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(−)-Linalool produces antinociception in two experimental models of pain

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Abstract

Linalool is a monoterpene compound commonly found as a major component of the essential oils of several aromatic plant species, many of which are used in traditional medical systems as analgesic and anti-inflammatory remedies. We previously reported that (−)-linalool, the natural occurring enantiomer, plays a major role in the anti-inflammatory activity displayed by different essential oils, suggesting that linalool-producing species are potentially anti-inflammatory agents. In this study, the antinociceptive activity of (−)-linalool was examined in two different pain models in mice: the acetic acid-induced writhing response, a model of inflammatory pain, and the hot plate test, a model of supraspinal analgesia. Moreover, the effect of (−)-linalool on spontaneous locomotor activity (25, 50, 75 and 100 mg/kg) was evaluated. The results show that this compound induced a significant reduction of the acid-induced writhing at doses ranging from 25 to 75 mg/kg. Such effect was completely reversed both by the opioid receptor antagonist naloxone and by the unselective muscarinic receptor antagonist atropine. In the hot plate test, only the dose of 100 mg/kg of (−)-linalool resulted in a significant effect. (−)-Linalool induced a dose dependent increase of motility effects, thus ruling out the confounding influence of a possible sedative effect. The more pronounced effect of (−)-linalool on the writhing test with respect to the hot plate test is consistent with the observation that (−)-linalool possesses anti-inflammatory activity. Finally, the activation of opioidergic and cholinergic systems appears to play a crucial role in (−)-linalool-induced antinociception.

Keywords: (−)-Linalool; Essential oil; Antinociceptive activity; Acetic acid-induced writhing response; Hot plate test; Locomotor activity

1. Introduction

(−)-Linalool is the natural occurring enantiomer monoterpene compound commonly found as a major volatile component of the essential oils of several aromatic plant species, many of which are used in traditional medicine to relieve symptoms and to cure a variety of acute and chronic ailments (Elisabetsky et al., 1995; Lis-Balchin and Hart, 1999; Ghelardini et al., 1999; Jia et al., 1999; Perry et al., 2000; Re et al., 2000). Among these species are *Salvia sclarea* and *Salvia desoleana*. We reported that *S. sclarea* and *S. desoleana* essential oils, after systemic administration to experimental animals, showed an anti-inflammatory and a peripheral analgesic activity (Moretti et al., 1997; Peana and Moretti, 2002). Moreover, we have shown that (−)-linalool and its corresponding ester linalyl acetate reduced carrageenin-induced oedema in rats at doses ranging from 25 to 75 mg/kg (Peana et al., 2002), thus suggesting a major role for (−)-linalool and its ester in the anti-inflammatory effect of these essential oils. To our knowledge, no studies on the possible antinociceptive action of (−)-linalool have been performed up to now.

The aim of this study was to evaluate the possible analgesic activity of (−)-linalool on two different pain models in mice: the acetic acid-induced writhing reaction and the hot plate test. The acetic acid-induced writhing reaction is a model of inflammatory pain, and it has long been used as a screening tool for evaluation of putative analgesic or anti-inflammatory agents (Ikeda et al., 2001; Collier et al., 1968; Koster et al., 1959). The hot-plate test is

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considered a model of supraspinal analgesia (Forman, 1999).

Linalool is a competitive antagonist of *N*-methyl-D-aspartate (NMDA) receptors (Elisabetsky et al., 1999; Silva Brum et al., 2001a,b) and the inhibition of NMDA receptor activity produces antinociception (Coderre and Van Empel, 1994; Chizh et al., 2001), partially mediated by brain opioids (Forman, 1999). Moreover, linalool possesses a weak in vitro cholinesterase inhibitory activity (Perry et al., 2000) and muscarinic neurotransmission is involved in mediating antinociception in the rat spinal cord (Naguib and Yaksh, 1997). The administration of (−)-linalool in the present study resulted in an analgesic effect, which was more pronounced in the writhing reaction test. In order to reveal the possible involvement of opioidergic and cholinergic mechanisms in this effect, we studied the effect of the opioid receptor antagonist naloxone and of the unselective muscarinic receptor antagonist atropine on (−)-linalool-induced analgesia in the writhing reaction test.

Several studies reported the ability of (−)-linalool to induce sedation in mice (Buchbauer et al., 1991; Elisabetsky et al., 1995; Jirovetz et al., 1991). In order to evaluate the possible confounding influence of this effect, the locomotor activity of mice after (−)-linalool administration was evaluated.

2. Materials and methods

The present study was carried out in accordance to the Italian law, which allows experiments on laboratory animals only after submission of a research project to the competent authorities, and in accordance to the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985).

2.1. Subjects

The experiments were performed on CD1 male mice weighing 20–25 g (Harlan, Italy). They were housed in groups of 10 per cage and maintained under controlled environmental conditions (temperature 22 ± 2 °C; humidity 60–65%; 12-h light–dark cycle). All animals were given standard laboratory diet and water ad libitum.

2.2. Drugs and treatments

(−)-Linalool (Sigma) was tested on 8/10 mice with 8/10 animals as controls, administered at doses of 25, 50, 75 and 100 mg/kg; for the locomotor activity increased doses from 25 to 100 mg/kg were used. The treatment was performed by subcutaneous (s.c.) injection of a constant volume of a solution (1 ml/100 g of body weight) containing the defined amount of active principle dissolved in PEG-200 (Sigma) (vehicle). All experiments were performed between 0900 and 1500 h.

2.3. Antinociceptive tests

2.3.1. Acetic acid writhing test

All doses of (−)-linalool were administered 30 min before the intraperitoneal (i.p.) injection of acetic acid (5 ml/kg as a 1.2% solution in distilled water v/v). The control animals received acetic acid and vehicle only. Immediately after the algic compound injection each animal was isolated in an individual observation chamber (25 × 20 × 25h cm). Separate groups of mice were pretreated with naloxone (5 mg/kg, i.p.) or atropine (5 mg/kg, i.p.) 15 min before the s.c. administration of (−)-linalool or vehicle. The cumulative number of writhing responses (abdominal constriction) was recorded for 30 min, after acetic acid injection.

The results were expressed as mean values ± S.E.M. and the activity was expressed as percentage of inhibition of writhings number compared with that of controls.

2.3.2. Hot plate test

The hot-plate test was used to measure response latencies according to the method described by Eddy and Leimbach (1953). Animals were placed on the hot plate maintained at 55 ± 0.5 °C; the time between placement of the animal on

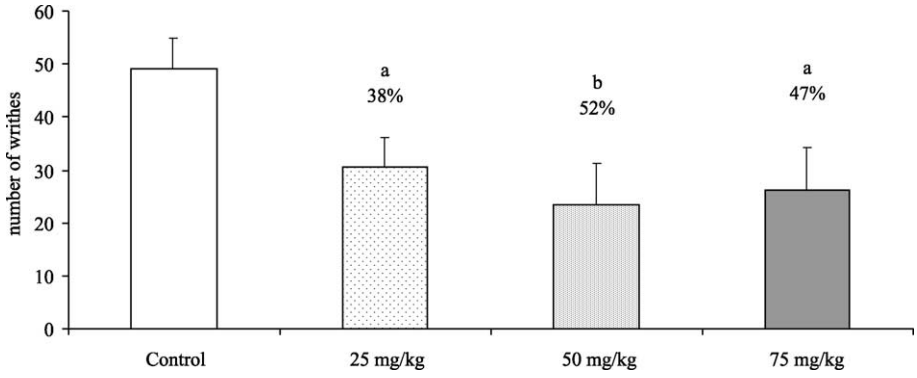

Fig. 1. Effect of (−)-linalool on acetic acid-induced writhing in mice. The doses are expressed as mg/kg. Data represent mean values (± S.E.M) and percent inhibition (%) compared to the control animals. Statistical differences vs. control group were calculated using ANOVA supplemented by LSD-test ($^{a}P<0.05$; $^{b}P<0.01$).

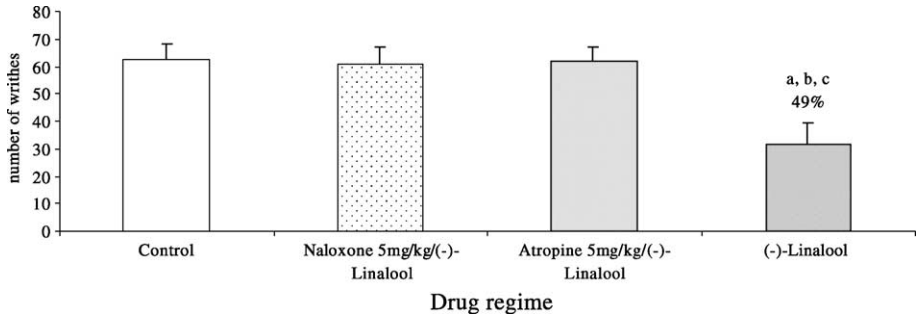

Fig. 2. Effect of naloxone or atropine on antinociception induced by (−)-linalool (50 mg/kg) on acetic acid-induced writhing in mice. Data represent mean values (± S.E.M). (%) Percent inhibition of (−)-linalool compared to the control animals. Statistical differences were calculated using ANOVA supplemented by LSD-test ($^{a}P<0.005$ vs. control; $^{b}P<0.005$ vs. naloxone/(−)-linalool; $^{c}P<0.005$ vs. atropine/(−)-linalool).

the hot plate and the occurrence of either the licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with baseline latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before (−)-linalool or vehicle administration (basal) and 1, 2 and 3 h after each treatment. The cut-off time for the hot plate latencies was set at 20 s and analgesic effect was expressed as percent increase quantified with the formula.

Percent increase = Latency after treatment

− Basal Latency/Basal Latency × 100

2.4. Locomotor activity

Motor activity was measured by an apparatus consisting of a mobile rack (height 180 cm, width 100 cm and depth 60 cm) with eight compartments (h 40 cm, w 45 cm, d 50 cm), into which a transparent perspex cage (height 19 cm, floor area 23 × 33 cm) was placed (Imetronic, Pessac, France). Motor activity is detected by a system of photocell infrared beams, dividing the cage area into two sectors, rear and front. In particular, the interruption of two photocell beams belonging to two different sectors is recorded as a "long movement" motility count. The apparatus was connected to a personal computer by an electronic interface. The motor response was recorded for the following 180 min after (−)-linalool injection. Data have been collected in 5-min time bins.

2.5. Statistics

The results were analysed by analysis of variance (ANOVA), supplemented by least significant difference (LSD)-test.

3. Results

3.1. The effect of (−)-linalool on acetic acid-induced writhing in mice

The results of the abdominal constriction test are shown in Fig. 1. Administration of 25, 50 and 75 mg/kg of (−)-

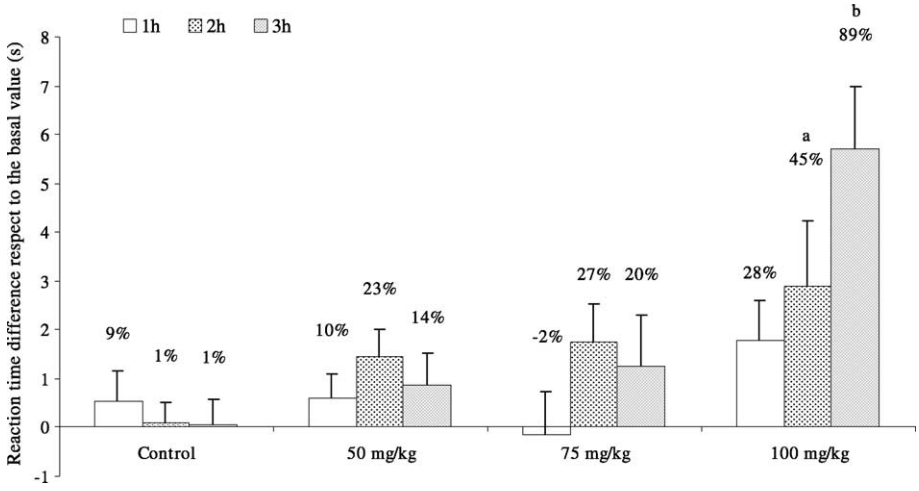

Fig. 3. Reaction time difference with respect to the mean basal value (s) in response to the hot plate after injection of (−)-linalool or vehicle (control). Each value represents the mean (± S.E.M.) and percent increase (%) with respect to mean basal value. The doses are expressed as mg/kg. Statistical differences were calculated using ANOVA (repeated measures) supplemented by LSD-test comparing each group to control groups ($^{a}P<0.005$; $^{b}P<0.0001$).

linalool elicited a pronounced analgesic response as demonstrated by a significant inhibition of constrictions in mice receiving acetic acid, with respect to control animals treated with PEG 200. (−)-Linalool produced a significant inhibition at the different doses used: 38% ($P=0.03$), 52% ($P=0.01$) and 47% ($P=0.03$), respectively. The dose of 50 mg/kg of (−)-linalool was selected to be used in the subsequent experiments (naloxone and atropine antagonism).

3.2. The effect of naloxone and atropine on (−)-linalool-induced analgesia

The antinociceptive effect of (−)-linalool, at a dose of 50 mg/kg (49% of inhibition with respect to control, $P=0.002$), was completely reversed by pretreatment with the opioid receptor antagonist naloxone (5 mg/kg, i.p., $P=0.004$, Fig. 2). Naloxone, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the number of acetic acid-induced abdominal constrictions (data not shown).

Treatment with the unselective muscarinic receptor antagonist atropine (5 mg/kg, i.p.) resulted in a complete reversal of the analgesic response to (−)-linalool ($P=0.004$, Fig. 2). Atropine, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the number of acetic acid-induced abdominal constrictions (data not shown).

3.3. The effect of (−)-linalool on the hot plate test

Administration of (−)-linalool, at the same doses shown to be effective in the writhing test (25, 50 and 75 mg/kg), did not produce any significant effect in the hot-plate test of analgesia. However, treatment with 100 mg/kg (−)-linalool elicited a pronounced analgesic response, as demonstrated by a significant increase in the reaction time, at the second and third hour after treatment (45%, $P=0.004$ and 89%, $P=0.0001$, respectively) (Fig. 3).

Table 1
Effect of (−)-linalool on spontaneous motility in mice

(−)-linalool dose mg/kg	Motility count mean ± S.E.M.	Percent variation vs. control group (%)
Control	67.16 ± 15	
25	76.60 ± 24	15
50	44.20 ± 11	−34
75	131.20 ± 18	95[a]
100	268.12 ± 46	300[b]

The motor response was recorded for the following 180 min after (−)-linalool injection.
Statistical differences vs. control group were calculated using ANOVA followed by LSD-test.
[a] $P<0.05$.
[b] $P<0.0005$.

3.4. Effect of (−)-linalool on locomotor activity

Administration of (−)-linalool resulted in a statistically significant increase of motility counts at the dose of 75 and 100 mg/kg. Lower doses failed to produce statistically significant effects (Table 1).

4. Discussion

The present results show that (−)-linalool produced an inhibition of the inflammatory pain in mice, as determined by a significant reduction in acetic acid-induced abdominal constrictions.

Moreover, at a higher dose and with an increased latency time, (−)-linalool was effective also in the hot plate test, a model of supraspinal analgesia.

Several studies reported the ability of (−)-linalool to produce sedative effects (Buchbauer et al., 1991; Elisabetsky et al., 1995; Jirovetz et al., 1991). Our results show that (−)-linalool, in the dose range examined in the present study, dose-dependently increased locomotor activity, thus ruling out the influence of a possible sedative effect in the results of analgesia tests.

The more pronounced effect of (−)-linalool on the writhing test with respect to the hot plate test is consistent with the observation that (−)-linalool possesses anti-inflammatory activity; indeed, (−)-linalool is active in the writhing test in the same dose range shown to be effective in carrageenin-induced oedema (Peana et al., 2002). These observations suggest the opportunity to investigate a possible effect of (−)-linalool on cyclooxygenase or some other enzyme of the arachidonic acid cascade. Moreover, a spasmolitic effect of (−)-linalool has been reported (Lis-Balchin and Hart, 1999), whose possible contribution in reducing the abdominal constriction response should be taken into consideration.

As for the different kinetics of analgesia development between the two pain models, no possible experimental evidence is available to provide a possible explanation.

Linalool is a competitive antagonist of NMDA receptors (Elisabetsky et al., 1999; Silva Brum et al., 2001a,b). This property might account both for the antinociceptive and motor stimulating effects observed in the present study. Indeed, NMDA receptor antagonists may induce analgesia (Chizh et al., 2001; Coderre and Van Empel, 1994; Chapman and Dickenson, 1992), attenuate carrageenin-induced behavioural hyperalgesia in rats (Ren et al., 1992) and stimulate locomotor activity (Waters et al., 1996; Ginski and Witkin, 1994; Ouagazzal et al., 1993).

Antinociception exerted by (−)-linalool in the writhing test appears to depend both on opioidergic and on cholinergic neurotransmission, since (−)-linalool effect was completely antagonised by the opioid receptor antagonist naloxone and by the muscarinic cholinergic receptor antagonist atropine. Endogenous opioids have been shown to mediate at least in

part the analgesic effect produced by NMDA receptor antagonists (Forman, 1999). Thus, it might be suggested that (−)-linalool might activate opioidergic transmission as a consequence of its ability to antagonise NMDA receptors. Since muscarinic neurotransmission is involved in mediating antinociception in the rat spinal cord (Naguib and Yaksh, 1997), and muscarinic receptor agonists and cholinesterase inhibitors have been shown to induce analgesia (Iwamoto and Marion, 1993; Naguib and Yaksh, 1997; Cozanitis et al., 1983; Miranda et al., 2002), the weak cholinesterase inhibitory activity of (−)-linalool (Perry et al., 2000) might also concur in determining its analgesic effect.

Finally, the results of this study confirm that (−)-linalool plays a major role in the analgesic and anti-inflammatory activity displayed by essential oils in which it is contained, and provide further evidence suggesting that linalool-producing plant species are potentially analgesic and anti-inflammatory agents.

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