Orofacial Analgesic-Like Activity of Carvacrol in Rodents

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Carvacrol (CARV) is a phenolic monoterpene present in the essential oil of several aromatic spices. The purpose of the present study was to evaluate the antinociceptive effect of CARV on formalin-, capsaicin-, and glutamate-induced orofacial nociception in mice. Male mice were pretreated with CARV [25, 50, and 100 mg/kg body weight (BW), intraperitoneal (i.p.)], morphine (5 mg/kg BW, i.p.), or vehicle (distilled water + one drop of 0.3% cremophor in distilled water), before formalin (20 μl, 2%), capsaicin (20 μl, 2.5 μg), or glutamate (40 μl, 25 μM) was injected into the right upper lip. Our results revealed that i.p. pretreatment with CARV was effective in reducing the nociceptive face-rubbing behaviour in both phases of the formalin test and also produced a significant antinociceptive effect at all doses in the capsaicin and glutamate tests. Further, we showed that the action of CARV on the central nervous system (CNS) did not affect these results, since this compound did not exert a significant CNS-depressant effect, as shown by the pentobarbital-induced hypnosis. Our results suggest that CARV might represent an important tool for the treatment of orofacial pain.

Key words: Monoterpene, Carvacrol, Orofacial Pain

Introduction

Face and mouth represent locations of the body where pains are felt most commonly. Many of the difficulties in the management of acute and chronic orofacial pain conditions stem from a lack in recognition and understanding of orofacial pain mechanisms. The management of pain continues to be a major challenge for medicine (Miranda *et al.*, 2009). In recent times there has been a constant search for alternative drugs that possess higher efficacy and safety in reducing inflammatory and neuropathic pain with a strategy to halt the transition from acute to chronic pain (Holanda-Pinto *et al.*, 2008). Although during the last two decades notable progress has been made in the development of natural therapies, there is an urgent need to discover effective and potent analgesic agents (Yunes *et al.*, 2005).

An increasing number of studies have demonstrated that plant-derived essential oils exhibit a variety of biological properties (De Sousa, 2011). Monoterpenes are the primary components of these essential oils, and the pharmacological effects of many medicinal herbs have been attributed to them (De Sousa, 2011). Carvacrol (5-isopropyl-2-methylphenol, CARV) is a monoterpene phenol with substantial anticancer (Zeytinoglu *et al.*, 2003), antioxidant (Guimarães *et al.*, 2010), and anti-inflammatory (Botelho *et al.*, 2009) properties. This compound is predominant in many essential oils of the family Lamiaceae, including the *Origanum* and *Satureja* species used through the ages as a source of flavour in food. Recent studies have shown CARV to be effective as an analgesic compound in various pain models (Guimarães *et al.*, 2010) and have demonstrated the antinociceptive property of monoterpenoid compounds (like citronellal, linalool, and *p*-cymene) in orofacial pain models in rodents (Quintans-Júnior *et al.*, 2010; Venâncio *et al.*, 2011; Santana *et al.*, 2011).

Since CARV, whose biological function has not been well studied, is the main component of the

medicinal plants used in orofacial pain conditions (Botelho *et al.*, 2007), we investigated the antinociceptive activity of CARV in formalin-, capsaicin-, and glutamate-induced orofacial nociception in rodents. This is the first study that has evaluated the effect of CARV using an orofacial pain approach.

Material and Methods

Chemicals

Glutamate, capsaicin, ethanol, dimethyl sulfoxide (DMSO), and carvacrol (98% purity) were purchased from Sigma (St. Louis, MO, USA). Morphine and sodium pentobarbital were obtained from União Química (Fortaleza, Brazil) and formaldehyde from Vetec (Duque de Caxias, Brazil). Capsaicin was dissolved in ethanol/DMSO/distilled water (1:1:8 v/v/v).

Animals

Male Swiss mice (25–33 g) were obtained from the Central Animal Facility of the Federal University of Sergipe (São Cristóvão, Brazil). Animals were randomly assigned to groups and maintained in plastic boxes at controlled room temperature [(21 ± 2) °C)] with free access to food and water, under a 12 h/12 h light/dark cycle. All experiments were carried out between 9:00 a.m. and 2:00 p.m. in a quiet room. All nociception tests were carried out by the same visual observer. Experimental protocols were approved by the Animal Care and Use Committee (CEPA/UFS # 18/10) at the Federal University of Sergipe.

Formalin-, capsaicin-, and glutamate-induced orofacial nociception

These tests were done as previously described by Quintans-Júnior *et al.* (2010) and Luccarini *et al.* (2006). Orofacial nociception was induced in mice by subcutaneous (s.c.) injection of formalin (20 μl, 2%), capsaicin (20 μl, 2.5 μg), or glutamate (40 μl, 25 μM) into the right upper lip (perinasal area). To assess the effects of the test drugs, groups of mice (n = 8 per group) were pretreated systemically with vehicle (0.3% cremophor in distilled water, the solvent for CARV) or CARV [(25, 50, and 100 mg/kg body weight (BW), intraperitoneal (i.p.)] 0.5 h before the local injection of the nociceptive solution. Morphine (MOR, 5 mg/kg BW, i.p.), administered 0.5 h before the algogen, was included as positive control. Nociception was quantified by measuring the time (s) that the animals spent face-rubbing the injected area with their fore- or hindpaws.

Pentobarbital-induced hypnosis

Pentobarbital-induced hypnosis was performed as described by Melo *et al.* (2010). A hypnotic dose of 50 mg/kg BW sodium pentobarbital was injected i.p. 30 min after pretreatment with vehicle or CARV (50, 100, and 200 mg/kg BW, i.p.). Then the latency (interval between injection of sodium pentobarbital and loss of the righting reflex) and duration of sleeping time (interval between loss and recovery of the righting reflex) were determined.

Statistical analysis

Data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. In all cases, differences were considered significant if $p < 0.05$. All statistical analyses were done using Graph Pad Prism 3.02 (Graph Pad Prism Software Inc., San Diego, CA, USA).

Results and Discussion

In the present study, we showed that acute treatment with CARV, a monoterpene phenol, plays a protective role in reducing behavioural pain when evaluated for formalin-, capsaicin-, and glutamate-induced orofacial nociception in mice.

The orofacial formalin test has a singular advantage among all single-parameter methods, the simplicity of the scoring technique. This simplicity, coupled to the fact that a 2% content of formalin was used, makes the model a useful tool for the study of nociceptive processes in the trigeminal region (Raboisson and Dallel, 2004). The use of formalin in the orofacial region (right upper lip) induces a biphasic nociceptive response (Luccarini *et al.*, 2006). The first phase (0–5 min) corresponds to the direct stimulation of nociceptors, predominantly C fibers, with the participation of substance P, bradykinin, and glutamate (Shibata *et al.*, 1989). According to Hunskaar *et al.* (1985), this phase is only sensitive to analgesics acting on the central nervous system (CNS). The first phase is followed by an interphase, which lasts about 15 min and results from active inhibition of the excitability of nocicep-

tors. The second phase, which starts 21 min after the formalin injection, is determined by two components: central sensitization of nociceptors and second-order neurons; and the action of inflammatory mediators such as histamine, serotonin (5-HT), bradykinin, and prostaglandins released as a result of tissue injury (Henry *et al.*, 1999). This second phase of nociception could be inhibited by both nonsteroidal anti-inflammatory drugs (NSAID) and opioid drugs.

Our results showed that pretreatment with CARV (at all doses tested) and morphine produced antinociception in the orofacial formalin test, evidenced by a statistically significant difference ($p < 0.05$ or $p < 0.001$) for the time that the animal remained rubbing the orofacial region in both phases of testing (Figs. 1A and B). The results of the orofacial formalin nociception test differ from those obtained in our previous study that did not reveal a robust antinociceptive effect for CARV in the formalin paw test in the first phase (Guimarães *et al.*, 2010). Apparently, CARV possesses a higher effectiveness in reducing neurogenic orofacial pain, even when administered systemically.

The pretreatment with CARV caused a dose-dependent decrease of the nociceptive behaviour induced by administration of capsaicin at all doses (Fig. 2A). The inhibitory effect observed with CARV on the capsaicin-induced orofacial nociceptive behaviour may be a result of its possible inhibition of substance P release or due to a direct blocking action of its receptor neurokinin-1 (NK-1). Additionally, the capsaicin-sensitive transient receptor potential vanilloid 1 (TRPV1) plays an important role in pain transduction and is one of the Ca^{2+} influx channels involved in cell migration (Waning *et al.*, 2007).

When injected in the right upper lip (perinasal area), glutamate elicited a noxious stimulus characterized by a behavioural response (licking or rubbing of the orofacial region) (Quintans-Júnior *et al.*, 2010). Glutamate is present in both central and peripheral terminals of trigeminal and dorsal root ganglion neurons. Noxious stimulation of primary afferent fibers results in the release of glutamate from the peripheral as well as central terminals of trigeminal and spinal afferent fibers (Keast and Stephensen, 2000; Lam *et al.*, 2005). All doses of CARV elicited a dose-dependent protection ($p < 0.01$ or $p < 0.001$), when compared with the control group (vehicle; Fig. 2B). Thus, the suppression of glutamate-induced nociception by CARV treatment can

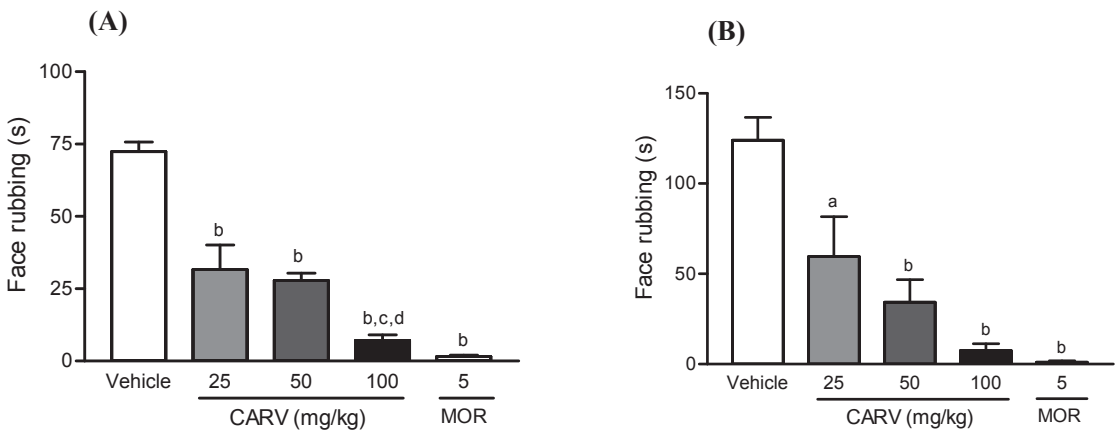

Fig. 1. Effects of carvacrol (CARV) or morphine (MOR) on the formalin-induced orofacial nociception in mice. Vehicle (control), CARV (25, 50, and 100 mg/kg BW), or MOR (5 mg/kg BW) were administered i.p. 0.5 h before formalin injection. (A) First phase (0–5 min) and (B) second phase (15–40 min). Each column represents means ± S.E.M. ($n = 8$ per group). [a]$p < 0.05$ and [b]$p < 0.001$ vs. control group; [c]$p < 0.01$ and [d]$p < 0.001$ compared to CARV 25 mg/kg BW and CARV 50 mg/kg BW group alone, respectively (ANOVA followed by Tukey's test).

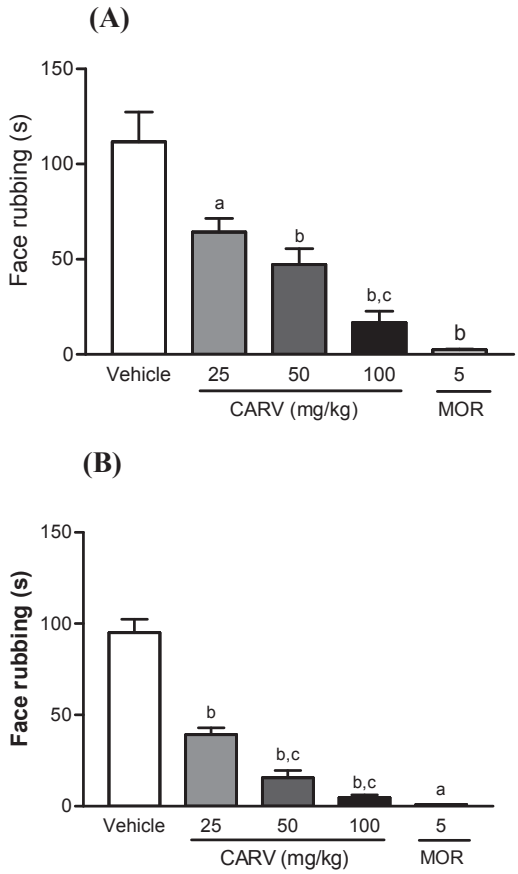

Fig. 2. Effects of carvacrol (CARV) or morphine (MOR) on the (A) capsaicin- and (B) glutamate-induced orofacial nociception in mice. Vehicle (control), CARV (25, 50, and 100 mg/kg BW), or MOR (5 mg/kg BW) were administered i.p. 0.5 h before capsaicin or glutamate injection. Each column represents means ± S.E.M. (n = 8 per group). $^{a}p < 0.01$ and $^{b}p < 0.001$ vs. control group; $^{c}p < 0.01$ compared to CARV 25 mg/kg BW group alone (ANOVA followed by Tukey's test).

be associated with its interaction with the glutamatergic system.

In the pentobarbital-induced hypnosis test, substances that depress the CNS generally increase the duration of sleep produced by sodium pentobarbital (Melo *et al.*, 2010). In this study, only the highest dose (200 mg/kg BW) of CARV was able to significantly increase the duration of sleep ($p < 0.05$) (Fig. 3). However, it is noteworthy that the compound did not show changes in motor coordination in previous studies (Guimarães *et al.*, 2010).

Taken together, these data lead to the hypothesis that carvacrol, a monoterpene phenol, has a protective role in orofacial nociception in rodents and a hypnotic effect at higher doses; however, further studies need to be performed to determine the precise mechanism of action.

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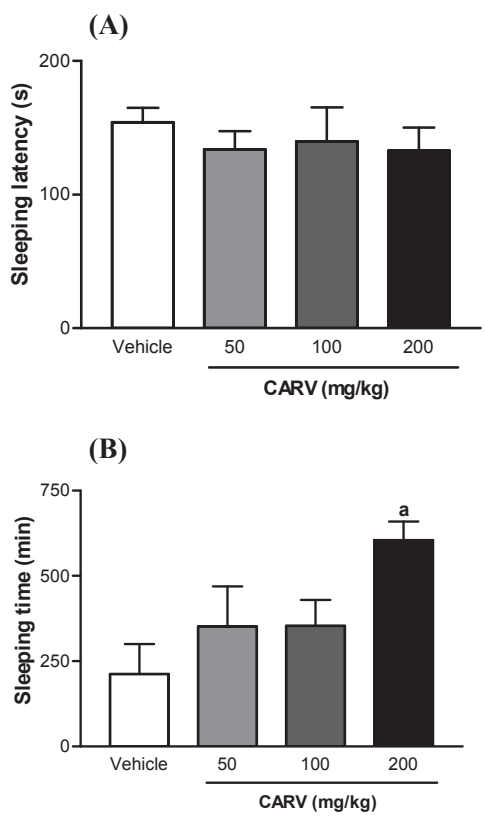

Fig. 3. Effect of carvacrol (CARV) on pentobarbital-induced hypnosis in mice. The parameters evaluated were the (A) onset of sleeping and (B) duration of sleeping. Values are means ± S.E.M. (n = 8 per group). $^{a}p < 0.05$ as compared to vehicle (control) (ANOVA followed by Tukey's test).

Botelho M. A., Nogueira N. A. P., Bastos G. M., Fonseca S. G. C., Lemos T. L. G., and Matos F. J. A. (2007), Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. Braz. J. Med. Biol. Res. **40**, 349–356.

Botelho M. A., Martins J. G., Ruela R. S., Santos J. A., Soares J. B., França M. C., Montenegro D., Ruela W. S., Barros L. P., Queiroz D. B., Araujo R. S., and Sampio F. C. (2009), Protective effect of locally applied carvacrol gel on ligature-induced periodontitis in rats: a tapping mode AFM study. Phytother. Res. **23**, 1439–1448.

De Sousa D. P. (2011), Analgesic-like activity of essential oils constituents. Molecules **16**, 2233–2252.

Guimarães A. G., Oliveira G. F., Melo M. S., Cavalcanti S. C. H., Antoniolli A. R., Bonjardim L. R., Silva F. A., Santos J. P. A., Rocha R. F., Moreira J. C. F., Araújo A. A. S., Gelain D. P., and Quintans-Júnior L. J. (2010), Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. Basic Clin. Pharmacol. Toxicol. **107**, 949–957.

Henry J. L., Yaspal K., Picher G. M., Chabot J. G., and Coderre T. J. (1999), Evidence for tonic activation of NK-1 receptors during the second phase of the formalin test in the rat. J. Neurosci. **19**, 6588–6598.

Holanda-Pinto S. A., Pinto L. M. S., Guedes M. A., Cunha G. M. A., Chaves M. H., Santos F. A., and Rao V. S. (2008), Antinoceptive effect of triterpenoid α,β-amyrin in rats on orofacial pain induced by formalin and capsaicin. Phytomedicine **15**, 630–634.

Hunskaar S., Fasmer O. B., and Hole K. (1985), Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Methods **14**, 69–76.

Keast J. R. and Stephensen T. M. (2000), Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. J. Comp. Neurol. **424**, 577–587.

Lam D. K., Sessle B. J., Cairns B. E., and Hu J. W. (2005), Neural mechanisms of temporomandibular joint and masticatory muscle pain: a possible role for peripheral glutamate receptor mechanisms. Pain Res. Manag. **10**, 145–152.

Luccarini P., Childeric A., Gaydier A. M., Voisin D., and Dallel R. (2006), The orofacial formalin test in the mouse: a behavioral model for studying physiology and modulation of trigeminal nociception. Pain **7**, 908–914.

Melo M. S., Sena L. C. S., Barreto F. J. N., Bonjardim L. R., Almeida J. R. G. S., Lima J. T., De Sousa D. P., and Quintans-Júnior L. J. (2010), Antinociceptive effect of citronellal in mice. Pharm. Biol. **48**, 411–416.

Miranda H. F., Sierralta F., and Prieto J. C. (2009), Synergism between NSAIDs in the orofacial formalin test in mice. Pharmacol. Biochem. Behav. **92**, 314–318.

Quintans-Júnior L. J., Melo M. S., De Sousa D. P., Araújo A. A. S., Onofre A. C. S., Gelain D. P., Gonçalves J. C. R., Araújo D. A. M., Almeida J. R. G. S., and Bonjardim L. R. (2010), Antinociceptive activity of citronellal in formalin-, capsaicin- and glutamate-induced orofacial pain in rodents and its action on nerve excitability. J. Orofac. Pain **24**, 305–312.

Raboisson P. and Dallel R. (2004), The orofacial formalin test. Neurosci. Biobehav. Rev. **28**, 219–226.

Santana M. F., Quintans-Júnior L. J., Cavalcanti S. C. H., Oliveira M. G. B., Guimarães A. G., Cunha E. S., Melo M. S., Santos M. R. V., Araújo A. A. S., and Bonjardim L. R. (2011), *p*-Cymene reduces orofacial nociceptive response in mice. Rev. Bras. Farmacogn. **21**, 1138–1143.

Shibata M., Ohkubo T., Takahashi H., and Inoki R. (1989), Modified formalin test: characteristic biphasic pain response. Pain **38**, 347–352.

Venâncio A. M., Marchioro M., Estevam C. S., Melo M. S., Santana M. T., Onofre A. S. C., Guimarães A. G., Oliveira M. G. B., Alves P. B., Pimentel H. C., and Quintans-Júnior L. J. (2011), *Ocimum basilicum* (Lamiaceae) leaf essential oil and (-)-linalool reduce orofacial nociception in rodents – a behavioral and electrophysiological approach. Rev. Bras. Farmacogn. **21**, 1043–1051.

Waning J., Vriens J., Owsianik G., Stüwe L., Mally S., Fabian A., Frippiat C., Nilius B., and Schwab A. (2007), A novel function of capsaicin-sensitive TRPV1 channels. Cell Calcium **42**, 17–25.

Yunes R. A., Cechinel Filho V., Ferreira J., and Calixto J. B. (2005), The use of natural products as sources of new analgesic drugs. Stud. Nat. Prod. Chem. **30**, 191–212.

Zeytinoglu H., Incesu Z., and Baser K. H. C. (2003), Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-RAS oncogene. Phytomedicine **10**, 292–299.