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Eugen J Verspohl

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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

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7 **Mode of action of peppermint oil and (-)-menthol with respect to 5-HT₃**
8 **receptor subtypes: binding studies, cation uptake by**
9 **receptor channels and contraction of isolated rat ileum**
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15 Heimes, Katharina, Hauk, Florian, Verspohl, Eugen J.
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18
19 ¹Department of Pharmacology, Institute of Pharmaceutical and Medicinal Chemistry,
20 Hittorfstr. 58-62, 48149 Münster, Germany
21
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28
29
30
31
32
33
34

35 Address for correspondence:
36
37

38 Dr. E.J. Verspohl
39

40 Dept. Pharmacology (Institute of Pharmaceutical and Medicinal Chemistry)
41

42 Hittorfstr. 58-62
43

44 48149 Münster, Germany
45

46 *49/(0)251/83-33339
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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-HT₃ receptors (serotonin receptor subtype): [¹⁴C]guanidinium influx into N1E-115 cells which express 5-HT₃ receptors, isotonic contractions of the isolated rat ileum and equilibrium competition binding studies using a radioactively labelled 5-HT₃ receptor antagonist ([³H]GR65630) (3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone). Both peppermint oil and (-)-menthol inhibited [¹⁴C]guanidinium influx through 5-HT₃ receptor channels as well as contractions of the ileum induced by serotonin. Neither the peppermint oil nor (-)-menthol, however, was able to displace [³H]GR65630 from 5-HT₃ binding sites. It may be concluded that peppermint oil and (-)-menthol exert their anti-emetic effect at least partly by acting on the 5-HT₃ receptor ion-channel complex, probably by binding to a modulatory site distinct from the serotonin binding site.

Index words: Peppermint oil, (-)-menthol, 5-HT₃ 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, cholagogue 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 applied 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-HT₃-receptor antagonists, 5-HT₄ 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-HT₃ mediated [¹⁴C]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-HT₃ receptors. Since 5-HT₃ 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-HT₃-receptor channel system.

2. MATERIALS AND METHODS

2.1 Materials

The peppermint oil was from Caelo, Hilden (Germany) and (-)-menthol 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, [¹⁴C]guanidinium HCl from Biotrend, Cologne (Germany) (specific activity: 55 mCi/mmol) and [³H]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 [¹⁴C]guanidinium influx into N1E-115 cells (Barann et al., 1995), and, therefore, 1 % ethanol was used in the experiments with [¹⁴C]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-HT₃ 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

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3 serum (PAA, Colbe, Germany). Cells were cultured in a humidified atmosphere containing 5 %
4 CO₂ at 37 °C in cell culture flasks (Sarstedt, Nuembrecht, Germany) and were sub-cultured
5 twice a week.
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10 2.4 [¹⁴C]Guanidinium Influx

12 Three days before starting equilibrium competition binding or [¹⁴C]guanidinium influx
13 experiments N1E-115 cells were sub-cultured in 24-well cell culture clusters (2.5 x 10⁴ cells
14 in 1000 µL/well) (Biochrom, Berlin, Germany). After removal of the growth medium, cells
15 were washed and pre-incubated for 20 min with 37 °C incubation buffer (300 µL/well)
16 containing (in mM): HEPES 25, Tris 25, KCl 5.4, MgSO₄ x 7 H₂O 0.98, D-glucose 5.5, choline
17 chloride 135 and bovine serum albumin 1 mg/mL and the drugs to be tested for inhibition of
18 [¹⁴C]guanidinium influx. Pre-incubation was carried out in a humidified atmosphere containing 5
19 % CO₂ at 37 °C. After pre-incubation, the cells were incubated for a further 2.5 min at room
20 temperature with the same incubation buffer, which, in addition, contained 5 µM
21 [¹⁴C]guanidinium chloride, the drug to be tested, and 100 µM serotonin creatinine sulphate
22 monohydrate (5-HT) or 300 µM veratridine. Incubation was terminated by removing the
23 incubation buffer and rapidly washing the cells twice with ice-cold washing buffer of the
24 following composition (mM): HEPES 25, Tris 25, KCl 5.4, MgSO₄ x 7 H₂O 0.98, D-glucose
25 5.5, NaCl 135. Thereafter, the cells were dissolved in 0.5 mL 0.1 % Triton X 100 and the
26 [¹⁴C]guanidinium content of the solution was determined by liquid scintillation counting using
27 a Beckman β-counter, LS 6000 LL (Beckman, Harbor, USA). All experiments were carried
28 out in duplicate or triplicate. 100 µM Tropicsetron was used as a positive control. From the
29 counts obtained, the cpms of blanks were subtracted and the resulting data expressed as a
30 percentage of the values obtained from serotonin - or veratridine-induced [¹⁴C]guanidinium
31 influx (100 µM or 300 µM, respectively).
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47 2.5 Equilibrium Competition Binding Experiments using N1E-115 Cells

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50 After removal of the growth medium, cells were washed and incubated with Krebs-Ringer-
51 HEPES buffer containing 0.4 nM of the selective 5-HT₃ receptor antagonist [³H]GR65630 and
52 the drug to be tested in a total volume of 200 µL/well for 30 min at room temperature. Non-
53 specific binding was determined using an excess of serotonin creatinine sulphate (10 mM).
54 The incubation was terminated by sucking off the incubation buffer and rapidly washing the
55 cells twice with ice-cold Krebs-Ringer-HEPES buffer. Thereafter, the cells were dissolved in
56 0.5 mL 0.1 % Triton X 100 and the [³H]GR65630 content of the sample was determined by
57 liquid scintillation counting using a β-counter. All experiments were carried out in duplicate.
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3 100 μ M Tropisetron was used as positive control. All results were expressed as a percentage
4 of maximum specific binding.
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7 8 2.6 Studies using isolated rat ileum 9

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11 Wistar rats of either sex, each weighing 240-350 g, were killed using ether anaesthesia. The
12 ileum was excised as rapidly as possible and flushed with warm Krebs-Henseleit solution.
13 Whole ileal segments, 3-4 cm in length, were suspended in a 10 mL organ bath under 1.0 g
14 tension in carbogen-aerated Krebs-Henseleit solution, maintained at pH 7.4 and 37° C. The
15 ileal segments were allowed to equilibrate for 30 to 60 min till a straight base line was
16 obtained. Before testing the antagonistic effect on serotonin receptors mediated by the oil
17 and compound under investigation, a concentration-response-curve with serotonin was
18 obtained for each segment and a submaximal concentration evoking 50-75 % of the maximal
19 response was chosen.
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28 The extract or substance to be tested was then added to the organ bath and left for a contact
29 time of 60 s, before repeating to add the chosen sub-maximal concentration of the agonist.
30 The ileum was washed several times after each drug addition till it returned to its previous
31 base line. The washing time was at least 20 min for serotonin. To abolish any effects
32 resulting from muscle tiring, 1 μ M acetylcholine was added twice at the beginning and the
33 end of each experiment and the response was recorded for control. Experiments in which the
34 average response at the end of the experiment was less than 90 % of the one at the
35 beginning were discarded. Tropisetron was used as a positive control.
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42 The effect of the different drugs was expressed as follows:

$$43 \frac{\text{response to the agonist after addition of the drug under investigation} \times 100}{44 \text{response to the agonist without previous addition of the drug under investigation.}} \\ 45 \\ 46 \\ 47 \\ 48 \\ 49 \\ 50$$

51 52 3. RESULTS 53

54 55 3.1 [¹⁴C]Guanidinium influx experiments 56 57

58 Experiments using the N1E-115 cell model showed that peppermint oil is able to completely
59 inhibit 5-HT₃ receptor mediated [¹⁴C]guanidinium influx into N1E-115 cells in a concentration-
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3 dependent manner (Fig. 1). The same is obvious when (-)-menthol was used (Fig. 1). 100
4 μM Tropisetron served as a positive control.
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8 In this figure the concentrations of (-)-menthol (lower x-axis) are shown as equivalents to the
9 concentrations of volatile peppermint oil. The closer the concentration-response curve of (-)-
10 menthol is located to the curve of peppermint oil, the more important would be the
11 participation of (-)-menthol on the main effect of the volatile oil.
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17 18 3.2 Veratridine experiments (with respect to validity of [^{14}C] guanidinium experiments)

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21 To ensure that this 5-HT₃ receptor channel effect (Fig. 1) is not based on a non-selective ion
22 channel blockade, the ability of peppermint oil and (-)-menthol to block the veratridine-
23 induced [^{14}C]guanidinium influx into N1E-115 cells was tested and compared to its effect on
24 the serotonin-induced [^{14}C]guanidinium influx. The peppermint oil at a concentration of
25 0.32 $\mu\text{L}/\text{mL}$ decreases the veratridine-induced influx to the same extent as serotonin (Table
26 1). Its main compound (-)-menthol, however, at a concentration of 1 mM shows no inhibition
27 of veratridine effect. At a concentration of 0.32 $\mu\text{L}/\text{mL}$ peppermint oil consists of approx.
28 750 μM (-)-menthol. (-)-Menthol is not involved in the nonspecific ion channel blockade of
29 peppermint oil which also holds for tropisetron (Table 1).
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38 3.2 5-HT₃ receptor binding

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41 Using equilibrium competition binding experiments, peppermint oil and (-)-menthol rather
42 failed to displace the radioligand [^3H]GR65630 (a selective 5-HT₃ receptor antagonist; 3-(5-
43 methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone) from the serotonin binding
44 site on 5-HT₃ receptors of N1E-115 cells (Fig. 2). Only at very high concentrations of 1 $\mu\text{L}/\text{mL}$
45 peppermint oil was able to decrease the specific binding to $85.4 \pm 4.8\%$ ($p = 0.0322$). This
46 effect could be mediated by an interaction of a compound other than (-)-menthol in the
47 peppermint oil or a general lipophilic effect of the volatile oil may be the reason.
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56 3.3 Isotonic contractions of the rat ileum

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59 Both peppermint oil and (-)-menthol were tested directly (curves starting at 0 %) and in the
60 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

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3 versus the contraction (Y-axis). Both peppermint oil and (-)-menthol relax the smooth muscle
4 in a concentration-dependent manner; the maximum effect is approx. -40 % (lower part of
5 Fig. 3). Both antagonize the serotonin-induced stimulation by approx. 80 % (upper part of
6 Fig. 3).
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10 11 12 13 **4. DISCUSSION**

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16 Peppermint oil and (-)-menthol are used for various indications in which 5-HT₃ receptors may
17 be involved. This possible mechanism via 5-HT₃ receptors was not clearly investigated yet.
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20 21 22 23 4.1 [¹⁴C]Guanidinium-Influx-Assay

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26 Experiments using the [¹⁴C]guanidinium influx assay show that both peppermint oil and (-)-
27 menthol inhibit the serotonin-induced cation influx into N1E-115 cells in a concentration-
28 dependent manner. The effect of peppermint oil at concentrations higher than 0.1 µL/mL is
29 partly mediated (40-57 %) by (-)-menthol. Especially at low concentrations of 0.032 µL/mL
30 volatile oil, (-)-menthol inhibits the cationic flux already by 19.8 % ± 6.5 %, whereas the oil
31 itself does not possess an inhibitory effect at this concentration. The rightward shift of the (-)-
32 menthol concentration-response curve can be interpreted in the way that in addition to
33 menthol other constituents are involved in the inhibitory effect of the peppermint oil.
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41 Peppermint oil, therefore, shows a 5-HT₃ receptor channel antagonistic effect which may
42 positively influence the disturbed motility and visceral sensitivity during symptoms of IBS and
43 emesis and may contribute besides other mechanisms to the overall effect of peppermint oil.
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49 50 4. 2 Specificity of [¹⁴C]guanidinium assay and validity of [¹⁴C]guanidinium approach

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53 It should be stressed that various compounds/extracts were already described to show a
54 non-specific inhibition of 5-HT₃ receptors (e.g. steroids, local anaesthetics) (Barann et al.,
55 1993 and 1999). In addition the N1E-115 cell line has been used extensively to also study
56 other compounds including NMDA receptor antagonists, barbiturates, cannabinoids,
57 chlorpromazine etc., which were able to inhibit 5-HT₃ receptor function, albeit by different
58 mechanisms (competitive and non-competitive antagonism, desensitisation, allosteric
59 modulation and through interaction with sigma-2-binding sites) (Molderings et al., 1996,
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3 Barann et al., 1993 and 1999). Two possible mechanisms have been proposed by Barann et
4 al. (1993) to explain this non-specific inhibition: 1. non-specific hydrophobic interaction with
5 membrane lipids or hydrophobic regions of the receptor proteins, in which case the potency
6 of the compounds is correlated with their lipophilicity and 2. nonspecific inhibition of ion
7 channels in general. Since the N1E-115 cells, being neuronal cells, are also endowed with
8 fast voltage-gated Na⁺ channels, which can be activated by veratridine and which are
9 permeable to [¹⁴C]guanidinium ions as well, the same cell line can be used to investigate this
10 type of (nonspecific) interaction by comparing the potency of compounds in inhibiting cation
11 influx through both channels (5-HT₃ receptor channels and fast voltage-gated Na⁺ channels).
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19 To investigate whether or not the presented data are a result of a non-specific inhibitory
20 effect on ion channels in general, the ability of peppermint oil and (-)-menthol to inhibit
21 veratridine-induced [¹⁴C]guanidinium influx into N1E-115 cells (mediated through fast
22 voltage-dependent Na⁺ channels) was compared to its inhibitory effect on serotonin-induced
23 influx (mediated through 5-HT₃ receptor channels). The peppermint oil at a concentration of
24 0.32 µL/mL decreases the veratridine-induced influx to the same degree as the serotonin-
25 induced cationic influx indicating a broad, nonselective effect on ion channels. Its main
26 compound (-)-menthol, however, at a concentration of 1 mM showed no inhibition of the
27 veratridine effect. At a concentration of 0.32 µL/mL peppermint oil contains approx. 750 µM (-
28)-menthol. (-)-Menthol, therefore, is not involved in the nonspecific ion channel blockade
29 induced by peppermint oil. This could be interpreted in the way that additional compounds of
30 peppermint oil contribute to the nonspecific blockade of ion channels and the 5-HT₃-receptor
31 channel.
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44 4.3. 5-HT₃ receptor binding site

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46 The specific binding of the receptor antagonist [³H]GR65630 as a radioligand is not
47 influenced by (-)-menthol up to concentrations of 1 mM, albeit peppermint oil at an extremely
48 high concentration of 1 µL/mL shows a small, significant inhibition of binding. Eventually
49 other substances in peppermint oil interact with the binding site of the radioligand. It can be
50 assumed that high oil concentrations may influence the binding of the radioligand by their
51 high lipophilic effects.
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58 Comparable to the volatile oil of ginger (Abdel-Aziz et al., 2006) it may be assumed that there
59 exists a 5-HT₃ receptor channel antagonistic effect of peppermint oil by an allosteric
60 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-HT₃

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3 receptor. Members of this family, which include the nicotinic acetylcholine receptor, the
4 GABA_A and the glycine receptor (Miller 2002), share many structural and functional
5 similarities. They all exist as homo- or hetero-pentamers with each subunit consisting of an
6 extracellular N-terminal domain which encodes the ligand-binding site and four
7 transmembrane regions. They also possess several modulatory binding sites for GABA_A
8 receptors, benzodiazepines, barbiturates, ethanol, neurosteroids, some general anaesthetics
9 (Mehta et al., 1999), and in extension for nicotinic acetylcholine receptors, substance P,
10 serotonin, methysergide, spiperone and quinacrine (Arias, 1998), and for anandamide and
11 other cannabinoid receptor agonists (Barann et al., 2002) as well as nimodipine (Hargreaves
12 et al., 1996). This includes that those compounds inhibit 5-HT₃ receptor function without
13 affecting the specific binding of [³H]GR65630 to its binding site. The results obtained in the
14 present work support the possible existence of modulatory binding sites on 5-HT₃ receptors.
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24 For other ion channel receptors it was already demonstrated that (-)-menthol interacts in this
25 way: Hall et al. (2004) have shown that (-)-menthol is a potent positively acting allosteric
26 modulator on GABA_A- and glycin receptors. Additionally (-)-menthol has a local anesthetic
27 (Galeotti et al., 2001) and an antinociceptive effect (Galeotti et al., 2002) which is presumably
28 mediated according to Haeseler et al. (2002) via blocking voltage-dependent neuronal Na⁺
29 channels. (-)-Menthol activates TRPM8 channels from the family of vanilloid receptors and
30 induces via the subsequent cationic influx a sensation of coldness (Peier et al., 2002).
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39 4.4 Antiserotonergic effects of peppermint oil and (-)-menthol on rat ileum

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42 The spasmolytic and antispasmodic effect of peppermint oil was established by various
43 investigations: for review see Grigoleit and Grigoleit (2005b). To confirm the results obtained
44 with the N1E-115 cell system, the isolated rat ileum was used as a second assay. This
45 preparation contains neuronally located 5-HT₃ receptors. In our experiments both the
46 spontaneous contraction and the serotonin-induced contraction were diminished. Most of the
47 effect was mediated by (-)-menthol. The shift of the curve to the right can only be
48 interpreted in the way that additional components of the oil contribute to the overall effect.
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55 Hills et al. (1991) observed in contraction studies in the isolated taenia coli of the guinea-pig
56 that serotonin-, histamine- and substance P-induced contractions are inhibited by peppermint
57 oil in a non-competitive manner. Peppermint oil inhibits the serotonin-induced motoric answer
58 much stronger than that of the other stimuli.
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3 Our experiments enlarged the outcome with respect to (-)-menthol and the 5-HT₃-receptor.
4 With respect to the results from [¹⁴C]guanidinium-influx experiments also a 5-HT₃-receptor
5 antagonistic effect could be involved in the effect of the volatile oil.
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10 4.5. Biological relevance of the peppermint oil concentrations

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12 In these experiments concentrations of peppermint oil of 0.01 and 1 µL/mL were used. In the
13 IBS therapy the recommended dose is 3 times daily 0.2–0.4 mL peppermint oil. The highest
14 dose would be 1.2 mL peppermint oil daily. Under the assumption of a distribution volume of
15 70 L/70 kg and a complete absorption of the compounds of the volatile oil, there will be
16 theoretically a concentration of volatile oil of 0.017 µL/mL in the body volume which would be
17 in the lower range of concentrations used in our experiments. Because of the acid-resistant
18 formulation of peppermint preparations much higher concentrations may exist in the
19 gastrointestinal tract than in plasma (body volume).
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29 4.6 Summary and conclusion:

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- 32 • Peppermint oil and its main constituent (-)-menthol inhibit the cationic influx through 5-
33 HT₃ receptor channels in a concentration-dependent manner. (-)-Menthol is responsible
34 for rather half of the effect of the volatile oil.
35
 - 36 • Both do not compete with the radioligand [³H]GR65630 for the 5-HT₃ binding site and,
37 therefore, does not act directly.
38
 - 39 • Peppermint oil shows nonspecific ion channel effect; (-)-menthol, however, does not.
40
 - 41 • Both the volatile oil as well (-)-menthol reduce the serotonin-induced contraction of the rat
42 ileum and posses a direct relaxant effect. The effect of the volatile oil is partly mediated
43 by (-)-menthol; additionally there are other components involved in the overall effect of
44 peppermint oil.
45
 - 46 • Their action, therefore, may be mediated by binding to a modulatory site distinct from that
47 of serotonin, an allosteric binding site not yet identified.
48
 - 49 • This binding site, once exactly identified, might represent a valuable future target for the
50 development of a new class of anti-emetics lacking the typical side effects caused by
51 setrones due to their direct action on 5-HT₅ receptors.
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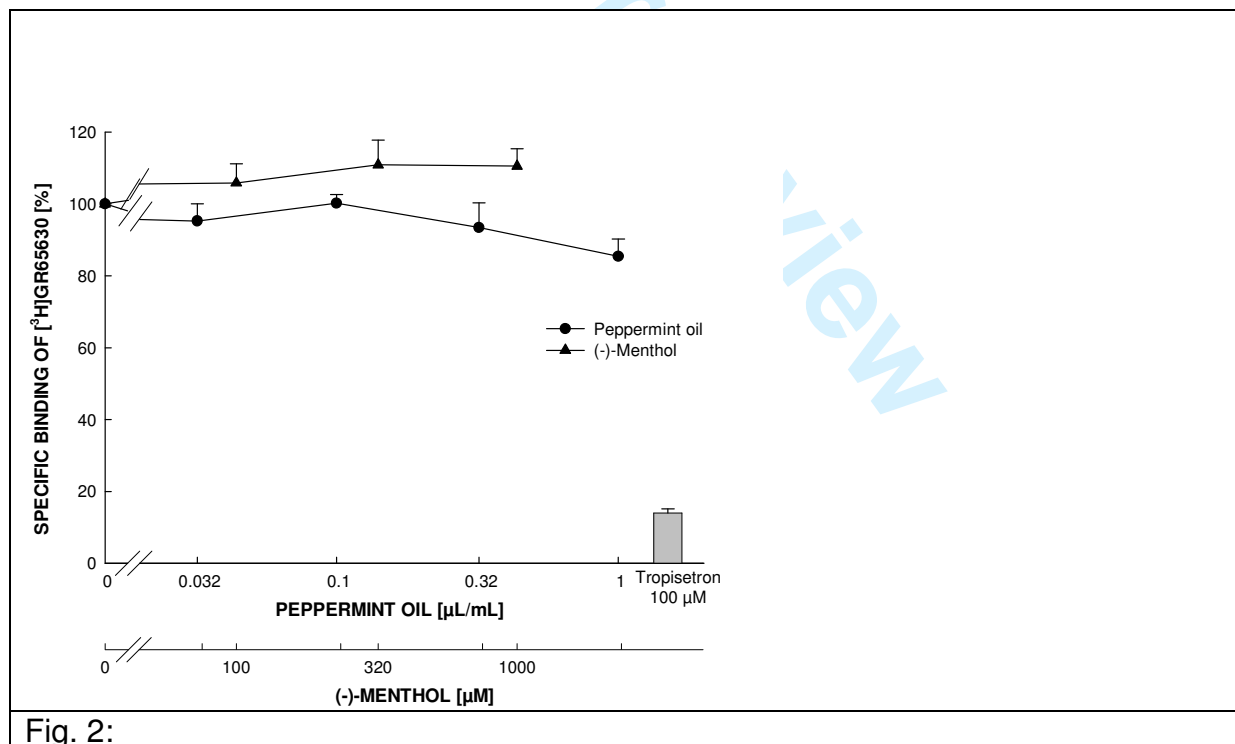
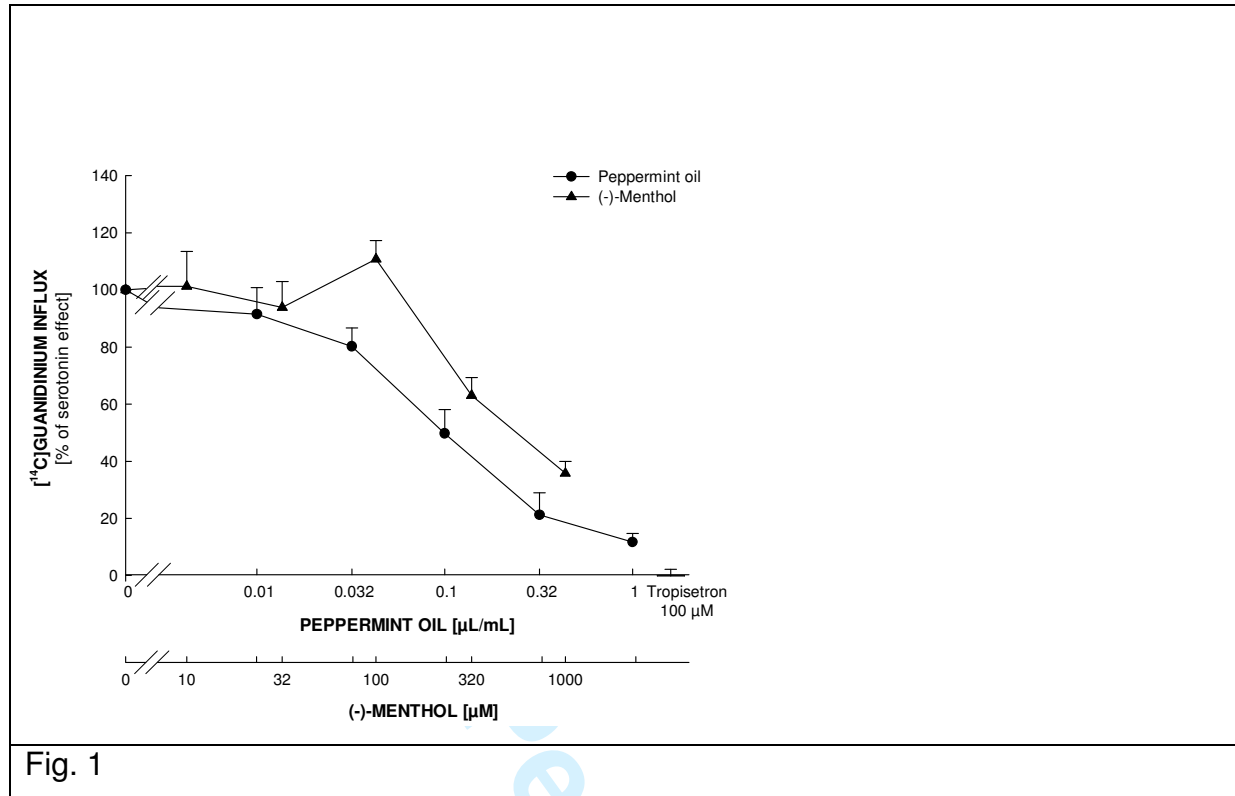
Table 1: The inhibitory effect of peppermint oil and (-)-menthol on either veratridine- or serotonin-induced [¹⁴C]guanidinium influx into N1E-115 cells. Methodological approach is as described for Fig. 1.

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)

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5 **Fig. 1: Concentration response curves for the effect of peppermint oil and (-)-menthol**
6 **and tropisetron on serotonin-induced [¹⁴C]guanidinium influx into N1E-115 cells.** Cells
7 were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of
8 various concentrations of indicated oil or compound or 100 μM tropisetron (as control). Prior
9 to stimulation with serotonin, cells were preincubated with the same concentration of oil or (-
10)-menthol for 20 min. Each point of the curves represents the mean value + S.E.M. from 4-7
11 independent experiments expressed as percentage of maximum [¹⁴C]guanidinium influx. The
12 scaling of (-)-menthol is an equivalent to the peppermint oil concentrations.
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21 **Fig. 2: Displacement of [³H]GR65630 from 5-HT₃ receptors on N1E-115 cells by**
22 **different concentrations of peppermint oil and (-)-menthol.** Cells were incubated for 30
23 min at room temperature with different concentrations of indicated oil or (-)-menthol or 100
24 μM tropisetron in the presence of 0.4 nM [³H]GR65630. Each point of the curves represents
25 the mean value ± S.E.M. of specific binding from 4-5 independent experiments expressed as
26 percentage of maximum [³H]GR65630 binding. The scaling of (-)-menthol is an equivalent to
27 the peppermint oil concentrations.
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36 **Fig. 3: Effect of different concentrations of peppermint oil and (-)-menthol on the**
37 **isotonic contractions of the isolated rat ileum to serotonin.** The compounds were left to
38 act for 60 s before measuring the response to 10 μM serotonin. Each point of the curves
39 represents the mean value ± S.E.M. from 3-7 experiments expressed as a percentage of the
40 response to 10 μM SR57227A.
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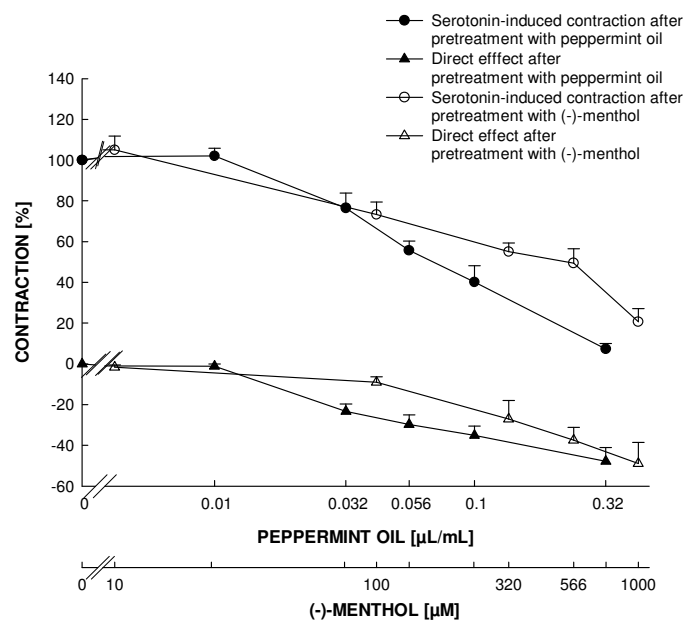
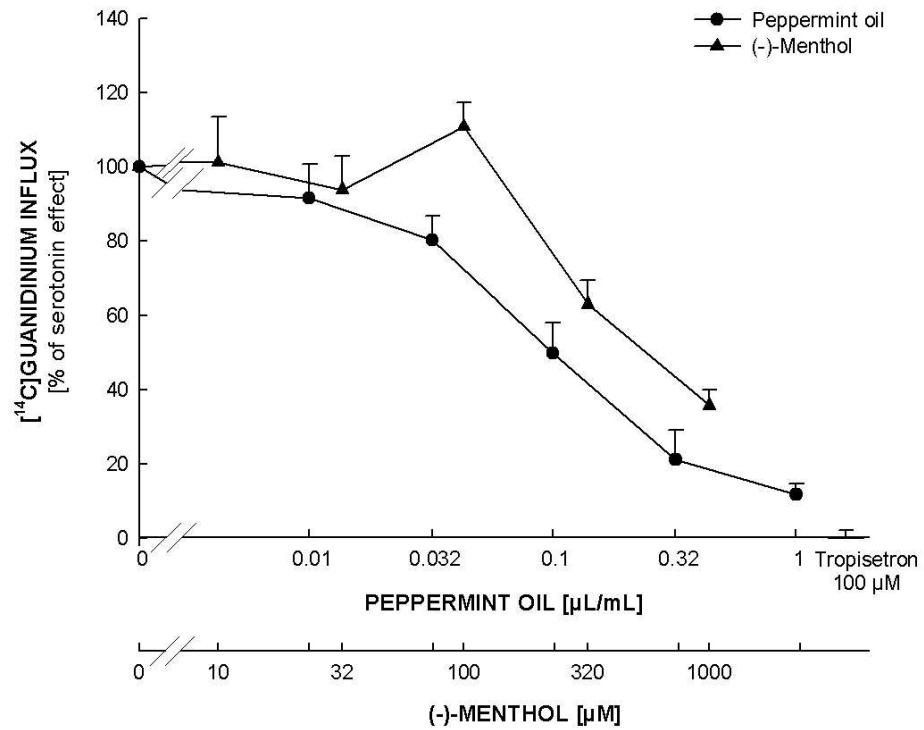
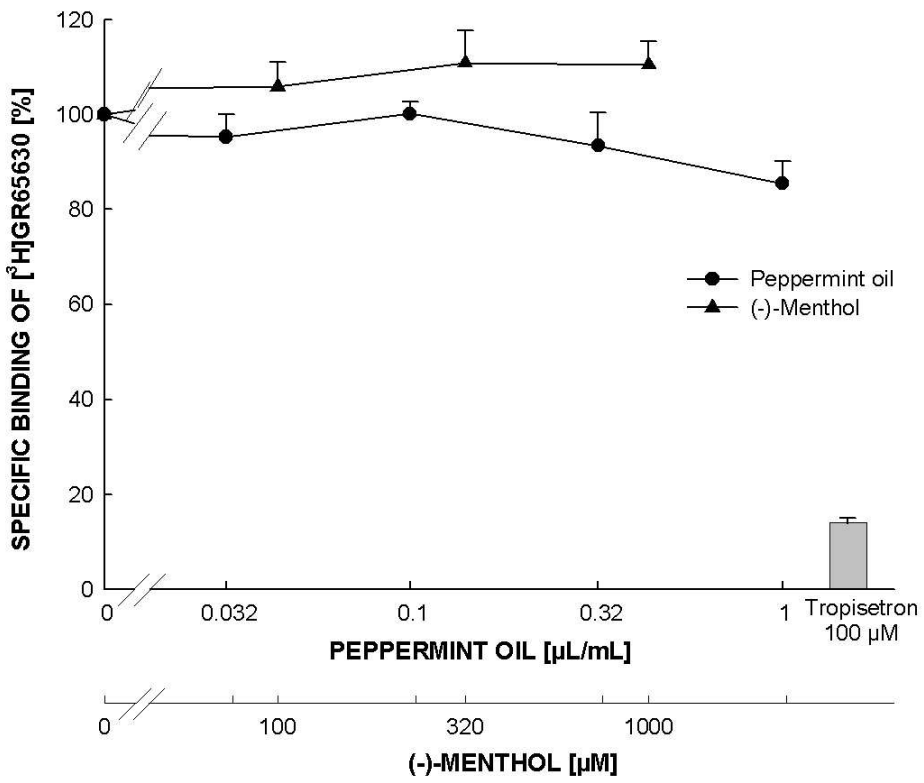


Fig. 3:

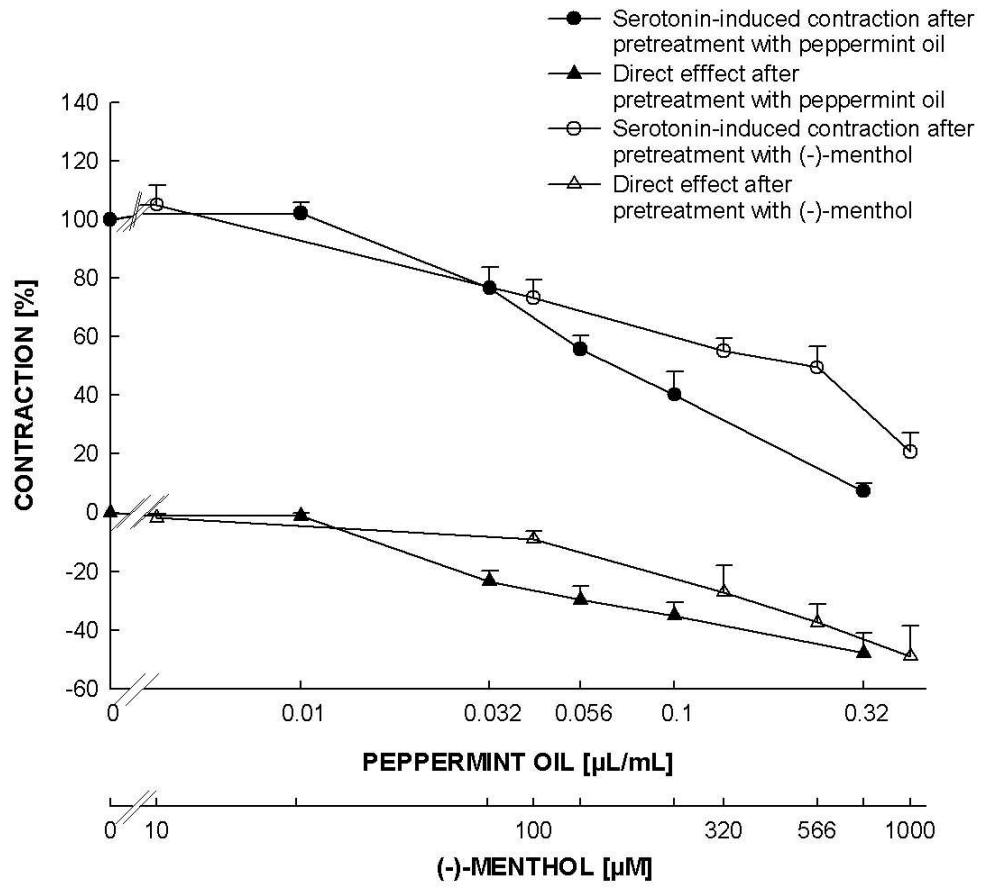


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