Antinociceptive Effects of Citronellal in Formalin-, Capsaicin-, and Glutamate-Induced Orofacial Nociception in Rodents and Its Action on Nerve Excitability

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Aims: *To evaluate the antinociceptive effects of citronellal (CTL) on formalin-, capsaicin-, and glutamate-induced orofacial nociception in mice and to investigate whether such effects might involve a change in neural excitability.* ***Methods:*** *Male mice were pretreated with CTL (50, 100, and 200 mg/kg, ip), morphine (5 mg/kg, ip), or vehicle (distilled water + one drop of Tween 80 0.2%) before formalin (20 µL, 2%), capsaicin (20 µL, 2.5 µg) or glutamate (40 µL, 25 µM) injection into the right vibrissa. Sciatic nerve recordings were made using the single sucrose gap technique in rats. The data obtained were analyzed by ANOVA followed by Dunnett's test for the behavioral analyses and by the Student* t *test for CAP evaluation.* ***Results:*** *Pretreatment with CTL was effective in reducing nociceptive face-rubbing behavior in both phases of the formalin test, which was also naloxone-sensitive. CTL produced significantly antinociceptive effect at all doses in the capsaicin- and glutamate- tests. Rota-rod testing indicated that such results were unlikely to be provoked by motor abnormality. Recordings using the single sucrose gap technique revealed that CTL (10 mM) could reduce the excitability of the isolated sciatic nerve through a diminution of the compound action potential amplitude by about 42.4% from control recordings.* ***Conclusion:*** *These results suggest that CTL might represent an important tool for management and/or treatment of orofacial pain.* J OROFAC PAIN 2010;24:305–312

Key words: antinociceptive, citronellal, neuronal excitability, orofacial pain, single sucrose gap technique

Pain is a complex, multidimensional experience that has a particular expression in the orofacial region since the face and mouth have a special biological, emotional, and psychological meaning for each individual. The orofacial region represents a region in the body where pain commonly occurs. Many of the difficulties in the management of acute and chronic orofacial pain conditions result from a lack of recognition and understanding of orofacial pain mechanisms.[1,2]

Although notable progress has been made in recent years in the development of natural therapies, there is an urgent need to discover effective and potent analgesic agents.[3] Essential oils are natural products that exhibit a variety of biological properties, such as analgesic,[4] anticonvulsant,[5] and anxiolytic.[6] These effects are attributed to the monoterpenes, which are the major chemical components of these essential oils. Citronellal (CTL) is a monoterpene, predominantly formed by the secondary metabolism of plants. It is typically

isolated as a nonracemic mixture of its R and S enantiomers by steam distillation or solvent extraction from the oils of *Corymbia citriodora* Hill and Johnson (former *Eucalyptus citriodora* Hook), *Cymbopogon nardus*, *C. citratus* and *C. winterianus* ("Java citronella").[5,7] It is also found in more than 50 other essential oils. Along with citral, geranial, linalool, and citronellol, CTL is one of the most important terpenes.[7] Until now, no data existed about the possible antinociceptive activity of this monoterpene. However, Holanda Pinto et al[8] have demonstrated the analgesic effect of another terpenoid, a triterpenoid α,β-amyrin, on orofacial nociception in rodents.

The aim of the present study was to evaluate the antinociceptive effects of CTL on formalin-, capsaicin-, and glutamate-induced orofacial nociception in mice and to investigate whether such effects might involve a change in neural excitability.

Materials and Methods

Animals

Male Swiss mice (30 to 36 g) and male Wistar rats (230 to 260 g), 2 to 3 months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 22 ± 2°C on a 12 hour light/dark cycle (lights on 06:00 to 18:00 hour) with free access to food and water. All experiments were carried out between 09:00 and 14:00 in a quiet room. All nociceptive tests were carried out by the same visual observer. Experimental protocols were approved by the Animal Care and Use Committee (CEPA/UFS # 12/08) at the Federal University of Sergipe.

Drugs

For all in vivo experiments, the following agents were used: CTL ([RS]-[±]-CTL, 98% purity, Dierberger), morphine hydrochloride (MOR) (União Química), naloxone hydrochloride (NAL) (Neoquímica), 37% formaldehyde (Vetec), Tween 80 (polyoxyethylene-sorbitan monolate), glutamate, and capsaicin (Sigma). Vehicle was one drop of Tween 80 0.2% dissolved in 0.9% saline solution and used to dilute the test drugs. In these protocols, the agents were injected intraperitoneally (ip) at a dose volume of 0.1 mL/10 g, except for the algesic chemicals formalin, glutamate, and capsaicin, which were injected subcutaneously (sc) into the right upper lip. The physiologic solution used for in vitro tests was composed of (in mM): NaCl 150; KCl 4; CaCl2 2; MgCl2 1; glucose 10; and [N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid] (HEPES) 10, adjusted to pH 7.4 with NaOH.

Formalin Test

Orofacial nociception was induced in mice by sc injection of 20 µL of 2% formalin into the right upper lip (perinasal area) using a 27-gauge needle.[9,10] This volume and percentage concentration of formalin was selected from pilot studies that showed a nociceptive-related biphasic behavioral response (face-rubbing) of great intensity at periods of 0 to 5 minutes (first phase) and 15 to 40 minutes (second phase). Nociception was quantified at these periods by measuring the time (seconds) that the animals spent face-rubbing in the injected area with its fore- or hindpaws.[10] To assess the effects of the test drugs, groups of mice (n = 8, each group) were pretreated systemically with vehicle (one drop of Tween 80 0.2% in distilled water, the solvent for CTL), CTL (50, 100, and 200 mg/kg, ip), 0.5 hours before the local injection of formalin. MOR (5 mg/kg, ip), administered 0.5 hours before the algogen, was included as a positive control. In separate experiments, the possible involvement of a µ-opioid mechanism was assessed in the antinociception produced by CTL or morphine with NAL (1.5 mg/kg, ip), a µ-opioid antagonist, injected simultaneously. To avoid unnecessary use of animals, NAL was applied only in this experiment.

Capsaicin Test

Orofacial nociception was induced by capsaicin as described earlier.[11] Mice (n = 8, each group) were injected with capsaicin (20 µL, 2.5 µg, sc) into the right upper lip (perinasal area) using a 27-gauge needle. Capsaicin was dissolved in ethanol, dimethyl sulfoxide, and distilled water (1:1:8). In pilot studies, rodents manifested nociceptive-related face-rubbing behavior with a high intensity for a 10- to 20-minute period following the injection of capsaicin. Therefore, quantification of nociception was performed at this period by measuring the time (seconds) that the animals spent face-rubbing the injected area with their fore- or hindpaws. CTL (50, 100, and 200 mg/kg, ip) or vehicle were given to animals as described for the formalin test, 0.5 hours before the local injection of capsaicin. MOR (5 mg/kg, ip), administered 0.5 hours before the algogen, was included as a positive control. An additional group received a similar volume of capsaicin vehicle.

Glutamate-Induced Nociception

In an attempt to provide more direct evidence concerning the interaction of the CTL with the glutamatergic system, the authors separately investigated whether the CTL was able to antagonize glutamate-induced orofacial nociception in mice. The procedure used was similar to that previously described by Beirith et al,[12] but with some alterations. A volume of 20 µL of glutamate (25 µM prepared in phosphate-buffered saline) was injected into the right upper lip (perinasal area) using a 27-gauge needle. Animals were observed individually for 15 minutes following the glutamate injection. Quantification of nociception was performed at this period by measuring the time (seconds) that the animals spent face-rubbing the injected area with their fore- or hindpaws. Animals (n = 8, per group) were treated with the CTL (50, 100, and 200 mg/kg, ip), MOR (5 mg/kg, ip), or vehicle 0.5 hours before the glutamate injection.

Evaluation of Motor Activity

To investigate if the treatments could influence the motor activity of the animals and consequently impair the assessment of the nociceptive behavior in the experimental models, the motor activity was evaluated in a Rota-rod apparatus.[13,14] Initially, the mice that were able to remain on the Rota-rod apparatus (AVS) longer than 120 seconds (9 rpm) were selected 24 hours before the test.[15] Then the selected animals were divided into five groups (n = 8) and treated ip with vehicle, CTL (25, 50, and 100 mL/kg, ip), or diazepam (1.5 mg/kg). At 30, 60, and 90 minutes later, each animal was tested on the Rota-rod apparatus and the time (seconds) it remained on the bar for up to 120 seconds.

Electrophysiologic Assays

Procedures for isolated nerve experiments have been described in previous papers.[16,17] Briefly, the sciatic nerves from rats (n = 4) were carefully removed and desheathed. One nerve bundle was positioned across the five compartments of the experimental chamber, which contained vaseline at the partitions to isolate them electrically. Compartments 1 and 2, at one end of the nerve bundle, were used to apply supramaximal stimulation, which consisted of 100 µs isolated rectangular voltage pulses (6 to 8 V) delivered by a stimulator (CF Palmer, Model 8048) that was triggered manually. These parameters were chosen to stimulate selectively fast-conducting myelinated fibers (Aα). All compartments were filled with a physiologic solution with the following composition (in mM): NaCl 150; KCl 4.0; CaCl2 2.0; MgCl2 1.0; HEPES 10, adjusted to pH 7.4 with NaOH, except for the fourth compartment, which was filled with isotonic sucrose solution (280 mM) that was continuously renewed in order to electrically isolate the neighboring recording compartments. CTL at different concentrations was introduced into the test (central) compartment. The potential difference between the test and the fifth (last) compartment was recorded every 10 minutes over a 30-minute period. Data were converted to digital form by a microcomputer-based 12-bit A/D converter at a rate of 10.5 kHz and later analyzed using a suite of programs (Lynux). To quantify the effects of CTL (10 mM), the authors measured the amplitude (the potential difference between the baseline and the maximal voltage of the evoked compound action potential, CAP) and the time constant of repolarization (τ) that was calculated with the equation $V = V0*\exp(t/\tau)$ by using nonlinear regression analysis applied to the repolarization phase of the CAP. All experiments were conducted at room temperature (25 ± 2°C).

Statistical Analysis

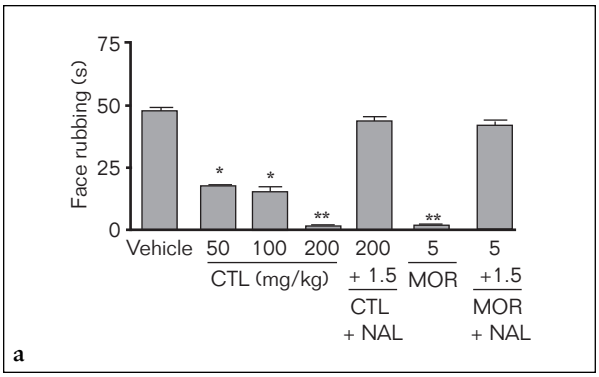
For the behavioral analyses, the data obtained were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. For the CAP data, values were evaluated using the two-tailed Student *t* test. Differences were considered to be statistically significant when $P < .05$. The percent of inhibition induced by an antinociceptive agent was determined by the following formula[18]:

Inhibition % = 100.(control–experiment)/control

Results

Administration of CTL produced a reduction in face-rubbing behavior induced by formalin (Fig 1). All tested doses of CTL significantly increased antinociception in both the first and second phase compared to control (vehicle). MOR was able to reduce nociceptive behavior in both phases. The effects of CTL and MOR were inhibited by naloxone.

Figure 2 shows that CTL significantly ($P < .001$) reduced the face-rubbing behavior induced by administration of capsaicin. The higher doses of CTL produced a similar effect to MOR. The group that received only the diluents of capsaicin (capsaicin vehicle group) did not present any nociceptive behavior.



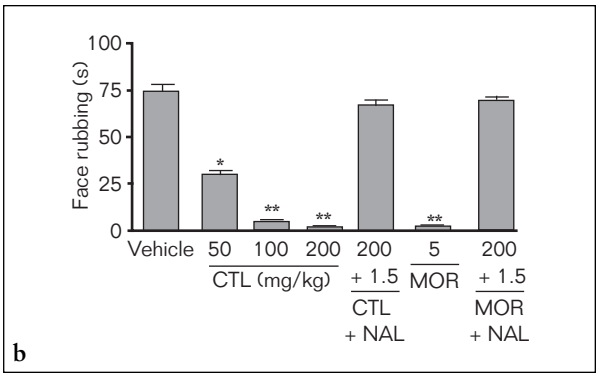


Fig 1 Effects of CTL or morphine (MOR) on formalin-induced orofacial nociceptive bahavior in the absence and presence of NAL (1.5 mg/kg) in mice. Vehicle (control), CTL (50, 100, and 200 mg/kg), or MOR (5 mg/kg) were administered ip 0.5 hours before formalin injection. *(a)* First phase (0 to 5 minutes) and *(b)* second phase (15 to 40 minutes) of the formalin test. Each column represents mean ± SEM (n = 8). $*P < .05$ or $**P < .001$ versus control (ANOVA followed by Dunnett's test).

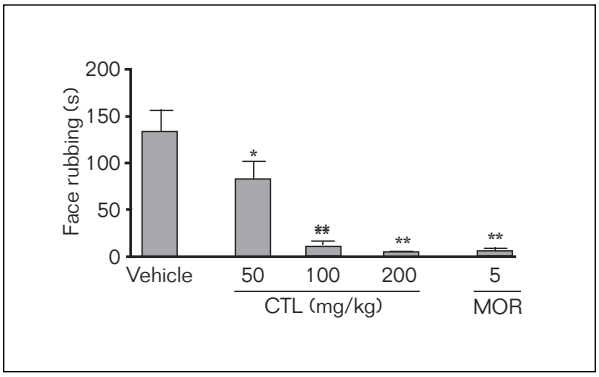


Fig 2 Effects of CTL on the capsaicin induced-orofacial nociceptive behavior in mice. Vehicle (control), CTL (50, 100, and 200 mg/kg), or MOR (5 mg/kg) were administered ip 0.5 hours before capsaicin injection. Each column represents mean ± SEM (n = 8, per group). $*P < .05$ or $**P < .001$ versus control (ANOVA followed by Dunnett's test).

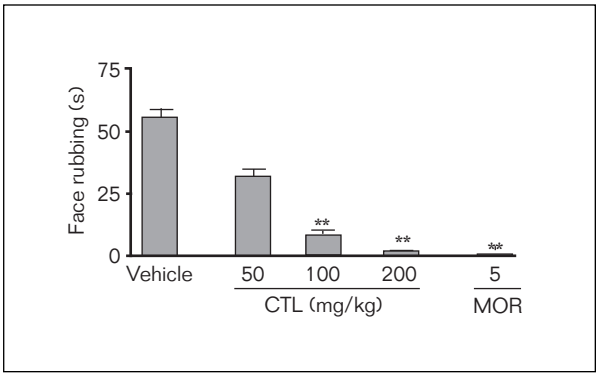


Fig 3 Effects of CTL or MOR on the glutamate-induced orofacial nociceptive behavior in mice. Vehicle (control), CTL (50, 100, and 200 mg/kg), or MOR (5 mg/kg) were administered ip 0.5 hours before glutamate injection. Each column represents mean ± SEM (n = 8, per group). $**P < .001$ versus control (ANOVA followed by Dunnett's test).

The results of the orofacial nociception induced by the glutamate test are represented in Fig 3. The higher doses of CTL (100 and 200 mg/kg, ip), as well as MOR, significantly decreased the face-rubbing behavior compared with the control group (vehicle).

In the Rota-rod test, CTL-treated mice did not show any significant alterations in motor performance (Fig 4); however, diazepam-treated mice had a significant reduction in time on the Rota-rod.

Figure 5a illustrates CTL (10 mM) effects on the CAP waveform of the isolated peripheral nerve, acquired by supramaximal stimulation (6 to 8V, 100 μs) every 10 minutes, using the single sucrose gap technique. After 30 minutes incubation, CTL produced a 42.4% CAP blockade, reducing the CAP amplitude from 49.3 ± 6.6 mV (mean values and SEM) to 28.4 ± 3.7 mV ($P < .05$) (Fig 5b).

Discussion

The objectives of this study were to evaluate the possible antinociceptive effects of CTL by using orofacial nociceptive tests and to investigate whether such effects might involve a change in neural excitability.

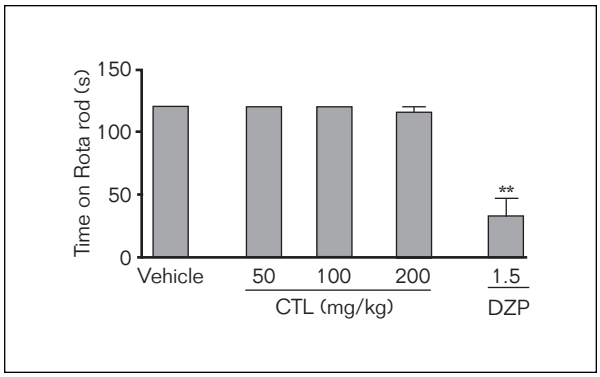


Fig 4 Time (seconds) on the Rota-rod observed in mice after ip treatment with vehicle (control), CTL (50, 100, and 200 mg/kg), or diazepam (DZP, 1.5 mg/kg). The motor response was recorded for the following 30, 60, and 90 minutes after drug treatment, and the time (seconds) the mouse remained on the bar for up to 120 seconds was recorded. Statistical differences versus control group were calculated using ANOVA, followed by Dunnett's test (n = 8, per group). $^{**}P < .001$.

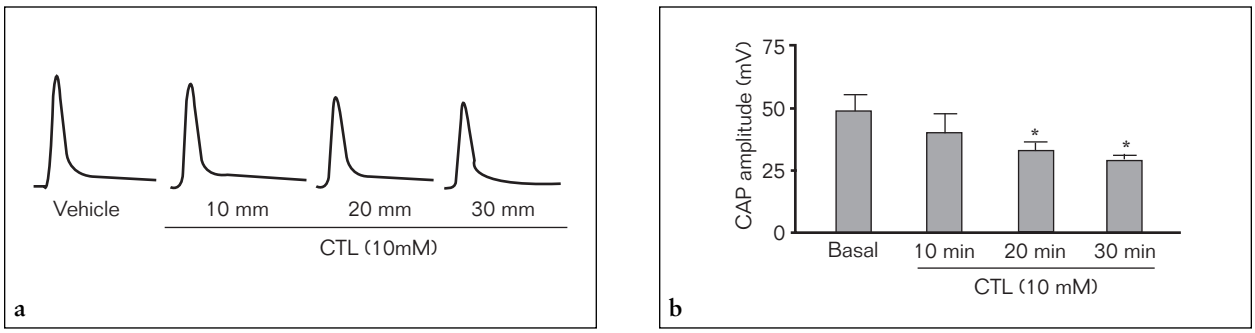


Fig 5 CTL reduced the CAP amplitude recordings in isolated rat nerves. *(a)* Representative CAP recordings obtained every 10 minutes (over a 30-minute period) in the presence of CTL (10 mM). Control records were acquired when the sciatic nerve was incubated with physiologic solution alone. *(b)* CAP amplitude decrease with CTL (10 mM) incubation (at 10, 20, and 30 minutes). Stimulation parameters were 6 to 8 V, 100 μs. Values are expressed as mean ± SEM (n = 4). $^{*}P < .05$ versus basal values (control) (ANOVA followed by Dunnett's test).

The orofacial formalin test in mice is a well-established preclinical model to investigate the efficacy of analgesic compounds in the facial region.[10,19] The test is based on a chemical stimulus (formalin) which induces tissue damage that mimics acute postinjury pain in humans. During the test, two phases can be observed that are associated with at least two partially distinct mechanisms of nociception. The first phase is associated with direct stimulation of C-nociceptors, whereas the second phase reflects integration between peripheral (nociceptors) and central (spinal/brainstem) signaling.[20,21] Furthermore, it has been reported that the development of hyperalgesia following the injection of formalin involves the glutamatergic system, mainly N-methyl-D-aspartate (NMDA) receptors.[10,12]

Acute administration of CTL caused pronounced antinociception as evidenced by decreased face-rubbing behavior in the first and second phases of the formalin test. These results suggest that CTL has a central analgesic effect. To confirm such an effect, the blocking effect of NAL, a specific antagonist of μ morphinomimetic receptors, was tested on both phases of the formalin test.[22] Its antagonistic effects suggest the participation of the opioid system in the modulation of nociception induced by CTL.

CTL also inhibited nociceptive behavior induced by capsaicin injection into the right upper lip. Capsaicin applied to skin, muscle, and other tissues has been shown to produce inflammation and to activate and to sensitize trigeminal and spinal small-diameter nociceptive afferents as well as dorsal horn neurons. It also evokes nociceptive behavior in animals and intense pain, hyperalgesia, and referred pain in humans.[23–26]

The inhibitory effect observed with CTL on capsaicin- and formalin-induced face-rubbing behavior may be a result of its possible inhibition of Substance P release or due to a direct blocking action on its receptor neurokinin-1 (NK-1).[8] In this

context, a previous study provided evidence for tonic activation of NK-1 receptors, through NK-1 receptor antagonist SR14033 administration, which blocked the second phase of the orofacial formalin test in rats.[27,28] Waning et al[29] demonstrated that the capsaicin-sensitive transient receptor potential vaniloid 1 (TRPV1), which plays an important role in pain transduction, is one of the Ca^{+2} influx channels involved in cell migration. Genetic approaches in worms, flies, and mice have demonstrated the involvement of TRPs in a variety of sensory processes that includes thermosensation, mechanosensation, and pain.[30] Moreover, Honda et al[26] have suggested that TRPV1 receptor mechanisms in rat facial skin influence nociceptive responses to noxious cutaneous thermal and mechanical stimuli by inducing neuroplastic changes in trigeminal brainstem subnucleus caudalis (Vc) and C1-C2 neurons. Microscopic studies have also revealed the expression of immunoreactivity for TRPV1 in the trigeminal ganglion.[31,32]

A number of monoterpenes have also been described as agonists or antagonists of different members of the TRPV channel family.[30,33] The ability of camphor, a naturally occurring monoterpene produced by the Camphor Laurel (*Cinnamomum camphora*), to modulate sensations of warmth in humans has been attributed to its ability to activate TRPV3.[34] TRPV3 is expressed in keratinocytes, the dorsal root ganglia, brain, and spinal cord.[30] It has been implicated in hyperalgesia, inflamed tissues, and possibly skin sensitization.[35] According to Vogt-Eisele et al,[30] some monoterpenes (such as camphor, carvacrol, thymol, and menthol) have been shown to activate TRPV3.

Additionally, the present results also showed that ip administration of the CTL produced a significant inhibition of the nociceptive response induced by right upper lip injection of glutamate into mice. 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.[36,37] In addition, glutamate injection into the rat masseter muscle or temporomandibular joint (TMJ) reflexly evokes a dose-dependent increase in jaw muscle electromyographic (EMG) activity[38–41] and central sensitization of trigeminal brainstem nociceptive neurons.[25] Similarly, glutamate injection into the human masseter muscle causes pain and mechanical hyperalgesia that may be attenuated by coinjection of an NMDA receptor antagonist.[39]

This nociceptive response induced by glutamate seems to involve peripheral, spinal, and supraspinal sites, and its action is mediated by NMDA and non-NMDA receptors.[12] Several studies have demonstrated that these excitatory amino acid receptors are involved in nociceptive primary afferent transmission and in the development and maintenance of pain conditions.[42] Thus, the suppression of glutamate-induced nociception by CTL treatment can be associated with the interaction of CTL with the glutamatergic system.[43] Batista et al[44] have demonstrated that linalool, a monoterpene compound prevalent in essential oils of various aromatic plant species, also possesses antinociceptive properties in mice. This effect involves peripheral and spinal sites of action and seems to be mediated by an interaction with ionotropic glutamatergic-dependent mechanisms, via NMDA receptors.

Previous studies have suggested that central nervous system depression and a nonspecific muscle relaxation effect can reduce motor coordination and might invalidate results of behavioral tests.[6,17] The present results revealed that all mice treated with CTL, at the doses evaluated, did not have any alteration in the performance in the Rota-rod test.

As inhibition of neuronal excitability is associated with blockade of the voltage-dependent Na^+ channels,[16] the present study used the single sucrose gap method to show that CTL could reduce the excitability of isolated nerves through a diminution of CAP amplitude. It is known that the single sucrose gap technique specifically records fast-conducting myelinated fibers (Aα) and not myelinated fibers (Aδ), which are the A fibers involved in conduction of nociceptive signals. Many studies have suggested that CAP evaluation using this technique allows for the analysis of the possible involvement of new substances, such as CTL, in the voltage-dependent Na^+ channels and in electrical conductance of signals in myelinated fibers.[16,17,45] Since the CTL reduced the CAP amplitude demonstrated in the present study, it is possible that the antinociceptive effects of CTL in the experimental models of nociception could be involved in the voltage-gated Na^+ channel blocking and may also have CNS effects.

In conclusion, the present results suggest that CTL modulates inflammatory pain as revealed in the tests of orofacial nociception induced by formalin (through a NAL-sensitive mechanism) and capsaicin. Indeed, the antinociceptive effect of CTL in the glutamate test may have a direct desensitizing effect on primary afferent fibers. The documented antinociceptive effects of CTL may be associated with decreased peripheral nerve excitability. However, more specific methodologies

are required to confirm this, such as the Patch-clump technique. The results also suggest that CTL may have therapeutic potential for painful facial and dental disorders.

Acknowledgments

The authors would like to thank the National Council of Technological and Scientific Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq/Brazil) and the Research Supporting Foundation of State of Sergipe (Fundação de Amparo à Pesquisa do Estado de Sergipe/FAPITEC-SE, Grant number 019.203-00791/2007-2) for their financial support.

References

1. Luccarini P, Cadet R, Saade M, Woda A. Antinociceptive effect of morphine microinjections into the spinal trigeminal subnucleus oralis. Neuroreport 1995;6:365–368.
2. Miranda HF, Sierralta F, Prieto JC. Synergism between NSAIDs in the orofacial formalin test in mice. Pharmacol Biochem Behav 2009;92:314–318.
3. Calixto JB, Beirith A, Ferreira J, Santos AR, Cechinel Filho V, Yunes RA. Naturally occurring antinociceptive substances from plants. Phytother Res 2000;14:401–418.
4. Almeida RN, Navarro DS, Barbosa-Filho JM. Plants with central analgesic activity. Phytomedicine 2001;8:310–322.
5. Quintans-Júnior LJ, Souza TT, Leite BS, et al. Phytochemical screening and anticonvulsant activity of Cymbopogon winterianus Jowitt (Poaceae) leaf essential oil in rodents. Phytomedicine 2008;15:619–624.
6. Almeida RN, Motta SC, Faturi CB, Catallani B, Leite JR. Anxiolytic-like effects of rose oil inhalation on the elevated plus-maze test in rats. Pharmacol Biochem Behav 2004; 77:361–364.
7. Lenardão EJ, Botteselle GV, Azambuja F, Perin G, Jacob RG. Citronellal as key compound in organic synthesis. Tetrahedron 2007;63:6671–6712.
8. Holanda Pinto SA, Pinto LMS, Guedes MA, et al. Antinoceptive effect of triterpenoid alpha, beta-amyrin in rats on orofacial pain induced by formalin and capsaicin. Phytomedicine 2008;15:630–634.
9. Clavelou P, Dallel R, Orliaguel T, Woda A, Raboisson P. The orofacial formalin test in rats: Effect of different formalin concentrations. Pain 1995;62:295–301.
10. Luccarini P, Childeric A, Gaydier AM, Voisin D, Dallel R. The orofacial formalin test in the mouse: A behavioral model for studying physiology and modulation of trigeminal nociception. Pain 2006;7:908–914.
11. Pellisier T, Pajot J, Dallel R. The orofacial capsaicin test in rats: Effects of different capsaicin concentrations and morphine. Pain 2002;96:81–87.
12. Beirith A, Santos ARS, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res 2002;924:219–228.
13. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 1957;46:208–209.
14. Vaz ZR, Cechinel Filho V, Yunes RA, Calixto JB. Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a novel xanthoxyline derivative on chemical and thermal models of nociception in mice. J Pharmacol Exp Ther 1996;278:304–312.
15. Rosland JH, Tjoisen A, Maehle B, Hole K. The formalin test in mice, effect of formalin concentration. Pain 1990; 42:235–242.
16. Cruz JC, Matavel ACS, Leão-Filho HM, Moraes-Santos T, Beirão PSL. Tityustoxin effect on nerve compound action potentials requires extracellular sodium. Neurosci Lett 2000;282:25–28.
17. De Sousa DP, Gonçalves JC, Quintans-Júnior LJ, Cruz JS, Araujo DAM, Almeida RN. Study of anticonvulsant effect of citronellol, a monoterpene alcohol, in rodents. Neurosci Lett 2006;401:231–235.
18. Reanmongkol W, Matsumoto K, Watanabe H, Subhadhirasakul S, Sakai SI. Antinociceptive and antipyretic effects of alkaloids extracted from the stem bark of Hunteria zeylanica. Biol Pharm Bull 1994;17: 1345–1350.
19. Raboisson P, Dallel R. The orofacial formalin test. Neurosci Biobehav Rev 2004;28:219–226.
20. Dallel R, Raboisson P, Clavelou P, Saade M, Woda A. Evidence for a peripheral origin of the tonic nociceptive response to subcutaneous formalin. Pain 1995;61:11–16.
21. Capuano A, Corato A, Treglia M, Tringali G, Russo CD, Navarra P. Antinociceptive activity of buprenorphine and lumiracoxib in the rat orofacial formalin test: A combination analysis study. Eur J Pharmacol 2009;605:57–62.
22. Belvisi MG, Chung KF, Jackson DM, Barnes PJ. Opioid modulation of non-cholinergic neural bronchoconstriction in guinea-pig in vivo. Br J Pharmacol 1998;95:413–418.
23. Hu JW, Fiorentino PM, Cairns BE, Sessle BJ. Capsaicin-induced inflammation within temporomandibular joint involves VR-1 receptor mechanisms. Oral Biosci Med 2005;4:241–248.
24. Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception I: Activation and peripheral sensitization of deep craniofacial nociceptive afferents. Brain Res 2009;1251:130–139.
25. Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception II: Activation and central sensitization in brainstem neurons with deep craniofacial afferent input. Brain Res 2009;1253:48–59.
26. Honda K, Kitagawa J, Sessle BJ, et al. Mechanisms involved in an increment of multimodal excitability of medullary and upper cervical dorsal horn neurons following cutaneous capsaicin treatment. Mol Pain 2008;4:59.
27. Henry JL, Yashpal K, Pitcher GM, Chabot JG, Coderre TJ. An evidence for tonic activation of NK-1 receptors during the second phase of the formalin test in the rat. J Neurosci 1999;19:6588–6598.
28. Luccarini P, Henry M, Alvarez Gaydier AM, Dallel R. Contribution of neurokinin 1 receptors in the cutaneous orofacial cutaneous pain. Naunyn Schmiedebergs Arch Pharmacol 2003;368:320–323.
29. Waning J, Vriens J, Owsianik G, et al. A novel function of capsaicin-sensitive TRPV1 channels. Cell Calcium 2007; 42:17–25.
30. Vogt-Eisele AK, Weber K, Sherkheli MA, et al. Monoterpenoid agonists of TRPV3. Br J Pharmacol 2007;151: 530–540.

31. Ichikawa H, Sugimoto T. VR1-immunoreactive primary sensory neurons in the rat trigeminal ganglion. Brain Res 2001;890:184–188.
32. Hou M, Uddman R, Tajti J, Kanje M, Edvinsson L. Capsaicin receptor immunoreactivity in the human trigeminal ganglion. Neurosci Lett 2002;330:223–226.
33. Xu HX, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 2005;25:8924–8937.
34. Moqrich A, Hwang SW, Earley MJ, et al. Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 2005;307:1468–1472.
35. Xu HX, Delling M, Jun JC, Clapham DE. Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP Channels. Nat Neurosci 2006;9:628–635.
36. Keast JR, Stephensen TM. Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. J Comp Neurol 2000;424:577–587.
37. Lam DK, Sessle BJ, Cairns BE, Hu JW. Neural mechanisms of temporomandibular joint and masticatory muscle pain: A possible role for peripheral glutamate receptor mechanisms. Pain Res Manag 2005;10:145–152.
38. Cairns BE, Sessle BJ, Hu JW. Evidence that excitatory amino acid receptors within the temporomandibular joint region are involved in the reflex activation of the jaw muscles. J Neurosci 1998;18:8056–8064.
39. Cairns BE, Hu JW, Arendt-Nielsen L, Sessle BJ, Svensson P. Sex-related differences in human pain and rat afferent discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2001;86:782–791.
40. Cairns BE, Sessle BJ, Hu JW. Characteristics of glutamate-evoked temporomandibular joint afferent activity in the rat. J Neurophysiol 2001;85:2446–2454.
41. Cairns BE, Gambarota G, Svensson P, Arendt-Nielson L, Berde CB. Glutamate-induced sensitization of rat masseter muscle fibers. Neuroscience 2002;109:389–399.
42. Coggeshall RE, Carlton SM. Receptor localization in the mammalian dorsal horn and primary afferent neurons. Brain Res Rev 1997;24:28–66.
43. Ferreira J, Santos ARS, Calixto JB. The role of systemic, spinal and supraspinal L-arginine-nitric oxide-cGMP pathway in thermal hyperalgesia caused by intrathecal injection of glutamate in mice. Neuropharmacol 1999;38:835–842.
44. Batista PA, Werner MFP, Oliveira EC, et al. Evidence for the involvement of ionotropic glutamatergic receptors on the antinociceptive effect of (-)-linalool in mice. Neurosci Lett 2008;440:299–303.
45. Gonçalves JCR, Oliveira FS, Benedito RB, De Sousa DP, Almeida RN, Araújo DAM. Antinociceptive activity of (-)-carvone. Biol Pharm Bull 2008;31:1017–1020.