	41.2 ± 5.7**
Linalool	2.5	16.0 ± 2.6
	5	15.8 ± 3.1
	10	13.0 ± 4.1
Linalyl acetate	10	17.7 ± 4.1
	20	16.7 ± 2.9

Each compound (20 µl/paw) was injected subcutaneously into the plantar surface of the hindpaw. The amount of time spent licking the injected paw was measured for a period of 5 min immediately after intraplantar injection.
** $P<0.01$ when compared to saline-treated group.

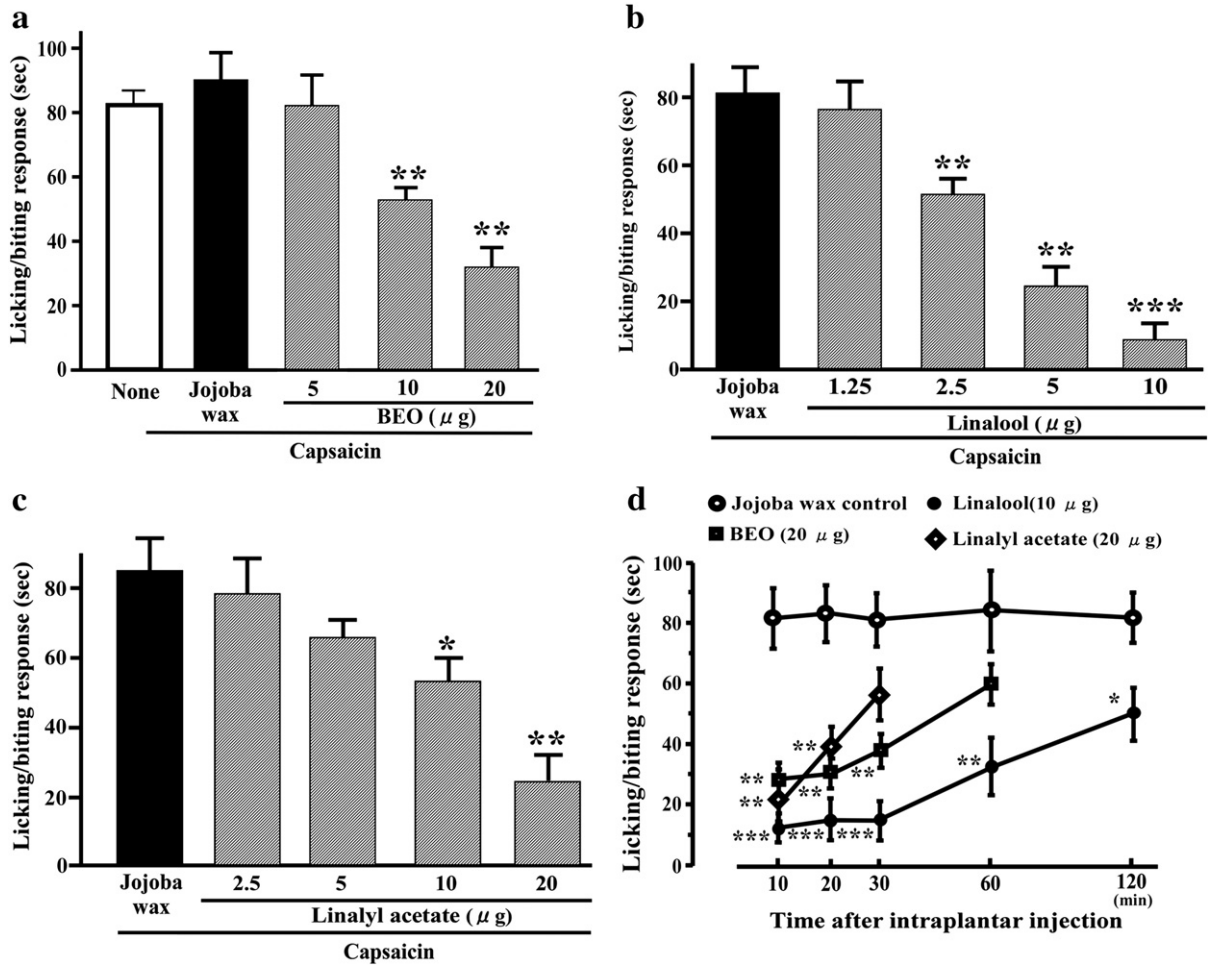

Fig. 1. Antinociceptive effect of intraplantar injection of BEO (a), linalool (b) or linalyl acetate (c) in the capsaicin test. Each compound was injected intraplantarly 10 min prior to capsaicin (1.6 μg/paw) injection. The time course of the antinociceptive effect of intraplantarly injected BEO, linalool or linalyl acetate in the capsaicin test. Each compound was injected into the plantar surface of the hindpaw 10 min, 20 min, 30 min, 60 min and 120 min before intraplantar injection of capsaicin (1.6 μg/paw) (d). The duration of licking/biting induced by capsaicin was determined using the 5-min period starting immediately after injection of capsaicin. Jojoba wax was used as a control and this failed to affect capsaicin-induced nociceptive response. Values represent the mean ± SEM for 10 mice per group. ***$P<0.001$, **$P<0.01$, *$P<0.05$, when compared to jojoba wax-treated control.

3.2. Antinociceptive effects of locally administered BEO, linalool and linalyl acetate in the capsaicin test

Injection of capsaicin (1.6 μg/paw) into the hindpaw plantar surface of normal mice produced an immediate and severe paw-licking behavior of the injected paw, as previously reported (Sakurada et al., 1992). This nociceptive behavior reached a maximum within 3 min after capsaicin injection, thereafter returned to normal level at 15 min (Sakurada et al., 2003). The antinociceptive effects of BEO, linalool and linalyl acetate were evaluated for dose-dependency (Fig. 1a–c) and peak time-effect of each compound after intraplantar injection to be studied (Fig. 1d). Nociceptive behavior following intraplantar injection of jojoba wax as control was not statistically different when compared with non-pretreated group (Fig. 1a). BEO, linalool, linalyl acetate or jojoba wax was injected 10 min before capsaicin at the same site to evaluate their peripheral antinociceptive effects. Intraplantar BEO (10 and 20 μg) produced a significant anti-nociceptive effect in mice; the lowest dose of BEO (5 μg) and jojoba wax, used as control, were ineffective (Fig. 1a). Linalool (2.5–10 μg) and linalyl acetate (10–20 μg) also inhibited significantly the nociceptive behavioral response to capsaicin (Fig. 1b and c).

Time course results show that the maximum effect of BEO (20 μg), linalool (10 μg) and linalyl acetate (20 μg) occurred at 10 min after capsaicin injection (Fig. 1d). Linalool-induced antinociception was observed potently throughout 10 min–30 min and there was a significant antinociceptive activity even at 120 min after intraplantar injection. Therefore, in further experiments, BEO and linalool were injected 10 min prior to intraplantar injection of capsaicin, and the induced paw-licking/biting was observed for 5 min after capsaicin injection.

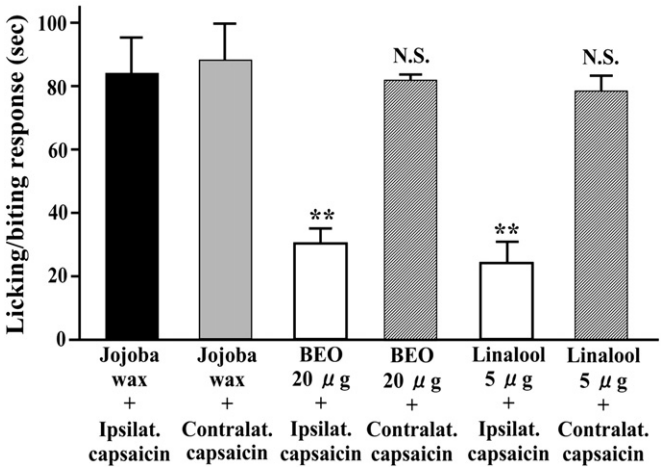

Fig. 2. Effects of BEO and linalool injected into the hindpaw ipsilateral and contralateral to the capsaicin injection. BEO or linalool was injected into the plantar surface of the hindpaw 10 min prior to ipsilateral and contralateral injection of capsaicin (1.6 μg/paw). Values represent the mean ± SEM for 10 mice per group. **$P<0.01$, when compared to jojoba wax-control. Ipsilat., Ipsilateral; Contralat., Contralateral.

3.3. Effects of BEO and linalool injected into the hindpaw contralateral to the capsaicin injection

To ensure that the effects of intraplantar injections of BEO and linalool were local and not due to systemic diffusion of each compound, BEO (20 μg) and linalool (5.0 μg) were injected into the hindpaw contralateral to the capsaicin injection. Nociceptive behavior following injection of either BEO or linalool in the contralateral hindpaw was not statistically different when compared with jojoba wax-treated control group (Fig. 2). Intraplantar injection of BEO and linalool into the hindpaw ipsilateral to the capsaicin injection reduced significantly nociceptive behavior when compared with the jojoba wax-treated control group.

3.4. Effects of naloxone hydrochloride on peripheral antinociception induced by BEO and linalool

To determine if antinociceptive effects of BEO and linalool were mediated by peripheral opioid systems, animals were pretreated i.p. with naloxone hydrochloride, 10 min before injection of BEO (10 μg) and 20 min prior to capsaicin injection. The opioid receptor antagonist naloxone hydrochloride (8.0 mg/kg, i.p.) reversed significantly the inhibitory effects of BEO and linalool on the capsaicin-induced behavioral response (Fig. 3a and b). In further experiments, naloxone hydrochloride was injected directly into the same site on the hindpaw before intraplantar injection of BEO and linalool. Intraplantar pretreatment with naloxone hydrochloride (32 and 64 μg) could also antagonize significantly antinociceptive effects of BEO (Fig. 3c). Linalool-induced antinociception was reversed significantly by intraplantar pretreatment with naloxone hydrochloride (32 μg) (Fig. 3d).

3.5. Effect of naloxone methiodide on peripheral antinociception induced by BEO, linalool, morphine and lidocaine

Naloxone methiodide is thought to act on the peripheral opioid receptors as an antagonist (Lewanowitsch and Irvine, 2002). Pretreatment with naloxone methioide (8 mg/kg, i.p.) resulted in a significant antagonistic effect on BEO- and linalool-induced antinociception (Fig. 4a and b). Lidocaine and morphine were used as reference drugs to compare the effect of naloxone methiodide on antinociception induced by intraplantar lidocaine and morphine. Antinociception induced by intraplantar lidocaine was not antagonized by pretreatment with

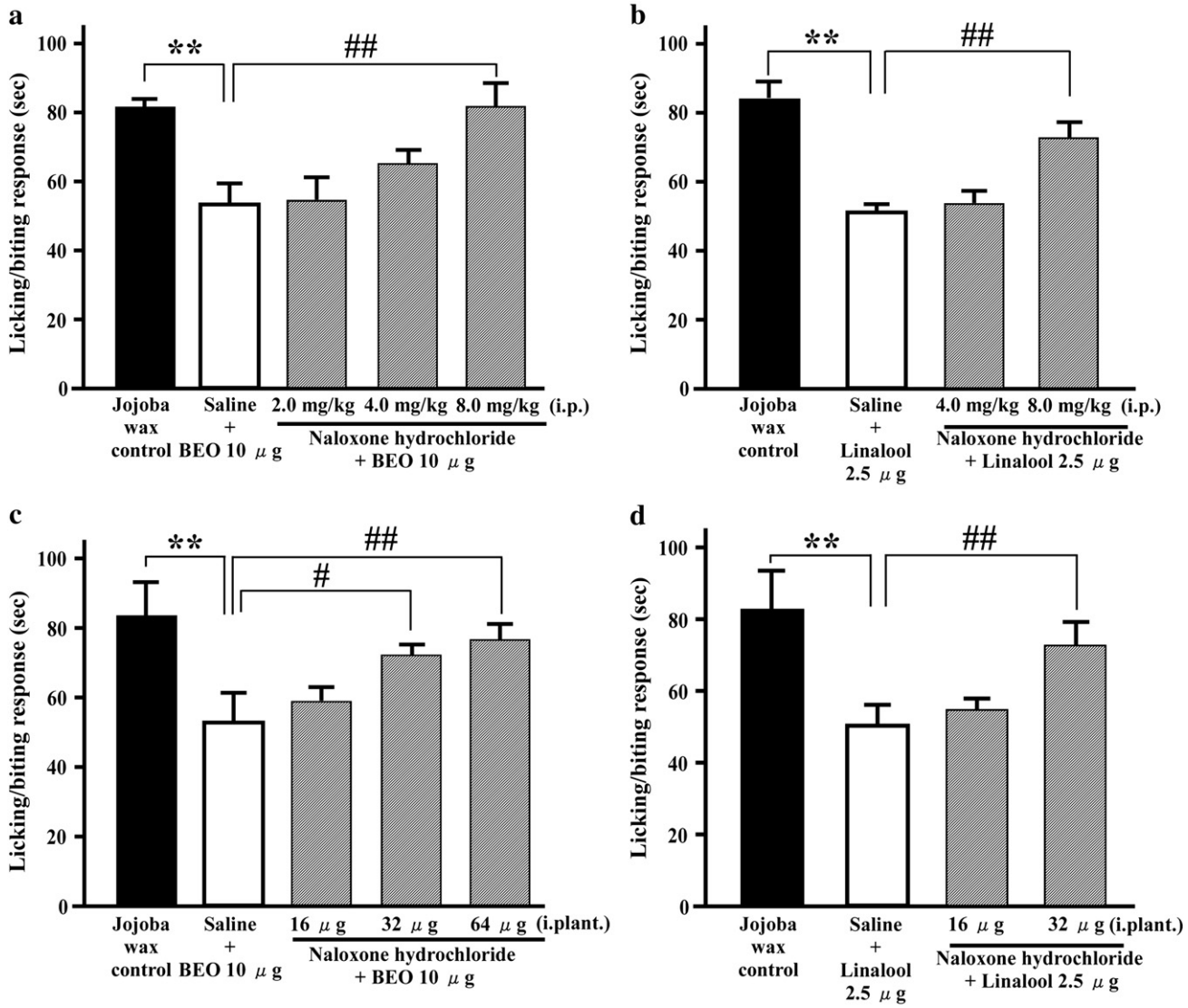

Fig. 3. Antagonism induced by i.p. and intraplantar injection of naloxone hydrochloride on the peripheral antinociception produced by BEO (a and c) and linalool (b and d) in the capsaicin test. Naloxone hydrochloride was injected i.p. and intraplantar 10 min prior to intraplantar injection of BEO or linalool. Capsaicin (1.6 μg/paw) was injected s.c. into the plantar surface of the hindpaw 10 min after BEO and linalool. Values represent the mean ± SEM for 10 mice per group. $^{**}P<0.01$, when compared to jojoba wax-control. $^{\#\#}P<0.01$, when compared to saline-treated control. i.plant., intraplantar.

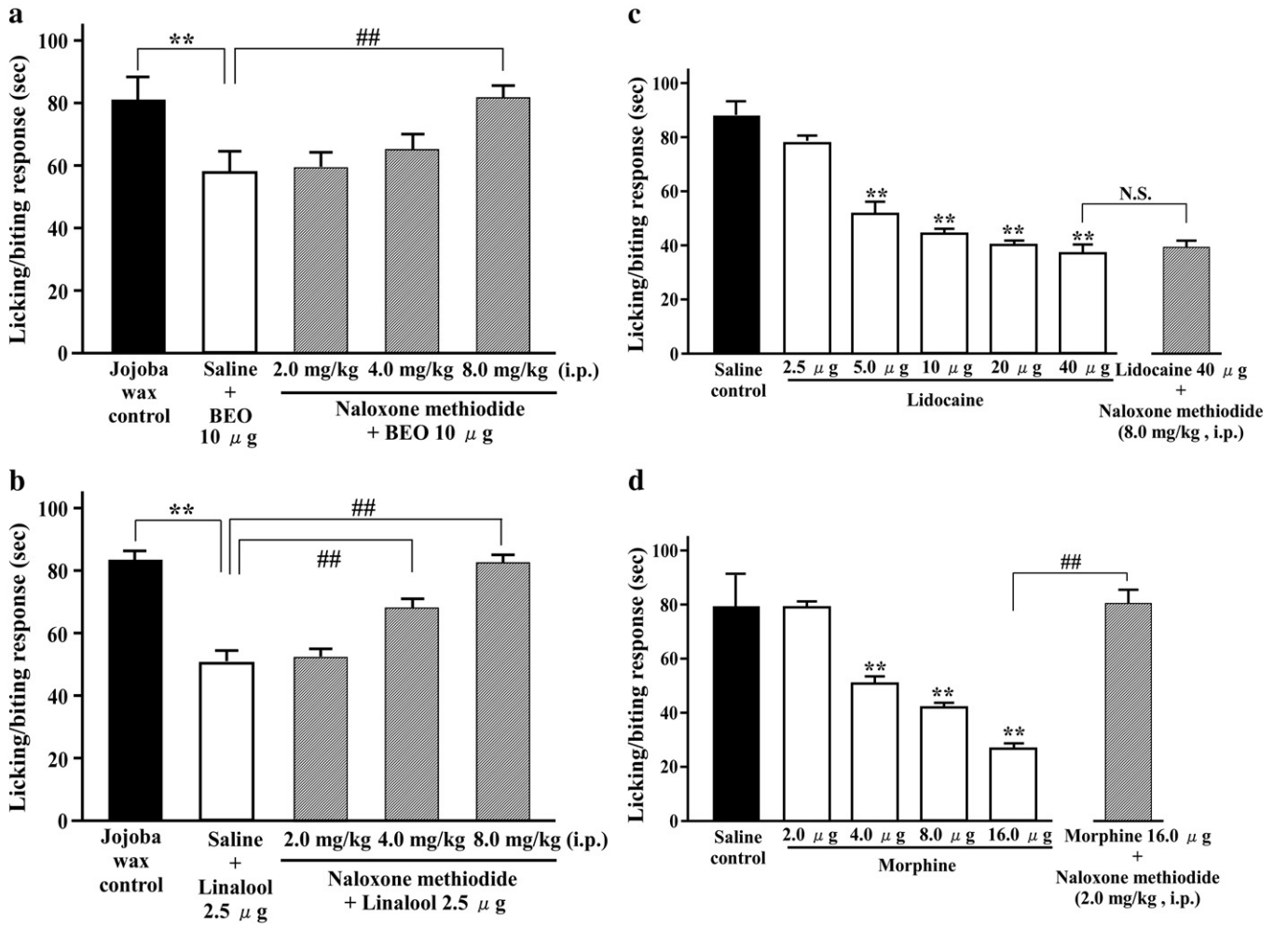

Fig. 4. Antagonism induced by i.p. administration of naloxone methiodide on the peripheral antinociception produced by BEO (a), linalool (b), lidocaine (c) and morphine (d) in the capsaicin test. Naloxone methiodide was administered i.p. 10 min prior to intraplantar injection of BEO, linalool, lidocaine or morphine. Capsaicin (1.6 μg/paw) was injected s.c. into the plantar surface of the hindpaw 10 min after each compound. Values represent the mean ± SEM for 10 mice per group. $^{**}P<0.01$, when compared to jojoba wax- or saline-treated control. $^{\#\#}P<0.01$, when compared to saline-treated control.

naloxone methiodide (Fig. 4c). The peripheral opioid receptor antagonist reversed readily the antinociceptive effect of intraplantar morphine given at a dose of 2.0 mg/kg (Fig. 4d).

3.6. Enhanced effects of BEO and linalool on morphine-induced antinociception

Intraplantar injection of BEO (5 μg) and linalool (1.25 μg) with saline or artificial CSF did not produce a significant effect on capsaicin-induced paw-licking/biting response (Fig. 5b, c, e and f). The i.p. injection of morphine in combination with BEO and linalool produced an enhanced effect on capsaicin-induced nociceptive response (Fig. 5a). The ID_{50} values of i.p. morphine in combination with BEO, linalool and jojoba wax as control were 1.16 (1.00–1.38) mg/kg, 1.23 (1.05–1.49) mg/kg and 2.34 (2.00–2.78) mg/kg, respectively. The antinociception induced by both compounds was reversed completely by a low dose (1.0 mg/kg, i.p.) of naloxone hydrochloride (Fig. 5b and c). When morphine was injected i.t., greater enhancement of antinociception was obtained in combination with BEO and linalool (Fig. 5d). Naloxone hydrochloride antagonized the combined effect of i.t. morphine with BEO or linalool (Fig. 5e and f). The ID_{50} values of i.t. morphine in combination with BEO, linalool and jojoba wax as control were 62.2 (52.2–77.0) pmol, 64.5 (54.5–80.0) pmol and 193.7 (165.7–225.6) pmol, respectively. On the basis of the ID_{50} values, i.t. morphine/BEO or linalool combination produced an approximately three-fold increase in antinociceptive potency as compared to jojoba wax used as control.

4. Discussion

The effects of a common aromatic essential oil, e.g. BEO, and of its main oxygenated monoterpenes, linalool and linalyl acetate, were investigated in a mouse capsaicin pain model. Intraplantar injection of BEO, linalool or linalyl acetate reduced behavioral signs of capsaicin-induced nociception in a dose-dependent manner. BEO and linalool injected into the contralateral paw were not antinociceptive at a sufficient antinociceptive dose used in the ipsilateral paw. This finding suggests that BEO- and linalool-induced antinociception may be mediated locally but not systemically. The present study further demonstrated that antinociception induced by intraplantar BEO or linalool was antagonized by pretreatment with locally (plantar surface of the paw) administered naloxone hydrochloride and by the peripherally acting opioid receptor antagonist naloxone methiodide (Lewanowitsch and Irvine, 2002). These data suggest that intraplantar injection of BEO or linalool could produce peripheral antinociception that is, at least in part, mediated through peripheral opioid mechanisms. In this study, we also demonstrate that an enhanced antinociceptive effect is obtained by combined administration of morphine (i.p. and i.t.), and BEO or linalool.

It is now established that the behavioral pattern observed in the capsaicin test is related to nociception or pain in mice and rats

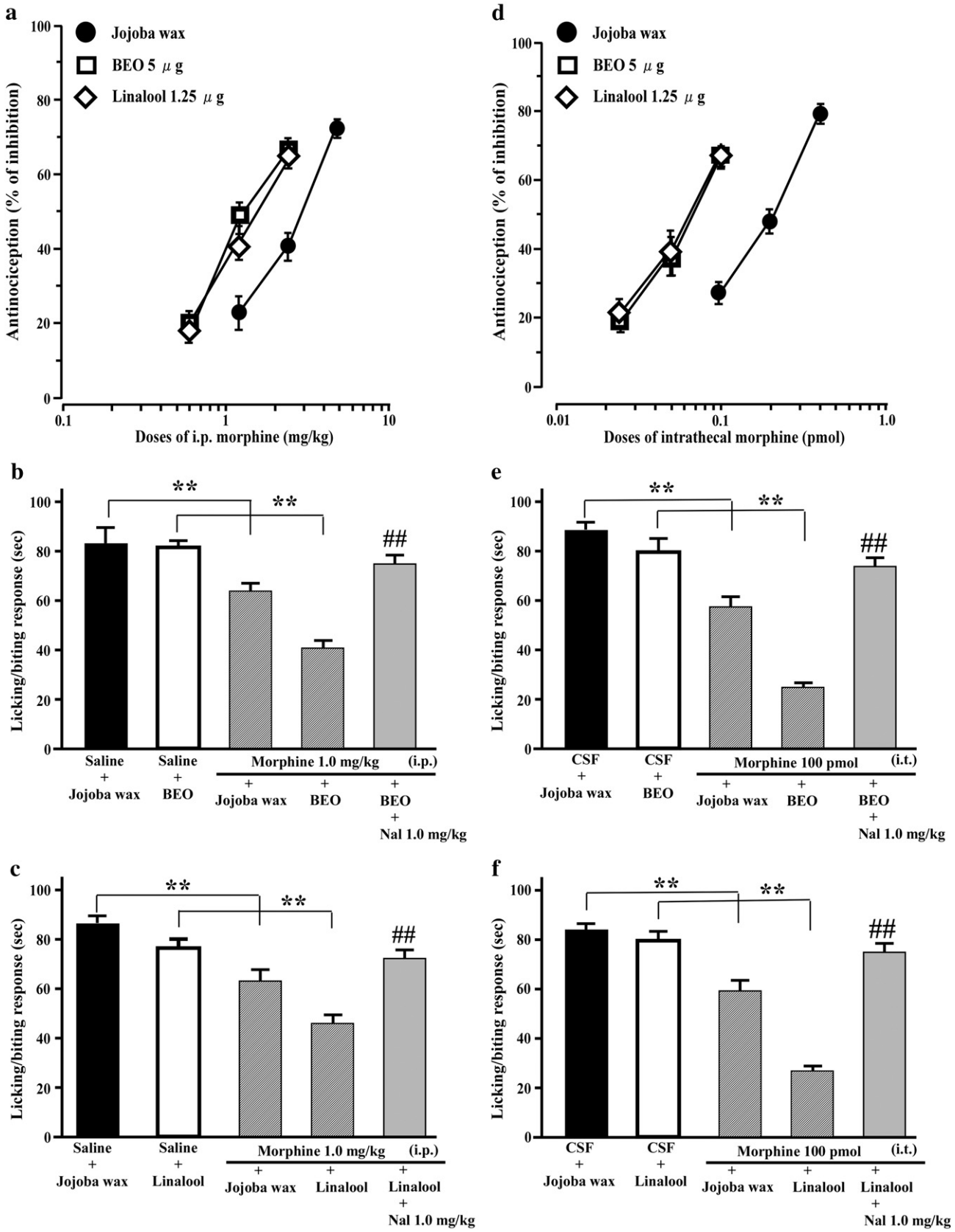

Fig. 5. Enhancement of i.p. and intrathecal morphine-induced antinociception by BEO and linalool in the capsaicin test (a and d), and antagonism of i.p. naloxone on enhanced antinociception induced by combined injection of BEO (b and e) and linalool (c and f). Morphine was injected i.p. immediately after intraplantar injection of BEO and linalool. Morphine was injected i.t. 5 min before intraplantar capsaicin. Naloxone hydrochloride was administered i.p. 10 min prior to intraplantar injection of BEO or linalool. Capsaicin was injected s.c. into the plantar surface of hindpaw 10 min after BEO and linalool. Values represent the mean ± SEM for 10 mice per group. $^{**}P<0.01$, when compared to saline-treated control. $^{\#\#}P<0.01$, when compared morphine plus BEO or linalool.

(Sakurada et al., 1992: Pelissier et al., 2002). In humans, the application of capsaicin into peripheral territories was found to evoke a tonic burning perception of monophasic time course (Hughes et al., 2002). BEO is widely used in the area of aromatherapy as one of the most common aromatic essential oil. Usually, BEO is applied to the skin of the whole body in aromatherapy massage in humans and can be absorbed into peripheral tissues. Aromatherapy massage has been shown to relieve pain (Ferrell-Torry and Glick, 1993; Glover et al., 1995) in addition to attenuation of anxiety and depression in patients with cancer (Wilkinson et al., 2007). Therefore, it is conceivable that there may be peripheral sites where BEO can exert its antinociceptive effect. In line with previous observations done by using a lower dose of capsaicin (50 ng/paw) (Sakurada et al., 2009), intraplantar BEO was efficacious in reducing pain behavior induced by a higher dose of capsaicin (1.6 μg/paw). Local injection of BEO or linalool into the contralateral hindpaw did not yield antinociceptive effects on capsaicin-induced nociception, strongly supporting a local effect of BEO and linalool on cutaneous nociceptors.

On the other hand, linalool has been shown to be effective as an antinociceptive compound in several nociceptive assays. Available evidence indicates that linalool could produce antinociception through the interaction with opioid, muscarinic M2 or adenosine A1 receptors and nitric oxide (NO) synthesis (Peana et al., 2003, 2004, 2006a,b). In addition to these pharmacological data, there are some findings of linalool supporting the modulation of glutamatergic neurotransmission through *N*-methyl-D-aspartate (NMDA) receptors both *in vitro* and *in vivo* conditions (Elisabetsky et al., 1995, 1999; Elisabetsky and Silva Brum, 2003; Batista et al., 2008).

Little information is available concerning the antinociceptive site of action of locally administered linalool. The present data, however, do support a peripheral site of action though this needs to be investigated further. Indeed, intraplantar injection of linalool significantly reduced the capsaicin-induced nociceptive behavior. Linalool was injected intraplantarly at a concentration (20 μl of 2.5–10 μg which translates to 0.125–0.5 mg/kg) much lower than the dose given systemically in other studies (Peana et al., 2004; Batista et al., 2008). This raises the possibility that local administration of BEO or linalool at doses that do not produce systemic effects could be used to reduce nociception in localized areas without producing unwanted side effects that are observed following systemic use.

In order to assess the possible participation of either opioid receptors or opioid peptides in intraplantar linalool-induced antinociception, the effect of opioid receptor antagonists on the antinociceptive activity of linalool was tested. The antinociceptive effect produced by linalool was reversed by i.p. and intraplantar pretreatment with naloxone hydrochloride. In addition, similar results were obtained in intraplantar BEO-induced antinociception. These results suggest that BEO- or linalool-induced antinociception may be mediated through opioid receptors in the periphery. This deduction is supported by the observation that pretreatment with the peripherally acting opioid receptor antagonist, i.e. naloxone methiodide (Lewanowitsch and Irvine, 2002), resulted in a significant antagonistic effect on antinociception induced by intraplantar BEO or linalool.

It is conceivable that intraplantar BEO or linalool might cause the local release of endogenous opioid peptides and these might be responsible for the peripherally mediated antinociceptive effect. It is worth noting that proopiomelanocortin, the precursor of a variety of neuropeptides including β-endorphin (Khodorova et al., 2003; Galiegue et al., 1995), is constitutively expressed by keratinocytes that are very abundantly present in the skin.

Following intraplantar injection of BEO or linalool, mediators other than β-endorphin, might also be released and these might contribute to their antinociceptive effect. This speculation is supported by the greater antagonism afforded by naloxone methiodide against morphine-induced antinociception as compared to the effect on BEO- or linalool-induced antinociception. In fact, pretreatment with 2.0 mg/kg, i.p. naloxone methiodide resulted in a significant antagonism on morphine-induced antinociception, but not on BEO- or linalool-induced antinociception. The results of the present study also show that antinociception induced by intraplantar linalool was reversed by pretreatment with a relatively high dose of naloxone (8 mg/kg, i.p.). These results suggest a possible participation of δ- and κ-opioid receptors in addition to μ-receptors. In fact, methionine-enkephalin and dynorphin A, which are endogenous opioids with preference for δ- and κ-receptors, respectively, are also produced and released from immune cells of animals with hindpaw inflammation (Cabot et al., 2001).

It has been previously shown that capsaicin can release glutamate from peripheral nerve endings (Jin et al., 2009), and i.t. injection of NMDA receptor antagonists reduces capsaicin-induced nociceptive behavior (Sakurada et al., 1998). There are data indicating that the antinociceptive effect of MK-801, a non-competitive NMDA receptor antagonist, can be blocked by the opioid antagonist, naloxone (Forman, 1999). Additionally, some results have shown that MPEP, an antagonist of the metabotropic glutamate receptor (mGluR) 5, and LY379268, an agonist of mGluR2/3) potentiate morphine-induced antinociception in a mouse model of neuropathic pain (Jones et al., 2005; Osikowicz et al., 2008). Altogether these data support the deduction that linalool-induced antinociception could be due to an interaction with glutamatergic system.

Different doses of intraplantar lidocaine (5.0–40 μg), known to produce a block of voltage-gated sodium channels, exhibited a significant dose-related attenuation of capsaicin-induced paw licking/biting behavior. Lidocaine-induced antinociception was not reversed by pretreatment with naloxone methiodide, indicating that antinociceptive effect of lidocaine is not mediated through the peripheral opioid system, but a peripheral, non-opioid receptor-mediated sodium mechanism (e.g. voltage-gated sodium channel blockade) may be involved in its antinociception.

In agreement with our previous studies (Sakurada et al., 1994; Komatsu et al., 2009), i.t. injection of morphine dose-dependently inhibited the nociceptive response in the capsaicin test. A combination of ineffective dose of BEO or linalool, and different doses of morphine given i.p. resulted in a supra-additive synergic antinociceptive action. The i.t. injection of different doses of morphine resulted in a greater antinociception in combination with BEO or linalool. However, the mechanism can hardly account for the dose-dependent effect of the combination of BEO or linalool with morphine. These results suggest that the supra-additive effect of morphine in combination with BEO or linalool could be due to a functional enhancement between the separate site of action with i.p. and i.t. morphine acting at the supraspinal and/or spinal level, thus binding mainly μ-opioid receptors, and BEO and linalool acting at the peripheral level further increasing opioid receptor-mediated events.

In conclusion, intraplantar BEO, linalool and linalyl acetate reduced the nociceptive response as assayed by the capsaicin test. The antinociceptive effects of BEO or linalool were antagonized by the direct injection of naloxone hydrochloride into the hindpaw and i.p. naloxone methiodide, an antagonist acting at the peripheral opioid receptors. Morphine-induced antinociception after i.p. and i.t. injections was enhanced markedly by the combined injection of intraplantar BEO or linalool. The present results indicate that the combination of morphine, and intraplantar BEO or linalool may be a promising therapeutic approach in treating nociception or pain, with a reduced risk of undesirable side effects.

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