British Journal of Pharmacology (1997) 121, 1645–1650 

# Capsaicin and neurokinin A-induced bronchoconstriction in the anaesthetised guinea-pig: evidence for a direct action of menthol on isolated bronchial smooth muscle

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**1** For many years menthol has been used in the treatment of respiratory disorders although, a bronchodilator effect of menthol has yet to be described. Using the bronchoconstrictors capsaicin (acting via stimulating the release of neuropeptides from sensory afferents) and neurokinin A (NKA) we have raised airways resistance in the guinea-pig (GP) and studied the effect of menthol on both capsaicin and NKA-induced bronchoconstriction *in vivo*. *In vitro* the effect of menthol on acetylcholine (ACh) and KCl precontracted GP bronchi was also studied.

**2** GP ($n=13$) were anaesthetized (urethane 1.5 g $kg^{-1}$, i.p.) and a bolus injection of capsaicin (7.5 $\mu$g $ml^{-1}$, i.v.) or infusion of NKA (1 $\mu$g $min^{-1}$, i.v.) was given either in the presence of air (0.81 $min^{-1}$) or air impregnated with menthol vapour (7.5 $\mu$g $l^{-1}$) freely breathed from a tracheal cannula via a T-piece. Airways resistance ($R_{aw}$) and ventilation were measured throughout. Bronchi of mean internal diameter (1029 + 73.6 $\mu$m; $n=24$) were removed from GP ($n=16$) and mounted in the Cambustion myograph. Bronchial rings were maximally precontracted with 80 mM KC1 or 2 mM ACh. Relaxation due to a cumulative dose of menthol (1–3000 $\mu$M) was measured.

**3** Menthol produced a significant ($P<0.05$) 51.3% reversal of the capsaicin-induced increase in $R_{aw}$, and also inhibited the significant ($P<0.05$) reduction in minute ventilation ($V_e$) associated with the capsaicin-induced increased in $R_{aw}$. Menthol also caused a significant ($P<0.05$) 41% reversal of the NKA-induced increase in $R_{aw}$. The NKA-induced decrease in $V_e$ was again significantly ($P<0.05$) reversed with menthol inhalation. Menthol caused a significant ($P<0.001$) dose-dependent relaxation of KCl and ACh precontracted bronchi.

**4** We have shown that menthol attenuates both capsaicin and NKA-induced bronchoconstriction *in vivo* and relaxes KCl and ACh preconstricted bronchi *in vitro*. Menthol inhibition of NKA and capsaicin-induced bronchoconstriction could be, in part, explained by a direct action of menthol on bronchial smooth muscle.

**Keywords:** Menthol; capsaicin; neurokinin A; airways resistance; bronchodilatation

## Introduction

Menthol has been used for many years in a wide range of over the counter medications. As a medicine, its most popular application is in the relief of common cold symptoms such as cough and chest congestion, although there is very little objective clinical evidence to show that menthol has any beneficial effects at the levels used in proprietary cough and cold products.

Antitussive activity of menthol has recently been demonstrated in both healthy volunteers (Morice *et al*., 1994) and in guinea-pigs (Laude *et al*., 1994) with the citric acid-induced cough model. It was suggested by Laude that the antitussive properties of menthol in the guinea-pig may be due to a direct inhibitory action on the cough reflex, although an action on other respiratory mechanisms associated with cough, such as bronchoconstriction and airways secretion could not be ruled out.

Evidence of an effect of menthol on pulmonary function is very limited. Cohen & Dressler (1982) have studied the effects of a mixture of aromatic vapours (including menthol) on the calibre of airways in volunteers suffering from the common cold. By measuring forced expiratory volumes, peak expiratory flow rate and lower as well as total airways resistance, an improvement in airway's calibre with a 20–60 min aromatic vapour inhalation was found. However, since a mixture of aromatic vapours was used it is hard to attribute any effects of the mixture to menthol alone. Tamaoki *et al*. (1995) have more recently demonstrated a reduction in airway hyperresponsiveness, measured as a shift in the methacholine dose-response curve, in patients with mild asthma following long-term treatment with menthol. However, this group did not find any improvement in measures of forced expiratory volume in 1 s ($FEV_1$) following menthol treatment, which suggests that menthol had no effect on airway's calibre in these patients.

Thus evidence of bronchodilatation following menthol inhalation has yet to be obtained, furthermore there is no evidence of a direct action of menthol on bronchial smooth muscle. However, menthol has been shown to relax ileal smooth muscle in guinea-pigs by Hawthorn *et al*. (1988) and a specific action of menthol on airways epithelium has been demonstrated by Chiyotani *et al*. (1994), in dog, where menthol application *in vitro* increased $Cl^-$ secretion.

In the present study, we have examined the effect of prior menthol inhalation on caspsaicin-induced increase in airways resistance ($R_{aw}$) in the guinea-pig. In view of the complex mechanism whereby capsaicin induced bronchoconstriction, involving sensory nerve stimulation and neuropeptide release, further studies have been designed to investigate the possible sites of action of menthol by use of exogenous neurokinin A *in vivo* and in preparations of isolated bronchi preconstricted with potassium chloride (KCl) and acetylcholine (ACh).

## Methods

### *Airways resistance*

Airways resistance was determined in Dunkin-Hartley guinea-pigs (420–645 g; $n=13$) anaesthetized with intraperitoneal

urethane (1.5 g $kg^{-1}$) as previously described (Bee *et al*., 1995). Blood pressure was continuously monitored from the carotid artery (Viggo-Spectromed pressure transducer with Lectromed amplification). Intravenous infusions or bolus injections were administered via a jugular vein cannula. Pleural pressure was measured by introducing a cannula connected to a pressure transducer, into the pleural cavity between the fifth and sixth ribs. Airflow ($\dot{V}$) was measured from a tracheal cannula which was connected to a pneumotachograph (Fleisch 0.6 V) and volume (V) was determined from the integrated flow. The animals were allowed to breathe spontaneously from an airflow of 0.8 l $min^{-1}$ passed across the tracheal cannula via a T-piece.

Airways resistance was measured from the pleural pressure-tracheal airflow relationship. A voltage which was proportional to lung volume was electronically subtracted from the pleural pressure and the resulting signal fed into one axis of an X-Y pen recorder, while the airflow signal was fed into the other axis. Signals were in phase. Total airways resistance ($R_{aw}$) was calculated from the slope of the pressure-flow loop.

### *Menthol*

Menthol (Sigma Chemicals, Poole, Dorset U.K.) vapours were administered to the animal by passing air at 0.8 l $min^{-1}$ through a glass tube containing a cotton wool plug impregnated with 2.5 g of menthol crystals. The animal was allowed to freely breath the aromatic vapours, from the tracheal cannula via a T-piece. The concentrtion of menthol delivered to the animal with this technique was (7.5 $\mu$g $l^{-1}$) at a rate of 0.8 l $min^{-1}$. The concentration of menthol administered was quantified by purging the output air through a dreschel bottle containing isopropyl alcohol. Analysis of the resulting solution was by gas chromatography Fisons GC-MS (GC8000 & MD800) with selected ion monitoring for menthol (Laude *et al*., 1994).

### *Capsaicin*

Repeated doses of capsaicin (7.5 $\mu$g $ml^{-1}$, i.v.) were given as 400 $\mu$l bolus injections via the jugular vein. Before the injection of capsaicin, air (0.8 l $min^{-1}$) or exposure to air impregnated with menthol (7.5 $\mu$g $l^{-1}$) was freely breathed by the animal for 5 min, the delivery of the air or menthol was promptly ceased post-injection.

Each capsaicin response was monitored for $>20$ min, with both Ve and $R_{aw}$ allowed to return to basal levels between injections.

### *NKA*

Because of the rapid metabolism of neuropeptides *in vivo* and the short duration of bolus injections, 1 $\mu$g $min^{-1}$ NKA was administered as an infusion. Post 20 min infusion, either air (0.8 l $min^{-1}$) or air impregnated with menthol (7.5 $\mu$g $l^{-1}$) was administered for 5 min. Values of $R_{aw}$ before, during and after air or menthol delivery were compared.

### *Myography*

Male Dunkin-Hartley guinea-pigs (400–750 g, $n=16$) were anaesthetized with intraperitoneal urethane (1.5 g $kg^{-1}$) and the lungs removed. Bronchi ($n=24$) of internal diameter ($1029\pm73.6$ $\mu$m), were dissected free of any connective tissue and mounted on two 40 $\mu$m wires. The effects of menthol upon bronchi preconstricted with KCl and ACh were studied in a myograph (Cambustion Biological, Cambridge) at a calculated transmural pressure of 20 mmHg, which in preliminary experiments had been shown to give the maximum contractile response to 80 mM KCl. The bronchi were bathed in a physiological saline solution (PSS), pH 7.4 of composition (in mM): NaCl 120, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.17, $NaHCO_3$ 35, $KH_2PO_4$ 1.18, EDTA 0.269 and glucose 5.5. The 5 ml organ bath was aerated with a 95% $O_2$/5% $CO_2$ gas mixture and the temperature held at 37°C. Bronchi were allowed to equilibrate for 30–40 min before the experimental procedures were commenced.

At the start of each experimental procedure 80 mM KCl was applied on at least three successive occasions or until the response was reproducible. The effect of menthol was then studied on bronchi precontracted with KCl (80 mM) or ACh (2 mM) by use of cumulative concentrations of menthol within the range 1–3000 $\mu$M. Each concentration was allowed to equilibrate for 15 min, by which time a maximum sustained relaxation was attained.

Control solutions of menthol vehicle (ethanol 12% in saline) were added to both ACh and KCl precontracted bronchial rings.

Smooth muscle relaxation associated with menthol application had an onset of action of between 5–10 min. Preliminary experiments showed that the dilatation induced by menthol could be maintained for between 10–15 min if the organ bath was left uncovered and for 35–40 min if covered. The difference in duration was probably due to loss of this volatile terpine alcohol to the atmosphere and thus subsequent experiments were carried out with the bath covered.

### *Chemicals*

Menthol, NKA and ACh were obtained from Sigma Chemicals (Poole, Dorset, U.K.). Menthol was dissolved in ethanol (20%) and subsequently diluted with saline to give a stock solution of 100 mM. NKA, ACh and KCl were dissolved in sterile, physiological saline. All other reagents were obtained from BDH Chemicals Ltd (Lutterworth Lecs, U.K.).

### *Statistics*

Results are expressed as mean ($\pm$s.e.mean). For comparison of airways resistance response paired *t* tests were used, calculation of regression lines and *r* values were by Microsoft Excel 5.0. For myography, $IC_{50}$ values were calculated by use of Grafit, plotting data as a sigmoid curve. Results were tested for significance by analysis of variance-single ANOVA. Values of $P<0.05$ were considered significant.

## Results

### *Airways resistance*

*Baseline $R_{aw}$* Menthol inhalation (5 min) had no significant effect upon basal $R_{aw}$. Calculated basal $R_{aw}$ with and without menthol inhalation were $2.64\pm0.27$ and $2.63\pm0.23$ mmHg $min^{-1}$, respectively.

*Capsaicin* Capsaicin (bolus injection, i.v.) consistently produced an increase in airways resistance which stabilized after 10 min. There was a within animal variation in sensitivity to capsaicin with air pretreatment (0.8 l $min^{-1}$) giving maximal increases in $R_{aw}$ from baseline ranging from 9.3 to 57.9 mmHg $min^{-1}$. Capsaicin injection with air pretreatment gave a mean increase in $R_{aw}$ from $9.1\pm2.6$ to $29.0\pm4.8$ mmHg $min^{-1}$, whereas prior menthol inhalation gave a mean increase in $R_{aw}$ from $8.4\pm2.2$ to $17.9\pm3.9$ mmHg $min^{-1}$, a statistically significant (51.3%, $P<0.01$) inhibition of the capsaicin-induced increase in $R_{aw}$ (Figure 1). In several animals, successive injections of capsaicin caused a potentiation of the maximal $R_{aw}$ response. However, this did not prove significant on analysis of the whole group.

*NKA* NKA (infusion, i.v.) 1 $\mu$g $min^{-1}$ consistently caused an increase in airways resistance, which attained 80% of maximum ($15.43\pm1.8$ mmHg $min^{-1}$) after 20 min. However,

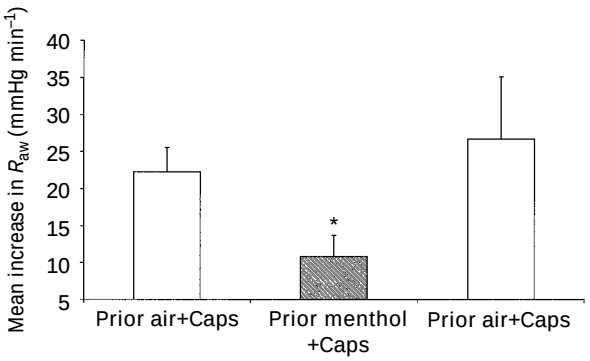


**Figure 1** Increase in airways resistance ($R_{aw}$; mean ± s.e.mean) following a 400 $\mu$l injection of capsaicin (Caps; 7.5 $\mu$g ml$^{-1}$, bolus i.v.) pretreated with either 5 min tracheal air administration (0.8 l min$^{-1}$), or 5 min air plus menthol vapour (7.5 $\mu$g l$^{-1}$). There was a significant difference between air and menthol pretreatment groups; *$P<0.01$.

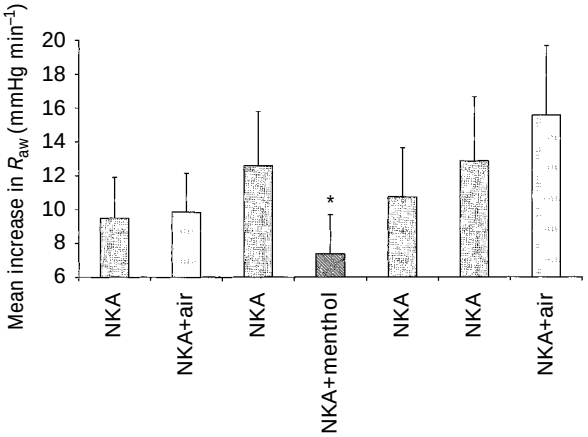


**Figure 2** Increase in airways resistance ($R_{aw}$; mean ± s.e.mean) following a neurokinin A (NKA) infusion (1 $\mu$g min$^{-1}$) with treatments of either 5 min tracheal air administration (0.8 l min$^{-1}$), or 5 min plus menthol vapour (7.5 $\mu$g l$^{-1}$). There was a significant difference between air and menthol pretreatment, *$P<0.01$.

subsequently there was a slow progressive potentiation of the NKA-induced increase in $R_{aw}$ during the course of the experiment, increasing from 12.07 ± 1.0 – 15.43 ± 1.8 mmHg min$^{-1}$.

The maximum increase in $R_{aw}$ from basal levels with NKA infusion over 50 min varied between animals (range 2.96 – 29.52 mmHg min$^{-1}$). Air inhalation during NKA infusion had little effect upon the NKA-induced increase in $R_{aw}$ 9.48 ± 2.4 to 9.84 ± 2.3. In contrast, menthol inhalation caused a statistically significant (41.3%, $P<0.01$) reversal in the NKA-induced increase in $R_{aw}$ from 12.6 ± 3.2 to 7.37 ± 3.0 (Figure 2), which was still evident 5 min after the initial exposure to menthol but returned to raised basal levels within 10 min.

## *Ventilation*

*Capsaicin bolus injection* Menthol inhalation had no significant effect on basal tidal volume ($V_t$) or frequency. Bolus intravenous injection of capsaicin with prior inhalation of air produced a significant (42.5%, $P<0.01$) decrease in minute ventilation corrected for 100 g body weight ($Ve_{Bt}$). The reduction in $Ve_{Bt}$ varied markedly between animals (range 0.45 – 15.1 ml min$^{-1}$ 100 g$^{-1}$) and was mostly due to a significant ($P<0.001$) fall in $V_t$ from 2.42 ± 0.20 to 1.6 ± 0.18 ml and to a lesser extent a non-significant decrease in respiratory rate from 57.1 ± 14.0 to 42.7 ± 11.2 breaths min$^{-1}$. The capsaicin-induced changes in $R_{aw}$ with air pretreatment correlated ($r^2=0.71$) closely with the changes in $Ve_{Bt}$. Inhalation of menthol, 5 min before capsaicin injection, prevented the capsaicin induced decrease in $Ve_{Bt}$.

Mean values of $Ve_{Bt}$ post-capsaicin were not significantly different from basal values pre-capsaicin (Figure 3). Approximately 30 min after menthol inhalation, a further capsaicin injection with prior air inhalation again significantly (36.4%, $P<0.01$) reduced $Ve_{Bt}$, which was not significantly different from that induced by the initial capsaicin injection (Table 1).

*NKA infusion* NKA-induced increase in $R_{aw}$ demonstrated a close correlation ($r^2=0.80$) with the reduction in $Ve_{Bt}$. The maximum reduction in $Ve_{Bt}$ from basal again varied between animals (range 0.83 – 24.3 ml min$^{-1}$ 100 g$^{-1}$). A 5 min inhalation of air (0.8 l min$^{-1}$) during the infusion had no effect upon the NKA-induced decrease in $Ve_{Bt}$. However, a 5 min inhalation of menthol vapour (7.5 $\mu$g l$^{-1}$) caused a significant (40%, $P<0.01$) reversal of the NKA-induced decrease in $Ve_{Bt}$ (Figure 4) correlating closely with changes in $R_{aw}$. This improvement in $Ve_{Bt}$ was due to a statistically significant ($P<0.01$) increase in both $V_t$ and respiratory rate (Table 1).

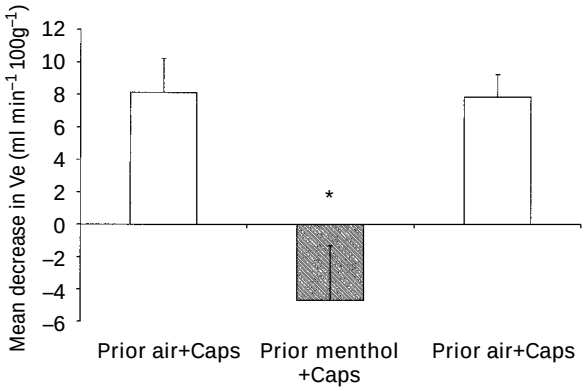


**Figure 3** Decrease in minute ventilation corrected for 100 g body weight ($Ve_{Bt}$; mean ± s.e.mean) following a 400 $\mu$l injection of capsaicin (Caps; 7.5 $\mu$g ml$^{-1}$, bolus i.v.) pretreated with either 5 min tracheal air administration (0.8 l min$^{-1}$), or 5 min air plus menthol vapour (7.5 $\mu$g l$^{-1}$). There was a significant difference between air and menthol pretreatment groups; *$P<0.01$.

**Table 1** A summary of the effect of menthol and air on capsaicin and NKA-induced changes in airway resistance and ventilation

| | *Decrease/increase* (%) | | | |
|---|---|---|---|---|
| | $R_{aw}$ | *Frequency* | $V_T$ | *Ve* |
| Caps with prior air | 424 | –14.6 | –33.5 | –41.2 |
| Caps with prior menthol | **170 | *9.5 | *8.9 | *23.8 |
| NKA and air | 507 | –25.4 | 34.9 | –3.14 |
| NKA and menthol | **302 | *–15.1 | *75.0 | *38.9 |

Results are expressed as % decrease (-ve values) or increase (+ve values) from baseline. Significance was calculated from mean absolute difference between control air and menthol inhalation by use of paired $t$ test. *$P<0.05$, **$P<0.01$. Caps, capsaicin and NKA, neurokinin A.

*Blood pressure* Five minutes after the capsaicin bolus injection there was a non-significant maximum decrease in blood pressure from basal value of 41.5 ± 6.4 to 37.2 ± 3.3 mmHg this returned to basal levels which coincided with the return to basal levels of $R_{aw}$. NKA infusion was also associated with a

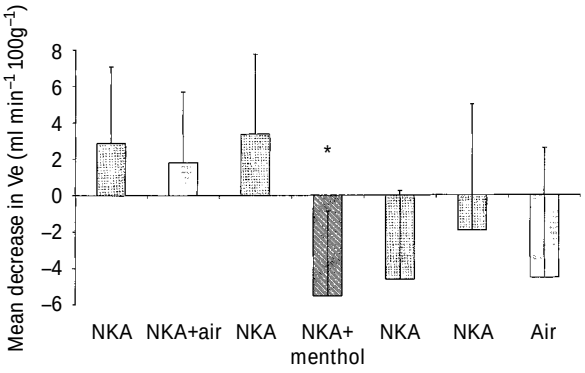


**Figure 4** Decrease in minute ventilation corrected for 100 g body weight ($Ve_{Bt}$; mean ± s.e.mean) following a neurokinin A (NKA) infusion (1 $\mu$g min$^{-1}$) with treatments of either 5 min tracheal air administration (0.8 l min$^{-1}$), or 5 min air plus menthol vapour (7.5 $\mu$g l$^{-1}$). There was a significant difference between air and menthol pretreatment groups; $*P<0.01$.

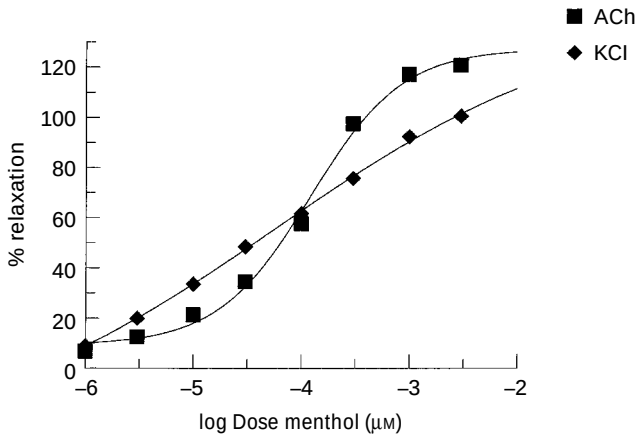


**Figure 5** Effects of menthol (1 – 3000 $\mu$M) on guinea-pig isolated bronchi preconstricted with either ACh (2 mM) or KCl (80 mM); $n=24$.

non-significant decrease in blood pressure from a basal value of 46.1 ± 4.7 to 35.8 ± 2.6 mmHg after 10 min of NKA infusion. Blood pressure returned to basal levels following cessation of the NKA infusion.

### *Myography*

The maximum dose of vehicle (ethanol 12% in saline) caused no significant effect on bronchi preconstricted with either ACh or KCl. In the case of ACh preconstricted bronchi the maximum concentration of vehicle caused 0.53% relaxation and, for bronchi preconstricted with KCl there was a 1.7% relaxation to the maximum concentration of vehicle.

ACh (2 mM) and KCl (80 mM) produced contractions of guinea-pig bronchi of 1.63 ± 0.30 and 2.14 ± 0.11 mN mm$^{-1}$, respectively. Menthol at the maximum concentration (3 mM) used in this study caused 101% relaxation of KCl preconstricted guinea-pig bronchi. A cumulative concentration-response curve to menthol gave an $IC_{50}$ of 58 $\mu$M. Menthol produced a highly significant ($P<0.001$) relaxation of KCl precontracted bronchi at all doses studied compared to vehicle.

Menthol (3 mM) caused 121% relaxation of ACh preconstricted guinea-pig bronchi. A concentration-response curve to menthol gave an $IC_{50}$ of 120 $\mu$M. Menthol produced a highly significant relaxation of ACh precontracted bronchi at all doses studied ($P<0.001$) (Figure 5).

The duration of the relaxation by menthol of both ACh- and KCl-induced contractions was >15 min.

## Discussion

In this study we have demonstrated that menthol vapour reduces both capsaicin and NKA-induced increases in $R_{aw}$ in spontaneously breathing anaesthetized guinea-pigs. The concomitant decrease in minute ventilation seen with these bronchoconstrictor agents was also found to be reduced by menthol inhalation.

*In vitro*, menthol caused a significant relaxation of bronchi preconstricted with either KCl or ACh, although menthol appeared to be more potent a dilator of KCl than ACh-induced constriction.

We have found no change in basal levels of $R_{aw}$ and minute ventilation with menthol inhalation *in vivo*. However, previous findings in both the dog (Sant'Ambrogio *et al*., 1992) and guinea-pig (Orani *et al*., 1991) have shown that laryngeal exposure to menthol reduces ventilation by decreasing respiratory rate and the authors concluded that this effect may be due to stimulation of upper airways cold receptors. In our study, inhalation of menthol was via a tracheal cannula, thus bypassing the laryngeal receptors. This together with the fact that no effect was found on basal respiratory rate or tidal volume, suggests that the observed improvement in minute ventilation was probably associated with a reduction in airways resistance.

We have demonstrated bronchodilatation both *in vitro* and *in vivo* on exposure to menthol, we suggest that *in vitro* and at least part of the action of menthol *in vivo* may be via inhibition of smooth muscle contraction. *In vivo*, airways resistance is mostly determined by the calibre of the large intrapulmonary airways (Pedley *et al*., 1977). In addition there is a high population of neurokinin receptors which lie predominantly in the bronchial smooth muscle of the larger airways (Strigas & Burcher, 1996). Thus our study was restricted to the larger bronchi 1029 ± 73.6 $\mu$m in diameter to determine a bronchodilator effect of menthol but, as different levels of the bronchopulmonary tree are structurally and functionally different (Daniel *et al*., 1986; Stephens, 1988), we cannot say whether the bronchodilator effect of menthol is common to all airway levels.

The effect of menthol on ACh and KCl preconstricted bronchi was carried out to establish a direct effect of menthol on bronchial smooth muscle. Since menthol relaxed both ACh and KCl preconstricted bronchi this suggests inhibition of both agonist- and depolarization-mediated contractions through a common mechanism of antagonism. Hills & Aaronson (1991) also observed a similar phenomenon in guinea-pig taenia coli where peppermint oil, containing menthol as its major active constituent was found to inhibit ACh, histamine, 5-hydroxytryptamine and substance P-induced contractions. Peppermint oil was found to reduce the amplitude of the potential dependant calcium current and increase the rate of the current's decay. This is a similar effect to that observed with menthol in neuronal preparations (Swandulla *et al*., 1986; 1987).

Thus we hypothesize that menthol may have a similar mechanism of action in bronchial smooth muscle as that in ileal smooth muscle and neuronal preparations, that is it acts upon cell membranes by regulating $Ca^{2+}$ efflux. Chiyotani *et al*. (1994) have shown a specific action of menthol on the bioelectric properties of cultured airway epithelial cells of the dog and this was associated with a specific increase in intracellular calcium concentration ($[Ca^{2+}]_i$).

A simple direct effect upon smooth muscle may not be the only mechanism whereby menthol inhibits capsaicin-induced bronchconstriction. Capsaicin by the stimulation of sensory afferents may affect a number of airway reflexes, including cough (Karlsson *et al*., 1988), mucociliarly clearance (Wong *et al*., 1990), mucus secretion (Davis *et al*., 1986; Kuo *et al*., 1990)

and bronchoconstriction (Forsberg & Karlsson, 1986). Menthol has been shown to attenuate chemically-induced cough (Packman & London, 1980; Morice *et al*., 1994; Laude *et al*., 1994) and, as cough is a neuronal reflex, an action of menthol upon sensory afferents or other neuronal elements is possible.

If it is assumed that capsaicin injection promotes the release of neuropeptides including NKA, then indirect evidence for a dual action of menthol on both sensory nerves and bronchial smooth muscle comes from analysis of the time course of the inhibition of the *in vivo* exogenous and endogenous neurokinin-induced bronchoconstriction. Menthol inhibition of NKA-induced constriction was short lived, lasting no longer than 10 min, whereas menthol inhibition of capsaicin-induced constriction was of a longer duration being still present 10 min after exposure to menthol, when in the case of exogenous NKA-induced bronchoconstriction the action of menthol would have been expected to have ceased.

The mode of action of capsaicin in inducing bronchoconstriction is thought to involve stimulation of C fibres (Fuller *et al*., 1985) culminating in the release of neuropeptides including substance P, calcitonin gene related peptide and neurokinins from sensory afferents via a peripheral axon-reflex mechanism (Holzer, 1991). Inhibition of $NK_2$ sites has been shown to reduce cough (Advenier *et al*., 1993; Girard *et al*., 1995) and it could be argued that menthol may have an effect on either the release of neurokinins by the tussive agents citric acid and capsaicin used in the cough studies, or on $NK_2$ receptor occupation. However, in the present study, we have shown menthol to be effective against exogenous NKA and assuming capsaicin induced the release of NKA, along with other neuropeptides, part of the action of menthol may be independent of the release or synthesis of neurokinins.

We hypothesize that menthol attenuates capsaicin-induced bronchoconstriction through a combined action on both sensory nerves and bronchial smooth muscle. Whereas its attenuation of exogenous NKA-induced bronchoconstriction is attributed predominantly to a direct effect upon bronchial smooth muscle. We suggest the mechanism of action of menthol at both the neuronal and smooth muscle sites could be due to a specific antagonism of $Ca^{2+}$ channels.

Because of the possible dual action of menthol on sensory nerves and smooth muscle, this compound may have a therapeutic role in upper respiratory tract infection, bronchitis and asthma. Menthol has been demonstrated to be an effective antitussive agent in human and guinea-pig cough models and, in this study, has also been shown to both inhibit and reverse chemically-induced bronchoconstriction in the guinea-pig. Cohen & Dressler (1982) demonstrated that acute inhalation of aromatic vapours including menthol improved airways calibre in volunteers suffering from respiratory tract infections. More recently, Tamaoki *et al*. (1995) have shown menthol to have beneficial effects in asthma. Despite this group finding an improvement in hyperresponsiveness of airways in patients with mild asthma, there was no change in the $FEV_1$, indicating no bronchodilator effect of menthol in these patients. This is in contrast to our study and that of Cohen & Dressler and may be due to the fact that the study of Tamaoki *et al*. involved chronic treatment with menthol, delivered as a nebulised solution over a period of 4 weeks, as opposed to acute treatment with vapours.

In either case the actual concentration of menthol delivered to the patient is unknown and as this is a highly volatile substance, the concentration of menthol in contact with the airways was probably very small and as supported by our study, it is unlikely menthol would have a long duration of action.

These properties of menthol point towards a compound of dual efficacy which may be an effective and well tolerated treatment for both respiratory tract infection and mild asthma by improving airways calibre and alleviating the associated cough.

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*(Received August 29, 1996*
*Revised May 12, 1997*
*Accepted May 22, 1997)*