Mode of action of peppermint oil and (-)-menthol with respect to 5-HT3 receptor subtypes: binding studies, cation uptake by receptor channels and contraction of isolated rat ileum

Eugen J Verspohl

Mode of action of peppermint oil and (-)-menthol with respect to 5-HT3 receptor subtypes: binding studies, cation uptake by receptor channels and contraction of isolated rat ileum

Journal:	*Phytotherapy Research*
Manuscript ID:	PTR-09-0443.R1
Wiley - Manuscript type:	Full Paper
Date Submitted by the Author:	24-Aug-2010
Complete List of Authors:	Verspohl, Eugen; Institute of Pharmaceutical and Medicinal Chemistry, Pharmacology
Keyword:	Peppermint oil, (-)-Menthol, 5-HT3 receptor

Mode of action of peppermint oil and (-)-menthol with respect to 5-HT_3 receptor subtypes: binding studies, cation uptake by receptor channels and contraction of isolated rat ileum

Heimes, Katharina, Hauk, Florian, Verspohl, Eugen J.

[1]Department of Pharmacology, Institute of Pharmaceutical and Medicinal Chemistry, Hittorfstr. 58-62, 48149 Münster, Germany

Address for correspondence:

Dr. E.J. Verspohl
Dept. Pharmacology (Institute of Pharmaceutical and Medicinal Chemistry)
Hittorfstr. 58-62
48149 Münster, Germany
*49/(0)251/83-33339

ABSTRACT

Peppermint oil (*Mentha x piperita* L. (*Lamiaceae*)) has been shown to exert potent anti-emetic properties, but its mode of action has not yet been elucidated. Among its active constituents (-)-menthol is most important. Three different *in vitro* models were used to investigate the effects on 5-HT_3 receptors (serotonin receptor subtype): [^{14}C]guanidinium influx into N1E-115 cells which express 5-HT_3 receptors, isotonic contractions of the isolated rat ileum and equilibrium competition binding studies using a radioactively labelled 5-HT_3 receptor antagonist ([^{3}H]GR65630) (3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone). Both peppermint oil and (-)-menthol inhibited [^{14}C]guanidinium influx through 5-HT_3 receptor channels as well as contractions of the ileum induced by serotonin. Neither the peppermint oil nor (-)-menthol, however, was able to displace [^{3}H]GR65630 from 5-HT_3 binding sites. It may be concluded that peppermint oil and (-)-menthol exert their anti-emetic effect at least partly by acting on the 5-HT_3 receptor ion-channel complex, probably by binding to a modulatory site distinct from the serotonin binding site.

Index words: Peppermint oil, (-)-menthol, 5-HT_3 receptor

1. INTRODUCTION

Peppermint (*Mentha x piperita* L. (Lamiaceae)) contains 0.5 – 4 % volatile oil with the main component (-)-menthol. The volatile oil should consist of 30 – 55 % (-)-menthol. Peppermint has numerous effects. A tea is used against acute and chronic gastritis, enteritis, disturbances of the GI-tract and gall bladder. An antispasmodic effect of an ethanolic peppermint extract was observed: contractions of guinea pig ileum induced by acetylcholine and histamine were inhibited (Forster et al., 1980). The mechanism of action of menthol was investigated (Della Loggia et al., 1990). For the antispasmodic effect of menthol partly a Ca-channel modifying effect was observed (Grigoleit and Grigoleit 2005a).

(-)-Menthol, menthon and similar compounds stereoselectively modulate the ionotropic channels of $GABA_A$- und glycin receptors (Hall et al., 2004). Since it influences gastric motility, it is used against IBS (irritable bowel syndrome) (Grigoleit and Grigoleit 2005a). Acid-resistant preparations (e.g. Colpermin®) are marketed and daily doses of 3 times 0.2 – 0.4 mL peppermint oil are used. It is combined in many preparations for its effect as a carminative, cholagoge or sedative. It is marketed as a prokinetic, e.g. Carminativum-Hetterich®, which includes additional constituents.

Topical (-)-menthol induces a sensation of coldness by which itching (urticaria and pruritus) and pain are reduced. This effect is mediated by the activation of TRPM8 receptors (Peier et al., 2002). Topically applicated peppermint oil is also used against migraine and tension headache.

In the context of this paper most importantly peppermint tea is used as an antiemetic; the mechanism of this action is not fully understood. It is a remedy in pregnant women besides ginger and cannabis (Westfall 2004). Interestingly 5-HT_3-receptor antagonists, 5-HT_4 receptor agonists and anticholinergics are not showing a better effect than peppermint oil.

Recently the ability was shown for ginger extracts and some of its compounds to inhibit 5-HT_3 mediated [^{14}C]guanidinium influx into N1E-115 cells (Abdel-Aziz et al., 2005). Based on these results, the present work describes similar experiments to elucidate the possible mode of antiemetic action of peppermint oil and (-)-menthol with respect to 5-HT_3 receptors. Since 5-HT_3 receptors are also involved in the pathogenesis of IBS, a disease already mentioned above, the outcome could also help to understand the peppermint oil effectiveness in this

disease. It was therefore, aimed to investigate a possible interaction of peppermint oil and of (-)-menthol with the 5-HT_3-receptor channel system.

2. MATERIALS AND METHODS

2.1 Materials

The peppermint oil was from Caelo, Hilden (Germany) and (-)-mernthol from Fluka, Buchs (Switzerland). Serotonin creatinine sulphate monohydrate (5-HT) was from Fluka, Germany, veratridine and acetylcholine from Sigma-Aldrich, Steinheim (Germany). Tropisetron was a gift from Novartis, Basel (Switzerland). SR57227A ((4-amino)-(6-chloro-2-pyridyl)L-piperidine hydrochloride) was purchased from Tocris, UK, [^{14}C]guanidinium HCl from Biotrend, Cologne (Germany) (specific activity: 55 mCi/mmol) and [^{3}H]GR65630 (3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone) from Perkin-Elmer, Boston (USA) (specific activity: 85.5 mCi/mmol).

2.2 Preparation of Solutions

The peppermint oil and (-)-menthol were dissolved in DMSO (dimethylsulfoxide) and then diluted to the required concentration with the respective assay buffer. The final concentration of DMSO in any of the experiments was less than 0.3 %. In this concentration (and in a 10-fold higher one) DMSO did not show any effect on either test system used. Veratridine, which was used as a control agent to activate fast voltage gated Na^+ channels, was dissolved in ethanol and then diluted to the required concentration with assay buffer (final concentration of ethanol was 1 %). Ethanol was shown not to affect the veratridine-induced [^{14}C]guanidinium influx into N1E-115 cells (Barann et al., 1995), and, therefore, 1 % ethanol was used in the experiments with [^{14}C]guanidinium and veratridine. All other substances were dissolved directly in the respective assay buffer.

2.3 N1E-115 Cell Culture

Mouse neuroblastoma cells of the clone N1E-115 express 5-HT_3 receptors (Bönisch et al., 1993). Passage numbers 40-70 were grown in Dulbecco's modified Eagle's medium (DMEM) containing sodium pyruvate, glucose (1000 mg/L) and pyridoxine; the growth medium was supplemented with penicillin (100 I.U./mL), streptomycin (100 µg/mL) and 10 % foetal calf

serum (PAA, Colbe, Germany). Cells were cultured in a humified atmosphere containing 5 % CO_2 at 37 °C in cell culture flasks (Sarstedt, Nuembrecht, Germany) and were sub-cultured twice a week.

2.4 [^{14}C]Guanidinium Influx

Three days before starting equilibrium competition binding or [^{14}C]guanidinium influx experiments N1E-115 cells were sub-cultured in 24-well cell culture clusters (2.5 x 10^4 cells in 1000 µL/well) (Biochrom, Berlin, Germany). After removal of the growth medium, cells were washed and pre-incubated for 20 min with 37 °C incubation buffer (300 µL/well) containing (in mM): HEPES 25, Tris 25, KCl 5.4, $MgSO_4$ x 7 H_2O 0.98, D-glucose 5.5, choline chloride 135 and bovine serum albumin 1 mg/mL and the drugs to be tested for inhibition of [^{14}C]guanidinium influx. Pre-incubation was carried out in a humified atmosphere containing 5 % CO_2 at 37 °C. After pre-incubation, the cells were incubated for a further 2.5 min at room temperature with the same incubation buffer, which, in addition, contained 5 µM [^{14}C]guanidinium chloride, the drug to be tested, and 100 µM serotonin creatinine sulphate monohydrate (5-HT) or 300 µM veratridine. Incubation was terminated by removing the incubation buffer and rapidly washing the cells twice with ice-cold washing buffer of the following composition (mM): HEPES 25, Tris 25, KCl 5.4, $MgSO_4$ x 7 H_2O 0.98, D-glucose 5.5, NaCl 135. Thereafter, the cells were dissolved in 0.5 mL 0.1 % Triton X 100 and the [^{14}C]guanidinium content of the solution was determined by liquid scintillation counting using a Beckman ß-counter, LS 6000 LL (Beckman, Harbor, USA). All experiments were carried out in duplicate or triplicate. 100 µM Tropisetron was used as a positive control. From the counts obtained, the cpms of blanks were subtracted and the resulting data expressed as a percentage of the values obtained from serotonin - or veratridine-induced [^{14}C]guanidinium influx (100 µM or 300 µM, respectively).

2.5 Equilibrium Competition Binding Experiments using N1E-115 Cells

After removal of the growth medium, cells were washed and incubated with Krebs-Ringer-HEPES buffer containing 0.4 nM of the selective 5-HT_3 receptor antagonist [^{3}H]GR65630 and the drug to be tested in a total volume of 200 µL/well for 30 min at room temperature. Non-specific binding was determined using an excess of serotonin creatinine sulphate (10 mM). The incubation was terminated by sucking off the incubation buffer and rapidly washing the cells twice with ice-cold Krebs-Ringer-HEPES buffer. Thereafter, the cells were dissolved in 0.5 mL 0.1 % Triton X 100 and the [^{3}H]GR65630 content of the sample was determined by liquid scintillation counting using a ß-counter. All experiments were carried out in duplicate.

100 µM Tropisetron was used as positive control. All results were expressed as a percentage of maximum specific binding.

2.6 Studies using isolated rat ileum

Wistar rats of either sex, each weighing 240-350 g, were killed using ether anaesthesia. The ileum was excised as rapidly as possible and flushed with warm Krebs-Henseleit solution. Whole ileal segments, 3-4 cm in length, were suspended in a 10 mL organ bath under 1.0 g tension in carbogen-aerated Krebs-Henseleit solution, maintained at pH 7.4 and 37° C. The ileal segments were allowed to equilibrate for 30 to 60 min till a straight base line was obtained. Before testing the antagonistic effect on serotonin receptors mediated by the oil and compound under investigation, a concentration-response-curve with serotonin was obtained for each segment and a submaximal concentration evoking 50-75 % of the maximal response was chosen.

The extract or substance to be tested was then added to the organ bath and left for a contact time of 60 s, before repeating to add the chosen sub-maximal concentration of the agonist. The ileum was washed several times after each drug addition till it returned to its previous base line. The washing time was at least 20 min for serotonin. To abolish any effects resulting from muscle tiring, 1 µM acetylcholine was added twice at the beginning and the end of each experiment and the response was recorded for control. Experiments in which the average response at the end of the experiment was less than 90 % of the one at the beginning were discarded. Tropisetron was used as a positive control.

The effect of the different drugs was expressed as follows:

$$\frac{\text{response to the agonist after addition of the drug under investigation}}{\text{response to the agonist without previous addition of the drug under investigation.}} \times 100$$

3. RESULTS

3.1 [^{14}C]Guanidinium influx experiments

Experiments using the N1E-115 cell model showed that peppermint oil is able to completely inhibit 5-HT_3 receptor mediated [^{14}C]guanidinium influx into N1E-115 cells in a concentration-

dependent manner (Fig. 1). The same is obvious when (-)-menthol was used (Fig. 1). 100 µM Tropisetron served as a positive control.

In this figure the concentrations of (-)-menthol (lower x-axis) are shown as equivalents to the concentrations of volatile peppermint oil. The closer the concentration-response curve of (-)-menthol is located to the curve of peppermint oil, the more important would be the participation of (-)-menthol on the main effect of the volatile oil.

3.2 Veratridine experiments (with respect to validity of [^{14}C] guanidinium experiments)

To ensure that this 5-HT$_3$ receptor channel effect (Fig. 1) is not based on a non-selective ion channel blockade, the ability of peppermint oil and (-)-menthol to block the veratridine-induced [^{14}C]guanidinium influx into N1E-115 cells was tested and compared to its effect on the serotonin-induced [^{14}C]guanidinium influx. The peppermint oil at a concentration of 0.32 µL/mL decreases the veratridine-induced influx to the same extent as serotonin (Table 1). Its main compound (-)-menthol, however, at a concentration of 1 mM shows no inhibition of veratridine effect. At a concentration of 0.32 µL/mL peppermint oil consists of approx. 750 µM (-)-menthol. (-)-Menthol is not involved in the nonspecific ion channel blockade of peppermint oil which also holds for tropisetron (Table 1).

3.2 5-HT$_3$ receptor binding

Using equilibrium competition binding experiments, peppermint oil and (-)-menthol rather failed to displace the radioligand [^{3}H]GR65630 (a selective 5-HT$_3$ receptor antagonist; 3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone) from the serotonin binding site on 5-HT$_3$ receptors of N1E-115 cells (Fig. 2). Only at very high concentrations of 1 µL/mL peppermint oil was able to decrease the specific binding to 85.4 ± 4.8 % ($p = 0.0322$). This effect could be mediated by an interaction of a compound other than (-)-menthol in the peppermint oil or a general lipophilic effect of the volatile oil may be the reason.

3.3 Isotonic contractions of the rat ileum

Both peppermint oil and (-)-menthol were tested directly (curves starting at 0 %) and in the presence of 10 µM serotonin (curves starting at 100 %). In Fig. 3 both the concentrations of peppermint oil and of the equivalent concentration of (-)-menthol (both X-axis) are plotted

versus the contraction (Y-axis). Both peppermint oil and (-)-menthol relax the smooth muscle in a concentration-dependent manner; the maximum effect is approx. -40 % (lower part of Fig. 3). Both antagonize the serotonin-induced stimulation by approx. 80 % (upper part of Fig. 3).

4. DISCUSSION

Peppermint oil and (-)-menthol are used for various indications in which 5-HT_3 receptors may be involved. This possible mechanism via 5-HT_3 receptors was not clearly investigated yet.

4.1 [^{14}C]Guanidinium-Influx-Assay

Experiments using the [^{14}C]guanidinium influx assay show that both peppermint oil and (-)-menthol inhibit the serotonin-induced cation influx into N1E-115 cells in a concentration-dependent manner. The effect of peppermint oil at concentrations higher than 0.1 µL/mL is partly mediated (40-57 %) by (-)-menthol. Especially at low concentrations of 0.032 µL/mL volatile oil, (-)-menthol inhibits the cationic flux already by 19.8 % ± 6.5 %, whereas the oil itself does not posses an inhibitory effect at this concentration. The rightward shift of the (-)-menthol concentration-response curve can be interpreted in the way that in addition to menthol other constituents are involved in the inhibitory effect of the peppermint oil.

Peppermint oil, therefore, shows a 5-HT_3 receptor channel antagonistic effect which may positively influence the disturbed motility and visceral sensitivity during symptoms of IBS and emesis and may contribute besides other mechanisms to the overall effect of peppermint oil.

4. 2 Specificity of [^{14}C]guanidinium assay and validity of [^{14}C]guanidinium approach

It should be stressed that various compounds/extracts were already described to show a non-specific inhibition of 5-HT_3 receptors (e.g. steroids, local anaesthetics) (Barann et al., 1993 and 1999). In addition the N1E-115 cell line has been used extensively to also study other compounds including NMDA receptor antagonists, barbiturates, cannabinoids, chlorpromazine etc., which were able to inhibit 5-HT_3 receptor function, albeit by different mechanisms (competitive and non-competitive antagonism, desensitisation, allosteric modulation and through interaction with sigma-2-binding sites) (Molderings et al., 1996,

Barann et al., 1993 and 1999). Two possible mechanisms have been proposed by Barann et al. (1993) to explain this non-specific inhibition: 1. non-specific hydrophobic interaction with membrane lipids or hydrophobic regions of the receptor proteins, in which case the potency of the compounds is correlated with their lipophilicity and 2. nonspecific inhibition of ion channels in general. Since the N1E-115 cells, being neuronal cells, are also endowed with fast voltage-gated Na^+ channels, which can be activated by veratridine and which are permeable to [^{14}C]guanidinium ions as well, the same cell line can be used to investigate this type of (nonspecific) interaction by comparing the potency of compounds in inhibiting cation influx through both channels (5-HT_3 receptor channels and fast voltage-gated Na^+ channels).

To investigate whether or not the presented data are a result of a non-specific inhibitory effect on ion channels in general, the ability of peppermint oil and (-)-menthol to inhibit veratridine-induced [^{14}C]guanidinium influx into N1E-115 cells (mediated through fast voltage-dependent Na^+ channels) was compared to its inhibitory effect on serotonin-induced influx (mediated through 5-HT_3 receptor channels). The peppermint oil at a concentration of 0.32 µL/mL decreases the veratridine-induced influx to the same degree as the serotonin-induced cationic influx indicating a broad, nonselective effect on ion channels. Its main compound (-)-menthol, however, at a concentration of 1 mM showed no inhibition of the veratridine effect. At a concentration of 0.32 µL/mL peppermint oil contains approx. 750 µM (-)-menthol. (-)-Menthol, therefore, is not involved in the nonspecific ion channel blockade induced by peppermint oil. This could be interpreted in the way that additional compounds of peppermint oil contribute to the nonspecific blockade of ion channels and the 5-HT_3-receptor channel.

4.3. 5-HT_3 receptor binding site

The specific binding of the receptor antagonist [^{3}H]GR65630 as a radioligand is not influenced by (-)-menthol up to concentrations of 1 mM, albeit peppermint oil at an extremely high concentration of 1 µL/mL shows a small, significant inhibition of binding. Eventually other substances in peppermint oil interact with the binding site of the radioligand. It can be assumed that high oil concentrations may influence the binding of the radioligand by their high lipophilic effects.

Comparable to the volatile oil of ginger (Abdel-Aziz et al., 2006) it may be assumed that there exists a 5-HT_3 receptor channel antagonistic effect of peppermint oil by an allosteric modulating binding site. The presence of such modulatory binding sites seems to be typical for receptors belonging to the superfamily of ligand-gated ion channels like the 5-HT_3

receptor. Members of this family, which include the nicotinic acetylcholine receptor, the $GABA_A$ and the glycine receptor (Miller 2002), share many structural and functional similarities. They all exist as homo- or hetero-pentamers with each subunit consisting of an extracellular N-terminal domain which encodes the ligand-binding site and four transmembrane regions. They also possess several modulatory binding sites for $GABA_A$ receptors, benzodiazepines, barbiturates, ethanol, neurosteroids, some general anaesthetics (Mehta et al., 1999), and in extension for nicotinic acetylcholine receptors, substance P, serotonin, methysergide, spiperone and quinacrine (Arias, 1998), and for anandamide and other cannabinoid receptor agonists (Barann et al., 2002) as well as nimodipine (Hargreaves et al., 1996). This includes that those compounds inhibit 5-HT_3 receptor function without affecting the specific binding of [^{3}H]GR65630 to its binding site. The results obtained in the present work support the possible existence of modulatory binding sites on 5-HT_3 receptors.

For other ion channel receptors it was already demonstrated that (-)-menthol interacts in this way: Hall et al. (2004) have shown that (-)-menthol is a potent positively acting allosteric modulator on $GABA_A$- and glycin receptors. Additionally (-)-menthol has a local anesthetic (Galeotii et al., 2001) and an antinociceptive effect (Galeotti et al., 2002) which is presumably mediated according to Haeseler et al. (2002) via blocking voltage-dependent neuronal Na^+ channels. (-)-Menthol activates TRPM8 channels from the family of vanilloid receptors and induces via the subsequent cationic influx a sensation of coldness (Peier et al., 2002).

4.4 Antiserotoninergic effects of peppermint oil and (-)-menthol on rat ileum

The spasmolytic and antispasmodic effect of peppermint oil was established by various investigations: for review see Grigoleit and Grigoleit (2005b). To confirm the results obtained with the N1E-115 cell system, the isolated rat ileum was used as a second assay. This preparation contains neuronally located 5-HT_3 receptors. In our experiments both the spontaneous contraction and the serotonin-induced contraction were diminished. Most of the effect was mediated by (-)-menthol. The shift of the curve to the right can only be interpreted in the way that additional components of the oil contribute to the overall effect.

Hills et al. (1991) observed in contraction studies in the isolated taenia coli of the guinea-pig that serotonin-, histamine- and substance P-induced contractions are inhibited by peppermint oil in a non-competitive manner. Peppermint oil inhibits the serotonin-induced motoric answer much stronger than that of the other stimuli.

Our experiments enlarged the outcome with respect to (-)-menthol and the 5-HT_3-receptor. With respect to the results from [^{14}C]guanidinium-influx experiments also a 5-HT_3-receptor antagonistic effect could be involved in the effect of the volatile oil.

4.5. Biological relevance of the peppermint oil concentrations

In these experiments concentrations of peppermint oil of 0.01 and 1 µL/mL were used. In the IBS therapy the recommended dose is 3 times daily 0.2–0.4 mL peppermint oil. The highest dose would be 1.2 mL peppermint oil daily. Under the assumption of a distribution volume of 70 L/70 kg and a complete absorption of the compounds of the volatile oil, there will be theoretically a concentration of volatile oil of 0.017 µL/mL in the body volume which would be in the lower range of concentrations used in our experiments. Because of the acid-resistant formulation of peppermint preparations much higher concentrations may exist in the gastrointestinal tract than in plasma (body volume).

4.6 Summary and conclusion:

- Peppermint oil and its main constituent (-)-menthol inhibit the cationic influx through 5-HT_3 receptor channels in a concentration-dependent manner. (-)-Menthol is responsible for rather half of the effect of the volatile oil.
- Both do not compete with the radioligand [^{3}H]GR65630 for the 5-HT_3 binding site and, therefore, does not act directly.
- Peppermint oil shows nonspecific ion channel effect; (-)-menthol, however, does not.
- Both the volatile oil as well (-)-menthol reduce the serotonin-induced contraction of the rat ileum and posses a direct relaxant effect. The effect of the volatile oil is partly mediated by (-)-menthol; additionally there are other components involved in the overall effect of peppermint oil.
- Their action, therefore, may be mediated by binding to a modulatory site distinct from that of serotonin, an allosteric binding site not yet identified.
- This binding site, once exactly identified, might represent a valuable future target for the development of a new class of anti-emetics lacking the typical side effects caused by setrones due to their direct action on 5-HT_5 receptors.

Acknowledgement: We thank Novartis Pharma, Basel, Switzerland for supplying tropisetron.

REFERENCES

Abdel-Aziz, H., Nahrstedt, A., Petereit, F., Windeck, T., Verspohl, E.J., 2005. 5-HT_3 receptor blocking activity of arylalkanes isolated from the rhizome of Zingiber officinale. Planta Med. 71, 609-616.

Abdel-Aziz, H., Windeck, T., Ploch, M., Verspohl, E.J., 2006. Mode of action of gingerols and shogaols on 5-HT_3 receptors: Binding studies, cation uptake by the receptor channel and contraction of isolated guinea-pig ileum. Eur. J. Pharmacology 530, 136-143.

Arias, H.R., 1998. Noncompetitive inhibition of nicotinic acetylcholine receptors by endogenous molecules. J. Neurosi. Res. 52, 369-379.

Barann, M., Göthert, M., Bruss, M., Bönisch, H., 1999. Inhibition of by steroids of [14C]-guanidinium flux through the voltage-gated sodium channel and the cation channel of the 5-HT3 receptor of N1E-115 neuroblastoma cells. Naunyn Schmiedebergs Arch. Pharmacol. 360, 234-241.

Barann, M., Göthert, M., Fink, K., Bönisch, H., 1993. Inhibition by anaesthetics of 14C-guanidinium flux through the voltage-gated sodium channel and the cation channel of the 5-HT_3 receptor of N1E-115 neuroblastoma cells. N.-S. Arch. Pharmacol, 347, 125-132.

Barann, M., Molderings, G., Bruss, M., Bönisch, H., Urban, B.W., Göthert, M., 2002. Direct inhibition by cannabinoids of human 5-HT_3A receptors: probable involvement of an allosteric modulatory site. Br. J. Pharmacol. 137, 589-596.

Barann, M., Ruppert, K., Gothert, M., Bönisch, H., 1995. Increasing effect of ethanol on 5-HT_3 receptor-mediated 14C-guanidinium influx in N1E-115 neuroblastoma cells. Naunyn Schmiedebergs Arch. Pharmacol. 352, 149-156.

Bönisch, H., Barann, M., Graupner, J., Göthert, M.,1993. Characterization of 5-HT_3 receptors of N1E-115 neuroblastoma cells by use of the influx of the organic cation [14C]-guanidinium. Br. J. Pharmacol., 108, 436-442.

Cappello, G., Spezzaferro, M., Grossi, L., Manzoli, L., Marzio, L. Peppermint oil (Mintoil) in the treatment of irritable bowel syndrome: a prospective double blind placebo-controlled randomized trial. Dig Liver Dis, 2007. 39(6): 530-536.

Della Loggia, R., Tubaro, A and Lunder, T.L., 1990. Evaluation of some pharmacological activities of a peppermint extract. Fitoterapia, 61, 215-221.

Diemunsch, P., Grelot, L., 2000. Potential of substance P antagonists as antiemetics. Drugs 60, 533-546.

Farber, L., Haus, U., Spath, M., Drechsler, S., 2004. Physiology and pathophysiology of the 5-HT_3 receptor. Scand. J. Rheumatol. (Suppl.), 2-8.

Forster, H.B., Niklas, H and S. Lutz, Antispasmodic Effects of Some Medicinal Plants. Planta Med, 1980. 40: 309-319.

Galeotti, N., Ghelardini, C., Mannelli, L., Mazzanti, G., Baghiroli, L., Bartolini, A., 2001. Local anaesthetic activity of (+)- and (-)-menthol. Planta Med. 67**,** 174-176.

Galeotti, N., Di Cesare Manelli, L., Mozzanti, G., Bartolini, a., Ghelardini, C., 2002. Menthol: a natural analgesic compound. Neurosci Lett. 322**,** 145-148.

Grigoleit, H.G. and P. Grigoleit, 2005a. Peppermint oil in irritable bowel syndrome. Phytomedicine 12, 601-606.

Grigoleit, H.G. and P. Grigoleit, 2005b. Pharmacology and preclinical pharmacokinetics of peppermint oil. Phytomedicine 12, 612-616.

Haeseler, G., Maue, D., Grosskretz, J., Bufler, J., Nentwig, B., Piepenbrock, S., Dengler, R., Leuwer, M., 2002. Voltage-dependent block of neuronal and skeletal muscle sodium channels by thymol and menthol. Eur. J. Anaesthesiol. 19**,** 571-579.

Halls, A.C., Turcotte, C.M., Betts, B.A., Yeung, W.Y., Agyeman, A.S., Burk, L.A., 2004. Modulation of human GABAA and glycine receptor currents by menthol and related monoterpenoids. Eur. J. Pharmacol. 506, 9-16.

Hargreaves, A.C., Gunthorpe, M.J., Taylor, C.W., Lummis, S.C.l., 1996. Direct inhibition of 5-hydroxytryptamine3 receptors by antagonists of L-type Ca2+ channels. Mol. Pharmacol. 50, 1284-1294.

Hawthorn, M., et al., 1988. The actions of peppermint oil and menthol on calcium channel dependent processes in intestinal, neuronal and cardiac preparations. Aliment Pharmacol. Ther. 2, 101-118.

Hills, J.M. and P.I. Aaronson, 1991. The mechanism of action of peppermint oil on gastrointestinal smooth muscle. An analysis using patch clamp electrophysiology and isolated tissue pharmacology in rabbit and guinea pig. Gastroenterology 101, 55-65.

Huang, Q.R., Iwamoto, M., Aoki, S., Tanaka, N., Tajima, K., Yamahara, J., Takaishi, Y., Yoshida, M., Tomimatsu, T., Tamai, Y., 1991. Anti-5-hydroxytryptamine3 effect of galanolactone, diterpenoid isolated from ginger. Chem. Pharm. Bull. (Tokyo), 39, 397-399.

Kawai, T., Kinoshita, K., Koyama, K., Takahashi, K, 1994. Anti-emetic principles of *Magnolia obovata* bark and *Zingiber officinale* rhizome. Planta Med. 60,17-20.

Liu, J.H., Chen, G.H., Yeh, H.Z., Huang, C.K., Poon, S.K., 1997. Enteric-coated peppermint-oil capsules in the treatment of irritable bowel syndrome: a prospective, randomized trial. J. Gastroenterol. 32, 765-768.

Liu, L., Simon, S.A., 1996. Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells. J. Neurophys. 76, 1858-1862.

Mehta, A.K., Ticku, M.K., 1999. An update on GABA A receptors. Brain Res. Rev. 29, 196-217.

Miller, K.W., 2002. The nature of sites of general anaesthetic action. Br. J. Pharmacol. 137, 589-596.

Molderings, G.I., Schmidt, K., Bönisch, H., Göthert, M., 1996. Inhibition of 5-HT_3 receptor function by imidazolines in mouse neuroblastoma cells: potential involvement of sigma 2 binding sites. N.S. Arch. Pharmacol. 354, 245-252.

Nagy, I., Sántha, P., Jancsá, G., Urbán, L., 2004. The role of vanilloid (capsaicin) receptor (TRPV!) in physiology and pathology. Eur. J. Pharmacol. 500, 351-369.

Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., Earley, T.J., Dragoni, I., McIntyre, P., Bevan, S., Patapoutian, A., 2002. A TRP channel that senses cold stimuli and menthol. Cell 108, 705-715.

Ramirez, M.J., Cenarruzabeitia, E., Del Rio, J., Lasheras, B., 1994. Involvement of neurokinins in the non-cholinergic response to activation of 5-HT_3 and 5-HT_4 receptors in guinea-pig ileum. Br. J. Pharmacol. 111, 419-424.

Sharma, S.S., Kochupillai, V., Gupta, S.K., Seth, S.D., Gupta, Y.K., 1997. Antiemetic efficacy of ginger (Zingiber officinale) against cisplatin-induced emesis in dogs. J. Ethnopharmacol. 57, 93-96.

Tate S. Peppermint oil: a treatment for postoperative nausea. J Adv Nurs 1997. 26: 543-549.

Westfall RE. Use of anti-emetic herbs in pregnancy: women`s choices, and the question of safety and efficacy. Complement Ther Nurs Midwifery 2004; 10: 30-36.

Yamahara, J., Huang, Q.R., Iwamoto, U., Kobayashi, G., Matsuda, H., Fujimura, H., 1989b. Active components of ginger exhibiting antiserotonergic action. Phytother. Res. 3, 70-71.

Yamahara, J., Rong, H.Q., Naitoh, Y., Kitani, T., Fujimura, H., 1989a. Inhibition of cytotoxic drug-induced vomiting in suncus by a ginger constituent. J. Ethnopharmacol. 27, 353-355.

Yang, Y., Kinoshita, K., Koyama, K., Takahashi, K., Kondo, S., Watanabe, K., 2002. Structure-antiemetic activity of some diarylheptanoids and their analogues. Phytomedicine 9, 146-152.

Table 1: The inhibitory effect of peppermint oil and (-)-menthol on either veratridine- or serotonin-induced [^{14}C]guanidinium influx into N1E-115 cells. Methodological approach is as described for Fig. 1.

Parameter	Blank	Veratridine (300 µM)/ Serotonin (100 µM)	Peppermint oil (0.32 µL/ml)	(-)-Menthol (1 mM)	Tropisetron (100 µM)
Veratridine-induced Influx [%]	0	100	20.60 ± 7.59 (n = 5)	111.6 ± 13.76 (n = 6)	94.46 ± 23.71 (n = 7)
Serotonin-induced Influx [%]	0	100	21.23 ± 7.78 (n = 5)	35.74 ± 4.24 (n = 4)	0.83 ± 2.49 (n = 15)

Fig. 1: Concentration response curves for the effect of peppermint oil and (-)-menthol and tropisetron on serotonin-induced [^{14}C]guanidinium influx into N1E-115 cells. Cells were incubated for 2.5 min at room temperature with 100 µM serotonin in the presence of various concentrations of indicated oil or compound or 100 µM tropisetron (as control). Prior to stimulation with serotonin, cells were preincubated with the same concentration of oil or (-)-menthol for 20 min. Each point of the curves represents the mean value + S.E.M. from 4-7 independent experiments expressed as percentage of maximum [^{14}C]guanidinium influx. The scaling of (-)-menthol is an equivalent to the peppermint oil concentrations.

Fig. 2: Displacement of [^{3}H]GR65630 from 5-HT$_3$ receptors on N1E-115 cells by different concentrations of peppermint oil and (-)-menthol. Cells were incubated for 30 min at room temperature with different concentrations of indicated oil or (-)-menthol or 100 µM tropisetron in the presence of 0.4 nM [^{3}H]GR65630. Each point of the curves represents the mean value ± S.E.M. of specific binding from 4-5 independent experiments expressed as percentage of maximum [^{3}H]GR65630 binding. The scaling of (-)-menthol is an equivalent to the peppermint oil concentrations.

Fig. 3: Effect of different concentrations of peppermint oil and (-)-menthol on the isotonic contractions of the isolated rat ileum to serotonin. The compounds were left to act for 60 s before measuring the response to 10 µM serotonin. Each point of the curves represents the mean value ± S.E.M. from 3-7 experiments expressed as a percentage of the response to 10 µM SR57227A.

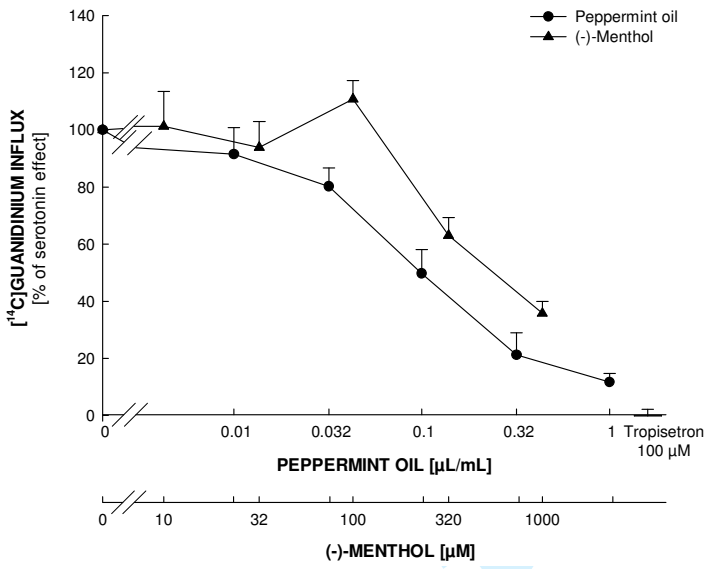

Peppermint oil
(-)-Menthol
[14C]GUANIDINIUM INFLUX
[% of serotonin effect]
140
120
100
80
60
40
20
0
0
0.01
0.032
0.1
0.32
1
Tropisetron
100 µM
PEPPERMINT OIL [µL/mL]
0
10
32
100
320
1000
(-)-MENTHOL [µM]

Fig. 1

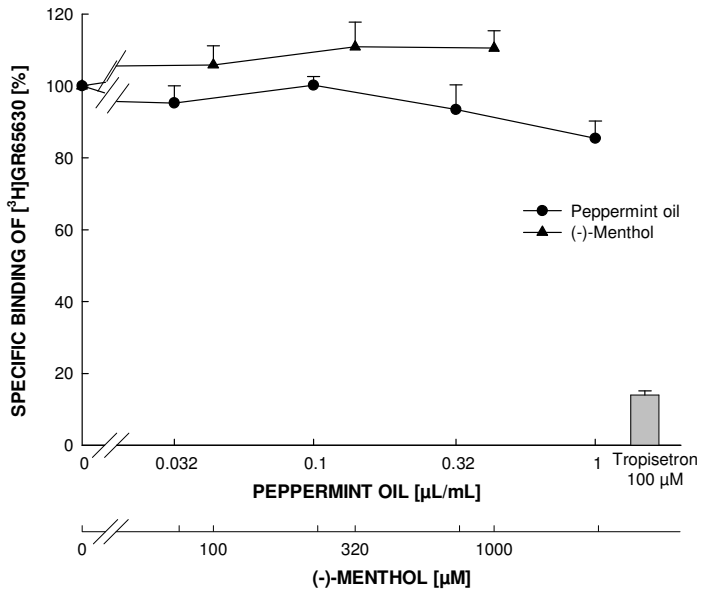

SPECIFIC BINDING OF [3H]GR65630 [%]
120
100
80
60
40
20
0
Peppermint oil
(-)-Menthol
0
0.032
0.1
0.32
1
Tropisetron
100 µM
PEPPERMINT OIL [µL/mL]
0
100
320
1000
(-)-MENTHOL [µM]

Fig. 2:

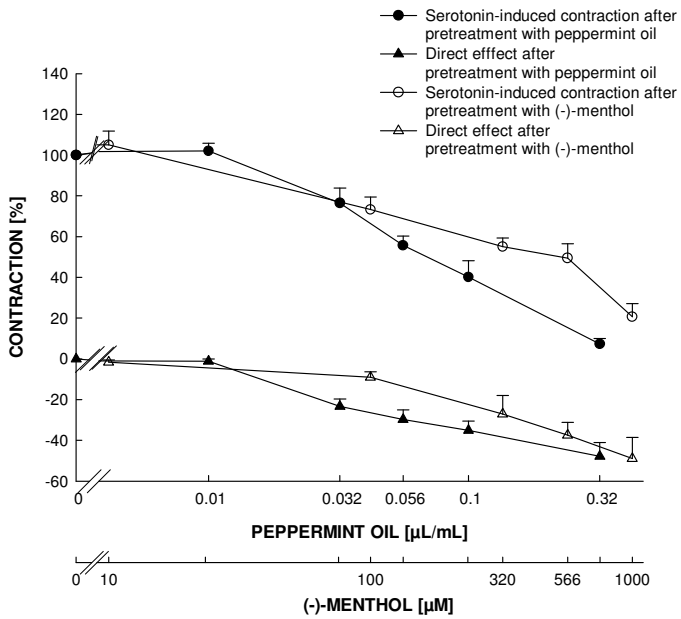
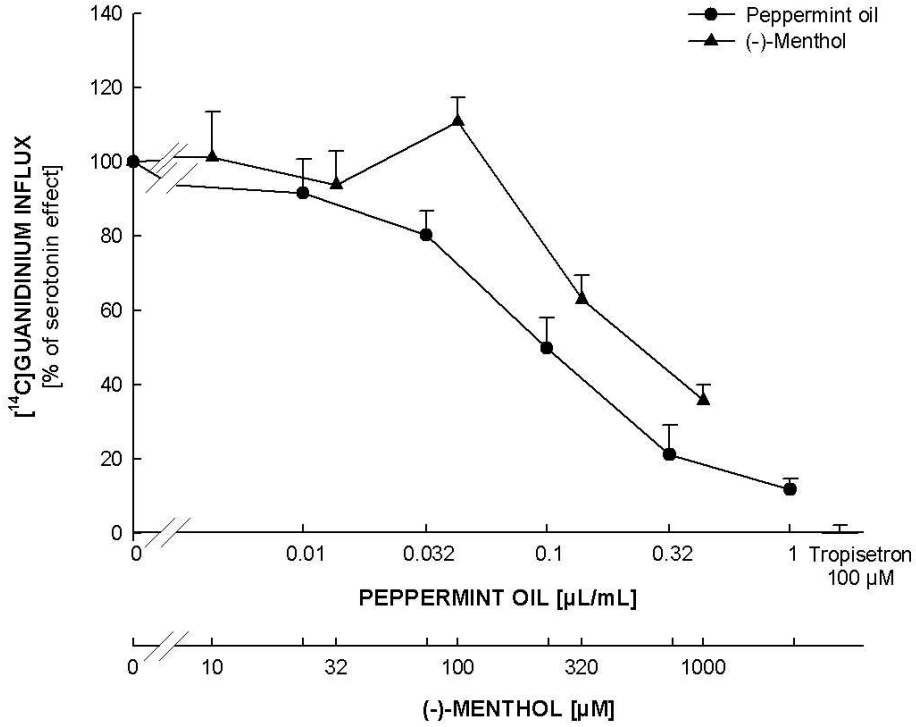
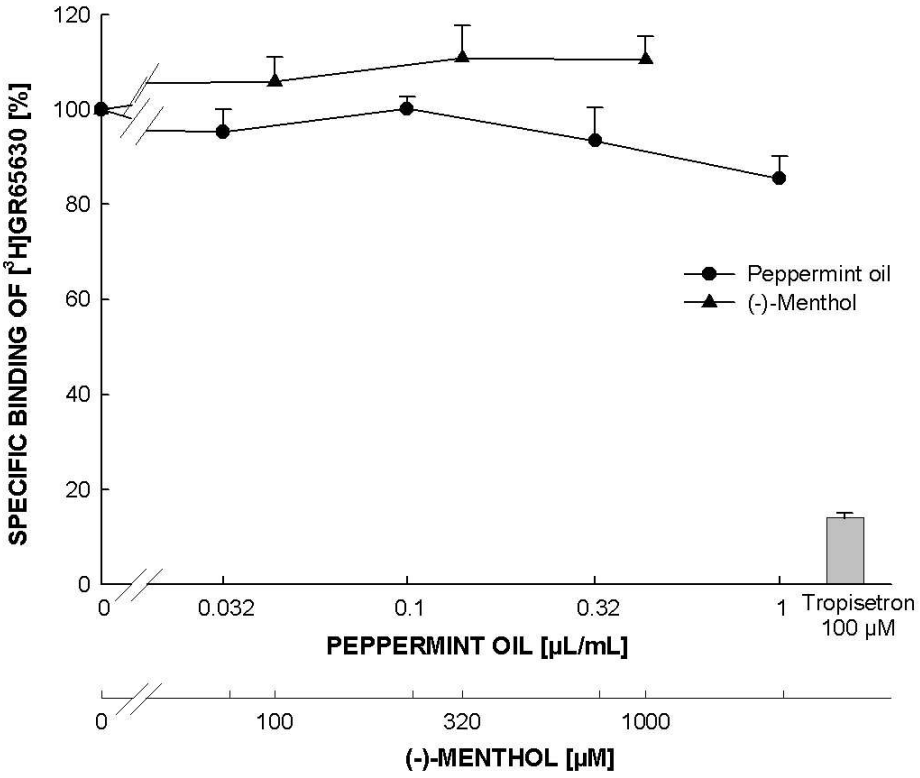


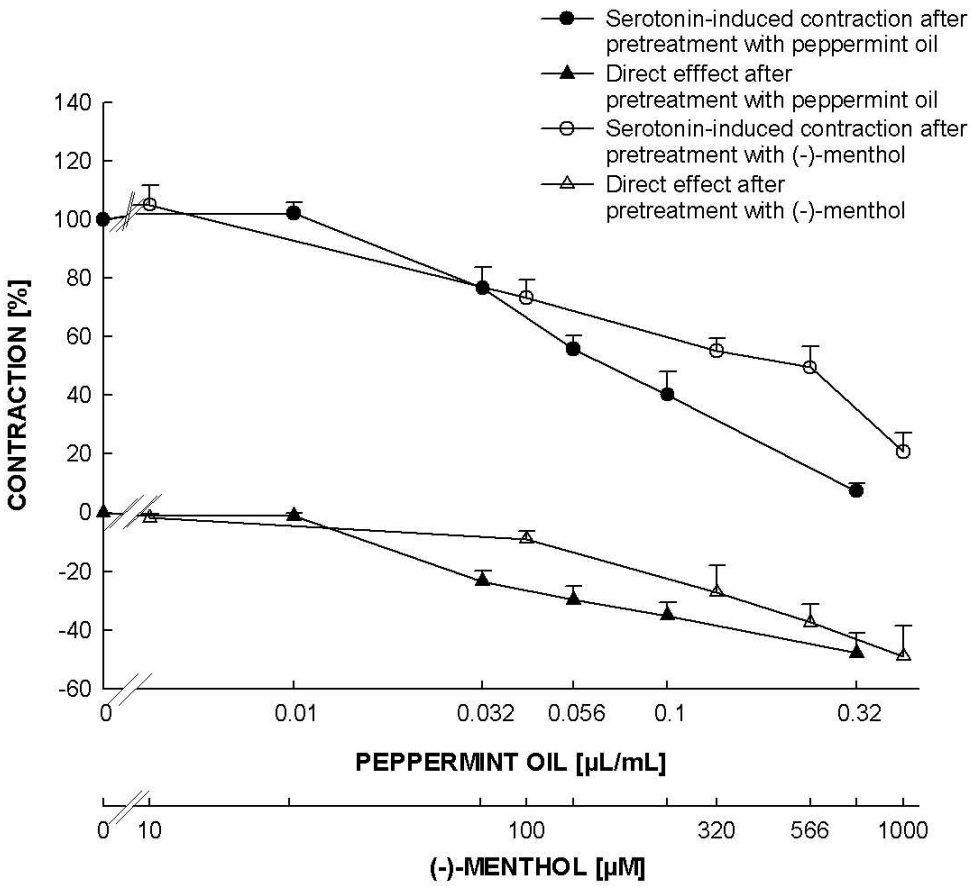
Fig. 3:



189x163mm (150 x 150 DPI)



187x173mm (150 x 150 DPI)



187x184mm (150 x 150 DPI)