LOCAL ANAESTHETIC ACTIVITY OF (+) AND (-) MENTHOL

Local Anaesthetic Activity of (+)- and (–)-Menthol

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Abstract: **In this work we studied the local anaesthetic activity of (+)- and (–)-menthol, a substance used after topical application to induce a feeling of coolness. We compared its activity to two chemically related compounds thymol and (–)-menthone. Anaesthetic activity was evaluated *in vivo* in the rabbit conjunctival reflex test and *in vitro* in a rat phrenic nerve hemidiaphragm preparation. Both enatiomers of menthol (10^{-4}–1 μg/ml), but not thymol and (–)-menthone, were able to drastically reduce, in a dose-dependent manner, the electrically evoked contractions of rat phrenic hemidiaphragm. In the rabbit conjunctival reflex test, treatment with a solution of (+)- and (–)-menthol (30 – 100 μg/ml) allowed a dose-dependent increase in the number of stimuli necessary to provoke the reflex, thus confirming *in vivo* the local anaesthetic activity observed *in vitro*. Similar to the *in vitro* results, thymol and (–)-menthone were ineffective also in the *in vivo* test. In conclusion, these data evidence the local anaesthetic activity of menthol, which appears to be strictly dependent on its chemical structure.**

Menthol is present in the volatile oil of several species of mint plants such as peppermint *Mentha piperita* L., (Labiatae). Peppermint oil, prepared by steam distillation from the fresh flowering tops of the plant, contains 30 – 40% of (–)-menthol, frequently more than 50% (1). Menthol can be also obtained from the essential oil of *M. arvensis* L. or by total synthesis; synthetic menthol is racemic (2). Applied topically, menthol causes a tingling sensation and a feeling of coolness due to stimulation of "cold" receptors (3). In pharmacy, it is part of topical antipruritic, antiseptic and cooling formulations. Moreover, (–)-menthol is included in eutectic formulations of local anaesthetic agents (4). Peppermint is traditionally used in the symptomatic treatment of digestive disorders; the antispastic, carminative, choleretic and colagogic properties attributed to it are referred to the presence of the essential oil rich in (–)-menthol (5). Menthol is also employed in external broncholytic and secretolytic preparations (6). Even though their wide use, ethereal oils are endowed with some toxic effects (7).

It has been suggested that, due to their high lipid solubility, the essential oils may interact with the lipid bilayer of the plasma membrane, inhibiting Ca^{++} influx, or preventing the increase of Na^{+} permeability, and thereby by blocking the

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neurotransmission (8). Therefore, the aim of the present study was to investigate the potential local anaesthetic activity of the two enantiomers of menthol.

Both (+)- and (–)-menthol, in the concentration range of 0.0001 – 0.1 μg/ml, were 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**). In the same experimental conditions, the classical local anaesthetic procaine (Fig. **1**) exhibited a similar profile to that shown by menthol enantiomers. On the contrary, (–)-menthone (oxidised derivative of menthol) and thymol (aromatic analogue of menthol) up to the dose of 1 μg/ml did not inhibit electrically evoked contractions (Fig. **1**). These two compounds did not reduce hemidiaphragm contractions even at concentrations of 1 mg/ml (data not shown). (+)- and (–)-menthol did not modify the contractions evoked through direct stimulation of the diaphragm muscle (data not shown).

In vivo the local anaesthetic activity of the above-mentioned enantiomers was confirmed in the conjunctival reflex test in the rabbit. Treatment with a solution of (+)-menthol (10 – 300 μg/ml) and (–)-menthol (10 – 300 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 thymol and menthone, in the range of doses of 3 – 1000 μg/ml, did not exhibit any local anaesthetic effect (Fig. **2**). The vehicle, constituted by a solution of DMSO in H_2O 1 : 4, was devoid of any effect when administered in the conjunctival sac alone (Fig. **2**). The local anaesthetic activity exhibited by (+)- and (–)-menthol was observed starting 5 min after administration, then quickly diminished and disappeared within 15 min (Fig. **3**). Both menthol enantiomers injected subcutaneously at concentrations of 300 μg/ml, also inhibited coutaneous muscle reflex in guinea-pig dorsal skin (data not shown).

Our experiments show that menthol has a strong local anaesthetic action. These results are in agreement with previous reports in which the anaesthetic activity on the gastric mucous membrane attributed to the essential oils containing menthol (7) and with use of menthol after application to the tympanic membrane during tympanocentesis (5). This pharmacological effect also explains the use of menthol in pain relief after application to the skin (12). It is interesting to note that, even if the natural enantiomer (which usually is the most effective) of menthol is (–), concerning the local anaesthetic activity both enantiomers are equiactive.

The local anaesthetic activity of menthol appears to be strictly dependent on its chemical structure since the oxidised derivative menthone is devoid of any effect. Furthermore, this action cannot be related to a general property of alcohols since the aromatic analogue of menthol did not show any local anaesthetic activity. 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, acetylsalicilic acid, were completely ineffective in the test of rabbit conjunctival reflex (data not shown). The only effect on this test observed using analgesic drugs is a potentiation of the effect induced by local anaesthetics (13).

Materials and Methods

Male Wistar rats (150 – 200 g), guinea pigs (300 – 400 g) and New Zealand albino rabbits (2.5 – 3.0 kg) from Morini (San Polo d'Enza, Italy) breeding farm were used. All experiments were carried out according to the guidelines of the European Community Council on animal care.

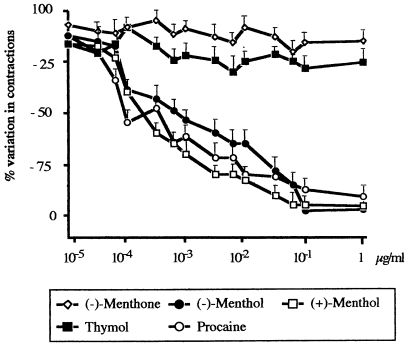

Fig. 1 Dose-response curve of (+)- and (–)-menthol, (–)-menthone and thymol in comparison with procaine on electrically-evoked contractions of phrenic nerve-hemidiaphragm. Each point represents the mean of 4 experiments. Vertical lines give s.e.m.

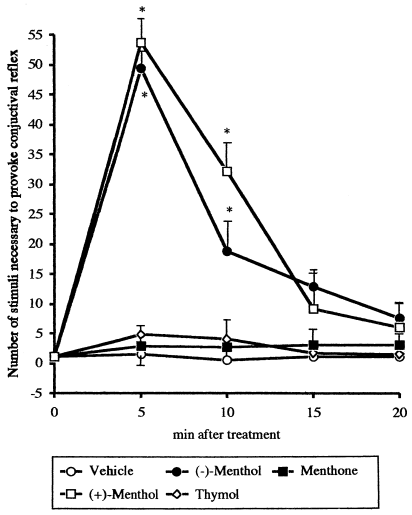

Fig. 2 Dose-response curves of (+)- and (–)-menthol, (–)-menthone and thymol in the rabbit conjunctival reflex test evaluated 5 min after administration. *P < 0.01. Each value represents the mean of 4 independent experiments. Vertical lines give s.e.m.

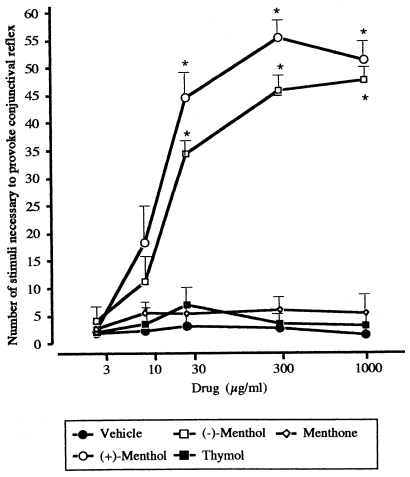

Fig. 3 Effect of (+)- and (–)-menthol, thymol and (–)-menthon in the rabbit conjunctival reflex test. All drugs were administered at the dose of 1000 μg/ml. *P < 0.01. Each value represents the mean of 4 independent experiments. Vertical lines give s.e.m.

Experiments on rat phrenic nerve-hemidiaphragm were performed according to Bülbring (9), and modified by Wessler and Kilbinger (10).

The rabbit conjunctival reflex test was conducted according to the method described by Donatelli and Buffoni (11). The external side of rabbit eye was stimulated with a cat whisker to induce conjunctival reflex and consequently the closure of the palpebrals.

The following drugs (purity higher than 90%) were used: (+)-menthol, (–)-menthone (Aldrich, Milan, Italy), (–)-menthol, thymol (Sigma, Milan, Italy); procaine hydrochloride (RBI, Milan, Italy). (+)- and (–)-menthol were solubilised in a vehicle of 1 : 4 solution of DMSO in H_2O at the highest concentration whereas successive dilutions were made by using only H_2O.

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