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Effects of 1.8-cineole (eucalyptol) on the spontaneous contractile activity of smooth muscles fibre

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Eucalyptus oil is frequently used in phytotherapy. The objective of the present paper was to investigate to what extent 1.8-cineole influences the activity of smooth muscle fibres. The effects of 1.8-cineole on the spontaneous contractile activity were investigated in *in vitro* experiments with circular smooth muscle fibres of guinea pig stomach. 1.8-cineole was found to have agonistic effects on the α_1 and α_2 adrenergic receptors. These effects can be registered at low concentrations of up to 3×10^{-7} to 2×10^{-5} M 1.8-cineole. At higher concentrations, the well-known spasmolytic effect appears. At concentrations above 4×10^{-4} M 1.8-cineole, the effect of 10^{-5} M acetylcholine is 100% suppressed. The results allow for the assumption that, besides the spasmolytic effects investigated to date and owing to its specific effects on the α_2 adrenergic receptors of the nerve cells, 1.8-cineole brings about an additional improvement to local blood circulation and alleviates pain.

Key words: Smooth muscle fibre, 1.8-cineole (eucalyptol), α_1 and α_2 adrenergic receptors, papaverine, pain, clonidine.

INTRODUCTION

1.8-cineole is found in many essential oils. 1.8-cineole is the main ingredient of both eucalyptus (70%) and rosemary oils, the concentration of which can exceed 45% (Schilcher et al., 2007). Through freeze drying and steam distillation, a 99% pure eucalyptus oil can be produced from which a pure form of 1.8-cineole can be attained, which is then used for the production of many herbal medicines. *In vitro* studies revealed secretomotoric, expectorant, antispasmodic (Forster et al., 1980; Wagner et al., 1986) local hyperaemic and anti-inflammatory qualities (Juergens et al., 2003) for the naturally isolated 1.8-cineole (synonym: eucalyptol). Because of its efficacy and excellent tolerance, 1.8-cineole has been successfully used for decades to treat rheumatic disorders topically, as well in encapsulated form to be orally applied against inflammatory diseases of the respiratory tract, sinusitis, acute and chronic bronchitis and as an add-on therapy in chronic obstructive pulmonary disease (COPD) and steroid-dependent asthma (Juergens et al. 2003; Worth et al., 2009).

So far there have been no studies on whether 1.8-cineole specifically affects, for example receptors. Such studies are complicated because of the fact that 1.8-

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cineole has spasmolytic, papaverine-like effects (Sagorchev et al., 2010). It is also well known that these specific effects usually appear at much lower concentration levels. The concentration interval between the specific and non-specific effects usually is in the range of two orders of magnitude. In our previous work (Beer et al., 2007) we have shown that another monoterpene, thymol, which also has strong anti-inflammatory and antispasmodic effects at concentrations less than 10^{-6} M, shows an agonistic effect on the α_1 and α_2 adrenoreceptors.

The aim of this study was to investigate whether 1.8-cineole exerts other effects on smooth muscle fibre besides the previously described spasmolytic effects (Cabo et al., 1986). Such additional effects, especially when applied externally, might better explain its versatility in treatment. Two types of tests have been conducted: First, studies were performed on the effects of 1.8-cineole on the $\alpha_{1,2}$ adrenoreceptors, the β-adrenergic and dopamine D_2 receptors because investigations of this kind with cineole have not yet been described in the literature. However, many clinical effects can only be described and explained by specific, that is receptor-mediated effects. Second, the well-known spasmolytic effect of 1.8-cineole was compared with that of papaverine.

MATERIALS AND METHODS

Substances

The following agonists and antagonists (Sigma, St. Louis, MO, USA) were used:

1. α_1 agonist: methoxamine (purity ≥ 98%).
2. α_1 antagonist: prazosine (purity ≥ 99%).
3. α_1 and α_2 antagonists: benextramine (purity ≥ 98%).
4. α_2 agonist: p-Iodoclonidine (purity ≥ 100%).
5. α_2 antagonist: rauwolscine (purity≥99%).
6. β antagonist: alprenolol (purity ≥ 98%).
7. D_2 dopamine agonist: quinpirole-dihydrochloride (purity ≥ 99%).
8. Selective antagonist of D_2 dopamine receptors: raclopride [S (-) L-Tartrate] (purity ≥ 98%).
9. Papaverine- HCl (purity ≥ 99 %, titration per chloric acid; Merck comp., Darmstadt, Germany).
10. Acetylcholine chloride (acetylcholine ophtalmicum dispersa®), Dispersa, Germering, Germany).
11. Substances for Krebs´ solution (Merck comp., Darmstadt, Germany): NaCl, KCl, $MgCl_2$ 6 H_2O, KH_2PO_4, $NaHCO_3$, $CaCl_2$.
12. 1.8-cineole Ph. Eur. - B: 1,015,309 (purity 99.6%; Klosterfrau Healthcare Group, Berlin).

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.

Measurements of spontaneous contractile activity (SCA) of smooth muscle fibre and method of preparation

The measurements including application of acetylcholine as positive control for the contractile activity of SMF were performed according to the standardized Golenhofen method (Golenhofen, 1976). The smooth muscle fibre used in the experiments was taken from the stomach of a male guinea pig whose weight was approximately 300 g (HsdPoc: DH 300 to 349 g Harlan Winkelmann GmbH, Borchen, Germany). All animal experiments were approved in advance by the Ruhr-University Bochum commission responsible for animal research (date and number of approval by the ethical committee: November 7th 2008; Nr. 8.87 to 50.10.42.08.253).

The muscle tissue had a length of between 12 to 14 mm and a width of between 1 to 2 mm and was taken from the corpus and antrum of the guinea pig stomach. Preparation of the muscle fibre 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 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). During the entire test series, the Krebs´ solution was aerated with carbogen gas. 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 ± 0.2°C. Measurements were conducted under isometric conditions (mn).

Statistics

To take into account the specific variations and alterations of the spontaneous contractile activity (SCA) preparation's measured values, the dose 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} M acetylcholine (ACh). The changes of the smooth muscle fibre (SMF) concerning spontaneous contractile activity (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.

RESULTS

The spasmolytic effect of 1,8-cineole in therapeutic concentrations is well known. Our objective was to investigate whether cineole in lower concentrations can specifically interact with receptors on smooth muscle fibre (SMF) as has been shown for thymol before (Beer et al., 2007). Figure 1A shows the effect of 0.025 µl cineole (1.5×10^{-5} M) (in 20 ml organ bath) on the spontaneous contractile activity (SCA) of smooth muscle fibre (SMF) from guinea pig stomach. After the addition of 1.8-cineole a pronounced excitation of the SCA of the SMF is clearly seen. In Figure 1B the effects of different concentrations

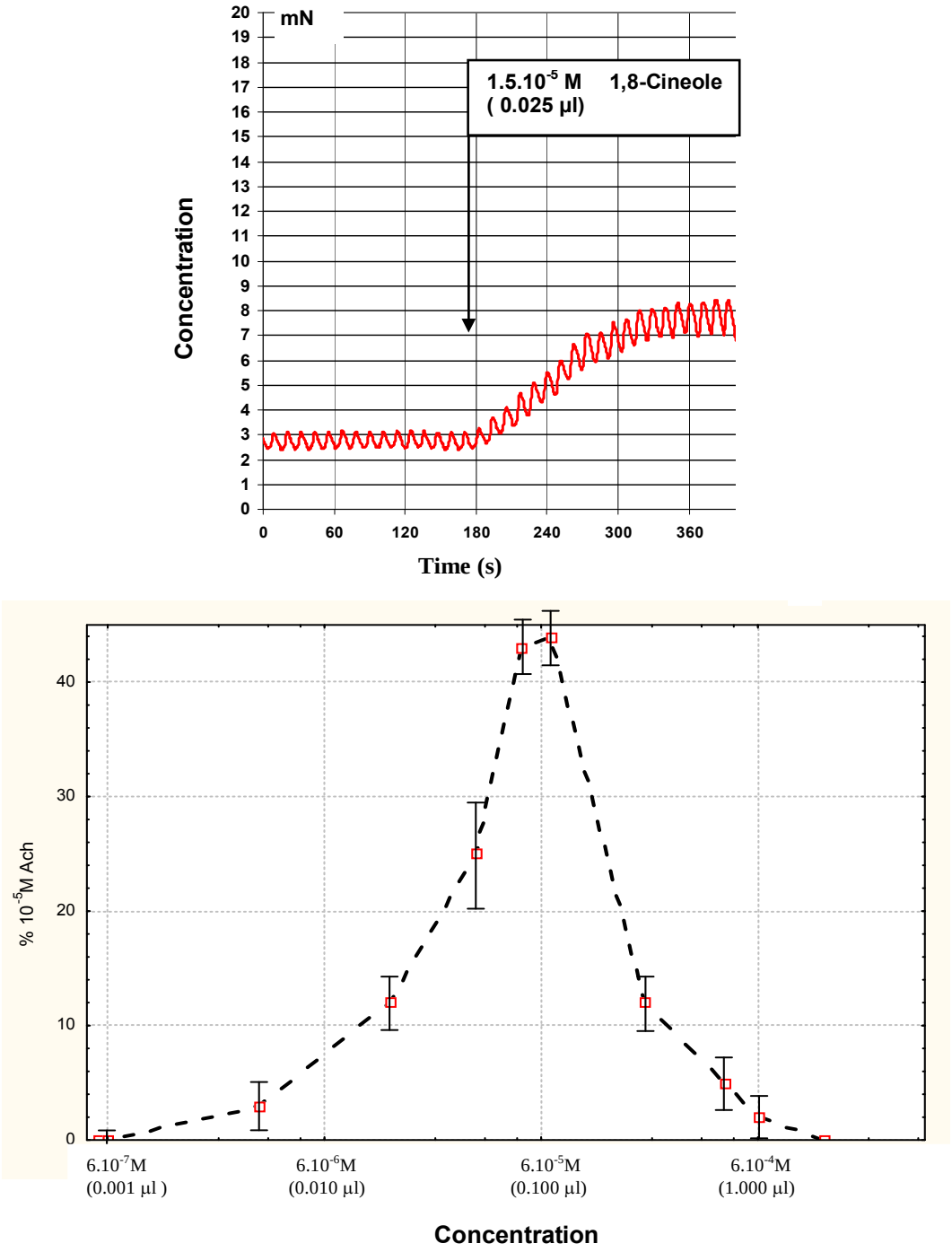

Figure 1. An effect of 1.5 × 10^{-5} M 1,8-Cineole (0.025 µl) on the spontaneous contractile activity of the smooth muscle strips of the guinea pig stomachs and a graphic representation of the effects of diverse concentrations of 1,8-Cineole von 6 × 10^{-7}M ÷ 1.5 × 10^{-3}M (0.001 µl ÷ 2.500 µl) on the same smooth muscles. Ach = acetylcholine.

of 1.8-cineole on the SCA of SMF are shown. Even with the addition of only 0.001 µl 1.8-cineole marked stimulation of the SCA of the SMF can still be induced. The maximum stimulating effect is achieved at a concentration of 0.07 µl. With a further increase in concentration of 1.8-cineole, an inhibition of the stimulating effects is achieved, and at a concentration of about 1 µl (0.5 µl cineole; 3 × 10^{-4} M) spontaneous contractile activity is completely suppressed.

Figure 2A shows that 1.8-cineole is not able to

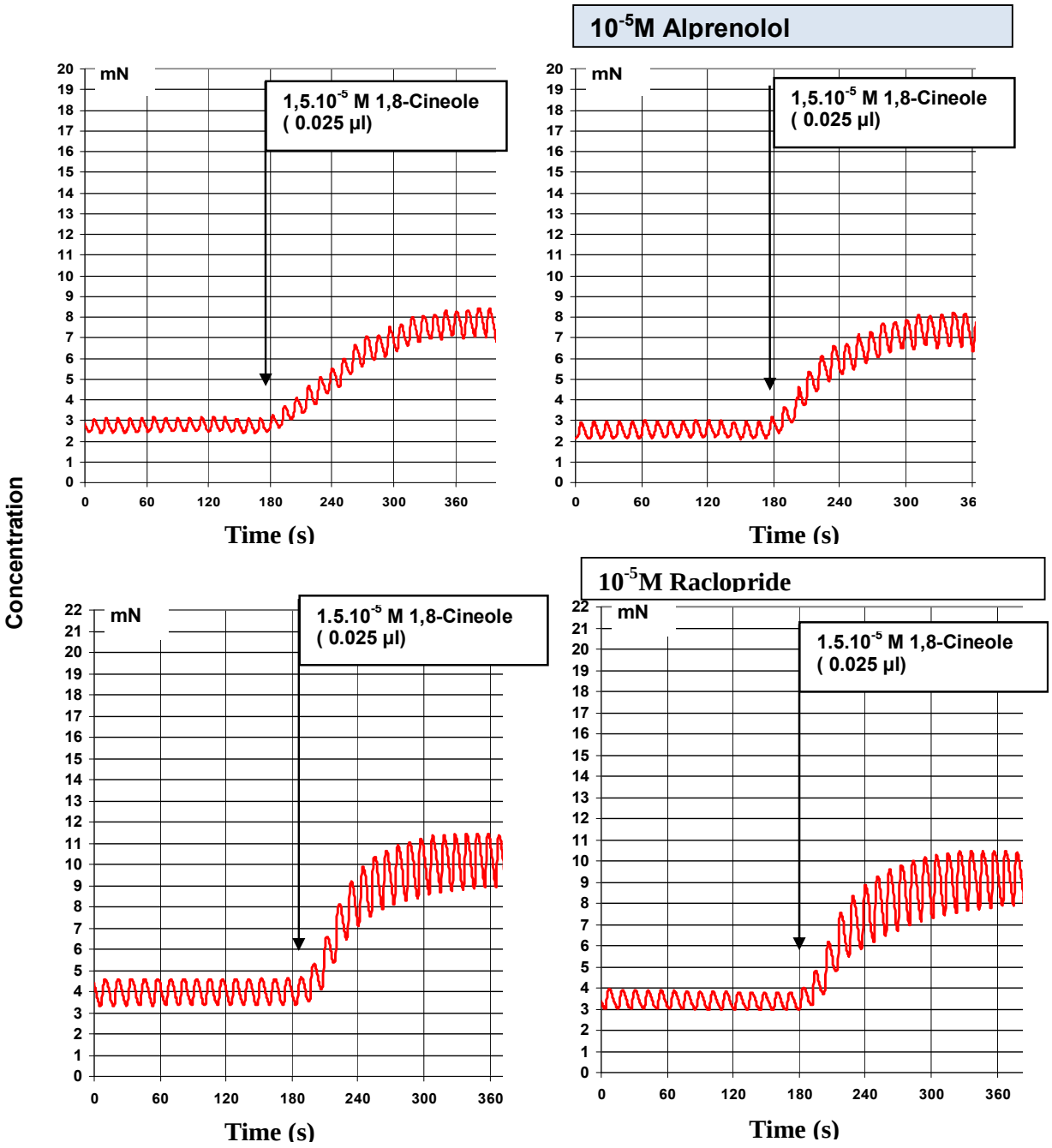

Figure 2. Isometrical records of the spontaneous smooth muscles-contractile activity alteration (guinea pig) after 0.025 µl Cineol (1.5×10^{-5} M) application in normal physiological conditions and after administration of 10^{-5} M Alprenolol and 10^{-5} M Raclopride.

influence β adrenoreceptors. With previous addition of 10 µM alprenolol, the stimulating effect of 1.8-cineole on the SCA of SMF remains unaffected. Figure 2B also shows that, even with an inhibition of D_2 dopamine receptors, the stimulating effect of 1.8-cineole on the SCA is not affected. Figure 3A shows the stimulating effects of 0.025 µl 1.8-cineol on the SCA of SMF under normal conditions (left) and after the addition of 10^{-5} M rauwolscine (α_2 adrenoreceptor antagonist) (Figure 3B). With previous inhibition of α_2 adrenoreceptors with rauwolscine, the stimulating effects of 1.8-cineole on the SCA the SMF are significantly lower. The effects of 0.025 µl cineole on the SCA of SMF are shown under normal conditions (Figure 3C) and after the addition of 10^{-5} M prazosine (Figure

3D). With prior inhibition of α_1 receptors with 10^{-5} M prazosine, the stimulating effects of 1.8-cineole are significantly reduced.

With simultaneous inhibition of the α_1 and α_2 adrenoreceptors with 10^{-5} M of rauwolscine and 10^{-5} M of prazosine, the stimulating effect of 1.8-cineole on the SCA of SMF (Figure 3E) is completely suppressed (Figure 3F). In Figure 4, the spasmolytic effects of 1.8-cineole and papaverine on the SCA of SMF are compared. The results show that 1.8-cineole has a very pronounced spasmolytic effect on the SCA of SMF. The complete suppression of the SCA of SMF and the stimulating effect of 10^{-5} M acetylcholine (ACh) is achieved with papaverine at a concentration of 10^{-4} M, the same effect with 1.8-cineole at a concentration of 4×10^{-4} M.

DISCUSSION

1.8-Cineole is a monoterpene 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 (2014). 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 (Beauchamp et al., 2010). Regarding the intestinal as well as the bronchial tract, 1.8-cineole also comes into contact with smooth muscle fiber (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, for example, in inflammatory bronchial diseases.

The results show that 1.8-cineole in low concentrations has a pronounced stimulating effect on the spontaneous contractile activity (SCA) of smooth muscle fibre (SMF). With 0.001 µl 1.8-cineole (about 2×10^{-7} M), a significantly stimulating effect on the SCA of SMF can be registered. This stimulating effect reaches its maximum at a concentration of 0.070 µl 1.8-cineole (3×10^{-5} M) (Figure 1B). Higher concentrations of 1.8-cineole lead to an inhibition of the SCA of SMF. The receptor-specific effect of 1.8-cineole on the SCA of SMF can only be observed in low concentrations up to 0.07 µl (2×10^{-5} M). Above this concentration the spontaneous contractile activity of smooth muscle fibre is fully suppressed by 1.8-cineole. Thus, the well-known spasmolytic effect of 1.8-cineole appears at higher, therapeutically relevant concentrations.

The effect of low concentrations of cineole on smooth muscle fibre is due to receptor-specific actions. The excitatory effect of 1.8 to cineole on the SCA of SMF remains unchanged with the prior addition of β adrenergic and D_2 dopaminereceptor antagonists (Figures 1 and 2 and Table 1). 1.8-cineole is a specific agonist at α_1 and α_2 adrenoreceptors (Figures 2 and 3). The maximum effect is between 40 and 50% of the maximal contractile activity of the SMF compared to the same effect achieved with acetylcholine (ACh) × 10^{-5} M (Figure 1B).

Due to the very pronounced spasmolytic effect of 1.8-cineole on smooth muscle fibre, no agonistic effect on α_1 and α_2 adrenoreceptors could be recorded after a few minutes with the applied method. The agonistic effect of 1.8-cineole on the neuronal receptors remained unchanged. The agonistic effects of 1.8-cineole on the α_1 and α_2 adrenergic receptors can have diverse effects on the organism. The following mechanisms of action may be postulated for 1.8-cineole: The activation of neuronal α_2 adrenoreceptors inhibits the release of noradrenaline. As a result, there are additional vasodilatatory effects, comparable to low doses of p-iodoclonidine, the inhibition of the release of noradrenaline leads to suppression of pain transmission. Thus, analgesic effects may be possible with the external use of 1.8-cineole (Sadjak et al., 2005), the agonistic effects of 1,8-cineole on α_1 and α_2 adrenoreceptors can still be observed at concentrations of 3×10^{-7} mM. The maximum stimulating effect is seen at concentrations of 2×10^{-5} M and is about 40% of maximal contractile activity of SMF, compared to the same effect reached with 10^{-5} M ACh (positive control for contractile activity).

On the other side, at concentrations greater than 2×10^{-5} M, 1.8-cineole displays its well-known spasmolytic effects on the spontaneous contractile activity of smooth muscle fibre. With 4×10^{-5} M 1.8-cineole, the stimulating effects of 10^{-5} M ACh are completely (100%) suppressed. The results are in line with former investigations indicating the spasmolytic effect of 1.8-cineole on SMF. In Ca^{2+} containing medium as well as in medium free of Ca^{2+} 1.8-cineole showed spasmolytic activity on the isolated rat duodenum against ACh- and calcium chloride-induced contractions (Gamez et al., 1990). From studies using rat and guinea pig tracheal rings it has been concluded that cineole relaxes the airway smooth muscle (non-sensitised as well as ovalbumin-sensitised) by a non-specific mechanism, most likely by blocking calcium influx across the membrane. Cineole also increased the relaxation rate of ovalbumin-sensitised bronchial rings following a Schultz-Dale reaction. This effect is regarded to be probably due to the established anti-inflammatory effects of 1.8-cineole as an inhibitor of leukotriene production (Nascimento et al., 2009).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 bronchoconstrictory symptoms, which has already been confirmed in clinical trials with patients suffering from

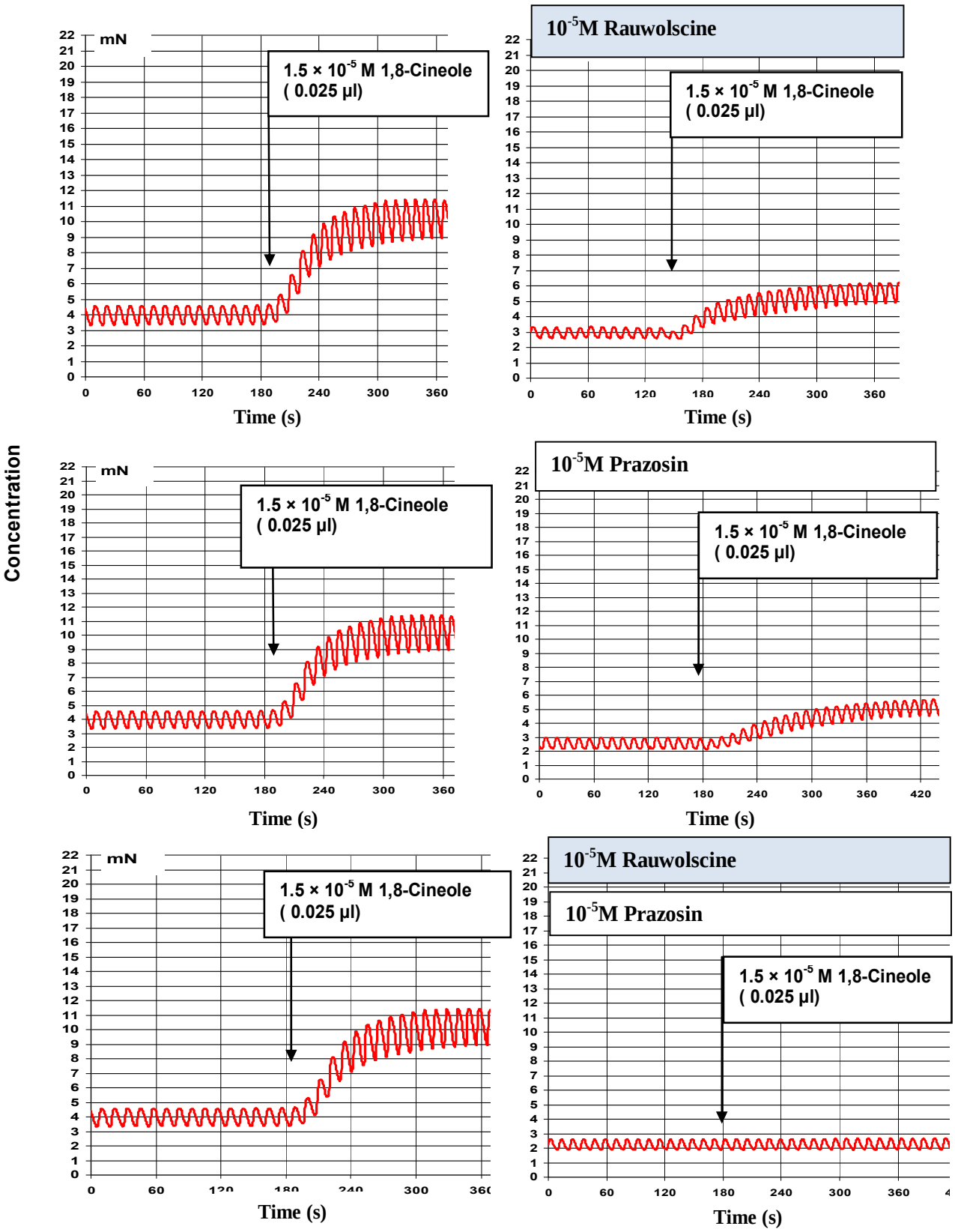

Figure 3. Effects of the administration of 0.025 µl 1,8-Cineole on the spontaneous contractile activity of the smooth muscle strips of the guinea pig stomach and after 20 min application of 10^{-5} M Rauwolscine (top), 10^{-5} M Prazosin (midle) and after both blockers (bottom)

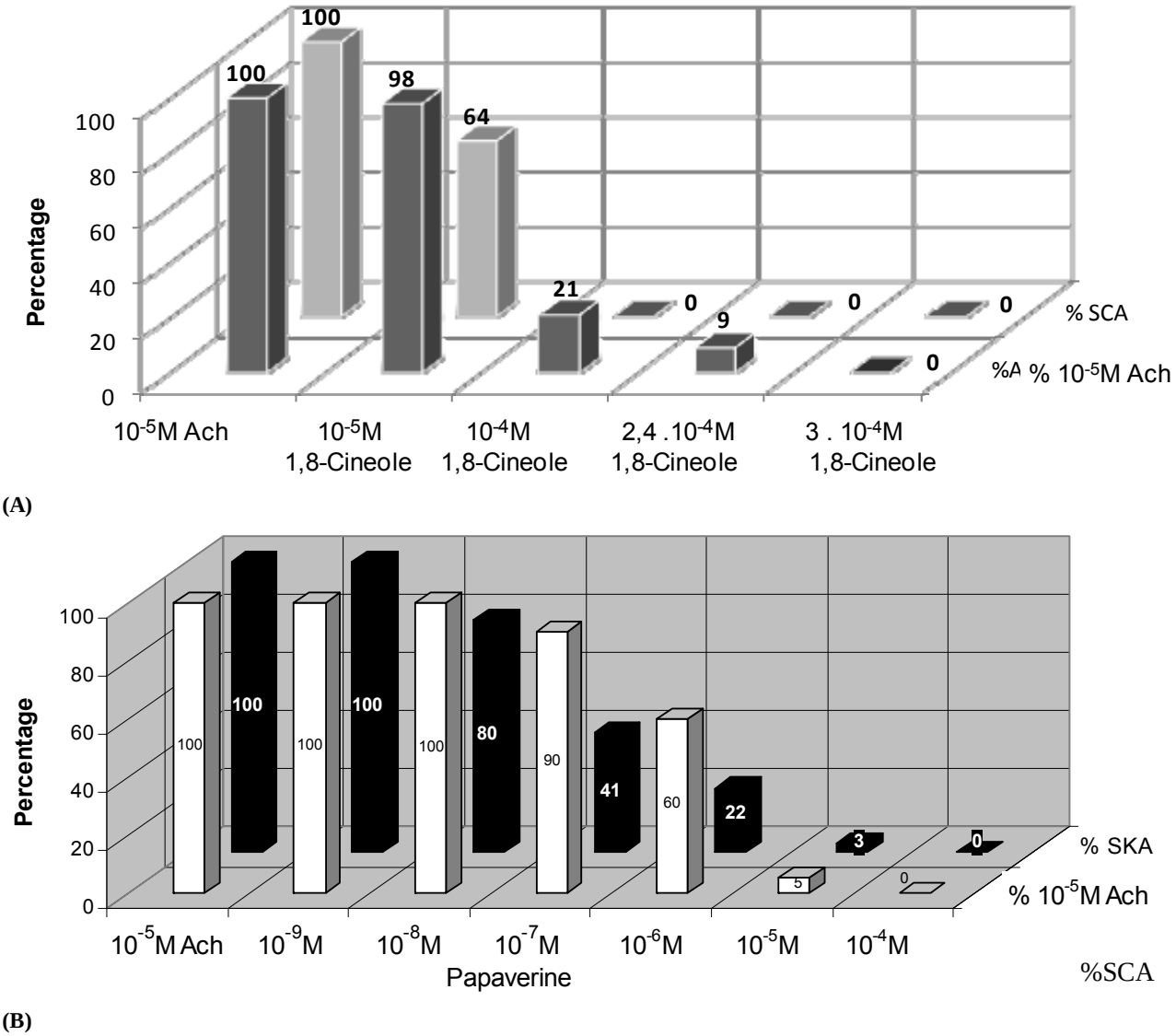

Figure 4. A comparison among effects of different concentrations of 1,8-Cineole (above) and Papaverine (below) on the spontaneous contractile activity (SCA) of the smooth muscle strips. (guinea pig stomach). Ach=Acetylcholine.

Table 1. Concentrations of 1,8-Cineole in the Krebs' solution (20 ml) in the organ bath.

№	Applicated volume of 1,8-Cineole in 20 ml Krebs' solution (µl)	Volume of 1,8-Cineole, dissolved in 20 ml Krebs' solution, rounded (µl)
1	0.005	0.005
2	0.050	0.050
3	0.100	0.100
4	0.500	0.455
5	1.000	0.495
6	3.000	0.735
7	5.000	1.056
8	10.000	1.257
9	50.000	5.277

asthma or chronic obstructive pulmonary disease (COPD) (Juergens et al., 2003; Worth et al., 2009; Worth and Dethlefsen, 2012).

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

Beauchamp J, Kirsch F, Buettner A (2010). Real-time breath gas analysis for pharmacokinetics: monitoring exhaled breath by on-line proton-transfer-reaction mass spectrometry after ingestion of eucalyptol-containing capsules. J. Breath Res. 4:26006.

Beer AM, Lukanov J, Sagorchev P (2007). Effect of Thymol on the spontaneous contractile activity of the smooth muscles. Phytomedicine 14:65-69.

Cabo J, Crespo ME, Jimenez J, Zarzuelo A (1986). The spasmolytic activity of various aromatic plants from the province of Granada. The activity of the major components of their essential oils. Plant Med. Phythother. 10(3):213-218.

Forster HB, Niklas H, Lutz S (1980). Antispasmodic effects of some medicinal plants. Planta Med. 40:309-319.

Gamez MJ, Jimenez J, Navarro C, Zarzuelo A (1990). Study of the essential oil of *Lavandula dentata* L. Pharmazie 45(1):69-70.

Golenhofen K (1976). Spontaneous activity and functional classification of mammalian smooth muscle. In: Bulbring E, Schuba MF, editors. Physiology of Smooth Muscle New York: Raven Press pp. 91-97.

Juergens UR (2014). Anti-inflammatory Properties of the Monoterpene 1.8-cineole: Current Evidence for Co-medication in Inflammatory Airway Diseases. Drug Res. 64:1-9.

Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H (2003). Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. Respir. Med. 97(3):250-256.

Nascimento NR, Refosco RM, Vasconcelos EC, Kerntopf MR, Santos CF, Batista FJ, De Sousa CM, Fonteles MC (2009). 1,8-Cineole induces relaxation in rat and guinea-pig airway smooth muscle. J. Pharm. Pharmacol. 61(3):361-366.

Sadjak A, Wintersteiger R, Zakel D, Wenzl T, Lankmayr EP, Gferer E, Likar R (2005). Periperal analgesic effect of intra-articularly applied clonidine. Schmerz. 20(4):293-4, 296-9.

Sagorchev P, Lukanov J, Beer AM (2010). Investigations into the specific effects of rosemary oil at the receptor level. Phytomedicine 17:693-697.

Schilcher H, Kammerer S, Wegener T (2007). Leitfaden Phytotherapie. 4. Auflage, München: Elsevier, Urban & Fischer.

Wagner H, Wierer M, Bauer R (1986). In vitro-Hemmung der Prostaglandin-Biosynthese durch ätherische Öle und phenolische Verbindungen. Planta Med. 52:184-187.

Worth H, Dethlefsen U (2012). Patients with Asthma Benefit from Concomitant Therapy with Cineole: A Placebo-Controlled, Double-Blind Trial. J. Asthma 49(8):849-853.

Worth H, Schacher C, Dethlefsen U (2009). Concomitant therapy with Cineole (Eucalyptole) reduces exacerbations in chronic obstructive pulmonary disease (COPD): A placebo-controlled double-blind trial. Respir. Res. 10:69.