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Antinociceptive effect of methyleugenol on formalin-induced hyperalgesia in mice

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Abstract

The effects of methyleugenol, an essential oil isolated from *Asiasari radix*, on antinociception were examined using the formalin test in mice. Oral administration of 10 mg/kg methyleugenol significantly decreased the duration of licking and biting behavior in the second phase without affecting that of the first phase, as did diclofenac, a non-steroidal anti-inflammatory drug. Methyleugenol also inhibited pain-related behaviors induced by intrathecal injection of *N*-methyl-D-aspartic acid (NMDA), while diclofenac did not affect these behaviors. These effects of methyleugenol were suppressed by bicuculline, a γ-aminobutyric acid$_A$ ($GABA_A$) antagonist. Muscimol, a $GABA_A$ agonist, displays the same action as methyleugenol with respect to the formalin test and NMDA-induced behaviors. Methyleugenol did not affect cyclooxygenase-1 and -2 activities. These results suggest that the antinociceptive effect of methyleugenol on the second phase of formalin-induced pain may be due to the inhibition of NMDA receptor-mediated hyperalgesia via $GABA_A$ receptors.

Keywords: Antinociceptive effect; Methyleugenol; $GABA_A$ receptor; NMDA receptor

1. Introduction

Methyleugenol (1-allyl-3, 4-dimethoxybenzene, Fig. 1), an alkenylbenzene compound, is a natural constituent of a number of essential oils including those of basil, nutmeg, mace, anise, clove, lemon grass, laurel leaf, and fruits. Furthermore, methyleugenol is used as a flavoring substance in a wide variety of dietary products such as cookies, ice cream, and nonalcoholic beverages, and is found in cosmetics, soaps, shampoos, fragrances, and herbal products in Europe, the USA, and other countries. In East Asia, methyleugenol is one of the substances found in the essential oil fraction of *Asiasari radix* (*Saishin* in Japanese). In Japan, it is prepared from *Asiasarum sieboldi* F. Maekawa or *A. heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa (Aristolochiaceae), which is one of the crude drugs in Kampo medicine (Yasuda et al., 1981; Hashimoto et al., 1994).

Many biological actions of methyleugenol have previously been reported. Hashimoto et al. (1994), who studied the anti-allergic activity of *A. radix* through in vivo and in vitro assays, reported that methyleugenol inhibited passive cutaneous anaphylaxis in rats, release of 5-lipoxygenase from RBL-1 cells, and inhibited leukotriene D4-induced constriction of the guinea pig ileum. Methyleugenol inhibited compound 48/80-induced systemic anaphylaxis and anti-dinitrophenyl IgE-induced local anaphylaxis in mice (Shin et al., 1997). Moreover, methyleugenol inhibited histamine release in mast cells activated by the compound 48/80 or anti-dinitrophenyl IgE and suppressed the expression of mRNA of L-histidine decarboxylase, a histamine-forming enzyme, in mast cells. These results suggest that the inhibitory effect of methyleugenol on anaphylaxis is based on inhibition of overexpression of the L-histidine decarboxylase gene (Shin et al., 1997). Methyleugenol inhibited constriction in the isolated guinea pig ileum induced by acetylcholine, histamine, and KCl through direct action on smooth muscle (Lima et al., 2000). Lahlou et al. (2004) reported the hypotensive action of methyleugenol in rats.

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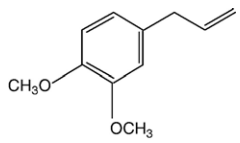

Fig. 1. Chemical structure of methyleugenol.

Carlini et al. (1981) reported the action of methyleugenol and its related compounds on the central nervous system. Intraperitoneal injection of 200–300 mg/kg of methyleugenol caused loss of the righting reflex and lack of sensitivity to tail pinching in rats and mice, and corneal reflex was lost in rabbits, suggesting that methyleugenol has an anesthetic action (Carlini et al., 1981; Sell and Carlini, 1976). Methyleugenol inhibited electroshock- or pentylenetetrazol-induced convulsions and demonstrated hypothermic and muscle relaxant actions (Dallmeier and Carlini, 1981, Dallmeier Zelger et al., 1983). Norte et al. (2005) reported that methyleugenol did not affect open-field, social interaction, elevated plus-maze, and holeboard parameters, which are related to anxiety, in rats, and that methyleugenol demonstrated an antidepressant action, i.e., reduction of the period of immobility in a forced swimming test.

Nevertheless, there was no evidence in these reports of the analgesic effect of methyleugenol. To clarify the analgesic effect of methyleugenol, we evaluated the antinociceptive effect of methyleugenol and its mechanism, using the formalin test in mice.

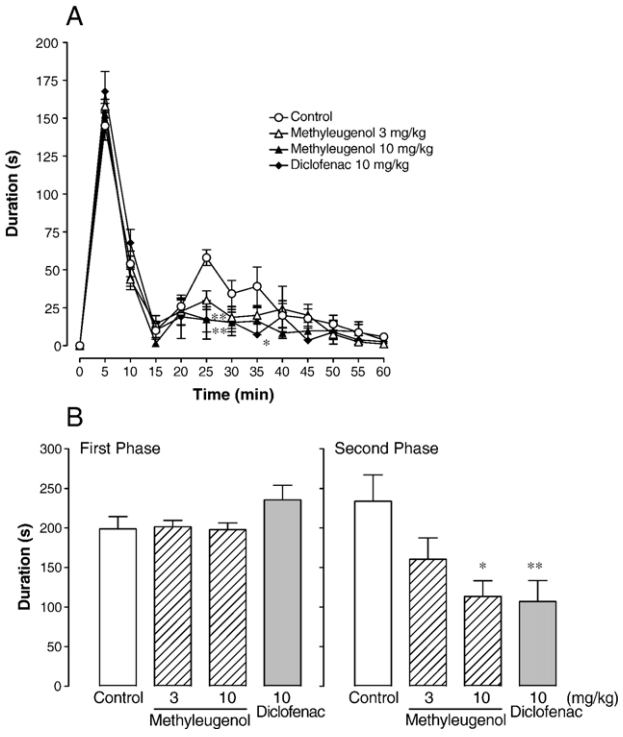

Fig. 2. A: Effects of pretreatment with methyleugenol (3 or 10 mg/kg, p.o.) or diclofenac (10 mg/kg, p.o.) on the time course of the formalin-induced biphasic nociceptive response in mice. B: Effects of methyleugenol or diclofenac on the total duration of response during the first (0–10 min) and second (15–60 min) phases. Each drug was administered 30 min before injection of formalin. Data are expressed as the total time spent licking and biting. Each point and column represents the mean±S.E.M. for 8 to 10 mice in each group. *$P<0.05$, **$P<0.01$ compared with the respective control group.

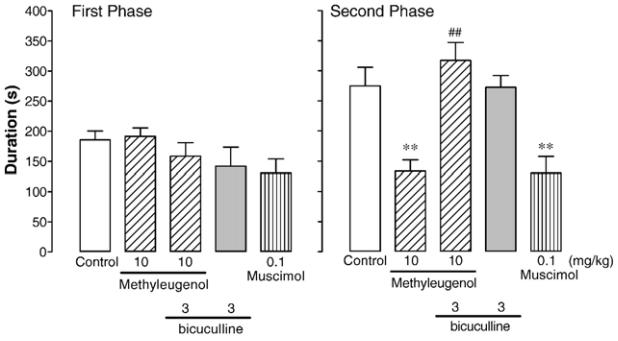

Fig. 3. Effect of bicuculline on methyleugenol-induced inhibition of formalin-induced nociceptive behavior in mice during the first and second phases. Methyleugenol (10 mg/kg, p.o.), bicuculline (3 mg/kg, s.c.), or muscimol (0.1 mg/kg, s.c.) was administered 30 min before injection of formalin. Data are expressed as the total time spent licking and biting. Each column represents the mean±S.E.M. for 7 to 10 mice in each group. **$P<0.01$ compared with the respective control group. ##$P<0.01$ compared with the methyleugenol-treated group.

2. Materials and methods

2.1. Animals

Male ddY strain mice weighing 20–30 g (Japan SLC, Inc., Hamamatsu, Japan) were used. They were maintained in an air-conditioned room with lighting from 7 a.m. to 7 p.m. The room temperature (23±3 °C) and humidity (50±20%) were controlled automatically. A laboratory pellet chow and water were given freely. All experimental procedures were performed according to the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee of Tsumura and Co.

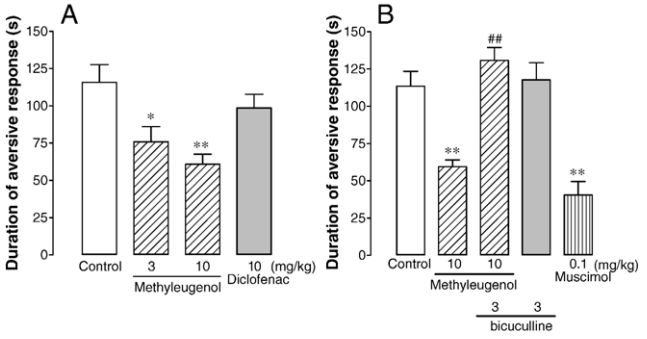

Fig. 4. A: Effects of pretreatment with methyleugenol (3 or 10 mg/kg, p.o.) or diclofenac (10 mg/kg, p.o.) on the total duration of NMDA-induced aversive behavior in mice. B: Effect of bicuculline (3 mg/kg, s.c.) on methyleugenol-induced inhibition of NMDA-induced aversive response. All drugs including muscimol (0.1 mg/kg, s.c.) were administered 30 min before injection of NMDA (0.25 nmol, i.t.). Data are expressed as the total time spent performing the aversive behavior described in the Materials and methods section. Each column represents the mean±S.E.M. for 10 or 11 mice in each group. *$P<0.05$, **$P<0.01$ compared with the respective control group. ##$P<0.01$ compared with the methyleugenol-treated group.