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Effect of the long-acting tachykinin NK_1 receptor antagonist MEN 11467 on tracheal mucus secretion in allergic ferrets

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1 We investigated the effect of MEN 11467 ((1*R*,2*S*)-2-*N*[1(*H*)indol-3-yl-carbonyl]-1-*N*-{N^{α}(*p*-tolylacetyl)-N^{α}(methyl)-D-3-(2-naphthyl)alanyl}diaminocyclohexane) on tachykinin-induced mucus secretion in ferret trachea *in vitro* and determined its effect on secretion by tracheae from allergic ferrets in response to allergen challenge.

2 Repeated administration of [Sar^9,$Met(O_2)^{11}$]-substance P ([Sar^9]SP, 1 μM) maintained mucus output above control values for at least 1.75 h. MEN 11467 inhibited secretion in a concentration-dependent manner with maximal inhibition at 10 μM and an approximate IC_{50} of 0.3 μM. Inhibition by MEN 11467 (0.1–10 μM) was maintained, to varying degree, for at least 1.75 h after washout in the continued presence of [Sar^9]SP.

3 In electrically stimulated tracheae, tachykininergic neural secretion was virtually abolished by 1 μM MEN 11467.

4 In tracheae from ovalbumin-sensitised animals, repeated administration of ovalbumin maintained mucus output above controls for 1.5 h. MEN 11467 inhibited ovalbumin-induced secretion in a concentration-dependent manner, with complete inhibition at 1 μM. Inhibition by MEN 11467 (1 and 10 μM) was maintained, to varying degree, after drug washout for the 1.5 h of ovalbumin stimulation.

5 MEN 11467 1 μM did not affect secretion induced by either acetylcholine or histamine, whereas 10 μM MEN 11467 did inhibit agonist-induced secretion.

6 We conclude that, in ferret trachea *in vitro*, MEN 11467 at concentrations of 0.1–1 μM is a long acting and selective inhibitor of tachykininergic-induced mucus secretion, and may have therapeutic potential for bronchial hypersecretion associated with allergic conditions, for example in asthma.
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Keywords: Airway; allergy; asthma; mucin; mucus; MEN 11467; respiratory tract; sensitisation; tachykinin receptor; tachykinin receptor antagonist

Abbreviations: ACh, acetylcholine; COPD, chronic obstructive pulmonary disease; [Sar^9]SP, [Sar^9,$Met(O_2)^{11}$]-substance P

Introduction

Mucus secretion is a vital component of airway homeostasis, providing the 'front-line' barrier to inhaled irritants. The rate of secretion is controlled by both humoral and neuronal mechanisms. In mammalian airways, the dominant neural control is cholinergic (Rogers, 2000a). Adrenergic neural mechanisms contribute little to control, particularly in human airways. Capsaicin-sensitive 'sensory-efferent' nerves also control secretion, although their relative contribution varies with species (Rogers, 2000a). Asthma and chronic obstructive pulmonary disease (COPD) are two severe respiratory conditions that are associated with airway mucus hypersecretion (Liu *et al*., 1998a; Rogers, 2000b). In both conditions, abnormalities in neural control are implicated in pathophysiology. Consequently, investigation of the neural control of airway secretion is linked to design of therapeutic drugs for bronchial mucus hypersecretion. Preclinical test systems have, therefore, been developed to assess compounds with potential to inhibit neurogenic secretion.

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One such system is the *in vitro* ferret trachea. Neurogenic secretion in ferret trachea is mediated *via* cholinergic nerves and capsaicin-sensitive 'sensory-efferent' nerves (Rogers, 2000a). Tachykinin receptor agonist studies (Geppetti *et al*., 1993; Meini *et al*., 1993) and tachykinin receptor antagonist studies using a range of peptide and non-peptide compounds (Ramarine *et al*., 1994; Khawaja *et al*., 1999) show that the sensory-efferent secretory response is mediated exclusively *via* tachykinin NK_1 receptors. Thus, mucus secretion from the *in vitro* ferret trachea is a useful experimental system for investigating drugs acting at tachykinin NK_1 receptors.

Allergy is one component of asthma. Consequently, antigen-sensitized animals are commonly employed as models of allergic asthma (Chung, 1995). In guinea-pigs, the late-phase of antigen-induced tracheal plasma exudation is mediated *via* tachykinin interaction with tachykinin NK_1 receptors (Bertrand *et al*., 1993a). The involvement of tachykininergic pathways in antigen-induced airway mucus secretion is not reported.

In the present study, we used the pseudopeptide tachykinin NK_1 receptor antagonist, MEN 11467 ((1*R*,2*S*)-2-*N*[1(*H*)in-

dol-3-yl-carbonyl]-1-*N*-{N^{α}(*p*-tolylacetyl)-N^{α} (methyl)-D-3-(2-naphthyl) alanyl}diaminocyclohexane) (Cirillo *et al*., 1998), to study tachykininergic involvement in antigen-induced mucus secretion in ferret trachea *in vitro*. We used this antagonist because its long duration of action was required if antigen-induced neurogenic secretory responses were late in onset (Bertrand *et al*., 1993a). Firstly, we determined the inhibitory profile and duration of action of MEN 11467 against secretion induced by the selective tachykinin NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P ([Sar^9]SP). Secondly, we examined inhibition by MEN 11467 of electrically stimulated tissue in the presence of adrenoceptor and cholinoceptor blockade (i.e. tachykininergic neural secretion). Thirdly, we assessed the inhibitory profile and duration of action of MEN 11467 in tracheae from ovalbumin-sensitised animals challenged with ovalbumin ('allergic' secretion). Finally, the selectivity of MEN 11467 for tachykininergic-induced mucus secretion was assessed using acetylcholine and histamine to induce secretion. We used $^{35}SO_4$ as a mucus marker because it localises to secretory structures and is released upon stimulation (Gashi *et al*., 1987), and the released material has a molecular weight and buoyant density characteristic of a mucus glycoprotein (Davies *et al*., 1990).

Methods

Tracheal preparation for measurement of mucus secretion

Our methodology for measurement of tracheal mucus secretion has been described in detail previously (Meini *et al*., 1993; Ramnarine *et al*., 1994). Male ferrets (Regal Rabbits, Great Bookham, Surrey, U.K.) weighing 1.5–2.0 kg were used throughout. They were kept 4–5 in a room with free access to food and water, and were allowed one week to acclimatize after delivery. They were terminally anaesthetized with pentobarbitone sodium (Sagatal; 60 mg kg^{-1}, i.p.), and bled by incising the left ventricle. The tracheae were removed and bathed in aerated (95% O_2, 5% CO_2) Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.5 and glucose 5.05, until required (~10 min). Tracheae were cut longitudinally through the dorsal membrane, opened flat and cut transversely to give four segments, each of which was pinned and clamped across the aperture separating the two halves of perspex Ussing-type chambers so that the tissue divided the chambers into a 'luminal' (i.e. mucus-producing) and 'submucosal' side. Each side of the tissue was bathed with 5 ml warmed (37°C) Krebs-Henseleit solution that was oxygenated and circulated using gas-lift pumps.

Radiolabelling of newly synthesized mucus

At time 0 h, $Na_2{}^{35}SO_4$ (0.1 mCi) was added to the submucosal half-chambers, in order to label newly synthesized intracellular mucus, where it remained throughout the experiment. At unit time intervals, the fluid in the luminal side of the chamber (containing secretions) was collected and replaced with fresh Krebs-Henseleit solution. Baseline stability of spontaneous output of $^{35}SO_4$-labelled macromolecules was reached after taking four 30 min collections followed by two 15 min collections (i.e. over 2.5 h following addition of radiolabel). After stabilization, drugs or control solutions were added and the tissues were stimulated electrically or challenged with antigen (see *Protocols* below).

Electrical stimulation

Tracheal segments were subjected to an electrical current to stimulate excitable tissues (e.g. nerves). Two pins piercing the tissue on either side were connected *via* outlet wires from the chambers to a Grass stimulator (model S88; Grass Instruments, Quincy, U.S.A.). Tissues were stimulated at 10 Hz, 50 V, 0.5 ms for the first 5 min of a 15 min incubation period.

Sensitization to ovalbumin in vivo

To determine the effect of MEN 11467 on 'allergic' secretion, ferrets were sensitized by a single i.p. injection of 100 μg ovalbumin in 2.5 ml saline containing 0.5 mg aluminium hydroxide as adjuvant. Dose and volume were increased proportionally from those used successfully in guinea-pigs (Anderson 1980; Liu *et al*., 1998b). Ferrets were left for 3 weeks to develop sensitivity to ovalbumin before tracheae were challenged *in vitro* with ovalbumin (see below).

Measurement of $^{35}SO_4$-labelled macromolecule secretion

Luminal fluid, approximately 4 ml and comprising secretions in Krebs-Henseleit solution, was drained into tubes containing 5 g guanidine hydrochloride, to dissolve the mucus. The final concentration of guanidine hydrochloride in the fluid was 6 M. Following this, each sample was dialysed exhaustively against distilled water containing excess Na_2SO_4 and sodium azide (to limit bacterial growth) using cellulose tubing allowing molecules of 12–14 kDa or less to pass through. The samples were recovered after at least six changes of distilled water when the radioactive count of the dialysis water was the same after dialysis as before dialysis (~20 disintegrations per minute (d.p.m.)). The recovered samples were weighed and the remaining radioactivity in 1 ml duplicates of each sample, mixed with 2 ml scintillant, was determined by scintillation spectrometry. The total radioactivity of each sample was estimated by multiplying the radioactivity present in a 1 ml aliquot of that sample by the total weight of the sample (assuming a 1 ml sample weighs 1 g).

Protocols

Following 2.5 h incubation, a 15 min collection was taken, representing baseline secretion. The tissue was then incubated for 1 h (i.e. four 15 min periods) with MEN 11467 or vehicle (DMSO) prior to stimulation with agonist, electrical pulses or allergen challenge. In the construction of inhibitory concentration-response curves, only one concentration of MEN 11467 was used per chamber.

To investigate the duration of effect of MEN 11467, increased tachykinin NK_1 receptor mediated secretion needed to be maintained. The duration of effect of a sub-maximal concentration of [Sar^9]SP (1 μM) was determined: (1) in

response to a single 15 min incubation, and (2) in response to replenishment with [Sar^9]SP after draining and refilling of the chambers following incubation. In both cases, secretion was followed for 1.75 h (i.e. eight 15 min incubation periods). Because the effect of the single application of [Sar^9]SP was short-lived (see Results), the duration of effect of MEN 11467 (1 nM – 10 μM) was determined on repeat application of [Sar^9]SP. MEN 11467 or vehicle was present for the one hour prior to stimulation, and for the 15 min stimulation period. MEN 11467 (or vehicle) was then removed and its effect examined in the continued presence of [Sar^9]SP.

To determine the effect of MEN 11467 on tachykininergic neural mucus secretion, tissues were incubated with MEN 11467 or vehicle prior to and during electrical stimulation (see above) in the presence of phentolamine, propranolol and atropine (10 μM for 30 min) to eliminate adrenergic and cholinergic neural influences.

Three weeks after *in vivo* ovalbumin sensitization, tracheae were challenged *in vitro* in Ussing chambers with 50 – 400 μg ml^{-1} ovalbumin to establish the optimal concentration of ovalbumin to elicit a secretory response. Subsequently, to determine optimal inhibition, the effect of MEN 11467 (0.1 – 10 μM) or vehicle was examined on secretion induced by 200 μg ml^{-1} ovalbumin. Lastly, to determine the duration of effect of MEN 11467 on maintained allergic secretion, tracheal segments were challenged repeatedly with 200 μg ml^{-1} ovalbumin at each incubation period for 1.75 h. MEN 11467 or vehicle was present for the 1 h prior to challenge, and for the 15 min challenge period. MEN 11467 (or vehicle) was then removed and its effect examined in the continued presence of ovalbumin.

To investigate the selectivity of MEN 11467 for NK_1 receptor-induced secretion, the effect of the antagonist (1 and 10 μM) or vehicle was examined on secretion induced by submaximal concentrations of acetylcholine or histamine (1 μM each).

Data analysis

In Results, data are the arithmetic mean and one standard error of the mean (s.e.mean), with n values the number of animals. Because baseline d.p.m. displayed variability between tracheal segments, responses obtained from individual segments were converted to percentage changes in radiolabel output for the difference between response to drug or electrical stimulation and the preceding collection. The concentration of MEN 11467 causing a 50% inhibition (IC_{50}) of [Sar^9]SP-induced secretion was calculated by non-linear regression using GraphPad Prism software (Microsoft, San Diego, U.S.A.). Significance of changes in $^{35}SO_4$ output, pre- and post-stimulation (agonist, electrical or challenge), were assessed using the Mann-Whitney U-test between two groups, or the Kruskal-Wallis test followed by Dunns multiple comparison test for multiple groups. The null hypothesis was rejected at $P<0.05$ (two-tail).

Drugs and chemicals

The following drugs and chemicals were used (Sigma Chemical Co., Poole, Dorset, U.K., except where stated): acetylcholine chloride, aluminium hydroxide, atropine sulphate (Phoenix Pharmaceuticals Ltd., Pharma Hameln, G.m.b.H., Germany), chicken egg albumin (ovalbumin, grade III), dimethylsulphoxide (DMSO), histamine, pentobarbitone sodium B.P. (Sagatal; RMB Animal Health Ltd., Dagenham, Essex, U.K.), phentolamine mesylate (Ciba Laboratories, Horsham, West Sussex, U.K.), propranolol hydrochloride (Imperial Chemical Industries Ltd., Macclesfield, Cheshire, U.K.), saline (0.9% sodium chloride BP for intravenous infusion, Travenol Laboratories, Thetford; Norfolk, U.K.), [Sar^9,Met(O$_2$)^{11}]-substance P ([Sar^9]SP) (Bachem (UK) Ltd., Saffron Walden, Essex, U.K.), $Na_2{}^{35}SO_4$ (Amersham International plc, Bucks., U.K.). Solutions of acetylcholine, histamine and ovalbumin, dissolved in saline, were made fresh on each experimental day. [Sar^9]SP was dissolved in saline at a concentration of 3 mM, and aliquots were stored frozen at -20°C. MEN 11467 was supplied by Menarini Ricerche spa, Rome, Italy (courtesy Dr Stefano Manzini), and was kept at -20°C as stock solution aliquots of 1 mM in DMSO.

Results

Median baseline radioactivity was of the order of 700 d.p.m. (402 – 987 d.p.m., depending upon the specific experiment). There were no significant differences between treatment groups.

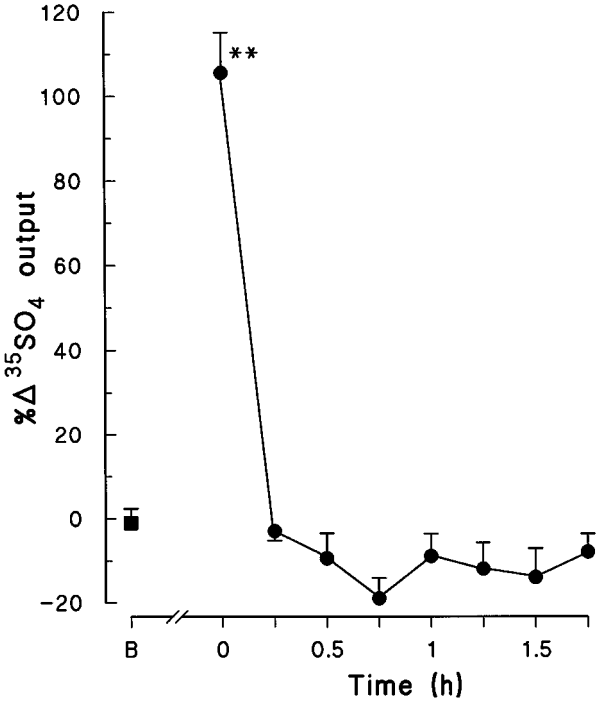

Figure 1 Time course of effect of a single administration of the selective tachykinin NK_1 receptor agonist [Sar^9,Met(O$_2$)^{11}]-substance P ([Sar^9]SP) on mucus secretion in ferret trachea *in vitro*. Tracheal tissues were incubated with [Sar^9]SP (1 μM) for 15 min (time 0 sample), after which [Sar^9]SP was absent. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for six animals per group. $^{**}P<0.01$ compared with baseline (B).

Effect of MEN 11467 on [Sar^9]SP-induced secretion

A single administration of [Sar^9]SP (present in the chambers for one 15 min incubation period) markedly increased $^{35}SO_4$ output (Figure 1). The increase in output lasted only for the one incubation period, returning to baseline by the following period and remaining there for the remainder of the experiment (1.5 h). In contrast, replenishment of [Sar^9]SP after each 15 min sample collection caused an initial increase in secretion (22 fold increase above controls), which was reduced by 48% after 30 min, and was maintained around this level for the remainder of the experiment (1.25 h) (Figure 2).

MEN 11467 (1 nM – 10 μM) inhibited [Sar^9]SP-induced $^{35}SO_4$, output in a concentration-dependent manner with an approximate IC_{50} of 0.3 μM (Figure 3). Inhibition was maximal at 10 μM MEN 11467, with secretion being reduced by 113% (i.e. to below baseline, albeit not significantly). Inhibition by MEN 11467 at 0.1, 1 and 10 μM (but not 1 or 10 nM) was maintained, to a greater or lesser extent, for the 1.75 h of the experiment: as an example, see Figure 2 for the inhibitory effect of 1 μM MEN 11467. Inhibition by MEN 11467 was maintained despite washout after the first 15 min of co-incubation with [Sar^9]SP. Neither 1 nor 10 μM MEN 11467 had any significant effect on baseline $^{35}SO_4$ output (Figures 2 and 3 respectively).

Effect of MEN 11467 on tachykininergic neurogenic secretion

In the presence of phentolamine, propranolol and atropine, electrical stimulation increased $^{35}SO_4$ output ~5 fold above sham stimulation controls, representing tachykininergic neurogenic secretion (Figure 4). MEN 11467 1 μM significantly inhibited tachykininergic neural secretion by 94%, without having any significant effect on $^{35}SO_4$ output in sham-stimulated controls (Figure 4).

Effect of MEN 11467 on ovalbumin-induced secretion in sensitized ferrets

Ovalbumin challenge (50 – 200 μg ml^{-1}) of tracheae from ovalbumin-sensitised ferrets increased $^{35}SO_4$ output in a concentration-dependent manner, with a ~4.5 fold increase above controls at 200 μg ml^{-1} (Figure 5). At the higher concentration of 400 μg ml^{-1} ovalbumin, there was no significant increase in secretion above controls, and this was associated with 'frothing' of the ovalbumin solution in the chamber. MEN 11467 (0.1 – 10 μM) inhibited ovalbumin-

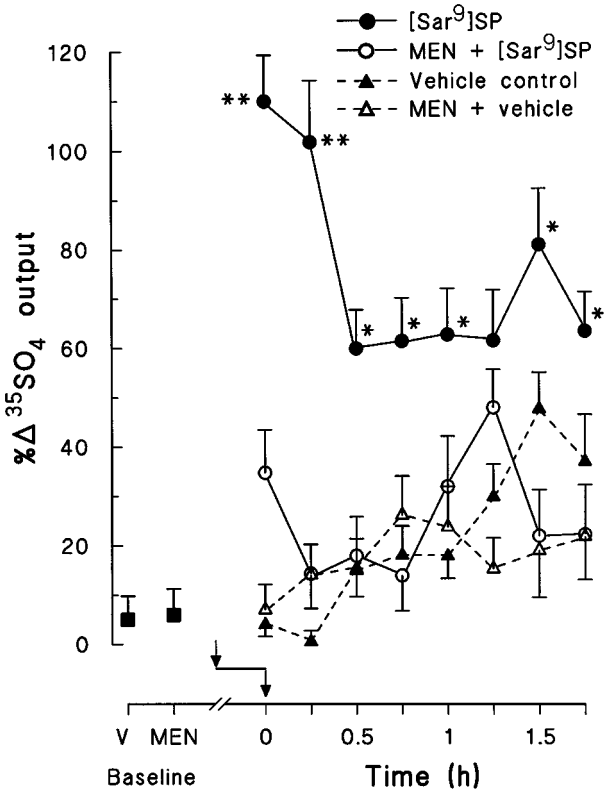

Figure 2 Inhibition by MEN 11467, a tachykinin NK_1 receptor antagonist, of [Sar^9]SP-maintained mucus secretion in ferret trachea *in vitro*. Tracheal tissues were incubated with [Sar^9]SP (1 μM) for 15 min periods with sample collections at the end of each period and replenishment with [Sar^9]SP. Tissues were pre-incubated with MEN 11467 (MEN; 1 μM) or vehicle (V) for 1 h prior to addition of [Sar^9]SP, and for the first 15 min incubation with [Sar^9]SP, after which it was absent (arrows and horizontal line). Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for 6 – 8 animals per group. $^*P<0.05$, $^{**}P<0.01$ compared with MEN 11467 at equivalent time point.

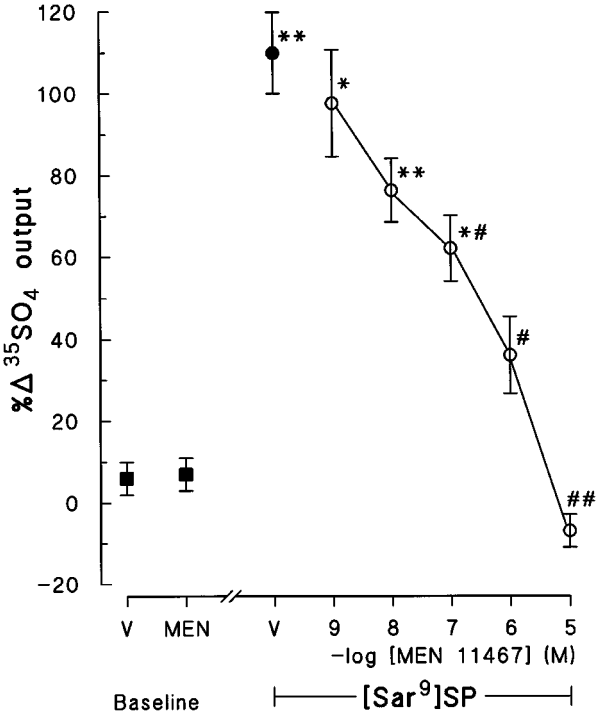

Figure 3 Inhibition by MEN 11467 of [Sar^9]SP-induced mucus secretion in ferret trachea *in vitro*. V = vehicle control; Baseline-MEN = effect of MEN 11467 (10 μM) on baseline secretion. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for 6 – 8 animals per group. $^*P<0.05$, $^{**}P<0.01$ compared with baseline vehicle control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with [Sar^9]SP vehicle control.

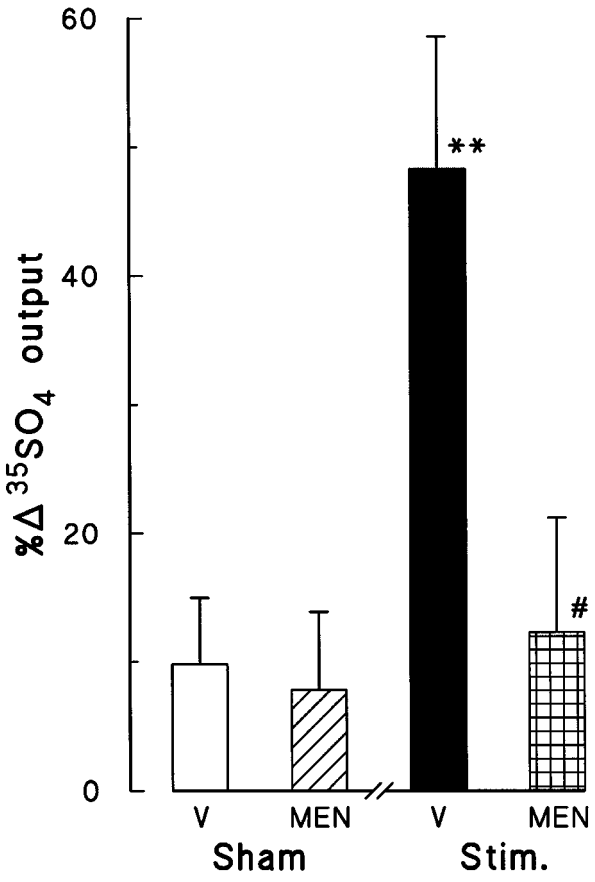

Figure 4 Inhibition by MEN 11467 of non-adrenergic, non-cholinergic (NANC) neurogenic mucus secretion in ferret trachea *in vitro*. Atropine, phentolamine and propranolol (APP, 10 μM each) were used to exclude cholinergic and adrenergic neural influences at stimulation parameters of 10 Hz, 50 V, 0.5 msec for 5 min (Stim.). Tracheal segments were incubated with MEN 11467 (1 μM) or vehicle (V) for 1 h prior to electrical stimulation. 'Sham': same time-point as stimulated secretion, but without electrical stimulation. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for eight animals per group. $^{**}P<0.01$ compared with vehicle + -sham; $^{\#}P<0.05$ compared with vehicle + stim.

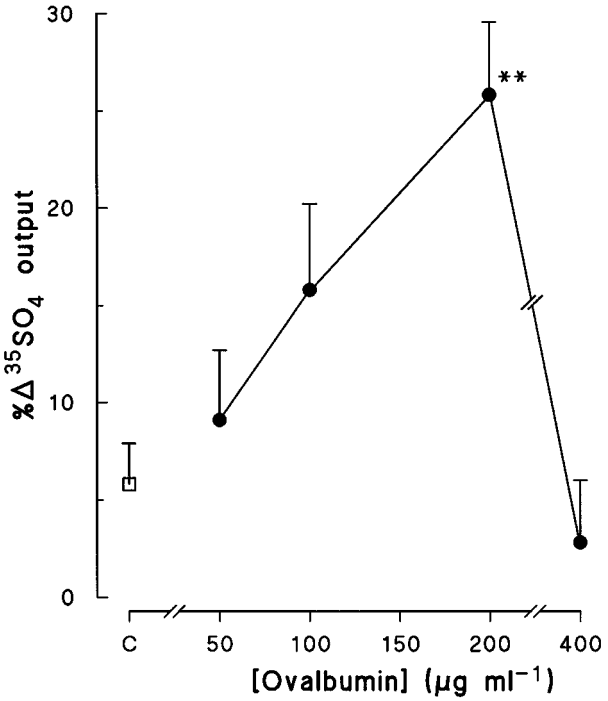

Figure 5 Ovalbumin-induced mucus secretion *in vitro* in tracheae from ovalbumin-sensitized ferrets. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for 7–9 animals per group ($n=3$ for 400 μg ml^{-1}). $^{**}P<0.01$ compared with control (C).

induced $^{35}SO_4$ output in a concentration-dependent manner with complete inhibition at 1 μM (Figure 6). At 10 μM, MEN 11467 reduced ovalbumin-induced secretion below baseline (129% inhibition), albeit not significantly. This same concentration of MEN 11467 had no significant effect on baseline $^{35}SO_4$ output (Figure 6).

Secretion induced by repeated replenishment of the chambers with 200 μg ml^{-1} ovalbumin was maintained for up to 1.5 h above vehicle control values (Figure 7). Inhibition by MEN 11467 at 1 and 10 μM (but not 0.1 μM) was maintained, to a greater or lesser extent, for the 1.5 h of ovalbumin stimulation: as an example, see Figure 7 for the inhibitory effect of 1 μM MEN 11467. Inhibition by MEN 11467 was maintained despite washout after the first 15 min of co-incubation with antigen. MEN 11467 1 μM had no significant effect on baseline $^{35}SO_4$ output (Figure 7).

Effect of MEN 11467 on agonist-induced secretion

Acetylcholine or histamine increased $^{35}SO_4$ output $\sim$5 fold above controls (Figure 8). The absolute increase above baseline ($\sim$25%) was matched to that induced by ovalbumin challenge (23%; see Figures 6 and 7). MEN 11467 1 μM did not significantly affect either ACh- or histamine-induced output (Figure 8). In contrast, at 10 μM, MEN 11467 significantly inhibited ACh-induced secretion by 97% and histamine-induced secretion by 56% (Figure 8).

Discussion

In the present study in ferret trachea *in vitro*, MEN 11467 inhibited mucus secretion induced by two stimulations that in this preparation are associated with selective activation of tachykinin NK_1 receptors, namely [Sar^9]SP-induced secretion (Meini *et al*., 1993) and electrical stimulation with adrenoceptor and cholinoceptor blockade (Ramnarine *et al*., 1994; Khawaja *et al*., 1999). Thus, it would appear that MEN 11467 is a tachykinin NK_1 receptor antagonist in this preparation. This contention is supported by the observation herein that MEN 11467, at 1 μM, did not inhibit secretion induced either by acetylcholine or histamine. In contrast, at 10 μM, the highest concentration used, MEN 11467 did inhibit both acetylcholine- and histamine-induced secretion. The later observation indicates that at this concentration MEN 11467 either acts as an antagonist at both muscarinic and histamine receptors, or has non-selective anti-secretory

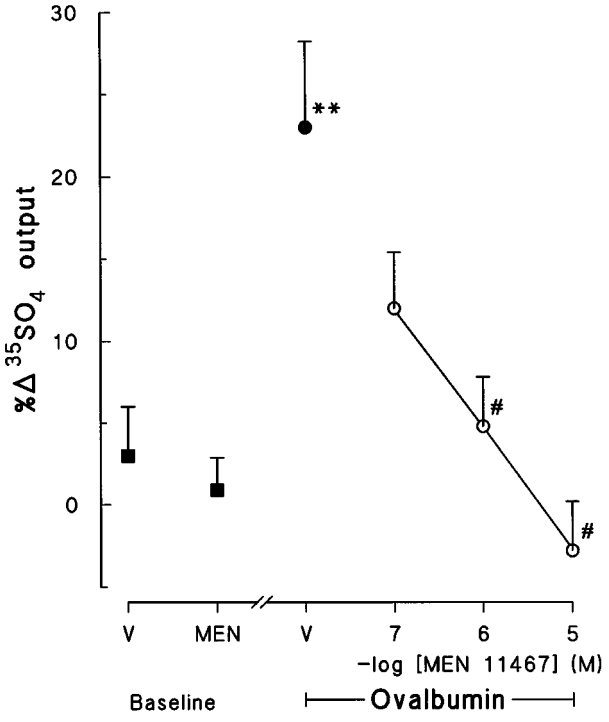

Figure 6 Inhibition by MEN 11467 of ovalbumin-induced mucus secretion *in vitro* in tracheae from ovalbumin-sensitized ferrets. Tracheal segments were incubated with MEN 11467 or vehicle (V) for 1 h prior to ovalbumin (200 μg ml^{-1}). Baseline-MEN = effect of MEN 11467 (10 μM) on baseline secretion. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for 7–9 animals per group ($n=3$ for 400 μg ml^{-1}). $^{**}P<0.01$ vehicle baseline; $^{\#}P<0.05$ compared with vehicle + ovalbumin.

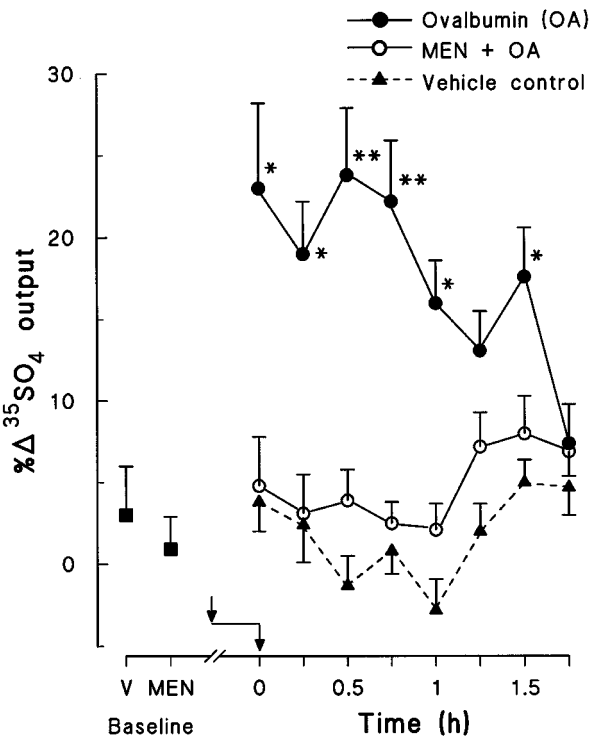

Figure 7 Inhibition by MEN 11467 of ovalbumin-maintained mucus secretion *in vitro* in tracheae from ovalbumin-sensitized ferrets. Tracheal tissues were incubated with ovalbumin (200 μg ml^{-1}) for 15 min periods with sample collections at the end of each period and replenishment with ovalbumin. Tissues were pre-incubated with MEN 11467 (MEN; 1 μM) or vehicle (V) for 1 h prior to addition of ovalbumin, and for the first 15 min incubation with ovalbumin, after which it was absent (arrows and horizontal line). Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for 5–6 animals per group. $^{*}P<0.05$, $^{**}P<0.01$ compared with MEN 11467 at equivalent time point.

activity, or a combination of the two. However, it should be noted that at concentrations of 1 μM and below, MEN 11467 is selective and effectively inhibits tachykininergic responses in the present experimental system. The IC_{50} of 0.3 μM for inhibition by MEN 11467 of [Sar^9]SP-induced secretion is somewhat less than that of 1 μM for inhibition of substance P-induced secretion by the structurally related peptide NK_1 receptor antagonist, FK888 (Ramnarine *et al*., 1994). This is consistent with data in a human astrocytoma cell line whereby MEN 11467 was considerably more potent than FK888 in inhibiting substance P-induced inositol monophosphate accumulation and interleukin-6 release (Palma *et al*., 1999). Of particular note in the present study was the long duration of action of MEN 11467 after wash-out. We found that MEN 11467 retained inhibitory activity for at least 1.5 h after removal from the incubation solution, despite continued stimulation with [Sar^9]SP or challenge with ovalbumin. This is consistent with a previous observation of continued inhibition by MEN 11467 of *in vitro* substance P-induced inositol monophosphate accumulation for up to 24 h after removal from the incubation medium (Palma *et al*., 1999). In contrast, in the latter study, FK888 activity was rapidly and completely lost after removal from the medium. The long duration of action of MEN 11467 is most likely due to its behaviour as an insurmountable antagonist, demonstrated in binding studies using membrane preparations (Cirillo *et al*., 1998) and whole cells (Palma *et al*., 1999). This form of antagonism can explain the prolonged inhibition of tachykininergic-induced mucus output following drug washout.

In the present study, inhibition by MEN 11467 of ovalbumin-induced mucus secretion is novel. Mucus secretion *in vitro* in response to allergen challenge of airway tissue from allergic animals has been demonstrated previously (for example see Liu *et al*., 1998b). In the latter study, in guinea-pig trachea, ovalbumin challenge increased mucus output in a concentration-dependent manner with a maximal effect at 200 μg ml^{-1} and a reduced effect at a higher concentration. Interestingly, the exact same phenomenon was observed herein in ferret trachea. 'Frothing' of the incubation medium with the higher concentrations indicates denaturation of the ovalbumin, possibly leading to a reduced final concentration in the baths and consequent reduced response to challenge. In the present study, repeated challenge with ovalbumin increased secretion and, although becoming reduced, was maintained at an elevated level for 1.5–1.75 h. Activation of sensory nerves, release of tachykinins and their interaction with tachykinin receptors are involved in a number of allergic systems in guinea-pigs, for example antigen-induced bronchospasm (Manzini *et al*., 1987; Bertrand *et al*., 1993b), bronchial hyperreactivity (Schuiling *et*

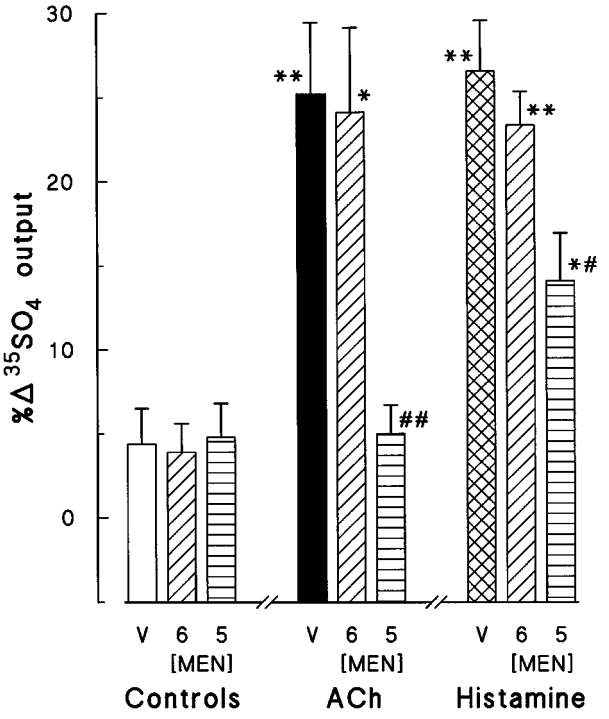

Figure 8 Effect of MEN 11467 (1 μM) on mucus secretion induced by acetylcholine (ACh) or histamine (1 μM each) in ferret trachea *in vitro*. V = vehicle controls. Data are mean per cent change (vertical bars = s.e.mean) in output of macromolecules labelled *in situ* with $^{35}SO_4$ (a marker for mucus) for six animals per group. $^{*}P<0.05$, $^{**}P<0.01$ compared with vehicle control; $^{\#}P<0.05$, $^{\#\#}P<0.05$ compared with vehicle + agonist.

al., 1999) and plasma exudation (Bertrand *et al*., 1993a). The bronchoconstrictor response is mediated *via* NK_1 and NK_2 receptors, with hyperreactivity and exudation mediated *via* NK_1 receptors. Thus, the present data and previous reports support a role for allergen-induced release of tachykinins and their subsequent interaction with NK_1 receptors in inducing a variety of airway responses, including mucus secretion.

Inhibition of tachykininergic mucus secretion has clinical implications. For example, although to date substantially unproven, tachykinins have been implicated in the pathophysiology of asthma and COPD (Rogers, 1997; Barnes, 1998), and in the development of bronchial hyperresponsiveness, an important clinical feature of asthma (Spina *et al*., 1998). In both asthma and COPD, airway mucus hypersecretion is a significant component of morbidity and mortality (Liu *et al*., 1998a; Rogers, 2000b). Inhibition of neurogenic mucus secretion is, therefore, a valid therapeutic target. Inhalation of the dual NK_1/NK_2 receptor antagonist FK 224 reduces cough and sputum production in patients with chronic bronchitis (Ichinose *et al*., 1993). Because FK 224 does not block NKA-induced bronchoconstriction in asthmatic subjects (Joos *et al*., 1996), its inhibitory effects on sputum production may be due to activity at NK_1 receptors. Formal clinical studies of the effects of tachykinin NK_1 receptor antagonists such as MEN 11467 on mucus secretion in asthma and COPD are, therefore, indicated. Determination of appropriate end-point markers for mucus hypersecretion will be a clinical challenge.

In summary, in ferret trachea *in vitro* MEN 11467, at concentrations of 0.1 and 1 μM, is a selective tachykinin NK_1 receptor antagonist with a long duration of action that effectively inhibits tachykininergic mucus secretion elicited by direct neural stimulation and by allergen challenge. Long-acting NK_1 receptor antagonists merit formal clinical investigation as inhibitors of mucus hypersecretion in airway conditions in which tachykininergic mechanisms have been implicated in pathophysiology, including asthma and COPD.

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