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# Profile of spinal and supra-spinal antinociception of (−)-linalool

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**Abstract**

We previously reported that administration of (−)-linalool, the naturally occurring enantiomer in essential oils, induced a significant reduction in carrageenin-induced oedema and in acetic acid-induced writhing. The latter effect was completely antagonised by the muscarinic receptor antagonist atropine and by the opioid receptor antagonist naloxone. To further characterise the antinociceptive profile of (−)-linalool, we studied its effect in the hot plate and the formalin in tests. In addition, to determine the possible involvement of the cholinergic, opioidergic and dopaminergic systems, we tested the effects of atropine, pirenzepine, a muscarinic M1 receptor antagonist, naloxone, sulpiride, a dopamine D2 receptor antagonist and (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390), a dopamine D1 receptor antagonist on (−)-linalool-induced antinociception. Moreover, since $K^+$ channels seem to play an important role in the mechanisms of pain modulation, we examined the effect of glibenclamide, an ATP-sensitive $K^+$ channel inhibitor on (−)-linalool-induced antinociception. The administration of (−)-linalool (100 and 150 mg/kg, s.c.) increased the reaction time in the hot-plate test. Moreover, (−)-linalool (50 and 100 mg/kg) produced a significant reduction in the early acute phase of the formalin model, but not in the late tonic phase. The highest dose (150 mg/kg) caused a significant antinociceptive effect on both phases. The antinociceptive effects of (−)-linalool were decreased by pre-treatment with atropine, naloxone, sulpiride and glibenclamide but not by pirenzepine and SCH-23390. These results are in agreement with the demonstrated pharmacological properties of linalool, mainly its cholinergic, local anaesthetic activity and its ability to block NMDA receptors. Furthermore, a key role seems to be played by $K^+$ channels, whose opening might be the consequence of a stimulation of muscarinic M2, opioid or dopamine D2 receptors.


*Keywords:* (−)-Linalool; Antinociception; Cholinergic; Opioid; Dopamine; $K^+$ channel; ATP-sensitive channel

## 1. Introduction

(−)-Linalool is the naturally occurring enantiomer monoterpene compound commonly found as a major volatile component of the essential oils of several aromatic plant species. The effects produced by this compound include a significant inhibition of carrageenin-induced oedema in rats (Peana et al., 2002) and a reduction in acetic acid-induced writhing in mice at doses ranging from 25 to 75 mg/kg. 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 unselective muscarinic cholinergic receptor antagonist atropine (Peana et al., 2003). It was also reported that linalool modulates glutamate activation expression in vitro (competitive antagonism of L-[$^3$H]glutamate binding) and in vivo (delayed subcutaneous *N*-methyl-D-aspartate (NMDA)-induced convulsions and blockade of intracerebroventricular quinolinic acid-induced convulsions) (Silva Brum et al., 2001a,b). Moreover, other authors have reported that linalool modifies the nicotinic receptor-ion channel kinetics at the mouse neuromuscular junction (Re et al., 2000), and possesses a local anaesthetic activity (Ghelardini et al., 1999), a spasmolitic effect (Lis-Balchin and Hart, 1999) and a weak in vitro cholinesterase inhibitory activity (Perry et al., 2000). Linalool also has been shown to possess antioxidant properties (Celik and Ozkaya, 2002).

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To further characterise the antinociceptive profile of (−)-linalool, we have examined in the present study the effects of systemic administration of (−)-linalool on thermal reactivity assessed by the hot-plate test in mice and on pain behaviour produced by an injection of a low concentration (1%) of formalin in rats. The hot-plate model is a nociception test very sensitive to opioids, used as a means of assessing acute antinociceptive activity depending upon spinal and supra-spinal mechanisms. The formalin test is widely used as a model of acute inflammatory pain. Formalin injection activates peripheral sensory nerves and produces pain responses that involve ongoing peripheral activity and peripheral and central sensitisation. Pain behaviours are related to formalin concentration and different mechanisms are involved at low and high concentrations. Thus, at low concentrations (<2%), there is a predominant activity of capsaicin-sensitive neurogenic components, while at high concentrations (5%), there is the additional involvement of more complex inflammatory elements and a peripheral release of glutamate (Sawynok and Reid, 2002).

Furthermore, to determine the possible participation of the cholinergic, opioidergic and dopaminergic systems, previously shown to be involved in the modulation of nociception, we tested the effects of atropine, pirenzepine, a selective muscarinic M1 receptor antagonist, naloxone, sulpiride, a selective dopamine D2 receptor antagonist and (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390), a selective dopamine D1 receptor antagonist, on (−)-linalool-induced antinociception both in the 1% formalin test and in the hot-plate test. Moreover, since $K^+$ channels play an important role in pain modulation, the effects of the ATP-sensitive $K^+$ channel inhibitor glibenclamide were examined on (−)-linalool-induced antinociception in the 1% formalin model and in hot-plate tests.

## 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 22–27 g and on naive male Wistar rats weighing 150–200 g (Harlan, Italy). They were maintained under controlled environmental conditions (temperature 22 ± 2 °C, humidity 60–65% and a regular light/dark cycle (08:00–20:00 h, light). All animals were given standard laboratory diet and water ad libitum. Eight out of ten animals were used in the control group and in each treatment group.

### 2.2. Drugs and treatments

(−)-Linalool (Sigma) was administered at the doses of 50, 100 and 150 mg/kg dissolved in polyethylene glycol (PEG)-200 (Sigma). The treatment was performed by abdominal subcutaneous (s.c.) injection. Atropine, pirenzepine (Sigma), naloxone (Sigma), sulpiride (Sigma) and SCH-23390 were dissolved in sterile saline; glibenclamide (Sigma) was dissolved in PEG-200. All drugs were administered by intraperitoneal (i.p.) injection 15 min before (−)-linalool administration. All experiments were performed between 08:30 and 15:00 h.

### 2.3. Antinociceptive tests

#### 2.3.1. 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 (Basile, Italy) maintained at 55 ± 0.5 °C; the time between placement of the animal on the hot plate and the occurrence of either the licking of the fore or hind paws, shaking or jumping off the surface was recorded as response latency. Mice with baseline latencies of more than 10 s were excluded 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 the antinociceptive effect was expressed as changes in thermal latency in seconds with respect to the basal value at each observation time point.

Separate groups of mice were pre-treated with atropine (5 mg/kg, i.p.), pirenzepine (75 mg/kg, i.p.), naloxone (5 mg/kg, i.p.), sulpiride (20 mg/kg, i.p.) or SCH-23390 (0.3 mg/kg, i.p.), 15 min before (−)-linalool administration. A separate group of rats was pre-treated with glibenclamide (1 mg/kg, i.p.), 15 min before (−)-linalool administration.

#### 2.3.2. Formalin test

The rats were individually placed into transparent Perspex cages (25 × 20 × 25 cm) and left for 30 min habituation to the new environment before formalin injection. Fifty microliters of diluted formalin (1%) were injected subcutaneously into the dorsal surface of a hind paw with a 30-gauge needle. Animals were then returned to the chambers and nocifensive behaviour was observed for the following 60 min and quantified as the number of flinches and/or licking/biting of the treated paw (Granados-Soto et al., 2002). Formalin-induced pain behaviour is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). The subjects were

treated with either (−)-linalool or vehicle 30 min before formalin treatment.

Separate groups of rats were pre-treated with atropine (5 mg/kg, i.p.), pirenzepine (75 mg/kg, i.p.), naloxone (5 mg/kg, i.p.), sulpiride (20 mg/kg, i.p.), SCH-23390 (0.3 mg/kg, i.p.) or glibenclamide (1 mg/kg, i.p.), 15 min before (−)-linalool administration.

### 2.4. Statistics

All data were expressed as the mean ± S.E.M. from each group. Formalin test data were analysed by one-way analysis of variance (ANOVA), followed by Least Significant Difference (LSD) test. Data of the early acute and late tonic phases were analysed separately.

Hot-plate test data were analysed by two-way ANOVA considering *treatment* as a between-groups factor and *time* as a within-groups factor, supplemented by LSD test. A level of probability of 0.05 or less was accepted as significant.

## 3. Results

### 3.1. Effect of (−)-linalool on the hot-plate test

ANOVA revealed a significant main effect of *treatment* ($F(2,96)=6.09$, $P=0.0032$) and *time* ($F(2,192)=6.47$, $P=0.0019$) but not a significant interaction between the two factors. LSD test, performed to investigate the differences between the groups, has shown a significant increase in latency time with respect to controls in animals treated with (−)-linalool at 100 mg/kg ($P=0.0051$) and 150 mg/kg ($P=0.0030$). No significant differences between the groups treated with 100 and 150 mg/kg (−)-linalool were observed (Fig. 1).

### 3.2. Effect of muscarinic receptor antagonists on (−)-linalool-induced antinociception in the hot-plate test

ANOVA concerning the effect of atropine and pirenzepine on (−)-linalool at the dose of 100 and 150 mg/kg showed a significant main effect of *treatment* ($F(3,85)=5.72$, $P=0.0013$ and $F(3,85)=7.55$, $P=0.00015$, respectively) but not a significant interaction between *time* and between the two factors. Further analyses (LSD test) has shown that at the dose tested, the unselective muscarinic receptor antagonist, atropine, produced a complete reduction of the antinociceptive effect of (−)-linalool at both doses tested (100 mg: $P=0.00035$, 150 mg/kg: $P=0.0047$) (Figs. 2 and 3). Moreover, atropine, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the latency time in the hot plate (data not shown).

Pre-treatment with 75 mg/kg of pirenzepine, a selective muscarinic M1 receptor antagonist did not prevent the analgesic effect of both active doses (100 and 150 mg/kg) of (−)-linalool in the hot plate (Figs. 2 and 3).

### 3.3. Effect of opioid receptor antagonist on (−)-linalool-induced antinociception in the hot-plate test

ANOVA relative to the effect of naloxone on (−)-linalool (100 and 150 mg/kg) revealed a significant main effect of *treatment* ($F(2,76)=4.68$, $P=0.012$ and $F(2,76)=4.57$, $P=0.013$, respectively) and *time* ($F(2,152)=3.15$, $P=0.045$ and $F(2,152)=10.82$, $P=0.00004$, respectively), and a significant interaction between the two factors only with 150 mg/kg dose ($F(4,152)=9.60$, $P=0.000001$). LSD test failed to show statistically significant differences between naloxone pre-treated groups and (−)-linalool 100 mg/kg (Fig. 2). However, a very significant difference with respect to (−)-linalool was demonstrated when naloxone was ad-

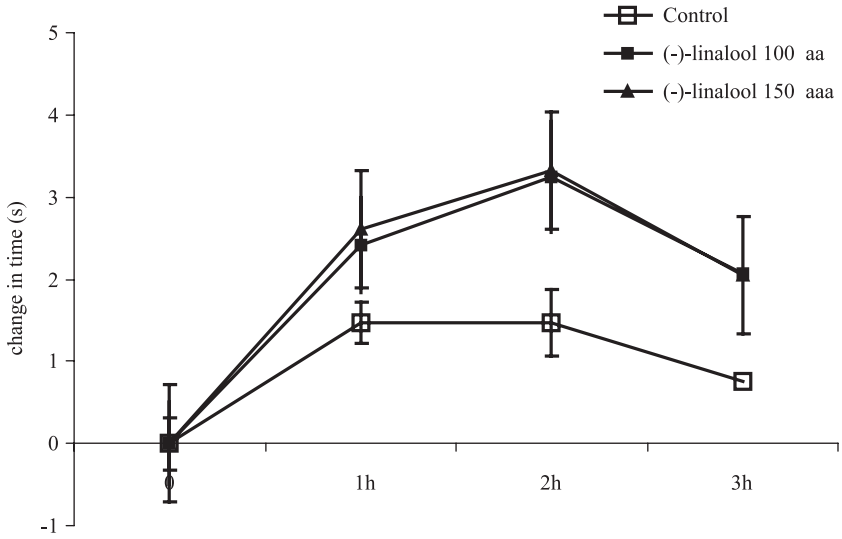


Fig. 1. Reaction time difference respect to the mean basal value (second) in response to the hot plate after (−)-linalool or vehicle (control) injection. The doses are expressed as milligrams per kilogram. Data represent mean values (± S.E.M). [a]Difference with respect to control group irrespective of time points. ([aa]$P<0.01$, [aaa]$P<0.005$, ANOVA followed by LSD test).

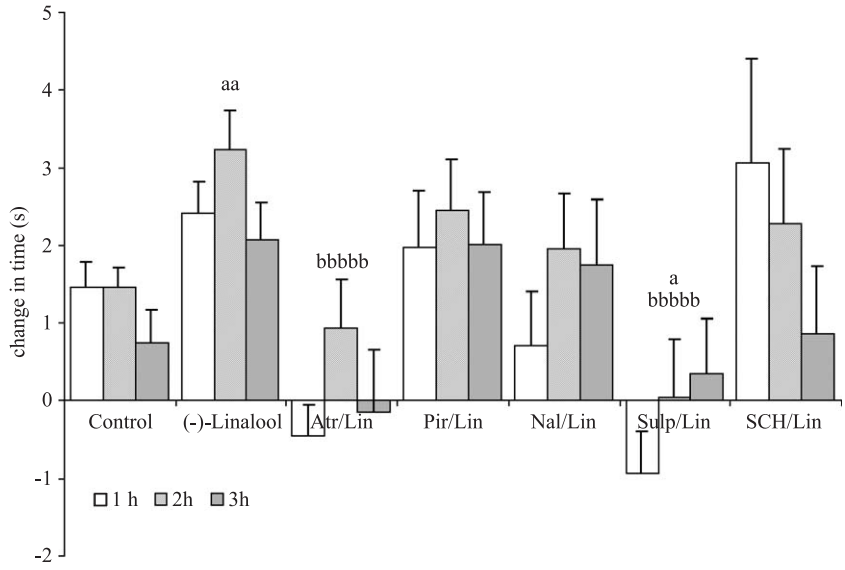


Fig. 2. Reaction time difference respect to the mean basal value (second) in response to antagonists on (−)-linalool-induced antinociception (100 mg/kg s.c.) on hot plate. Data represent mean values (± S.E.M). [a]Difference with respect to control group irrespective of time points. [b]Difference with respect to (−)-linalool group irrespective of time points. ([a]$P<0.05$, [aa]$P<0.01$, [bbbbb]$P<0.0005$, ANOVA followed by LSD test).

ministered to rats treated with (−)-linalool at 150 mg/kg only at the first hour of observation ($P=0.0000001$) (Fig. 3). Moreover, naloxone, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the latency time in the hot plate (data not shown).

### *3.4. Effect of dopamine receptor antagonists on (−)-linalool-induced antinociception in the hot-plate test*

ANOVA relative to the effect of dopamine receptor antagonists on (−)-linalool (100 and 150 mg/kg) revealed a significant main effect of *treatment* ($F(3,84)=6.79$, $P=0.00037$ and $F(3,85)=4.47$, $P=0.0057$, respectively). Further analysis (LSD test), performed to investigate the differences between the treatment groups, has shown that sulpiride not only inhibited ($P=0.00008$) but even reversed the antinociceptive effect of (−)-linalool at the dose of 100 mg/kg ($P<0.05$) (Fig. 2). In contrast, sulpiride failed to block the effect of the highest dose of (−)-linalool (150 mg/kg) (Fig. 3+).

Pre-treatment with SCH-23390, a selective dopamine D1 receptor antagonist failed to antagonise (−)-linalool-induced antinociception at both doses (100 and 150 mg/kg) (Figs. 2 and 3).

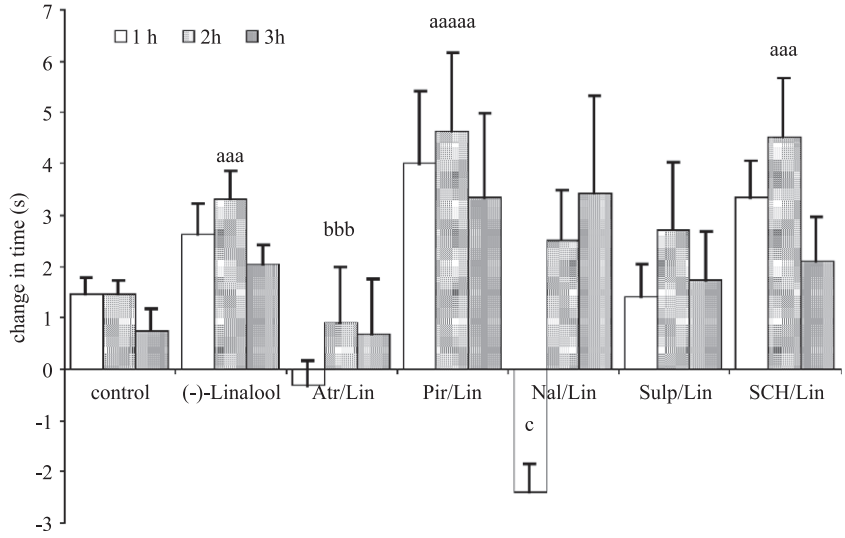


Fig. 3. Reaction time difference respect to the mean basal value (second) in response to antagonists on (−)-linalool-induced antinociception (150 mg/kg s.c.) on hot plate. Data represent mean values (± S.E.M). [a]Difference with respect to control group irrespective of time points. [b]Difference with respect to (−)-linalool group irrespective of time points. [c]Difference with respect to (−)-linalool group relative to single time points. ([b,c]$P<0.05$, [aaa,bbb]$P<0.005$, [aaaaa]$P<0.0005$, ANOVA followed by LSD test).

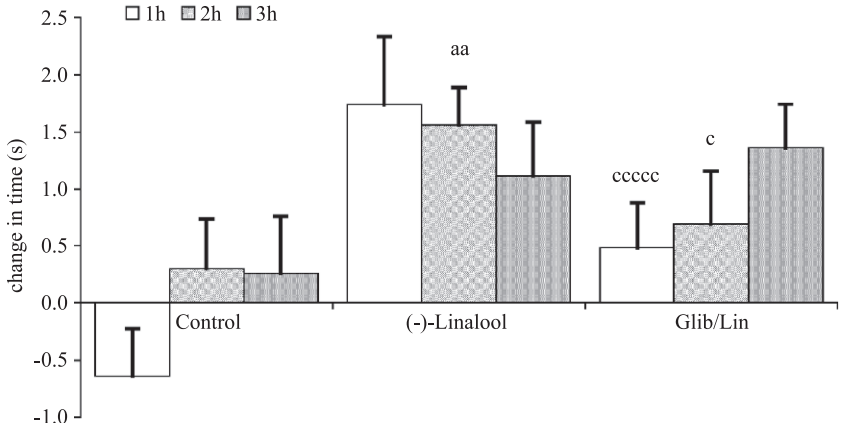


Fig. 4. Reaction time difference respect to the mean basal value (second) in response to glibenclamide on (−)-linalool-induced antinociception (100 mg/kg s.c.) on hot plate. Data represent mean values (± S.E.M). [a]Difference with respect to control group irrespective of time points. [c]Difference with respect to (−)-linalool group relative to single time points. ([c]$P<0.05$, [aa]$P<0.01$, [ccccc]$P<0.0005$, ANOVA followed by LSD test).

### 3.5. Effect of glibenclamide on (−)-linalool-induced antinociception in the hot-plate test

Pre-treatment with the ATP-sensitive $K^+$ channel inhibitor glibenclamide (1 mg/kg) was able to prevent only the antinociceptive effect of 100 mg/kg of (−)-linalool. In this set of data, ANOVA revealed a significant effect of *treatment* ($F(2,21)=4.01$, $P=0.033$) and a significant interaction between *treatment* and *time* ($F(4,42)=2.66$, $P=0.046$). Further analyses (LSD test) showed that this blockade was apparent only after 1 h ($P=0.0048$) and 2 h ($P=0.043$) after (−)-linalool administration (Fig. 4).

### 3.6. Effect of (−)-linalool in the 1% formalin test in rats

Formalin administration produced a typical pattern of pain responses. The first phase (early, acute phase) started immediately after administration of formalin and then diminished gradually in about 10 min. The second phase (late, tonic phase) started at about 15 min and lasted for 1 h. ANOVA of early acute phase data revealed a significant main effect ($F(3,58)=3.16$, $P=0.031$). Further analysis (LSD test) showed that (−)-linalool induced an antinociceptive effect in the early acute phase when compared with vehicle-treated control animals ((−)-linalool 50: $P=0.013$; (−)-linalool 100: $P=0.040$; (−)-linalool 150: $P=0.048$) with no significant differences among the groups treated with 50, 100 and 150 mg/kg (Fig. 5).

ANOVA of late tonic phase data revealed a significant main effect ($F(3,58)=3.86$, $P=0.014$). Further analysis (LSD test) showed that (−)-linalool at 150 mg/kg produced a reduction (32%) of the formalin-induced flinching/licking/biting response with respect to control group ($P=0.0058$) No effect was observed with 50 and 100 mg/kg doses (Fig. 6).

### 3.7. Effect of muscarinic receptor antagonists on (−)-linalool-induced antinociception in the 1% formalin test

ANOVA of early acute phase data relative to the effect of the atropine (5 mg/kg) and pirenzepine (75 mg/kg) receptor antagonists on 50 mg/kg (−)-linalool-induced antinociception revealed a significant main effect ($F(3,47)=3.27$, $P=0.029$). Further analysis (LSD test) showed that pretreatment with the unselective muscarinic receptor antagonist atropine decreased the antinociceptive effect of (−)-linalool ($P=0.038$) (Fig. 7). Atropine did not show any

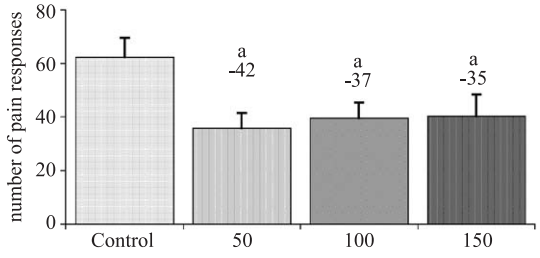


Fig. 5. The dose effect (mg/kg s.c.) of (−)-linalool on the acute phase of formalin test. Data represent mean values (± S.E.M) and percent inhibition compared to the control animals. Significant differences from control group are indicated by [a]. ([a]$P<0.05$, ANOVA followed by LSD test).

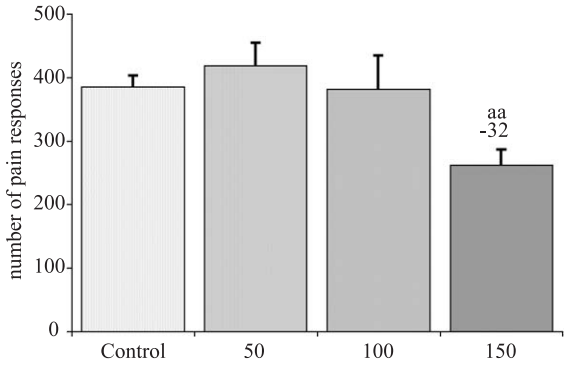


Fig. 6. The dose effect (mg/kg s.c.) of (−)-linalool on the tonic phase of formalin test. Data represent mean values (± S.E.M) and percent inhibition compared to the control animals. Significant differences from control group are indicated by [a]. ([aa]$P<0.01$, ANOVA followed by LSD test).

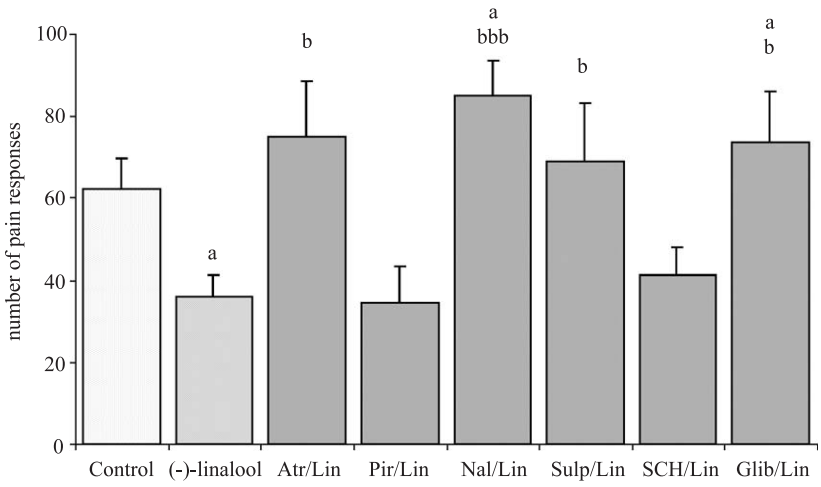


Fig. 7. Effect of antagonists on (−)-linalool-induced antinociception (50 mg/kg s.c.) on the acute phase of formalin test. Each value represents the mean (± S.E.M.). Significant differences from control group are indicated by [a] while from (−)-linalool group by [b]. ([a,b]$P<0.05$, [bbb]$P<0.005$, ANOVA followed by LSD test).

significant effect with higher doses (100 and 150 mg/kg) of (−)-linalool (Table 2; Fig. 8).

ANOVA of late tonic phase relative to the effect of the atropine and pirenzepine on the active dose of (−)-linalool (150 mg/kg) in the formalin test revealed a significant main effect ($F(3,43)=2.80$, $P=0.051$). LSD test showed that atropine pre-treatment produced a complete prevention of the (−)-linalool-induced antinociception of ($P=0.029$) (Fig. 9). Atropine, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the number of pain behaviours after formalin injection (data not shown). Pirenzepine, a selective muscarinic M1 receptor antagonist failed to antagonise the antinociceptive effect of (−)-linalool at all the doses tested in both phases of the formalin response (Figs. 7–9; Tables 1–3). By contrast, pirenzepine, when administered with linalool at the dose of 100 mg/kg, resulted in an increase of the pain response in the late phase ($P<0.005$) (Table 3).

### 3.8. Effect of opioid receptor antagonist on (−)-linalool-induced antinociception in the 1% formalin test

ANOVA of early acute phase data relative to the effect of opioid receptor antagonist naloxone (5 mg/kg) on 50 mg/kg (−)-linalool-induced antinociception showed a significant main effect ($F(2,43)=5.36$, $P=0.0083$). Further analysis (LSD test) revealed that pre-treatment of animals with naloxone, resulted in a complete reversal of the antinociception-induced by (−)-linalool ($P=0.003$) (Fig. 7). Groups treated with naloxone and higher doses of (−)-linalool (100 and 150 mg/kg) showed a mean number of pain response similar to the control group; however the differences between these groups

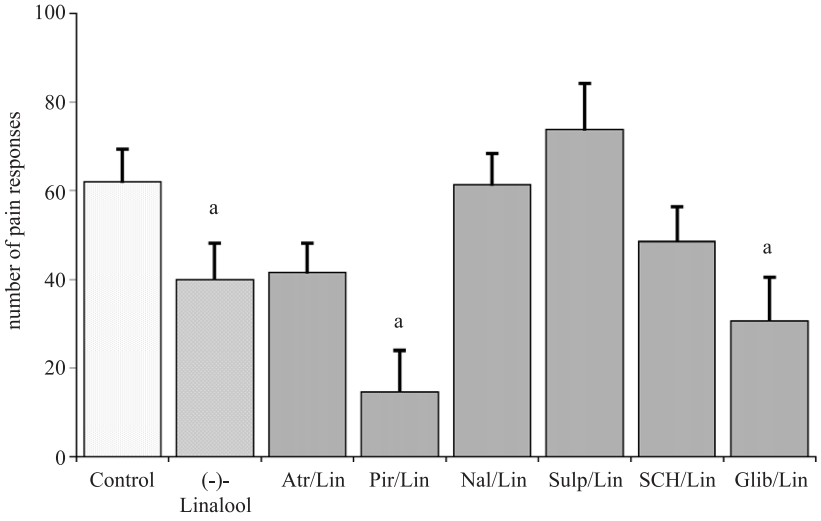


Fig. 8. Effect of antagonists on (−)-linalool-induced antinociception (150 mg/kg s.c.) on the acute phase of formalin test. Each value represents the mean (± S.E.M.). Significant differences from control group are indicated by [a]. ([a]$P<0.05$, ANOVA followed by LSD test).

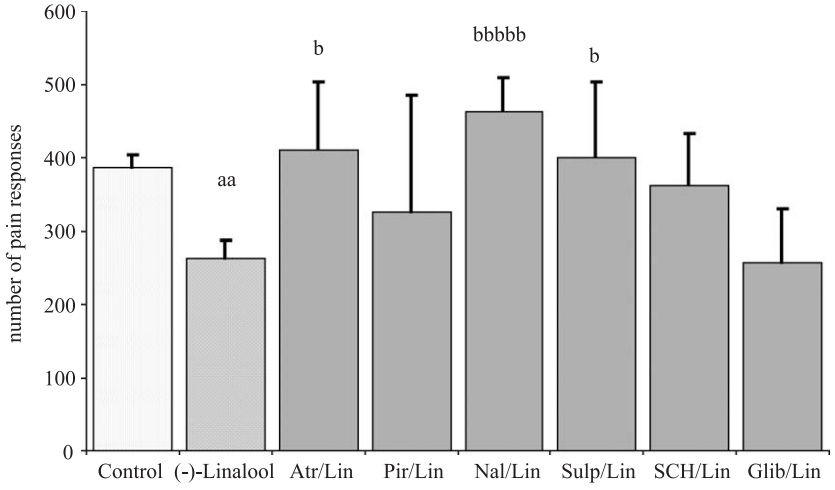


Fig. 9. Effect of antagonists on (−)-linalool-induced antinociception (150 mg/kg s.c.) on the tonic phase of formalin test. Each value represents the mean (± S.E.M.). Significant differences from control group are indicated by [a] while from (−)-linalool group by [b]. ([b]$P<0.05$, [aa]$P<0.01$, [bbbbb]$P<0.001$, ANOVA followed by LSD test).

and the groups treated with the corresponding dose of (−)-linalool did not reach statistical significance (Table 2; Fig. 8).

Nevertheless, ANOVA relative to the effect of naloxone on active dose of (−)-linalool (150 mg/kg) during the late, tonic phase of the test revealed a significant main effect ($F(2,41)=10.29$, $P=0.00024$). LSD test showed that naloxone decreased the antinociceptive effect of (−)-linalool ($P=0.00015$) (Fig. 9). In addition, naloxone resulted in an increased pain response in the late phase ($P<0.05$), with linalool (50 mg/kg) being devoid of effect per se (Table 1). Moreover, naloxone, at the dose shown to be effective in antagonising (−)-linalool effect, failed to influence the number of pain behaviours after formalin injection (data not shown).

### 3.9. Effect of dopamine receptor antagonists on (−)-linalool-induced antinociception in the 1% formalin test

ANOVA of early acute phase data relative to the effect of dopamine receptor antagonists on 50 mg/kg (−)-linalool-induced antinociception revealed a significant main effect ($F(3,50)=2.65$, $P=0.050$). Further analysis (LSD test) revealed that pre-treatment with sulpiride, resulted in a complete antagonism of the antinociception induced by (−)-linalool ($P=0.031$) (Fig. 7). Groups treated with sulpiride and higher doses of (−)-linalool (100 and 150 mg/kg) showed a mean number of pain response similar to the control group; however, the differences between these groups and the groups treated with the corresponding dose of (−)-linalool did not reach statistical significance (Fig. 8; Table 2).

ANOVA of late tonic phase data relative to the antagonism of sulpiride on the active dose (150 mg/kg) of (−)-linalool revealed a significant main effect ($F(2,41)=4.30$, $P=0.020$). LSD test highlighted that pre-treatment with sulpiride antagonised the (−)-linalool-effect ($P=0.033$) (Fig. 9).

Pre-treatment with SCH-23390, a selective dopamine D1 receptor antagonist, failed to antagonise (−)-linalool effects on both phases of the test (Figs. 7–9; Tables 2 and 3). In contrast, ANOVA relative to the effect of SCH-23390 on a

Table 1
Effects of (−)-linalool (50 mg/kg) in the presence or absence of antagonist drugs on the tonic phase of formalin test

| Treatment | Time after formalin injection (min) | |
|---|---|---|
| | 15–60 | $P$ |
| Control | 385 ± 18.50 | |
| (−)-Linalool | 418 ± 36.57 | |
| (−)-Linalool + atropine | 469 ± 77.10 | |
| (−)-Linalool + pirenzepine | 361 ± 55.34 | |
| (−)-Linalool + naloxone | 566 ± 52.72 | aaa, b |
| (−)-Linalool + sulpiride | 423 ± 51.26 | |
| (−)-Linalool + SCH-23390 | 228 ± 36.27 | aaa, bbbb |
| (−)-Linalool + glibenclamide | 483 ± 12.33 | a |

[a]$P<0.05$; [aaa]$P<0.005$, different from control group; [b]$P<0.05$, [bbbb]$P<0.005$ different from (−)-linalool group.

Table 2
Effects of (−)-linalool (100 mg/kg) in the presence or absence of antagonist drugs on the acute phase of formalin test

| Treatment | Time after formalin injection (min) | |
|---|---|---|
| | 0–10 | $P$ |
| Control | 62 ± 7.35 | |
| (−)-Linalool | 39 ± 6.02 | a |
| (−)-Linalool + atropine | 58 ± 10.97 | |
| (−)-Linalool + pirenzepine | 36 ± 11.95 | |
| (−)-Linalool + naloxone | 51 ± 14.16 | |
| (−)-Linalool + sulpiride | 59 ± 13.49 | |
| (−)-Linalool + SCH-23390 | 51 ± 9.97 | |
| (−)-Linalool + glibenclamide | 59 ± 16.18 | |

[a]$P<0.05$, different from control group.

Table 3
Effects of (−)-linalool (100 mg/kg) in the presence or absence of antagonist drugs on the tonic phase of formalin test

| Treatment | Time after formalin injection (min) | |
|---|---|---|
| | 15–60 | *P* |
| Control | 385 ± 18.50 | |
| (−)-Linalool | 381 ± 53.75 | |
| (−)-Linalool + atropine | 518 ± 51.87 | a |
| (−)-Linalool + pirenzepine | 590 ± 101.81 | aaa, bb |
| (−)-Linalool + naloxone | 386 ± 48.02 | |
| (−)-Linalool + sulpiride | 449 ± 63.54 | |
| (−)-Linalool + SCH-23390 | 390 ± 41.32 | |
| (−)-Linalool + glibenclamide | 530 ± 95.78 | a |

[a]$P<0.05$, [aaa]$P<0.005$ different from control group; [bb]$P<0.01$, different from (−)-linalool group.

low dose of (−)-linalool (50 mg/kg) unable to induce antinociception by itself in the late phase of the test showed a significant main effect ($F(2,43)=6.89$, $P=0.0025$) with an increased response of (−)-linalool effect ($P=0.00077$) (Table 1).

### *3.10. Effect of the ATP-sensitive $K^+$ channel inhibitor on (−)-linalool-induced antinociception in the 1% formalin test*

ANOVA relative to the effect of glibenclamide (1 mg/kg) on (−)-linalool (50 mg/kg) in the early phase of formalin test showed a significant main effect ($F(2,45)=3.82$, $P=0.029$). In this phase, glibenclamide reverted (−)-linalool-induced antinociception ($P=0.043$, LSD test) (Fig. 7). In contrast, glibenclamide failed to modify the effect induced by the higher doses (100 and 150 mg/kg) of (−)-linalool (Table 2; Fig. 8). Moreover, in the late phase glibenclamide, when administered to rats treated with the ineffective dose of linalool 100 mg/kg, resulted in an increase of the pain response ($P<0.05$) (Table 3). In addition, in the late tonic phase of the test, glibenclamide was not able to antagonise the effect of the active dose (150 mg/kg) of (−)-linalool (Fig. 9).

## 4. Discussion

The present results show the efficacy of (−)-linalool in antagonising different pain responses generated either by the exposure to a thermal nociceptive stimulus applied in the hot-plate test or by a tissue injure produced by formalin injection (Haley et al., 1990). The antinociceptive effects of (−)-linalool occurred at doses that did not produce any visible modification of the animal's gross behaviour.

In the present study, a significant antinociceptive response in the hot-plate test was observed in mice at the doses of 100 and 150 mg/kg of (−)-linalool, as revealed by the increased latency times. The unselective muscarinic receptor antagonist atropine prevented the effect of linalool at 100 and 150 mg/kg, suggesting an involvement of cholinergic transmission, while the failure of pirenzepine, a selective muscarinic M1 receptor antagonist, rules out the involvement of this receptor subtype in this effect. The effects of the other antagonists were different depending on the dose of (−)-linalool examined. (−)-Linalool effect at 100 mg/kg was not only inhibited but even reversed by the dopamine D2 receptor antagonist sulpiride, while the dopamine D1 receptor antagonist SCH-23390 failed to show any effect. Pre-treatment with the ATP-sensitive $K^+$ channel inhibitor, glibenclamide produced an inhibition of (−)-linalool-induced antinociception. These antagonists were unable to influence the effect of the higher dose of linalool. By contrast, the opioid receptor antagonist naloxone was effective only when administered to animals treated with the higher dose (150 mg/kg). These observations suggest that (−)-linalool activates different mechanisms depending on the dose, with dopaminergic transmission, via D2 receptors, and $K^+$ channels playing a major role in the effect of the lower dose, and with opioidergic transmission playing a major role in the effect of the higher dose. The ability of sulpiride to reverse (−)-linalool effect, taken together with the evidence that both the dopamine D2 agonist quinpirole and the dopamine D2 receptor antagonist sulpiride at certain doses may exert an analgesic effect on their own (Zarrindast et al., 1999), support the view of a complex role for the dopaminergic system in pain modulation.

In the formalin test, (−)-linalool at the dose of 50 and 100 mg/kg was found to be effective in preventing the neurogenic nociception elicited by formalin in the early acute phase, but not in the late tonic phase, while at the highest dose (150 mg/kg), it reduced significantly the pain response both in the early and in the late phase. Since inflammatory mechanisms play a predominant role in the late phase, the latter result is in agreement with the observations that (−)-linalool possesses anti-inflammatory activity in carrageenin-induced oedema in rats (Peana et al., 2002) and inhibited paw swelling induced by 1% formalin (unpublished data).

Blockade of muscarinic receptors with atropine, but not with the selective muscarinic M1 receptor antagonist pirenzepine, resulted in an inhibition of the effect of the lowest dose of (−)-linalool (50 mg/kg) in the early phase and of (−)-linalool effect (150 mg/kg) in the late phase of the formalin response. These results suggest an involvement of muscarinic transmission in the mechanism of action of (−)-linalool. However, at variance with these results is the observation that pirenzepine, when administered with (−)-linalool at the dose of 100 mg/kg, which by itself was devoid of any effect, resulted in an increase of the pain responses in the late phase.

Blockade of opioid receptors by naloxone, per se devoid of effects at the dose tested, reversed the effect of the dose of 50 mg/kg of (−)-linalool in the early phase, and resulted in an increased pain response in the late phase, with (−)-linalool devoid of effect per se. Moreover, naloxone inhibited the antinociceptive effect of (−)-linalool 150 mg/kg in the late phase. These results

suggest the involvement of opioidergic transmission in (−)-linalool effect in both phases of formalin response. However, the hypothesis of a functional antagonism by naloxone, although improbable due to its lack of effect per se, cannot be ruled out.

Blockade of dopamine D2 receptors with sulpiride inhibited the effect of (−)-linalool in the early phase ((−)-linalool 50 mg/kg) and in the late phase ((−)-linalool 150 mg/kg). By contrast, blockade of dopamine D1 receptors by SCH-23390 failed to antagonize (−)-linalool effects, and, in rats treated with the ineffective dose of 50 mg/kg (−)-linalool, resulted in an antinociceptive effect in the late phase. These results suggest an involvement of dopaminergic transmission in (−)-linalool-induced antinociception, with dopamine D1 and D2 receptors playing opposite roles.

Glibenclamide resulted in the reversal of the effect of the dose of 50 mg/kg (−)-linalool in the early phase of formalin test. When administered to rats treated with the ineffective dose of (−)-linalool 100 mg/kg, it resulted in an increase of the pain response in the late phase. These results suggest an involvement of $K^+$ channels in the (−)-linalool antinociceptive effect.

It is generally accepted that NMDA transmission is involved in the nociceptive responses (Chizh et al., 2001; Coderre and Van Empel, 1994; Haley et al., 1990). Thus, linalool, which has been shown to be a competitive receptor antagonist of NMDA receptors (Elisabetsky et al., 1999; Silva Brum et al., 2001a,b), might exert the antinociceptive effect in the hot-plate test by virtue of its antagonist activity on NMDA receptors. Consistently, the inhibition of NMDA receptor activity produces supraspinal analgesia mediated by the stimulation of central opioid receptors and by dopamine D1/D2 receptors (Bernardi et al., 1996; Forman, 1999). Thus, it might be suggested that (−)-linalool activate opioidergic and dopaminergic D2 transmissions, indirectly, as a consequence of its ability to antagonise NMDA receptors. It cannot be ruled out that blockade of NMDA receptors, by itself not sufficient to account for the antinociceptive effect in 1% formalin test (Sawynok and Reid, 2002), might nonetheless play an important role concurring with other pharmacological actions.

Further support to the involvement of opioidergic mechanism in (−)-linalool antinociceptive effects, in the hot plate and in the formalin test, is provided by several lines of experimental evidences showing a striking similarity between the pharmacological profile of (−)-linalool and opioids. It has been shown that opioid agonists such as morphine are effective in increasing latency times in the hot-plate test (Chiang and Zhuo, 1989) as well as in suppressing nociceptive responses such as those produced during acute and tonic phases of formalin test (Zarrindast et al., 1999). The antinociceptive effect of morphine is reduced not only by the opioid receptor antagonist naloxone but also by glibenclamide (Santos et al., 1999) and by intrathecal administration of atropine (Chiang and Zhuo, 1989) and it is influenced by dopamine agents (Zarrindast et al., 1999). Furthermore, SCH-23390, which in the present study showed the ability to exert an antinociceptive effect in the late tonic phase of formalin test if co-administered with a sub threshold dose of (−)-linalool, showed the same effect in combination with an inactive dose of morphine in the same experimental condition (Zarrindast et al., 1999).

Since muscarinic neurotransmission is involved in mediating antinociception in the rat spinal cord, and muscarinic receptor agonists and cholinesterase inhibitors have been shown to induce analgesia (Naguib and Yaksh, 1997; Miranda et al., 2002), the weak cholinesterase inhibitory activity of linalool (Perry et al., 2000) might also concur in determining its analgesic effect. Moreover, the antinociceptive effect produced by muscarinic agonists and acetylcholinesterase inhibitors might be mediated by opioids and $K_{ATP}^+$ channel activity (Cozanitis et al., 1983; Hartvig et al., 1989; Iwamoto and Marion, 1993). In addition, it is well documented that systemic administration of local anaesthetics induces antinociception depending by a central cholinergic mechanism (Bartolini et al., 1987) since pre-treatment with muscarinic receptor antagonists reduces this effect (Abelson and Höglund, 2002); thus, the local anaesthetic properties of linalool (Ghelardini et al., 1999) might also concur in determining its antinociceptive effect.

The observation that glibenclamide prevents (−)-linalool antinociception effect is consistent with previous work showing that $K^+$ channels activity influences various pain responses and that the opening of $K^+$ channels in the cell membrane plays an important role in the positive modulation of the analgesic effect of opioid and muscarinic agonists (Yamazumi et al., 2001). Moreover, opioid, muscarinic M2 and dopaminergic D2 receptors are coupled to $G_i/G_o$ proteins and one of the effects elicited by these G proteins is the opening of $K^+$ channels and the consequent cellular hyperpolarisation (Childers, 1991).

The present results are in agreement with the demonstrated pharmacological properties of linalool, mainly its cholinergic and local anaesthetic activity and its ability to block NMDA receptors. Furthermore, a key role seems to be played by $K^+$ channels, whose opening might be the consequence of a stimulation of muscarinic M2, opioid or dopamine D2 receptors.

The antinociceptive and antiinflammatory properties of linalool, shown in the present and in previous studies performed in our laboratory, suggest an interesting therapeutic potential for this compound and might account for the use of (−)-linalool-containing plant species in traditional medicine from different countries. Due to its very complex and unselective pharmacological profile, an extensive investigation on the possible untoward effects is granted.

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