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Effects of terpineol on the compound action potential of the rat sciatic nerve

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Abstract

Terpineol, a volatile terpenoid alcohol of low toxicity, is widely used in the perfumery industry. It is an important chemical constituent of the essential oil of many plants with widespread applications in folk medicine and in aromatherapy. The effects of terpineol on the compound action potential (CAP) of rat sciatic nerve were studied. Terpineol induced a dose-dependent blockade of the CAP. At 100 µM, terpineol had no demonstrable effect. At 300 µM terpineol, peak-to-peak amplitude and conduction velocity of CAP were significantly reduced at the end of 180-min exposure of the nerve to the drug, from 3.28 ± 0.22 mV and 33.5 ± 7.05 m/s, respectively, to 1.91 ± 0.51 mV and 26.2 ± 4.55 m/s. At 600 µM, terpineol significantly reduced peak-to-peak amplitude and conduction velocity from 2.97 ± 0.55 mV and 32.8 ± 3.91 m/s to 0.24 ± 0.23 mV and 2.72 ± 2.72 m/s, respectively (N = 5). All these effects developed slowly and were reversible upon 180-min washout.

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- Compound action potential
- Nerve conduction velocity

Terpineol, a relatively nontoxic, volatile monoterpenoid alcohol, is a major component of the essential oil of many plants, such as *Ravensara aromatica* (Ravensara), *Melaleuca qinquenervia* (Niaouli), *Myrtus communis* (Myrtle), *Laurus nobilis* (Laurel), *Croton sonderianus* (Marmeleiro preto, in Northeastern Brazil), and *Eucalyptus globulus* (Eucalyptus), which are widely used in folk medicine and aromatherapy (1,2). This compound is also a constituent of some inhalants and syrups marketed over-the-counter (3). Some of the biological actions of terpineol have been studied. The compound has been reported to have insecticidal, antimicrobial, antispasmodic and immunostimulant properties and to enhance the permeability of the skin to lipid-soluble compounds (2,4,5).

Many essential oils and their chemical constituents are known to have potent local anesthetic activity (6-8). Because no previous pharmacological studies with terpineol had examined its effects on peripheral nerve electrophysiology, the present study aimed at characterizing the actions of terpineol on the compound action potential (CAP).

Wistar rats (*Rattus norvegicus*; 300-350 g) were sacrificed by cervical dislocation and the sciatic nerves were carefully dissected. One nerve was then mounted in a moist chamber (9) and one of its ends was stimulated at a frequency of 0.2 Hz with electric pulses of 50-100-µs duration at 10-20 V delivered by a stimulus isolation unit

(Model SIU4678, Grass Instruments Co., Quincy, MA, USA), connected to a stimulator (Model S48, Grass). Evoked CAP were recorded with platinum electrodes placed 4 to 5 cm from the stimulating electrodes. For continuous monitoring, these electrodes were connected through a high input impedance amplifier (Model P15, Grass) to an oscilloscope (Model 547, Tektronix, Inc., Portland, OR, USA). Digidata 1200 computer acquisition hardware (Axon Instruments, Inc., Foster City, CA, USA) and AxoScope software (Axon) were used for data capture and analysis. A 15- to 20-mm segment of the nerve suspended between the stimulating and recording electrodes was immersed in Locke's solution which was employed to maintain chamber humidity. Pharmacological agents were dissolved in Locke's solution and administered via the bathing solution.

After a stabilization period of 60 min, during which stable peak-to-peak CAP amplitude recording was performed for at least 30 min, the bathed nerve segment was exposed to pharmacological agents for 180 min. This interval was usually sufficient to allow steady-state action potential amplitude to be reached during terpineol administration. This period was followed by a washout and a 180-min recovery period. Experiments were carried out at room temperature (24°-26°C).

Modified Locke's solution (140 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, and 10 mM Tris-(hydroxymethyl)aminomethane), pH 7.4, was thoroughly aerated before use in the chamber. Nerves were normally used on the day of dissection; however, in some experiments they were stored overnight in cold (5°C) Locke's solution for use on the following day. This storage period did not affect nerve electrophysiological control parameters.

Stock solutions of terpineol in dimethylsulfoxide (DMSO, vehicle) were prepared daily. This stock solution was added to the chamber Locke's solution so as to provide the desired terpineol concentration with a final vehicle concentration always below 0.25% (v/v). At such concentration DMSO did not alter CAP parameters. All salts and drugs, including terpineol, were purchased from Sigma (St. Louis, MO, USA), or Reagen (Rio de Janeiro, RJ, Brazil) and were of analytical grade.

The results are reported as mean ± SEM, with (N) indicating the number of experiments. The values were analyzed using the

Table 1. Effects of terpineol (TER) on the peak-to-peak amplitude and conduction velocity of the compound action potential (CAP) of the rat sciatic nerve.

TER concentration (µM)	Peak-to-peak amplitude (mV)			Velocity of CAP conduction (m/s)		
	Control	At 180th min in TER[a]	Recovery	Control	At 180th min in TER[a]	Recovery
0	4.5 ± 0.12 (5)	-	4.4 ± 0.13[b] (5)	33.3 ± 2.70 (5)	-	32.9 ± 1.45 (5)
100	3.3 ± 0.12 (5)	3.4 ± 0.17 (5)	-	32.5 ± 3.99 (5)	32.8 ± 5.56 (5)	-
300	3.3 ± 0.22 (5)	1.9 ± 0.51 (5)	3.0 ± 0.27 (2)	33.5 ± 7.05 (5)	26.2 ± 4.55 (5)	30.5 ± 4.71 (2)
600	3.0 ± 0.55 (5)	0.2 ± 0.23 (5)	1.9 ± 0.06 (2)	32.8 ± 3.91 (5)	2.7 ± 2.72 (4)[c]	20.0 ± 2.55 (2)

Data are reported as mean ± SEM. The number of observations is given in parentheses.
[a]Quantification at 180 min.
[b]Control (0.25% DMSO), at the 360th min after stabilization.
[c]N = 4 because in one experiment CAP blockade was total after 120 min.

Student *t*-test, or ANOVA followed by a contrast test, or a nonparametric test, as appropriate. Results were considered significant at P≤0.05.

At 100 µM, terpineol did not alter the CAP (Table 1 and Figure 1), but at doses of 300 and 600 µM, peak-to-peak amplitude and conduction velocity were significantly reduced in a dose-dependent manner (Table 1 and Figure 1). These effects developed slowly and were reversible within the 180-min recovery period.

Terpineol has been previously shown to increase the absorption of lipid-soluble substances through the skin, and to possess antimicrobial, antispasmodic and immunostimulant properties (1,2). The present finding that terpineol blocks the CAP propagation of rat sciatic nerve is novel. This terpineol-induced blockade of nerve action potentials occurs at relatively low concentrations (300 µM). This concentration is approximately equivalent to a 0.005% (v/v) solution. In aromatherapy massages a volume of 5-25 ml of a concentrated (1 to 5%, v/v) solution of essential oil in an oily vehicle is commonly used (3). As much as 25% of the essential oil may be absorbed through the skin during the massage (3) and terpineol comprises up to 30% of some oils (10). Thus, terpineol concentrations in the range of 300 to 600 µM might be reached in dermal tissues during massages with essential oils rich in terpineol.

Since terpineol is an important constituent of many essential oils, some of them used in aromatherapy, it is important to understand the physiological effects of oil constituents and of the potential for accidental intoxication. Blockade of action potentials in peripheral nerves raises the question of whether this blocking effect might be induced by aromatherapy in peripheral nerve terminals or other nerve segments and, if so, what contribution it might add to treatment. Moreover, the effect of terpineol on nerve action potentials suggests that it may also affect other excitable tissues such as muscle. The effect of terpineol on excitable tissues thus deserves further investigation.

Action potential blockade was demonstrated with the quantification of the CAP parameters, conduction velocity and peak-

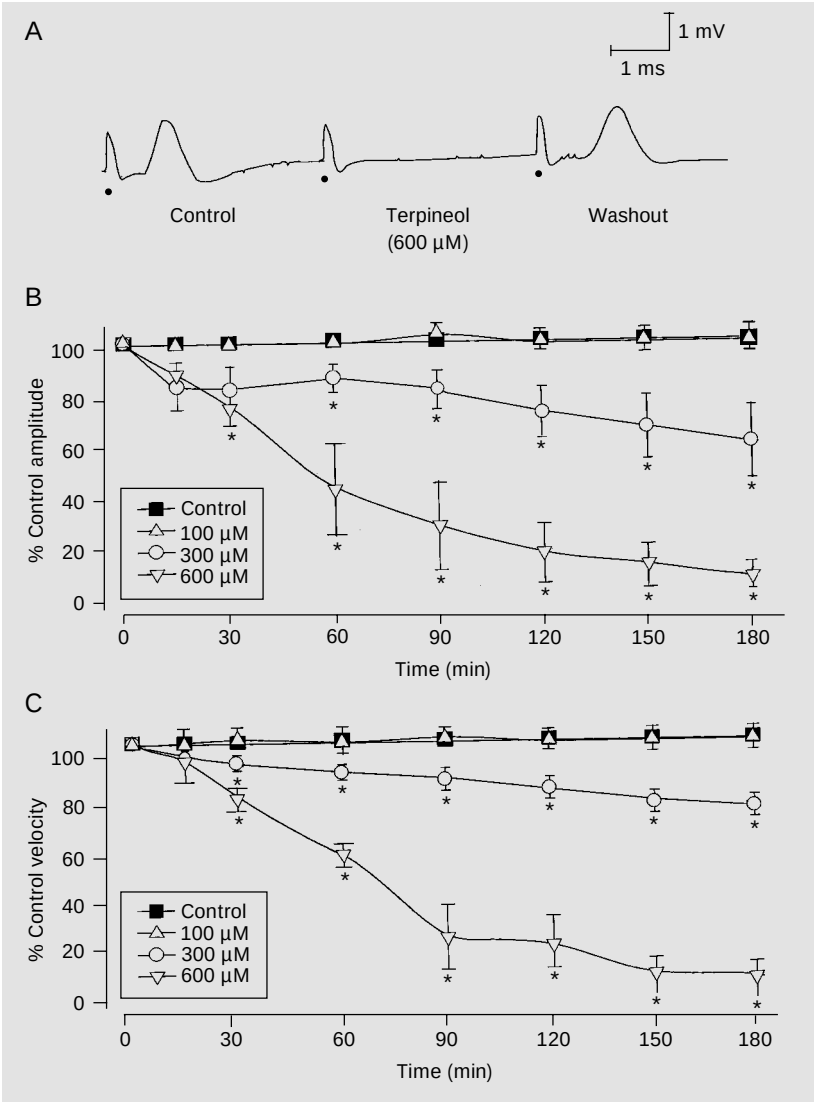

Figure 1. Time course of the effects of terpineol on peak-to-peak amplitude and conduction velocity of the compound action potential (CAP) of rat sciatic nerve. *Panel A* shows representative CAP tracings under control conditions and at the 180th min of nerve exposure to 600 µM terpineol and at 180 min washout. The dot below each tracing shows the time of stimulation. Quantification of terpineol-induced effect on peak-to-peak amplitude and conduction velocity is shown in *panels B* and *C*, respectively. Data are reported as mean ± SEM. The number of experiments was 5 in all cases, except for conduction velocity for 600 µM terpineol at 150 and 180 min where N was 4 since blockade was complete in one of the experiments. *P<0.05 compared to control (ANOVA and contrast technique test).

to-peak amplitude. Control values reported here for both parameters are well within the range reported by others in peripheral nerve recordings *in vitro* at room temperature and *in vivo* at 37ºC, which ranged from 100 to 16 m/s (11-14). The preparation showed great stability (6 h) which permitted us to demonstrate that, at concentrations ≥300 µM, terpineol induced a reversible blockade of the action potential conduction. Another important monoterpenoid constituent of essential oils, eugenol, whose molecular structure bears similarity to terpineol, also blocks the nerve action potential with a similar potency, but with a reversibility that has been questioned (15). Terpineol blocked the CAP and this fulfills one criterion for local anesthetic activity (16), but other criteria, like direct drug interaction with voltage-gated Na^+ channels and absence of significant or consistent alteration of resting potential, need to be investigated to characterize this substance as a local anesthetic agent.

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