Effects of 1.8-cineol (eucalyptol) on the Activity of Histamine H1 Receptors

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1. Introduction

For many years, medications containing 1.8-cineol (eucalyptol) have been used for the treatment of bronchial complaints, sinusitis and colds. It has been previously shown that 1.8-cineol, a monoterpene (C-10) [1], has a very pronounced spasmolytic effect on smooth muscle fibre. In nearly all clinical applications with 1.8-cineol, especially when used in conjunction with allergic symptoms, histamine receptors prove to be crucial for treatment (use of histamine inhibitors).

The aim of this study was to determine to what extent 1.8-cineol influences the effect of histamine receptors. The contractile effect of histamine using various concentrations of 1.8-cineol on smooth muscle fibre from guinea pig stomach, was investigated. The results showed that even at concentrations of 10^-8 M histamine, an excitation of the contractile effect of smooth muscle fibre occurred. The concentration response curve of the effects of histamine shows that the maximum excitation effects are achieved at a concentration of 5 x 10^-6 M histamine.

The results of the interactions between 1.8-cineol and histamine indicate that 1.8-cineol is a reversible antagonist for histamine receptors. Increasing the “concentration” of 1.8-cineol by 16-fold (from 0.005 up to 0.080 ul) increases this inhibition from approx. 25% to approx. 66%.

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Keywords

Smooth Muscle Fibre; 1.8-Cineol (Eucalyptol); Histamine H1 Receptors; Bronchitis; Monoterpene

1. Introduction

For many years, medications containing 1.8-cineol (eucalyptol) have been used for the treatment of bronchial complaints, sinusitis and colds. It has been previously shown that 1.8-cineol, a monoterpene (C-10) [1], has a very pronounced spasmolytic effect on smooth muscle fibres (SMF), similar to that of papaverine [2]. 1.8-cineole was found to have agonistic effects on the α1 and α2 adrenergic receptors [3]. These effects can be registered at low concentrations of up to 3 x 10^-7 M to 2 x 10^-5 M 1.8-cineole. At higher concentrations, the well-known spasmolytic effect appears. At concentrations above 4 x 10^-4 M 1.8-cineole, the effect of 10^-5 M acetylcholine is 100 % suppressed.
In almost all clinical applications with preparations containing 1.8-cineol, the H1 histamine receptor is essential, especially in the treatment of allergic symptoms. In the treatment of various respiratory tract diseases the application of histamine H1 inhibitors plays an important role. So far there are no studies on the extent and specific effects of 1.8-cineol, e.g. exerts on receptors. Such studies are particularly difficult because of the fact that 1.8-cineol has spasmolytic, papaverine-like effects [2]. It is also known that these specific effects usually appear at much lower concentration levels. So we obtain a concentration interval between the specific and non-specific effects, which is usually in the range of 2 orders of magnitude.

The aim of this study was to determine what effect 1.8-cineol has on the effect of histamine H1 receptors. The histamine H1 receptor is a receptor which is activated by the biogenic amine histamine. It is expressed in smooth muscles, on vascular endothelial cells, in the heart, and in the central nervous system. The H1 receptor is linked to an intracellular G-protein (Gq) that activates phospholipase C and the phosphatidylinositol (PIP2) signalling pathway [4, 5].

2. Materials and Methods

In vitro experiments on smooth muscle fibre of guinea pig stomach (GPS), the influence of histamine under normal conditions and using different concentrations of 1.8-cineol on the spontaneous contractile effect (SCA) of the SMF was investigated.

2.1 Measurement of the Spontaneous Contractile Activity of Smooth Muscle Fibre and Method of Preparation

The measurements were performed according to the standardized Golenhofen method [6]. The smooth muscle fibre used in the experiments was taken from the stomach of a male guinea pigs whose weight was approximately 300g (HsdPoc: DH 300-349g Harlan Winkelmann GmbH, Borchen, Germany). All animal experiments were approved in advance by the Ruhr-University Bochum commission responsible for animal research.

The muscle tissue had a length of between 12-14 mm and a width of between 1-2 mm and was taken from the corpus and antrum of the guinea pig stomach. The preparation of muscle tissue was carried out in a circular direction, starting from the serosa along the greater curvature, running along the direction of the fibres.

The organ baths (20 mL volume) contained a Krebs’ solution (KS) with the following composition: mmol/L: Na\(^+\) (143), K\(^+\) (5.94), Mg\(^{2+}\) (1.19); Ca\(^{2+}\) (2.5), Cl\(^-\) (133), HCO\(^-\) (16.7), HPO\(_4^{2-}\) (11.9) and glucose (11.5). A pH-value within physiological limits of 7.2 to 7.3 (7.2 ± 0.8) was maintained. The organ baths of Krebs’ solution were kept at 35 °C (± 0.2 °C). Throughout the experiments the Krebs’ solution was carbonated with carbon dioxide-gas. The response to histamine was measured under isometric conditions (mN).

2.2 Substances
- Acetylcholine chloride (acetylcholine ophtalmicum dispersa®), Dispersa, Germering, Germany
- Substances for Krebs’ solution (Merck comp., Darmstadt, Germany): NaCl, KCl, MgCl\(_2\) 6 H\(_2\)O, KH\(_2\)PO\(_4\), NaHCO\(_3\), CaCl\(_2\)
- 1.8-cineole Ph. Eur. - B: 1,015,309 (purity 99.6 %; Klosterfrau Healthcare Group, Berlin).
- H1 agonist (2 - (2-pyridyl) - ethylamine), Sigma-Aldrich Co, USA
- H1 inhibitors (olopatadine), Sigma-Aldrich Co, USA
- Histamine dihydrochloride, Sigma-Aldrich Co, USA

2.3 Cineole Solution

At room temperature, 10 µL 1.8-cineole were added to 2 mL of ethanol (98 %). The sample was homogenized with a vortex for 2 min. The transparency of the samples was determined using standardized methodology at an illumination of 1500 lux. After proper solubility was established, 1.8-cineole was progressively diluted to 100 µL 1.8-cineole in 2 mL of ethanol (98 %) (5 % solution of cineole in 98 % ethanol). Further dilution with Krebs’ solution was performed. Thereafter, direct comparisons of the absorption spectra of the concentrations of 1.8-cineole in Krebs’ solution were determined.

2.4 Statistics

To take into account the specific variations and alterations of the SCA preparation’s measured values, the concentration-response curves in each case of N=10 individual experiments, always measured the excitation in % of maximal contractile activity of smooth muscle.
tissue when acted upon by $10^{-5} \text{ M acetylcholine (ACh)}$. The changes of the smooth muscle fibre (SMF) concerning SCA with various substances are given in Newtons (N).

The processing of experimental data was performed by using the Statistica 4.5 (StatSoft, Inc. Microsoft, USA) program. For comparison between two groups, the t-Test (student) for unpaired samples was implemented. For comparisons between three or more groups, variance analysis (ANOVA) was used. The statistical comparisons were performed at the 5% significance level. The results are expressed as mean ± standard deviation. In each case $n = 7$ measurements per experiment were performed.

**Figure 1:** The effects of different concentrations of histamine ($5 \times 10^{-9} \text{ M} – 10^{-5} \text{ M}$) on the SCA of the SMF of guinea pig stomach under normal conditions (above) and with prior addition of $10^{-5} \text{ M}$ H1 inhibitors (olopatadine) (below)

Figure 2 shows the effect of $10^{-6} \text{ M}$ histamine on the SCA of the SMF (above, Tracing A) and after 45 minutes (below, tracing B) of contact time with 0.005 µl 1.8-cineol (in a 10 ml organ bath) solution. It is clearly evident that the stimulating effects of histamine on the SCA of the SMF have been suppressed by about 25-30%.

The tracing in B (showing the histamine response after 45 min incubation with 1.8-cineol) obtained immediately after tracing in A (Figure 2-7). Figure 3 illustrates that the reactivity of the histamine H1 receptors finally normalizes after 3 hours of repeated change of solution with KS.

The results in Figure 5 show that after 3 hours and repeated solution replacement with KS, the effect of the histamine H1 receptors is almost completely restored.

In Figure 6, the stimulating effect of $10^{-6} \text{ M}$ histamine on the SCA of the SMF under normal conditions (above) and with previous 45 minute exposure time with 0.08 µl 1.8-cineol and solution replacement (below) is shown. The stimulating effect of $10^{-6} \text{ M}$ histamine on the SCA of the SMF with the prior addition of 0.08 cineol can be seen to be about 66% lower than under normal conditions.

Figure 7 shows that after 3 hours and repeated changes of KS, the effect of histamine H1 receptors is no longer measurable. The stimulating effects of $10^{-6} \text{ M}$ histamine on the SCA of the SMF achieve only about 80% of their maximum effect. This is due to the fact that at concentrations of 1.8-cineol (0.08 µl), cineol has a very pronounced spasmolytic effect.
**Figure 2:** The effects of $10^{-6}$ M histamine on the SCA of the SMF of guinea pig stomach (above) and after 45 minutes (below) of contact time with 0.005 µl 1.8-cineol (in 10 ml organ bath) solution and solution change.

**Figure 3:** The effects of $10^{-6}$ M histamine on the SCA of the SMF of guinea pig stomach (above) and after 45 minutes of contact time with 0.005 µl 1.8-cineol and after 3 hours of contact time with frequent solution change (below).

**Figure 4:** The effects of $10^{-6}$ M histamine on the SCA of the SMF of guinea pig stomach (above) and after 45 minutes of contact time with 0.01 µl 1.8-cineol and solution change (below).
Figure 5: The effects of $10^{-4}$ M histamine on the SCA of the SMF of guinea pig stomach (above) and after 45 minutes of contact time with 0.01 μl 1.8-cineol and 3 hours of contact time (below).

Figure 6: The effects of $10^{-6}$ M histamine on the contractile effect of histamine of smooth muscle fibre guinea pig stomach (above) and after 45 minutes of contact time with 0.08 μl 1.8-cineol and solution change (below).

Figure 7: The effects of $10^{-8}$ M histamine on the SCA of the SMF of guinea pig stomach (above) and after 45 minutes of contact time with 0.01 μl 1.8-cineol and 3 hours of contact time (below).
4. Discussion

1.8-Cineole is a monoterpane known to be a component of various essential oils, e.g. of the genus Eucalyptus, Salvia, Rosmarinus, but is mainly isolated from Eucalyptus species which produce essential oil rich in cineole. This saturated terpene has a number of medicinally useful anti-inflammatory, anti-oxidative and antimicrobial effects, as recently presented in an overview by Juergens [7]. 1.8-cineole is used as an active ingredient in medicinal products and can be inhaled, topically applied or be taken orally. After resorption of 1.8-cineole in the small intestine part of it is eliminated unchangedly by exhalation [8]. Regarding the intestinal as well as the bronchial tract, 1.8-cineole also comes into contact with smooth muscle fibre (SMF). Investigations of the effects of 1.8-cineole on SMF, whether receptor-specific or receptor-independent, are expected to contribute to the rationale of clinical efficacy of 1.8-cineole, e.g. in inflammatory bronchial diseases [9].

The results reported in this manuscript shows that the mechanisms of this inhibition are due to an effect on the histamine H1 receptors. In order to hypothesize an action on histamine H1 receptors, at least the effects on concentration-response curve to histamine was studied and a rightward shift evidential (Figure 1). It would also be important to see whether the contractile response to other agonists in inhibited by 1.8-cineol [3] (Figure1.), because the inhibition was seen could equally be due to activations of adrenergic receptors. In this context, we point out that increasing the “concentration” of 1.8-cineole by 16-fold (from 0.005 µl up to 0.080 µl) increases this inhibition from approx. 25% to approx. 66%.

The results show that histamine contracted of the GPS. The maximum excitation effects on the SCA of the SMF have registered at concentrations of 10^-6 M. These effects achieve up to 50% of the maximum possible excitation effects of 10^-5 M ACh. Figure 1 shows that these stimulating effects of histamine on the SCA of the SMF are dependent exclusively on the effect of the histamine H1 receptors. With previous receptor suppression, the stimulating effects of histamine on the SCA of the SMF are completely inhibited.

The test results presented in figures 2-7 show that a 1.8-cineole test solution with a volume of up to 0.08 µl suppresses effect of histamine H1 receptors. The maximum amount of suppression on the stimulating effects of histamine on the SCA to the SMF is over 80%. Investigations with higher concentrations of 1.8-cineol are not possible because at concentrations of 0.1 µl (in a 10 ml organ bath) or more, cineol develops a pronounced spasmolytic effect on the SCA of the SMF and histamine stimulated effects can no longer be registered.

These results are in line with former investigations indicating the spasmolytic effect of 1.8-cineole on SMF in our results [3]. A paper by Wagner [10] showed however, that terpenes, when administered to patients with bronchial asthma, displayed paradoxical bronchoconstriction reactions. We believe that these contradictory reports are based on the special properties of terpenes: Monoterpenes are known to have both specific and non-specific effects on smooth muscle fibre, dependent on the dose. Some monoterpenes, such as 1.8-cineole and thymol [11], at concentrations of up to 5.10^-7 M lead to constricting effects on almost all smooth muscle fibre. These effects are due to the agonistic effects on α1 and α2 adrenoreceptors. However, they can only be observed up to an administered dose of a concentration of 5.10^-7 M. Exceeding this amount, the pronounced spasmolytic effect of cineol appears, which no longer allows the registration of specific effects. Because of this, we have only investigated the effects of 1.8-cineole on the H1 receptors up to concentrations of 0.08 µl (in a 10 ml organ bath). Above this concentration it is not possible to measure specific effects. That does not mean that 1.8-cineole cannot inhibit histamine H1 receptors at higher concentrations. The methodology we used does not permit observation of measurements above this concentration, making the properties of thymol and 1.8-cineole as simply not yet investigated.

Low concentrations of 1.8-cineole allow observations of bronchoconstriction. Here, we recorded the stimulating effects of 1.8-cineole on smooth muscle fibre (due to the excitation effects on H1 receptors). At higher concentrations very pronounced spasmolytic effects can be observed and of course the inhibition of H1 receptor activity.

The concentration-response curve of the effects of histamine indicates that the maximum excitation effects are achieved at a concentration of 5.10^-6 M. The results of the interaction between 1.8-cineole and histamine indicate that 1.8-cineole is a reversible antagonist of the histamine H1 receptors. Regarding the clinical relevance of the use of 1.8-cineole, the well-known spasmolytic effect of 1.8-cineole in therapeutic concentrations could be particularly valuable in inflammatory airway diseases with bronchoconstriction symptoms, which has already been confirmed in clinical
trials with patients suffering from asthma or COPD [7].

In the first step of our investigations we were choosing stomach muscle rather than human airway muscle, because the ultimate goal of this paper was not to address the bronchodilatory of this chemical, but rather the mechanism of action of H1 receptors in general. To show a possible role for 1.8-cineol in airway diseases it is needed to use human airway smooth muscle because the pharmacological properties of airway smooth muscle could be in several aspects different from those of gastric and other types of smooth muscle.

Conflicts of Interest: Prof. J. Lukanov reported receiving financial support by Klosterfrau Company used for reagents and animals to perform the study. All other authors declare that they have no conflict of interest.

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References


