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Effect of myrcene on nociception in mice

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Abstract—Myrcene, a monoterpene isolated from lemon grass oil (*Cymbopogon citratus*) has been investigated for antinociception in mice by a low temperature (51·5 ± 0·5°C) hot plate method and by the acetic acid-induced writhing test. Significant inhibition of nociception was seen in the tests with myrcene at doses of 10 and 20 mg kg^{-1} (i.p.) or at 20 and 40 mg kg^{-1} (s.c.), respectively. The antinociceptive effect was significantly antagonized by naloxone (1 mg kg^{-1}) or yohimbine (2 mg kg^{-1}). The results suggest that myrcene is capable of inducing antinociception in mice, probably mediated by α_2-adrenoceptor stimulated release of endogenous opioids.

Essential oil from *Cymbopogon citratus* (D.C) Stapf (Graminеae), commonly known as lemon grass oil, is used as a folk remedy in Brazil and elsewhere for the treatment of gastrointestinal disturbances (Alves et al 1960). The reported major constituents are citral (80%), an aldehyde, and myrcene (16%), a monoterpene (Silva & Bauer 1971). Despite its widespread use no reports on the pharmacological activity of this oil or its major constituents appear to have been published. In this study we show that the constituent myrcene exerts an antinociceptive effect in the mouse.

Materials and methods

Swiss male mice, 25–28 g, were kept at an ambient temperature of 25 ± 2°C and fed on standard pellet chow with water freely available.

Antinociceptive activity. This was measured by a low temperature (51·5 ± 0·5°C) hot plate method of Eddy & Leimbach (1953) and by the acetic acid-induced writhing test of Koster et al (1959).

In the hot plate test, each mouse received two trials on the hot plate, separated by a 30 min interval. The first trial familiarized the animal with the test procedure and the second trial served as the control reaction time (licking of hind feet or jumping) for the animal. Mice were preselected, any showing a reaction time greater than 10 s were not used. Immediately after the second trial, groups of mice (10 per group) were given myrcene (10 or 20 mg kg^{-1}) or vehicle (0·9% NaCl solution containing 2% Tween 80) intraperitoneally (i.p.) in a volume of 10 mL kg^{-1}. The reaction time for each mouse was determined on the hot plate surface at 15 min intervals after drug administration for a total of 120 min. To avoid possible injury, there was a cut off period of 45 s while measuring the reaction time. In a few experiments, the influence of simultaneously administered naloxone (1 mg kg^{-1} s.c.) or yohimbine (2 mg kg^{-1} s.c.) on myrcene-induced antinociception was assessed.

In the acetic acid test, each mouse was injected i.p. with 0·6% (v/v) aqueous acetic acid (10 mL kg^{-1}) 30 min after s.c. administration of myrcene (20 or 40 mg kg^{-1}) or vehicle. Groups of six to eight animals were used. Five minutes after acetic acid treatment the mice were observed for 20 min and the number of writhes counted. The effect of simultaneous administration of naloxone (1 mg kg^{-1} s.c.) or yohimbine (2 mg kg^{-1} s.c.) was also verified on the nociceptive activity of 20 mg kg^{-1} myrcene.

Drugs used were: myrcene (CEPEQ, Brazil), morphine (CEME, Brazil), naloxone (Sigma) and yohimbine (Sigma). Myrcene was suspended in 0·9% NaCl solution containing 2% Tween 80. All other drugs were diluted in 0·9% NaCl solution. The data were evaluated by use of ANOVA and *t*-independent test. Statistical significance was assigned for $P < 0·05$.

Results

Results of the hot plate test are shown in Fig. 1. Myrcene (10 or 20 mg kg^{-1}) administered i.p., produced a dose related elevation in the reaction time lasting about 120 min with a peak effect between 45 and 60 min. The antinociceptive response, however, was inferior to that produced by morphine (5 mg kg^{-1}, i.p.). Myrcene (20 mg kg^{-1})-induced analgesia was partially atte-

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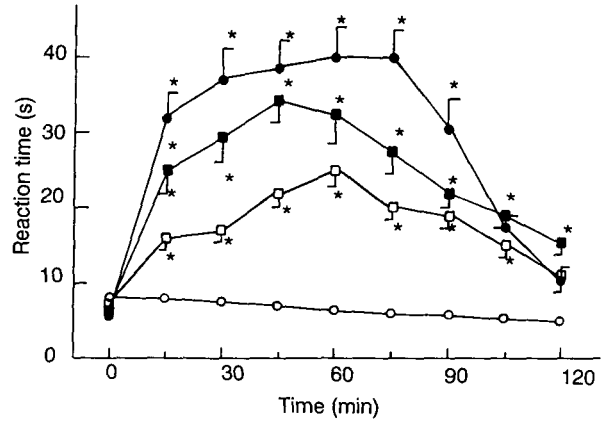

FIG. 1. Time course of reaction time changes induced by thermal stimuli (hot plate) in mice treated i.p. with vehicle (○——○), morphine 5 mg kg^{-1} (●——●), myrcene 10 mg kg^{-1} (□——□) or 20 mg kg^{-1} (■——■). n = 10 mice per group. * $P < 0.05$.

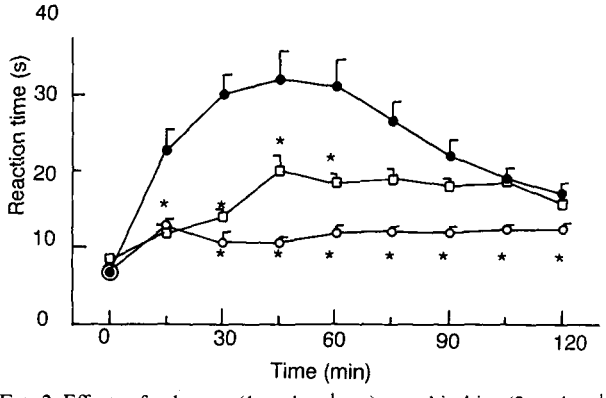

FIG. 2. Effects of naloxone (1 mg kg^{-1} s.c.) or yohimbine (2 mg kg^{-1} s.c.) on the antinociception induced by i.p. myrcene (40 mg kg^{-1}) in mice. Myrcene (●——●), myrcene + naloxone (□——□), myrcene + yohimbine (○——○). n = 10 mice per group. * $P < 0.05$.

nuated by naloxone (1 mg kg^{-1}) significantly at 30, 45 and 60 min, and by yohimbine (2 mg kg^{-1}) which almost completely reversed the terpene's analgesic effect (Fig. 2).

In the acetic acid writhing test, myrcene produced a dose-dependent reduction in the mean number of writhes per mouse. From the mean number for vehicle-treated mice of 59·3 ± 4·8 to 39 ± 3.2 (34·2%) and 27 ± 2.4 (55·1%) at 20 and 40 mg kg^{-1}, respectively. Simultaneous treatment with naloxone or yohimbine completely reversed the antinociceptive effect of 40 mg kg^{-1} myrcene.

Discussion

The results show that myrcene, a monoterpene from lemon grass oil, exerts analgesia by acting at both central and peripheral sites as evidenced by an increase in reaction time of mice to thermal stimuli in the hot plate test and the decrease in the number of writhes to chemical stimuli in the acetic acid test. Myrcene-induced analgesia was reversed by pretreatment with naloxone in both tests suggesting the mediation of endogenous opioids in its mechanism. Several reports indicate that stress (Hart et al 1983), electroconvulsive shock (Abbiati et al 1985), and pharmacologically diversified compounds such as tricyclic antidepressants (Takahashi & Paz 1987), anti-histamines (Yeh 1986) and antihypertensives like clonidine (Pettibone & Mueller 1981) produce naloxone-reversible opioid-like antinociception. In the hot plate test, the effect of naloxone on myrcene-induced antinociception was significant only up to 60 min post injection. This may imply either a short lived action of naloxone or different mechanisms underlying the antagonism of endogenous opioid and morphine.

Presynaptic α-adrenoceptor stimulation at both central and peripheral sites has been shown to enhance β-endorphin levels in the circulation (Pettibone & Mueller 1981). There have been reports that local or systemic administration of clonidine, an α_2-adrenergic agent, produces analgesia (Fielding et al 1978; Nakamura & Ferreira 1988) and this effect could be inhibited by yohimbine, an α_2-adrenoceptor antagonist (Bentley et al 1983; Portugal-Santana & Nakamura 1987). These observations prompted us to explore the possibility of presynaptic α_2-adrenoceptor participation in the antinociceptive effect of myrcene using yohimbine, at a dose that blocks clonidine analgesia (Portugal-Santana & Nakamura 1987). Since yohimbine effectively blocked the analgesic effect of myrcene, it implies presynaptic α_2-adrenoceptors are involved in its action.

The present data establish the basis for the use of lemon grass oil in folk remedies to alleviate gastrointestinal disturbances.

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