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Linalool blocks excitability in peripheral nerves and voltage-dependent Na^+ current in dissociated dorsal root ganglia neurons

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ABSTRACT

Linalool is a terpene that occurs as a major constituent of essential oils of many plants of widespread distribution. It possesses several biological and pharmacological activities, including depressant effects on the central nervous system and olfactory receptors. The present study investigated whether linalool affects the excitability of peripheral components of the somatic sensory system. We used sciatic nerve and preparations of intact and dissociated neurons of dorsal root ganglion for extracellular, intracellular and patch-clamp recordings. Linalool concentration-dependently (0.3–2.0 mM) and reversibly blocked the excitability of the sciatic nerve. It inhibited peak-to-peak amplitude of the compound action potential (IC_{50} was 0.78 ± 0.04 mM). At 0.8 mM, it reversibly increased rheobase and chronaxy (from 3.2 ± 0.1 V and 52.4 ± 4.1 μs to 4.2 ± 0.3 V and 71.2 ± 5.5 μs (n = 5), respectively) and inhibited with greater pharmacological potency the amplitude of the compound action potential components corresponding to axons with slower velocity of conduction. In a similar concentration range (0.1–6 mM), linalool concentration-dependently and reversibly blocked the generation of action potentials of intact dorsal root ganglion neurons without alteration of resting membrane potential and input resistance, and inhibited the voltage-gated Na^+ current of dissociated dorsal root ganglion neurons. In conclusion, we demonstrated that linalool acts on the somatic sensory system with local anesthetic properties, since it blocked the action potential by acting on voltage-dependent Na^+ channels. This finding is important in showing the potential usefulness of linalool as a pharmacotherapeutic agent.

1. Introduction

Nature has provided us with a variety of chemotherapeutic agents (Gurib-Fakim, 2006). Terpenes are a class of secondary metabolites that have a distinct structure and function and are considered important agents in the medicinal use of aromatic plants (Bakkali et al., 2008; Dorman and Deans, 2000). Linalool is one example of this class of chemical compounds which occur naturally as major constituents of the essential oils of many plant species distributed throughout the world (Randrianarivelo et al., 2009; Sibanda et al., 2004).

Linalool has been reported to have a great diversity of biological and pharmacological activities in *in vivo* and *in vitro* preparations (Bickers et al., 2003; Celik and Ozkaya, 2002; Peana et al., 2002; Sugawara et al., 1998), used in exploring the putative medicinal properties for this substance. Interestingly, linalool did not show significant mutagenic and/or toxicological effects (Bickers et al., 2003; Di Sotto et al., 2008). On the other hand, linalool showed anti-inflammatory activity by reducing paw edema induced by carrageenin (Peana et al., 2002) and also antagonized different responses to nociception, either by exposure to noxious thermal stimulation (using the hot plate test) or by injection of formalin (inflammatory model of acute pain) (Peana et al., 2004). In addition, linalool has shown depressant actions on the central nervous system (Silva Brum et al., 2001), sedative effects in humans (Sugawara et al., 1998), and antioxidant properties (Celik and Ozkaya, 2002).

Recently, Narusuye et al. (2005) in studying newt olfactory receptor and rat cerebellar Purkinje cells provided evidence that linalool suppresses non-selective voltage-gated channels. Still, the pharmacological effects of linalool on somatic sensory neurons have not yet been reported.

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In the present study, we were interested in finding out whether the reported depressant effects of linalool on the nervous system are related to changes in neuronal excitability and if these changes are a consequence of the modulation of voltage-dependent Na^+ channels.

2. Materials and methods

2.1. Animals, tissue dissection and dissociation protocol

Wistar rats (200–300 g) of both sexes were used. They were kept under conditions of constant temperature (22 ± 2 °C) with a 12 h light/12 h dark cycle and free access to food and water. All animals were handled in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996; http://www.nap.edu/readingroom/books/labrats/index.html), and all efforts were made to minimize animal suffering. All procedures described herein were first reviewed and approved by the local animal ethics committee.

The sciatic nerve and dorsal root ganglia were dissected from rats sacrificed by cerebral concussion. For intracellular and extracellular recordings, the tissues were immediately placed in a container containing modified Locke's solution, and the tissues were used on the same day of dissection. For patch-clamp recording, the dorsal root ganglion were placed in Ca^{2+}-, Mg^{2+}-free Hanks' balanced salt solutions followed by dissociation solution which consisted of 1 mg/mL collagenase type I for 75 min and 2.5 mg/mL trypsin for 15 min, both in Hanks' balanced salt solutions at 37 °C. After exposure to the dissociation solutions, the dorsal root ganglion neurons were freed from tissue by gentle trituration in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, 100 U/mL streptomycin and 0.1 mg/mL penicillin. The cells were plated on coverslips coated with poly-D-lysine 0.01%. The neurons were incubated in air atmosphere containing 5% CO_2 maintained at 37 °C and were used within 48 h.

2.2. Solutions and drugs

For intracellular recording, we used modified Locke's solution whose composition was (in mM): NaCl 140, KCl 5.6, $MgCl_2$ 1.2, $CaCl_2$ 2.2, tris(hydroxymethyl-aminomethane) 10, and glucose 10. We used the same Locke's solution above for extracellular recording, and for Na^+ removal experiments, this ion was replaced by N-methyl-D-glucamine on an equimolar basis. We also used 500 nM tetrodotoxin dissolved in Locke's solution for some extracellular recordings. Hanks' balanced salt solution used in the dissociation protocol had the following composition (in mM): NaCl 137.93, KCl 5.33, KH_2PO_4 0.44, $NaHCO_3$ 4.0, Na_2HPO_4 0.3, and glucose 5.6. For patch-clamp recording, the composition of bath solution was (in mM): NaCl 140, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, Hepes 5, and glucose 5. The composition of the external test solution was (in mM): NaCl 20, choline-Cl 90, CsCl 10, $CaCl_2$ 2, $MgCl_2$ 1, tetraethylammonium-Cl 20, $CdCl_2$ 0.2, Hepes 10, and glucose 10. The pH of all the above solutions was adjusted to 7.4 with HCl. The pipette internal solution to measure Na^+ currents contained (in mM): NaCl 10, CsCl 100, Hepes 10, ethylene glycol tetraacetic acid 11, tetraethylammonium-Cl 10, $MgCl_2$ 5, and pH adjusted to 7.2 with CsOH. Choline served as the non-permeant monovalent cation in place of external Na^+ and was used to reduce the amplitude of the Na^+ current in patch-clamp experiments. Cs^+ and tetraethylammonium were used to block K^+ channels and Cd^{2+} was used to block Ca^{2+} channels.

Linalool was dissolved in dimethylsulfoxide with a final concentration or equal to or less than 0.2% v/v. At this concentration, dimethylsulfoxide did not alter electrophysiological parameters (Schauf, 1987), and stock solutions were prepared daily. To provide the desired drug concentration, the stock solutions were added to the chambers containing modified Locke's solution for extracellular and intracellular recordings and external solution for patch-clamp recordings. The concentrations of linalool used in extracellular were 0.3, 0.6, 0.8, 1 and 2 mM, for intracellular 0.1, 0.6, 2 and 4 mM, and for patch-clamp recordings 0.6 and 6 mM.

Experiments were carried out at room temperature (18 to 22 °C), and all salts and drugs were of analytical grade and were purchased from Sigma Chemical (St. Louis, MO, USA) or Reagen (Rio de Janeiro, RJ, Brazil).

2.3. Electrophysiology

2.3.1. Extracellular recording

The sciatic nerve was mounted and evoked compound action potential (extracellular recording of the overall electric activity which results from the action potential of nerve axons) was recorded as described by Lima-Accioly et al. (2006). Briefly, the sciatic nerve was mounted in a moist chamber and one of its ends was stimulated with a stimulus isolation unit connected to a stimulator (Model S48, Grass Instruments Co., Quincy, MA, USA). Evoked compound action potential was recorded with platinum electrodes placed 40 to 50 mm from the stimulating electrodes and continuously monitored using an oscilloscope (Model 547, Tektronix, Inc., Portland, OR, USA). Computer acquisition hardware was used for data storage and analysis.

A 15 to 20 mm segment of the sciatic nerve was suspended between the stimuli and recording electrodes and immersed in Locke's solution which was used to maintain chamber humidity and to administer the drug. Sciatic nerve exposure to linalool was performed only when stable peak-to-peak compound action potential amplitude was achieved for at least 30 min, and the drug exposure time was set to 180 min. This interval was usually sufficient to allow steady-state compound action potential amplitude to be reached during linalool administration and this period was followed by a 180 min washout/recovery period.

The electrophysiological parameters measured in extracellular recording were rheobase, chronaxy, peak-to-peak amplitude, positive amplitude of compound action potential components and conduction velocity. Strength–duration curves with voltage square waves were used to determine rheobase and chronaxy. Rheobase was measured as the threshold stimulus voltage for an active response with a long-duration pulse (1000 μs) and chronaxy as the threshold pulse–duration at a pulse–voltage corresponding to twice the rheobase.

2.3.2. Intracellular recording

The intact dorsal root ganglion was fixed and transmembrane responses recorded as described by Ferreira-da-Silva et al. (2009). Briefly, the ganglia were fixed in an acrylic chamber designed to permit superfusion with Locke's solution or linalool-containing solution. The chamber was placed on a magnifying glass and the microelectrode movement and impalement was done with a hydraulic micromanipulator (MWO-3; Narishige International, Long Island, NY, USA). The microelectrode was filled with 3 M KCl solution, connected to an Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA, USA) via a silver–silver chloride wire, the response signal was visualized continuously on an oscilloscope, and data storage was performed by computer acquisition hardware for further analysis.

The cells were acceptable for study when neurons were stabilized with a resting potential more negative than −48 mV and with input resistance greater than 10 MΩ for 3 to 5 min after impalement. The intact ganglia were perfused with linalool for at least 5 min or until full action potential blockade, followed by a washout/recovery period.

The electrophysiological parameters measured in intracellular recording were resting potential, input resistance, action potential amplitude and duration, maximum ascendant inclination and maximum descendant inclination, that is, the absolute value of the minimum value of negative inclination.