Local anaesthetic activity of beta-caryophyllene.

ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 56 (2001) 387–389

IL FARMACO

Local anaesthetic activity of β-caryophyllene☆

C. Ghelardini [a,*], N. Galeotti [a], L. Di Cesare Mannelli [a], G. Mazzanti [b], A. Bartolini [a]

[a] *Department of Pharmacology, University of Florence, Viale G. Pieraccini 6, I-50139 Florence, Italy*
[b] *Department of Pharmacology of Natural Substances and General Physiology, University 'La Sapienza', Piazzale A. Moro 5, I-00185 Rome, Italy*

Received 31 October 2000; accepted 10 January 2001

Abstract

In this work we studied the local anaesthetic activity of β-caryophyllene, one of the main components of clove oil obtained from the dried flower-buds of *Syzygium aromaticum* (Myrtaceae family). We compared its activity to a chemically related compound, caryophyllene oxide. Anaesthetic activity was evaluated in vivo in the rabbit conjunctival reflex test and in vitro in a rat phrenic nerve-hemidiaphragm preparation. β-Caryophyllene (10^{-4}–1 μg/ml), but not caryophyllene oxide, was able to reduce drastically, in a dose-dependent manner, the electrically evoked contractions of the rat phrenic hemidiaphragm. In the rabbit, conjunctival reflex test treatment with a solution of β-caryophyllene (10–1000 μg/ml) allowed a dose-dependent increase in the number of stimuli necessary to provoke the reflex. As in the in vitro results, caryophyllene oxide was ineffective also in the in vivo test.

In conclusion, these data evidence the local anaesthetic activity of β-caryophyllene, which appears to be strictly dependent on its chemical structure. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: β-Caryophyllene; Caryophyllene oxide; Local anaesthetic; Rat hemidiaphragm; Rabbit corneal reflex

1. Introduction

β-Caryophyllene is a sesquiterpenoid occurring in essential oils, especially in clove oil obtained from the dried flower-buds of *Syzygium aromaticum* (Myrtaceae family). Clove oil is the best-known herbal product used as a local analgesic and it has long been employed to obtain transient relief from toothache. The local analgesic activity of clove oil is ascribed to its main constituent eugenol which is believed to act on contact to depress the sensory receptors involved in pain perception [1]. Moreover, some findings suggest that eugenol is a local anaesthetic [2]. β-Caryophyllene, the other main component of clove oil, showed anti-inflammatory activity in several animal models, including carrageenan- and PGE-induced hindpaw edema; this activity does not need the integrity of adrenal glands [3]. Furthermore, unlike non-steroidal anti-inflammatory agents, which have gastric side effects, β-caryophyllene seems to have gastric cytoprotective effects in rats [4]. In this work we investigated the local anaesthetic activity of β-caryophyllene and compared its activity to a chemically related compound, caryophyllene oxide.

2. Experimental

2.1. Animals

Male Wistar rats (150–200 g) and New Zealand albino rabbits (2.5–3.0 kg) from the Morini breeding farm (San Polo d'Enza, Italy) were used. All experiments were carried out according to the guidelines of the European Community Council on animal care.

2.2. Rat phrenic nerve-hemidiaphragm preparation

Experiments were performed according to Bülbring [5] and modified by Wessler and Kilbinger [6]. The effect of drugs in the presence of electrical stimulation (0.2 Hz, 0.5 ms, double threshold voltage) was calculated as the percentage variation of electrically evoked

☆ Giornata di Studio SIPHAR (Italian Society for Pharmacognosy), Assisi (Italy), 19 Sept. 2000.

* Corresponding author.
E-mail address: ghelard@server1.pharm.unifi.it (C. Ghelardini).

0014-827X/01/$ - see front matter © 2001 Éditions scientifiques et médicales Elsevier SAS
PII: S0014-827X(01)01092-8

contractions in the presence of drug- versus pre-drug-evoked efflux.

2.3. Rabbit conjunctival reflex test

The test was conducted according to the method described by Donatelli and Buffoni [7]. The external side of the rabbit eye was stimulated with a cat whisker to induce conjunctival reflex and, consequently, the closure of the palpebrals. The local anaesthetic activity of the drug dropped in the rabbit conjunctival sac is evidenced by the necessity of a higher number of stimuli to provoke the palpebral closure.

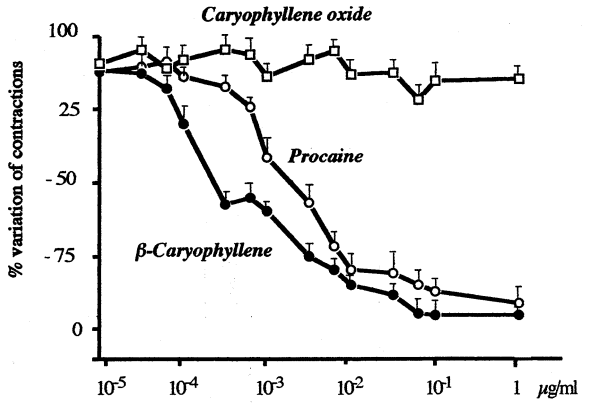

Fig. 1. Dose–response curves of β-caryophyllene and caryophyllene oxide in comparison with procaine on electrically evoked contractions of phrenic nerve hemidiaphragm. Each point represents the mean of four experiments. Vertical lines give the SEM.

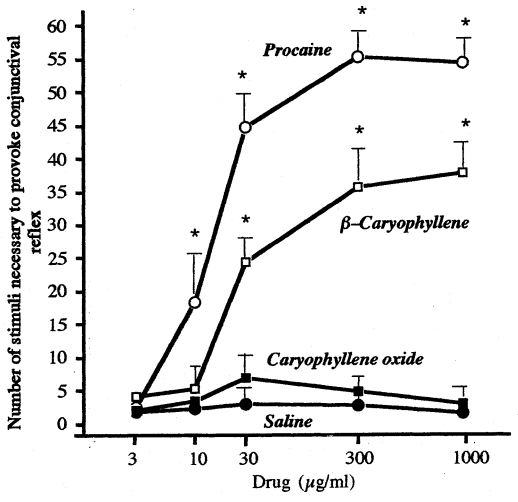

Fig. 2. Dose–response curves of β-carophyllene and caryophyllene oxide in comparison with procaine in rabbit conjunctival reflex test evaluated 5 min after administration. $P < 0.01$. Each value represents the mean of four independent experiments. Vertical lines give the SEM.

2.4. Statistical analysis

Results are given as the mean ± SEM; analysis of variance, followed by Fisher's protected least significant difference procedure for post-hoc comparison, was used to verify the significance between two means. P values of less than 0.05 were considered significant. Data were analysed with the StatView for the Macintosh (1992) computer program.

3. Results and discussion

β-Caryophyllene, in the concentration range of 0.0001–1 μg/ml, was able to reduce, in a dose-dependent manner, the electrically evoked contractions of rat phrenic hemidiaphragm in vitro, up to complete abolishment of contractions (Fig. 1). Under the same experimental conditions the classical local anaesthetic procaine (Fig. 1) exhibited a similar profile to that shown by β-caryophyllene. On the contrary, under the same experimental conditions caryophyllene oxide, up to the dose of 1 μg/ml, did not inhibit the electrically evoked contractions (Fig. 1). β-Caryophyllene did not modify the contractions evoked through direct stimulation of the diaphragm muscle (data not shown).

In vivo the local anaesthetic activity of β-caryophyllene was confirmed in the conjunctival reflex test in the rabbit. Treatment with a solution of β-caryophyllene (10–1000 μg/ml), administered in the conjunctival sac, permitted a dose-dependent increase in the number of stimuli necessary to provoke the reflex (Fig. 2). The application of a solution of caryophyllene oxide, in the range of doses of 3–1000 μg/ml was ineffective (Fig. 2).

The local anaesthetic activity exhibited by β-caryophyllene was observed starting 5 min after administration; it then quickly diminished and disappeared within 15 min (data not shown).

Our experiments show that β-caryophyllene has a strong local anaesthetic action that appears to be strictly dependent on its chemical structure since the oxidised derivative caryophyllene oxide is devoid of any effect. The reduction of the number of the palpebral closures cannot be attributed to an increase of the pain threshold since even drugs able to induce a strong analgesia, such as morphine, diphenhydramine, amitriptyline and acetylsalicilic acid, were completely ineffective in the test of rabbit conjunctival reflex (data not shown).

Acknowledgements

This work was supported by a grant from MURST

References

[1] V.E. Tyler, Nervous system disorders, in: Michey C. Smith (Ed.), Herbs of Choice, Pharmaceuticals Products Press, New York, 1994, pp. 117–134 (chap. 8).
[2] P. Brodin, A. Roed, Effects of eugenol on rat phrenic nerve and phrenic nerve-diaphragm preparations, Arch. Oral Biol. 29 (1984) 611–615.
[3] S. Martin, E. Padilla, M.A. Ocete, J. Galvez, J. Jiménez, A. Zarzuelo, Anti-inflammatory activity of the essential oil of *Bupleurum fruticescens*, Planta Med. 59 (1993) 533–536.
[4] Y. Tambe, H. Tsujiuchi, G. Honda, Y. Ikeshiro, S. Tanaka, Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophyllene, Planta Med. 62 (1996) 469–470.
[5] E. Bülbring, Observation on the isolated phrenic nerve diaphragm preparation on the rat, Br. J. Pharmacol. 1 (1946) 38–61.
[6] I. Wessler, H. Kilbinger, Release of [3*H*] acetylcholine from a modified rat phrenic nerve-hemidiaphragm prepration, Naunyn-Schmiedebergs Arch. Pharmakol. 334 (1986) 357–364.
[7] L. Donatelli, F. Buffoni, Potenziamento dell'anestesia locale operato da mefenidina, Arch. It. Soc. Farmacol. 1 (1948) 4–20.