Br. J. Pharmacol. (1994), **113**, 1183–1190 © Macmillan Press Ltd, 1994

'Sensory-efferent' neural control of mucus secretion: characterization using tachykinin receptor antagonists in ferret trachea *in vitro*

Sean I. Ramnarine, Yoshitaka Hirayama, Peter J. Barnes & [1]Duncan F. Rogers

Department of Thoracic Medicine, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY

1 We characterized the tachykinin receptor(s) mediating 'sensory-efferent' neural control of release of $^{35}SO_4$-labelled macromolecules (mucus) from ferret trachea *in vitro* in Ussing chambers using selective tachykinin antagonists. Secretion was induced by substance P (SP), neurokinin A (NKA), capsaicin, the NK_1 tachykinin receptor agonist [Sar^9, $Met(O_2)^{11}$]substance P ([Sar^9]SP), or acetylcholine (ACh), or by electrical stimulation of nerves. Antagonists used were FK888 and L-668,169, selective for the NK_1 receptor, SR 48968, selective for the NK_2 receptor, and FK224, a dual antagonist at NK_1 and NK_2 receptors. The selectivity of FK888 and SR 48968 was examined on NKA-induced contraction of ferret tracheal smooth muscle *in vitro*.

2 SP (1 μM) increased mucus secretion by 695% above vehicle controls. FK888 (0.1 μM–30 μM) inhibited SP-induced secretion in a dose-dependent manner, with complete inhibition at 10 μM and an IC_{50} of 1 μM. L-668,169 (1 μM) also completely inhibited SP-induced secretion.

3 NKA (1 μM) significantly increased mucus secretion by 271% above baseline, a response which was completely inhibited by FK888 (10 μM) or L-668,169 (μM). Secretion induced by ACh (10 μM: 317% above baseline) was not inhibited by FK888 but was inhibited by atropine. Capsaicin (10 μM)-induced secretion (456% above vehicle controls) was significantly inhibited by FK888 and by L-668,169 (111% and 103% inhibition respectively).

4 Electrical stimulation (50 V, 10 Hz, 0.5 ms, 5 min) increased mucus output above baseline (increased by 12 to 26 fold), a response blocked by tetrodotoxin (0.1 μM). FK888 (10 μM) inhibited the increase in secretion due to electrical stimulation by 47%. Atropine, propranolol and phentolamine in combination (APP) inhibited the response to electrical stimulation by 48%. The remaining NANC response, i.e. in the presence of APP, was further reduced by 66% with FK888. FK224 (10 μM) inhibited neurally-evoked secretion by 73%. SR 48968 (0.1 μM) had no effect on electrically-stimulated or [Sar^9]SP-induced secretion.

5 NKA (10 nM–10 μM: in the presence of DMSO control vehicle) induced tracheal smooth muscle contraction in a concentration-dependent manner with a maximal contraction of 30% of the maximal response to ACh (10 mM) and an EC_{50} of 0.3 μM. SR 48968 (0.1 μM in DMSO) inhibited the NKA-induced contraction whereas FK888 did not. Neither antagonist had any inhibitory effect on ACh-induced contraction.

6 We conclude that 'sensory-efferent' neurogenic mucus secretion in ferret trachea *in vitro* is mediated via tachykinin NK_1 receptors with no involvement of NK_2 receptors. Potent and selective tachykinin antagonists may have therapeutic potential in bronchial diseases such as asthma and chronic bronchitis in which neurogenic mucus hypersecretion may be aetiologically important.

Keywords: NK_1 receptors; NK_2 receptors; FK888; FK224; SR 48968; mucus secretion; tachykinin antagonists; tachykinin receptors; mucus; sensory nerves

Introduction

Secretion of high molecular weight mucous glycoproteins (mucus) onto the internal surface of the airways is a protective mechanism which can be induced by irritants and by humoral and neuronal mechanisms. Electrical stimulation of nerves both *in vivo* and *in vitro* induces mucus secretion in the airways of a number of animal species and a proportion of the secretion persists despite adrenoceptor and cholinoceptor blockade (Peatfield & Richardson, 1983; Borson *et al.*, 1984; Fung *et al.*, 1992a,b). Two principle neural pathways mediate the latter non-adrenergic, non-cholinergic (NANC) component of neurogenic secretion (Ramnarine & Rogers, 1994). The first pathway comprises an orthodromic system of adrenergic and cholinergic nerves which contain neuropeptides colocalized with the classical neurotransmitter including vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY), both of which may increase or decrease mucus secretion or modulate stimulus-evoked secretion, depending upon preparation. The second pathway comprises a system of capsaicin-sensitive nerves which subserve a motor function and may be termed 'sensory-efferent' nerves (Maggi & Meli, 1987). The neurotransmitters of the latter neural system are collectively termed sensory neuropeptides and include calcitonin gene-related peptide (CGRP) and the tachykinins substance P (SP) and neurokinin A (NKA) and NKB. Of these, the tachykinins elicit significant mucus secretion (Lundgren *et al.*, 1989; Mizoguchi & Hicks, 1989; Rogers *et al.*, 1989; Webber, 1989; Gentry, 1991), whereas CGRP is a weak secretagogue (Webber *et al.*, 1991). In the absence of selective receptor antagonists for VIP or NPY, the relevance of these peptides to NANC neural control of mucus secretion is difficult to assess. In contrast, potent and selective tachykinin receptor antagonists are now available, and we investigated a number of these in the present study.

Currently three classes of tachykinin receptor are recognised and are designated NK_1, NK_2 and NK_3 according to

[1] Author for correspondence.

their preferential affinities for SP, NKA and NKB respectively (Guard & Watson, 1991). The order of potency of the natural tachykinins (i.e. SP > NKA > NKB: Lundgren *et al.*, 1989; Mizoguchi & Hicks, 1989; Rogers *et al.*, 1989; Webber, 1989; Gentry, 1991), and of synthetic agonists selective for the three classes of tachykinin receptor (Geppetti *et al.*, 1993; Meini *et al.*, 1993) in inducing mucus secretion indicate that tachykinin NK_1 receptors mediate tachykinin-induced secretion. However, the relevance of tachykinins to control of mucus secretion induced by stimulation of sensory-efferent nerves and the tachykinin receptor(s) involved in the response is not reported. Consequently, in the present study we determined the effects of selective tachykinin antagonists not only on agonist-induced secretion, but more particularly on secretion induced by electrical stimulation of nerves. We used the selective NK_1 receptor antagonists, FK888 (Fujii *et al.*, 1992; Hirayama *et al.*, 1993) and L-668,169 (McKnight *et al.*, 1988; Williams *et al.*, 1988), the NK_2 receptor antagonist SR 48968 (Advenier *et al.*, 1992a,b; Edmonds-Alt *et al.*, 1992; Martin *et al.*, 1992), and the dual NK_1/NK_2 receptor antagonist FK224 (Morimoto *et al.*, 1992; Murai *et al.*, 1992; Hirayama *et al.*, 1993). We used the ferret trachea to study sensory-efferent neural control of mucus secretion because: (a) mucus secretion can be induced in this preparation via NANC neural mechanisms (Borson *et al.*, 1984); (b) the source of mucus is identifiable because the trachea of this species contains abundant submucosal glands and very few epithelial goblet cells (Robinson *et al.*, 1986; Meini *et al.*, 1993); (c) the ferret trachea is supplied by axons containing sensory neuropeptides (Luts & Sundler, 1989); and (d) the airway submucosal glands express SP receptors (Meini *et al.*, 1993). We used $^{35}SO_4$ as the mucus label, because it is a marker predominately for tracheal submucosal gland mucous glycoprotein in the ferret (Gashi *et al.*, 1987). The selectivity of FK888 and SR 48968 in the ferret was investigated by determining their activity on NKA-induced tracheal smooth muscle contraction *in vitro*, a preparation which synthetic agonists indicate as essentially an NK_2 receptor system (Geppetti *et al*, 1993; Meini *et al.*, 1993). The selectivity of SR 48968 was further investigated against secretion induced by $[Sar^9Met(O_2)^{11}]$ substance P ($[Sar^9]$), a synthetic agonist selective for the NK_1 receptor (Maggi *et al.*, 1990) which induces secretion in the ferret trachea (Meini *et al.*, 1993). Some of these results have been published in abstract form (Ramnarine *et al.*, 1993).

Methods

Tissue preparation for secretory studies

Male ferrets (Regal Rabbits, Great Bookham, Surrey) weighing 1.0–2.0 kg were anaesthetized with pentobarbitone sodium (Sagatal: 60 mg kg^{-1}, i.p.). The tracheae were removed, 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, $NaHCO_2$ 25.5 and glucose 5.05, and cut longitudinally through the dorsal membrane, opened flat and cut transversely to give four segments. Each segment 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. The chambers were rectangular in cross-section to ensure optimal use of the tissue. The exposed surface area of each segment was 1.12 cm^2. Each side of the tissue was bathed with 9 ml warmed (37°C) Krebs-Henseleit solution which was oxygenated (95% O_2:5% CO_2) and circulated with gas-lift pumps. The tracheal segments could be subjected to an electrical current (not alternating in polarity) to stimulate excitable tissues (e.g. nerves). Two pins piercing the tissue on either side were connected via outlet wires through the chambers to a Grass stimulator (model S88; Grass Instruments, Quincy, U.S.A.) (Borson *et al.*, 1984). Tissues were stimulated at 10 Hz, 50 V and 0.5 ms for the first 5 min of a 15 min collection period.

We have previously established optimal conditions under which collections are taken in order to define baseline secretion and to maximize detection of the secretory response to drug addition (Meini *et al.*, 1993). At time 0 h, $Na_2{}^{35}SO_4$ (0.1 mCi; Amersham International plc, Aylesbury, Bucks.) was added to the submucosal half-chambers, in order to label newly-synthesised 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 achieved by taking four 30 min collections followed by two 15 min collections (i.e. over 2.5 h following addition of radiolabel). After stabilisation, drugs or control solutions were added and the tissues were electrically stimulated (see *Protocols for secretory studies* below).

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

Luminal fluid, approximately 9 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 exhaustively dialysed against distilled water containing excess Na_2SO_4 and sodium azide (10 mg l^{-1}) using cellulose tubing (Medicell International Ltd., London) which allowed molecules of 12000–14000 Da or less to pass through. The sodium azide was used to limit bacterial contamination. 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 (Ultima Gold XR, Canberra Packard Ltd., Pangbourne, Berks.) was determined by scintillation spectometry (model 1900CA Spectrophotometer, Canberra Packard Ltd.). 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 for secretory studies

To examine the effects of antagonists on baseline and stimulated secretion, drugs were added to the luminal half chambers for varying times before stimulation: atropine, phentolamine and propranolol (APP) in combination (all 10 μM) for 45 min (i.e. present for three 15 min collections), SR 48968 (0.1 μM) for 45 min prior, and FK888 (0.1 μM–30 μM), FK224 (10 μM) or L-668,169 (1 μM) for 30 min prior to stimulation (i.e. present for two 15 min collections). Antagonists were also present in the chambers during the period of stimulation.

We have found previously that there is no significant tachyphylaxis of secretion to consecutive additions of SP and methacholine when additions are separated by 1.25 h (Meini *et al.*, 1993). In the present study we determined whether there was tachyphylaxis between consecutive electrical stimulations. We found that using at least a 1 h interval there was no significant difference in magnitude of secretory response to consecutive stimulations: mean increase (and s.e.mean) above baseline in radiolabel output for the first stimulation was 106.1% ± 52.6% (n = 8) and for the second stimulation was 118.9% ± 38.8% (n = 8). Thus, for the four chambers, eight possible combinations of stimulations could be carried for each experiment.

The effect of FK888 or L-668,169 on SP-induced secretion was tested to establish inhibitory concentrations for the antagonists. At time 3 h, SP was added at a final concentra-

tion of 1 μM to tissues pretreated with either FK888 (0.1 μM–30 μM), L-668,169 (1 μM), or their vehicle, dimethylsulphoxide (DMSO), the latter given at a concentration equivalent to the highest concentration of antagonist used. Collections were made so that data for baseline secretion, the effect of DMSO, FK888 or L-668,169 on baseline, the effect of SP, and the effects of DMSO, FK888 or L-668,169 on the response to SP were assessed.

The effects of single concentrations of FK888 (10 μM) or L-668,169 (1 μM) on secretion induced by NKA (1 μM) or capsaicin (10 μM) were also examined, as were those of FK888 and atropine (10 μM) on ACh-evoked mucus secretion. In addition, the effect of SR 48968 (0.1 μM) on secretion induced by [Sar^9]SP (10 μM) was determined.

Single concentrations of FK888 or SR 48968 upon secretion induced by electrical stimulation of the tissues was investigated. The effect of each tachykinin antagonist was investigated on secretion induced both in the absence of autonomic receptor blockade and in the presence of autonomic blockade by APP (the latter representing the remaining NANC neural response). Responses to six combinations of treatment were obtained, namely (1) sham stimulation (i.e. collections in the absence of electrical stimulation but at the same time-point as other tissues were electrically stimulated); (2) electrical stimulation alone (i.e. without antagonist or vehicle, DMSO); (3) electrical stimulation with antagonist vehicle (i.e. to determine the effect of vehicle on the magnitude of the secretory response); (4) electrical stimulation with FK888 or SR 48968 (i.e. to determine the effect of the antagonists on the total secretory response to cholinergic, adrenergic and NANC stimulation); (5) electrical stimulation with APP (to determine the magnitude of the NANC neurogenic component of secretion); and (6) electrical stimulation with APP and either FK888 or SR 48968 (to determine how much of the NANC response is reduced by these antagonists). The neural composition of the electrically-induced increase in secretion was investigated by use of tetrodotoxin, 0.1 μM given for 45 min before stimulation.

Contractility studies

Studies of smooth muscle contraction in ferret tracheal strips were performed in order to assess the specificity of SR 48968 and FK888. Ferret tracheae were cut longitudinally through the cartilage and then cut transversely into 3–4 mm segments (approximately 6 cartilage rings). On each day of experimentation, three segments from each of two animals were suspended from isometric force transducers connected to a six channel polygraph (model 7D, Grass Instruments Co., Quincy, U.S.A.) in 5 ml organ baths containing warmed (37°C), aerated (95% O_2 + 5% CO_2) Krebs-Henseleit solution (composition as for secretory studies) containing 10 μM indomethacin to inhibit the effects on tracheal tension of spontaneously-released prostaglandins. The polygraph was calibrated to record a 4 g resting tension on the segments for equilibration during which time the Krebs solution was changed every 5–10 min until a stable baseline was established (approximately 1 h). A supramaximal dose of ACh (10 mM) was added to all tissues to establish tissue viability and responsiveness. Once the contraction peak to ACh had plateaued the tissues were washed repeatedly until baseline tension was restored. Following this a cumulative concentration-response to NKA (10 μM–10 mM) for smooth muscle contraction was carried out, with each sequential addition of the tachykinin added after the effect of the previous addition had plateaued. Tissues were then washed again until baseline tension was restored after which they were incubated with either drug vehicle (DMSO) or the tachykinin antagonists before carrying out a second dose-response to NKA (in the same concentration-range as used in the initial dose-response determination). Thus, the effect of vehicle or tachykinin antagonist on NKA-induced tracheal contraction was investigated for each animal. Incubation with DMSO or FK888 was for 45 min before NKA. With SR 48968, preincubation was for either 120 min ($n = 2$) or 45 min ($n = 4$). Where incubation with SR 48968 was for 120 min before the second administration of NKA, the two remaining tracheal strips from that animal were left untreated for the additional 75 min before administration of either DMSO or FK888. Finally, ACh (10 mM) was added again to the tissues to assess the responsiveness of the tissue at the end of the experiment compared with the initial response to ACh.

Drugs

The following drugs were used: SP, NKA, capsaicin, indomethacin, tetrodotoxin and DMSO (Sigma Chemical Company Ltd., Poole, Dorset), atropine sulphate (Phoenix Pharmaceuticals Ltd., Pharma Hameln, G.m.b.H., Germany), phentolamine mesylate (Ciba Laboratories, Horsham, West Sussex), propranolol hydrochloride (Imperial Chemical Industries Ltd., Macclesfield, Cheshire), pentobarbitone sodium B.P. (Sagatal; RMB Animal Health Ltd., Dagenham, Essex), [Sar^9]SP (Bachem (UK) Ltd., Saffron Waden, Essex).

Tachykinin receptor antagonists were kind gifts from the Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan (FK888 and FK224), Cambridge Research Biochemicals Limited, Northwich, Cheshire (L-668,169), and Sanofi Recherche, Montpellier, France (SR 48968). FK888 is a synthetic dipeptide with the structure N^2-[(4R)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-benzyl-*N*-methyl-*N*-phenylmethyl-3-(2-napthyl)-L-alaninamide). FK224 is a modified cyclic peptide with the structure N-(N^2-(*N*-(*N*-(*N*-(2,3-didehydro-*N*-methyl-*N*-(*N*-(3-(2-penthlyphenyl)-propionyl)-L-threonyl]tyrosyl-L-leucynyl]-D-phenylalanyl)-L-*allo*-threonyl)-L-asparaginyl-L-serine-ν-lactone. L-668,169 is a cyclic dimer: cyclo(Gln-D-Trp(NMe)Phe(R)Gly[ANC-2]LeuMet)$_2$. SR 48968 is a non-peptide with the structure (S)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl] benzamide.

FK888, FK224 and L-668,169 were dissolved in DMSO (stock solutions of 10 mM), SR 48968 was dissolved in distilled water (stock of 100 μM), and SP, NKA, ACh and [Sar^9]SP were dissolved in distilled water (stock solutions of 10 mM). These stock solutions were divided into aliquots, stored at − 20°C. Capsaicin was dissolved in ethanol (stock solution of 10 mg ml^{-1}), stored at 4°C. All stock solutions were diluted in Krebs-Henseleit on each day of experimentation. Indomethacin was prepared in phosphate buffer (pH 7.8) of composition: 20 mM KH_2PO_4 and 120 mM Na_2HPO_4.

Data analysis

Data in Results are the arithmetic mean and one standard error of the mean (s.e.mean), with *n*-values being the number of animals. Responses obtained from individual tissue segments were calculated to give percentage changes in radiolabel output for the differences between response to drug or electrical stimulation and the preceding collection. The latter are presented in Results as mean ± s.e.mean, whereas changes compared with another group are given only as a percentage. Drug-induced changes in tension were calculated as a percentage of the contraction to ACh (10 mM). The concentration of antagonist causing 50% inhibition (IC_{50}) in secretion or contraction was calculated by non-linear regression. Significance of changes in secretion pre and post-drug or electrical stimulation were assessed with the Wilcoxon sign-rank sum test. The significance of differences between groups were assessed by the Mann-Whitney U-test. The null hypothesis was rejected at $P<0.05$ (two-tail). Inhibition of stimulated secretion by an antagonist was considered complete when the value for stimulations with

antagonist were significantly different from stimulations without antagonist and were not significantly different from the value for sham stimulation.

Results

Effects of tachykinin NK_1 antagonists on SP-induced secretion

Median baseline radioactivity in this series of experiments was 51 d.p.m. (range 10–57 d.p.m.; $n = 14$) and median SP-stimulated radioactivity was 184 d.p.m. (range 57–382 d.p.m.; $n = 5$). SP (1 μM) increased radiolabel output by 695% above vehicle controls (Figure 1). FK888 inhibited this response in a concentration-dependent manner with an IC_{50} of 1 μM ($pIC_{50} = 6$), and abolished the secretory response at concentrations 3 μM and greater (Figure 1). FK888 alone caused minimal changes in basal secretion (range of − 4% to + 18% change in radiolabel output compared to baseline at 0.1 μM–3 μM FK888) which were not significantly different from the response to DMSO (7.5% ± 3.7% increase). L-688,169 (10 μM) had no significant effect on baseline secretion (increased by 9.0% ± 10.8%, $n = 5$) but inhibited SP-induced secretion by 125% (i.e. reduced below baseline values: baseline value was a 9.0% ± 10.4% increase above previous collection, $n = 8$; response to SP was 58.0% ± 9.7% increase above previous collection, $n = 8$, L-668,169 reduced the response to SP to − 14.5% ± 15.1% below baseline value, in separate tracheal segments).

Effects of NK_1 antagonists on NKA-, acetycholine- or capsaicin-induced secretion

Median baseline radioactivity in this series of experiments was 47 d.p.m. (range 7–143 d.p.m.; $n = 13$). Median stimulated radioactivity was similar in all three agonist groups (combined median of 88 d.p.m., range 42–382 d.p.m.; $n = 16$). The DMSO vehicle increased secretion by 15% ± 9% above baseline, although this was not significant ($P = 0.14$; Figure 2). NKA (1 μM), capsaicin (10 μM) or ACh (10 μM) induced significant increases above vehicle controls in radiolabel output (271%, 456% and 317% increases respectively) (Figure 2). FK888 (10 μM) completely inhibited NKA-induced and capsaicin-induced increases in secretion (by 103% and 111% respectively), whereas the ACh-evoked secretion was not significantly inhibited by FK888 (reduced by 8%: Figure 2). Increase in radiolabel output due to ACh was significantly inhibited by atropine (by 87%). L-668,169 also significantly ($P < 0.05$) inhibited the secretion evoked by both NKA and capsaicin by 107% and 103% for each stimulant respectively ($n = 5$, for NKA, and $n = 6$).

Effects of tachykinin antagonists on secretion induced by electrical stimulation

The magnitude of the response to electrical stimulation differed by 127% between the two series of experiments. Median baseline radioactivity in the first series of experiments was 146 d.p.m. (range 52–330 d.p.m.; $n = 7$) which was not significantly different ($P = 0.6$) from that of 69 d.p.m. (range 7–378 d.p.m.; $n = 8$) in the second series of experiments. The median stimulated radioactivity was 402 d.p.m. (range 67–624 d.p.m.; $n = 7$) in the first series of experiments and 134 d.p.m. (range 31–443 d.p.m.; $n = 8$) in the second series of experiments.

In the first series of experiments (Figure 3), electrical stimulation of the tissue at 50 V, 10 Hz, 0.5 ms for 5 min resulted in a 26 fold increase in radiolabel output over sham-stimulated values. The antagonist vehicle (DMSO) had no significant effect on the stimulated increase in radiolabel output. Adrenoceptor and cholinoceptor blockade with APP

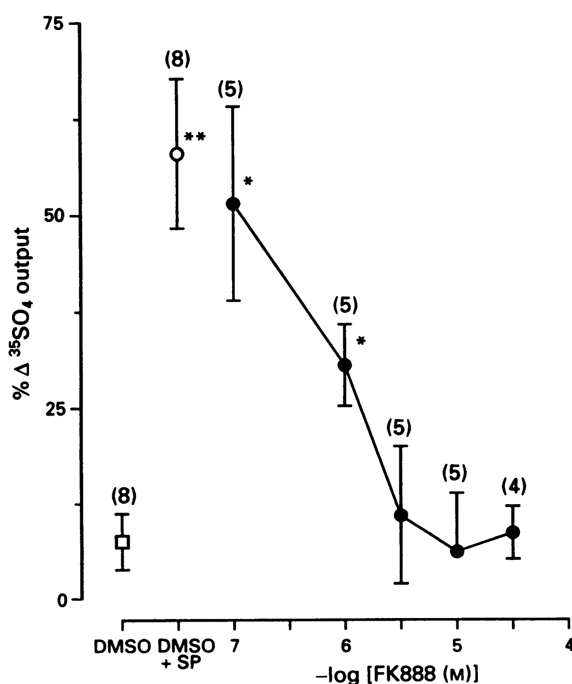

Figure 1 Effect of a tachykinin NK_1 receptor antagonist, FK888 (●), on mucus secretion induced by substance P (SP: 1 μM; O) in ferret trachea *in vitro*. Data are mean percentage changes in output of macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus) for the number of animals indicated in parentheses for each group; s.e.mean are shown. *$P < 0.05$, **$P < 0.01$ compared with DMSO group (dimethylsulphoxide, the vehicle for FK888, □).

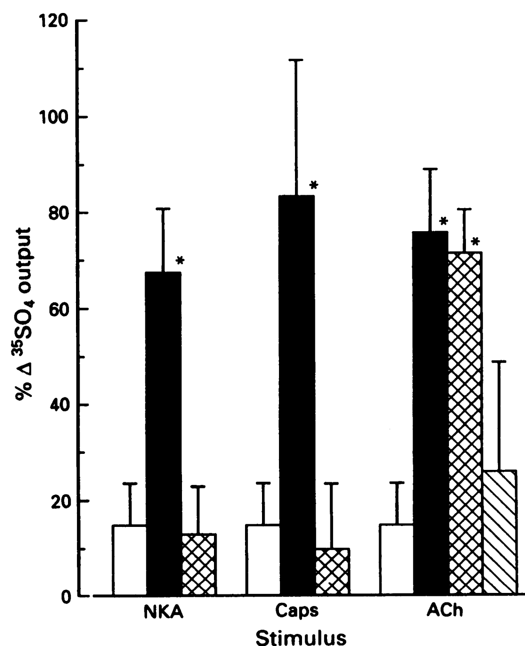

Figure 2 Effects of a tachykinin NK_1 receptor antagonist, FK888 (10 μM), on mucus secretion induced *in vitro* in ferret trachea by neurokinin A (NKA, 1 μM), capsaicin (Caps. 1 μM) or acetylcholine (ACh 10 μM). Data are mean percentage change in output of macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus); s.e.mean are shown. Open columns: DMSO (vehicle for FK888; $n = 11$ for all three sets); solid colums: DMSO = mucus stimulant ($n = 5$ for NKA and ACh, $n = 6$ for capsaicin); cross-hatched columns: effect of FK888 on stimulated secretion ($n = 5$ for NKA and ACh, $n = 6$ for capsaicin); hatched column: atropine (10 μM) + ACh ($n = 5$). *$P < 0.05$ compared with DMSO group.

in combination significantly though partially inhibited the latter increase in secretion by 48% leaving 52% of the response due to NANC innervation. FK888 (10 μM) alone significantly inhibited the total neurogenic response by 47%. In combination with adrenoceptor and cholinoceptor blockade, FK888 significantly inhibited the remaining NANC response by 66% (Figure 3): thus, blockade of adrenoceptors, cholinoceptors and tachykinin NK_1 receptors accounted for 83% of the total secretory response to electrical stimulation, leaving 17% of the response which was insensitive to blockade of the latter autonomic receptors. FK888 had no significant effect on baseline secretion (mean increase of 17.9% ± 6.4% above baseline, $n = 7$). FK224 (10 μM) significantly inhibited NANC neurogenic secretion by 73%.

In the second series of experiments (Figure 4), electrical stimulation (50 V, 10 Hz, 0.5 ms for 5 min) resulted in a 12 fold increase in radiolabel output. The antagonist vehicle (DMSO) had no significant effect on the stimulated increase in radiolabel output. Adrenoceptor and cholinoceptor blockade significantly reduced the latter response by 70%. SR 48968 (0.1 μM) did not inhibit the total neurogenic response nor did it inhibit the remaining NANC neurogenic response. SR 48968 had no significant effect on baseline secretion (5.4% increase, $n = 7$). In this series of experiments, tetrodotoxin (0.1 μM) significantly ($P < 0.01$) reduced electrically-stimulated secretion from 114% ± 20.8% (a significant increase above baseline, $n = 10$) to 27.3% ± 8.2%, the latter value not being a significant increase above baseline ($P = 0.06$, $n = 5$).

The lack of effect on tachykinin-induced secretion of SR 48968 was further investigated with [Sar⁹]SP. [Sar⁹]SP (1 μM) evoked a 42.3% ± 8.4% ($n = 6$) increase above baseline in radiolabel output, which was not inhibited by SR 48968 (increase of 53.8% ± 12.5% above baseline, $n = 8$).

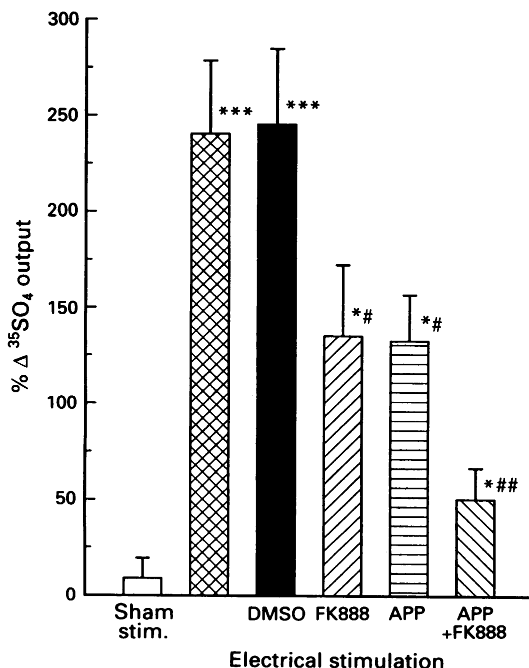

Figure 3 Effect of a tachykinin NK_1 receptor antagonist, FK888 (10 μM), on mucus secretion induced in ferret trachea *in vitro* by electrical stimulation (50 V, 10 Hz, 0.5 ms, 5 min). Data are mean percentage change in output of macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus); s.e.mean are shown ($n = 8-10$ for each experimental group). APP = adrenoceptor and cholinoceptor inhibition with atropine, propranolol and phentolamine in combination (all at 10 μM); DMSO = dimethylsulphoxide (vehicle for FK888). $^*P < 0.05$, $^{***}P < 0.001$ compared with sham stimulation; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with DMSO and electrical stimulation respectively.

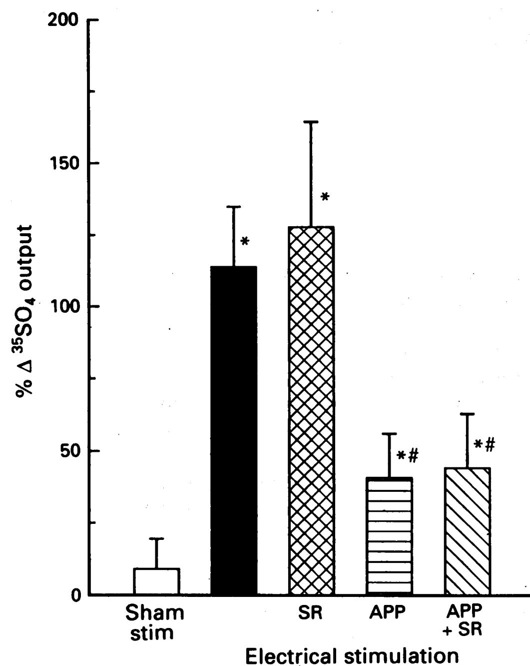

Figure 4 Effects of an NK_2 tachykinin receptor antagonist, SR 48968 (0.1 μM), on mucus secretion induced in ferret trachea *in vitro* by electrical stimulation (50 V, 10 Hz, 0.5 ms, 5 min). Data are mean percentage change in output of macromolecules labelled *in situ* with $^{35}SO_4$ (representing mucus); s.e.mean are shown. Open column: sham stimulation ($n = 8$); solid column: electrical stimulation ($n = 10$); cross-hatched column: effect of SR 48968 on stimulated secretion ($n = 5$); lined column: effect of adrenoceptor and cholinoceptor inhibition with atropine, propranolol and phentolamine in combination (APP, all at 10 μM) on stimulated secretion ($n = 10$); hatched column: effect of adrenoceptor, cholinoceptor and NK_2 receptor inhibition on stimulated secretion ($n = 12$). $^*P < 0.05$ compared with sham stimulation; $^{\#}P < 0.05$ compared with electrical stimulation without receptor antagonists.

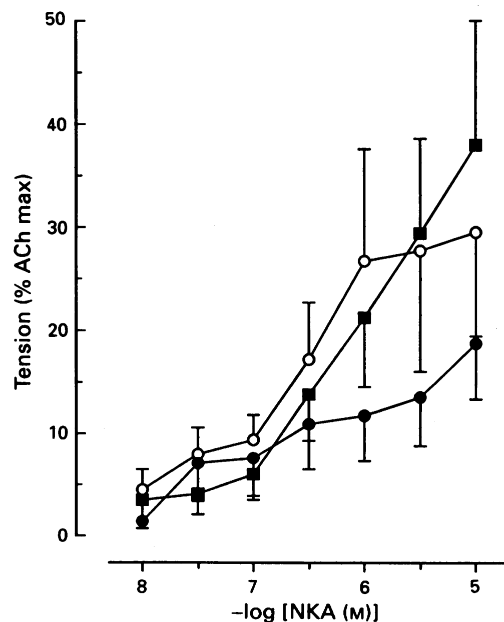

Figure 5 Effect of a tachykinin NK_1 receptor antagonist, FK888 (■; 10 μM) or a tachykinin NK_2 receptor antagonist, SR 48968 (●; 0.1 μM), on ferret tracheal smooth muscle contraction induced by neurokinin A (○) *in vitro*. All drugs were dissolved in dimethylsulphoxide (DMSO). Data are mean tension developed expressed as a percentage of the contraction to 10 mM ACh (ACh max) for $n = 6$ per group; s.e.mean are shown.

Effect of FK888 and SR 48968 on tracheal smooth muscle contraction

NKA (10 nM–10 μM), given after tension had returned to baseline following contraction to a supramaximal concentration of ACh, concentration-relatedly increased tracheal smooth muscle tone (maximal contraction of 45% of the ACh maximum and an EC_{50} of 0.47 μM). The second dose-response to NKA, although reduced, was not significantly affected following 45 min preincubation with DMSO, the drug vehicle (maximal contraction of 30% of the ACh maximum and an EC_{50} of 0.31 μM). No evidence was obtained for a difference in magnitude of inhibition by SR 48968 (0.1 μM) when tissues were incubated for 45 min ($n = 4$) or 120 min ($n = 2$) and the data for the two groups were pooled. SR 48968 (0.1 μM) inhibited NKA-induced tracheal contraction at concentrations of NKA above 0.3 μM (Figure 5). FK888 (10 μM) had no significant effect on the contractile response over the concentration-range of NKA used (Figure 5). None of the treatments had any significant effect on the magnitude of contraction to ACh before or after drug administration: mean percentage differences between initial and final administrations of acetylcholine were 14.4% (s.e.mean 5.2%) for the DMSO group, − 2.2% (s.e.mean 4.3%) for the FK888 group, and − 7.7% (s.e.mean 4.7%) for the SR 48968 group ($n = 6$ each).

Discussion

In the present study, electrical stimulation *in vitro* of ferret trachea increased secretion of $^{35}SO_4$-labelled macromolecules. The increased output of $^{35}SO_4$ is consistent with increased mucus secretion from submucosal glands because: (1) ferret trachea has few surface epithelial goblet cells (Robinson *et al*., 1986; Meini *et al*., 1993) indicating that the main source of mucus is the submucosal glands; (2) $^{35}SO_4$ is relatively selective for labelling intracellular mucin in submucosal glands of cat trachea (Davies *et al*., 1990); and (3) autoradiographic localisation of $^{35}SO_4$ demonstrates preferential loss of label from ferret tracheal glands in response to neuromimetic drugs (Gashi *et al*., 1987).

The secretory response observed herein was blocked by tetrodotoxin indicating that the increase in $^{35}SO_4$ output was via stimulation of nerves associated with submucosal glands. A proportion of the secretory response (30%–50%) remained after inhibition of cholinergic and adrenergic influences, an observation consistent with previous studies (Fung *et al*., 1992a,b; Peatfield & Richardson, 1983; Borson *et al*., 1984). Partial inhibition of neurogenic mucus secretion in the present study in unlikely to be due to use of insufficiently high concentrations of autonomic receptor antagonists (10 μM) because in a similar preparation concentrations of 1–100 μM of these antagonists gave comparable degrees of partial inhibition of neurogenic secretion (Borson *et al*., 1984). In the present study the sensory nerve stimulant capsaicin (Holzer, 1991) induced mucus secretion. Capsaicin also induces mucus secretion in human bronchi *in vitro* (Rogers & Barnes, 1989). Thus, a portion of the remaining NANC response may be due to activation of sensory-efferent nerves and release of tachykinins.

In the present study, the NK_1 receptor antagonist, FK888, partially inhibited secretion evoked by electrical stimulation. In rodents, binding assays, bioassays and *in vivo* functional studies demonstrate that FK888 has a high degree of selectivity for the tachykinin NK_1 receptor (Fujii *et al*., 1992; Hirayama *et al*., 1993; Miyayasu *et al*., 1993; Wang *et al*., 1994). In the present study, FK888 inhibited SP-induced mucus secretion with a pIC_{50} of 6. The concentration of SP used is submaximal for secretion under the present experimental conditions (Meini *et al*., 1993). The pIC_{50} found herein is higher than the K_1 of 0.69 nM or pA_2 of 9.3 reported for inhibition of [^{3}H]-SP binding to guinea-pig lung membranes or SP-induced contraction of guinea-pig ileum *in vitro* (Fujii *et al*., 1992). However, it is consistent with the pIC_{50} of 6.6 for inhibition of electrically-evoked contraction of rabbit iris sphincter (Wang *et al*., 1994). The reasons for the discrepancy between data in the guinea-pig and the data in the rabbit and ferret are unclear but may be due to the recently-reported species and tissue differences in affinities of NK_1 receptor antagonists (Maggi *et al*., 1993; Hall *et al*., 1993). In the present study the selectivity of FK888 was established for the NK_1 receptor in the ferrėt trachea. It has been demonstrated previously that only the selective synthetic tachykinin NK_1 receptor agonist [Sar^9]SP induces mucus secretion in this preparation (Geppeti *et al*., 1993, Meini *et al*., 1993). In the present study, FK888 inhibited SP-induced mucus secretion in a dose-dependent manner with maximal inhibition at 10 μM. The selectivity of FK888 at this concentration was investigated further in ferret tracheal smooth muscle *in vitro* where only a synthetic selective NK_2 agonist induces contraction. FK888 did not inhibit NKA-induced tracheal contraction which indicates that FK888 does not interact with NK_2 receptors in this preparation. FK888 did not exhibit non-selective activity in inhibiting stimulated secretion because it did not inhibit ACh-induced mucus output, a response which was blocked by atropine. Another NK_1 antagonist, L-668,169 (McKnight *et al*., 1988; Williams *et al*, 1988), inhibited $^{35}SO_4$ labelled macromolecule secretion evoked by SP which is further evidence for the involvement of the NK_1 receptor in tachykinin-induced mucus secretion. Both FK888 and L-688,169 blocked secretion evoked by capsaicin and NKA, the latter indicating that NKA interacts with NK_1 receptors in the *in vitro* ferret tracheal preparation. The concentration of NKA used is submaximal for secretion *in vitro* in ferret trachea (Mizoguchi & Hicks, 1989, Webber, 1989). It should be noted that in the isolated submucosal gland of the cat trachea, secretion and glandular contraction induced by SP are not blocked by peptide SP receptor antagonists (Shimura *et al*., 1987). The reasons why the latter antagonists failed to block SP-induced responses whereas SP-induced secretion in the present study was blocked by two different tachykinin NK_1 receptor antagonists is unclear but may be related to the activity of the drugs used, to a species difference, or to differences in use of an isolated glandular preparation compared with the present intact preparation.

In contrast to previous studies using natural and synthetic tachykinins (see above), a preliminary report on isolated cat tracheal submucosal glands found an order of potency of NKA > NKB > SP in inducing secretion of trichloroacetic acid-predictable glycoconjugates (Nagaki *et al*., 1991) which indicated that the tachykinin NK_2 receptor mediated secretion. In the present study, SR 48968 failed to inhibit neurally-evoked secretion, either in the absence or presence of adrenoceptor and cholinoceptor inhibition. In rodent binding assays, bioassays and *in vivo* functional studies, and in human airway smooth muscle *in vitro*, SR 48968 exhibits a high degree of selectivity for the NK_2 receptor (Advenier *et al*., 1992a,b; Edmonds-Alt *et al*., 1992; Martin *et al*., 1992). In the present study, SR 48968, at a dose of 0.1 μM which inhibits NK_2-mediated but not NK_1-mediated responses (Advenier *et al*., 1992b; Edmonds-Alt *et al*., 1992), inhibited NKA-induced tracheal smooth muscle contraction (NK_2 receptor system: see above). In the guinea-pig, SR 48968 has activity at tachykinin NK_3 receptors and μ-opioid receptors (Boyle *et al*., 1993). The involvement of the latter two receptors in NKA-induced tracheal contraction is unlikely because of the minimal effect of a selective NK_3 agonist in inducing contraction in this preparation (Geppetti *et al*., 1993; Meini *et al*., 1993) and because a μ-opioid antagonist does not inhibit SP-induced contraction of guinea-pig airway smooth muscle (Belvisi *et al*., 1990). In contrast to inhibition of NKA-induced tracheal contraction, SR 48968 did not inhibit ACh-induced contraction or [Sar^9]SP-induced mucus secretion. The latter observations provide further evidence for the

selectivity of SR 48968 for the ferret tracheal NK_2 receptor.

In the present study, a small secretory response remained (~17%) after inhibition of adrenoceptors, cholinoceptors and tachykinin NK_1 receptors. The neurotransmitter(s) of the response are unknown. It is unlikely to be due to activation of tachykinin NK_2 receptors because the magnitude of the inhibition by FK224, a tachykinin antagonist which is active at both NK_1 and NK_2 receptors (Morimoto *et al.*, 1992; Murai *et al.*, 1992; Hirayama *et al.*, 1993), was not greater than that induced by the NK_1 antagonist (FK888). The remaining response may be mediated by vasoactive intestinal polypeptide (VIP) and related peptides, or NPY (Ramnarine & Rogers, 1994). The relevance of these and other neuropeptides to NANC neural control of mucus secretion awaits development of selective antagonists.

In the present study, we found variability in secretion of $^{35}SO_4$-labelled mucins. Variation in secretion is related to uptake of label and its incorporation into mucus, to the amount of gland present in different tissue segments, and to the tenacity of the mucus, which affects its release from the luminal surface and dispersion into the bathing medium. The basal condition of the tissue does not appear to contribute greatly to variability because baseline absolute counts of released radioactivity did not differ significantly between experimental groups. Variation in secretory response to capsaicin has been noted previously (Rogers & Barnes, 1989). The reasons for this are unclear but relate to accessibility of capsaicin to the submucosa (the drug is applied luminally), and to variation in distribution of sensory-efferent nerves, tachykinin receptors and degradative enzymes between tissue segments. Difference in magnitude of secretion between the two nerve-stimulated groups is most probably due to variability between batches of ferrets because baseline secretion was not different between the groups.

The present data have clinical implications. Although sensory-efferent neural control of mucus secretion can be readily demonstrated in the airways of a number of non-human animal species (Ramnarine & Rogers, 1994), the involvement of these nerves in control of mucus secretion in human airways is difficult to demonstrate (Baker *et al.*, 1985) and any increase in secretion is comparatively small (Rogers & Barnes, 1989). It is possible that sensory-efferent neural pathways become important in control of mucus secretion in certain diseases of the airways. In asthmatics, the number of SP-immunoreactive nerves associated with airway submucosal glands is increased above that in non-asthmatics (Ollerenshaw *et al.*, 1991) and mRNA levels for NK_1 receptors are increased in asthmatic airways (Adcock *et al.*, 1993), although this increase is not associated with increased receptor number (Goldie, 1990). SP immunoreactivity is increased in sputum of patients with asthma and chronic bronchitis compared with healthy subjects (Tomaki *et al.*, 1993). The dual NK_1/NK_2 antagonist, FK224, has been found to reduce sputum production and cough in patients with chronic bronchitis (Ichinose *et al.*, 1993). Clarification of whether NK_1 receptors are relevant to human bronchial hypersecretory disease requires information on whether reduction in sputum represents a reduction in mucus production and the dual activity of FK224 requires that inhibition of sputum production be confirmed using a selective NK_1 antagonist.

In conclusion, the present study provides evidence that sensory-efferent neural control of mucus secretion *in vitro* in ferret trachea is via activation of tachykinin NK_1 receptors whereas the involvement of NK_2 receptors is minimal. Use of selective and potent tachykinin antagonists will be invaluable in elucidating the involvement of tachykinins in secretory processes in the airways. However, the activity of the dual antagonist FK224 demonstrates that although of value in determining the role of tachykinins in disease, selective tachykinin antagonists may be of limited use therapeutically whereas development of compounds with multiple antagonism at selected receptors may have greater therapeutic potential.

We thank Pfizer Central Research (Sandwich, Kent), the Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan), and the Clinical Research Committee of the Royal Brompton National Heart & Lung Hospitals for financial support. We wish to acknowledge the technical skill of Mr Bill Clack in modifying the Ussing chambers for electrical stimulation. We also thank Susie Lee for her assistance with the secretory studies during her work experience placement from North East Surrey College of Technology(NESCOT), and Drs. M.A. Giembycz and M.G. Belvisi for helpful discussion concerning the contractility studies.

References

ADCOCK, I.M., PETERS, M., GELDER, C., SHIRASAKI, H., BROWN, C.R. & BARNES, P.J. (1993). Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids. *J. Mol. Endocrinol.*, **11**, 1–7.

ADVENIER, C., NALINE, E., TOTY, L., HAYASSAM, B., EDMONDS-ALT, X., VILAIN, P., BRELIÈRE, J.-C. & LE FUR, G. (1992a). Effects on the isolated human bronchus of SR 48968, a potent and selective nonpeptide antagonist of the neurokinin A (NK_2) receptors. *Am. Rev. Respir. Dis.*, **146**, 1177–1181.

ADVENIER, C., ROUISSI, N., NGUYEN, Q.T., EDMONDS-ALT, X., BRELIÈRE, J.-C., NELIAT, G., NALINE, E., & REGOLI, D. (1992b). Neurokinin A (NK_2) receptor revisited with SR 48968, a potent non-peptide antagonist. *Biochem. Biophys. Res. Commun.*, **184**, 1418–1424.

BAKER, B., PEATFIELD, A.C. & RICHARDSON, P.S. (1985). Nervous control of mucin secretion into human bronchi. *J. Physiol.*, **365**, 297–305.

BELVISI, M.G., STRETTON, C.D. & BARNES, P.J. (1990). Modulation of cholinergic neurotransmission in guinea-pig airways by opioids. *Br. J. Pharmacol.*, **100**, 131–137.

BORSON, D.B., CHARLIN, M., GOLD, B.D. & NADEL, J.A. (1984). Neural regulation of $^{35}SO_4$-macromolecule secretion from tracheal glands of ferrets. *J. Appl. Physiol.*, **57**, 457–466.

BOYLE, S.J., MANLEY, S., TANG, K.-W., MEECHAM, K., GUARD, S., WATLING, K.J., WOODRUFF, G.M. & MCKNIGHT, A.T. (1993). Affinity of the NK_2 antagonist SR 48968 at NK_3-tachykinin and μ-opioid receptors. *Br. J. Pharmacol.*, **108**, 24P.

COLES, S.J., NEILL, K.H. & REID, L.M. (1984). Potent stimulation of glycoprotein secretion in canine trachea by substance *P. J. Appl. Physiol.*, **57**, 1323–1327.

DAVIES, J.R., CORBISHLEY, C.M., RICHARDSON, P.S. (1990). The uptake of radiolabelled precursors of mucus glycoconjugates by secretory tissues in the feline trachea. *J. Physiol.*, **420**, 19–30.

EDMONDS-ALT, X., VILAIN, P., GOULAOUIC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., LE FUR, G. & BRELIÈRE, J.C. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci.*, **50**, PL-101–PL-106.

FUJII, T., MURAI, M. MORIMOTO, H., MAEDA, Y., YAMAOKA, M., HAGIWARA, D., MIYAKE, H., IKARI, N. & MATSUO, M. (1992). Pharmacological profile of a high affinity dipeptide NK_1 receptor antagonist, FK888. *Br. J. Pharmacol.*, **107**, 785–789.

FUNG, D.C.K., ALLENBY, M.I. & RICHARDSON, P.S. (1992a). NANC nerve pathways controlling mucus glycoconjugate secretion into feline trachea. *J. Appl. Physiol.*, **73**, 625–630.

FUNG, D.C.K., BEACOCK, D.J. & RICHARDSON, P.S. (1992b). Vagal control of mucus glycoconjugate secretion into the feline trachea. *J. Physiol.*, **453**, 435–447.

GASHI, A.A., BORSON, D.B., FINKBEINER, W.E., NADEL, J.A. & BASBAUM, C.B. (1987). Neuropeptides degranulate serous cells of ferret tracheal glands. *Am. J. Physiol.*, **251**, C2223–C2229.

GENTRY, S.E. (1991). Tachykinin receptors mediating airway macromolecular secretion. *Life Sci.*, **48**, 1609–1618.

GEPPETTI, P., BERTRAND, C., BACCI, E., HUBER, O. & NADEL, J. (1993). Characterisation of tachykinin receptors in ferret trachea by peptide agonists and nonpeptide antagonists. *Am. J. Physiol.*, **265**, L164–L169.

GOLDIE, R.G. (1990). Receptors in asthmatic airways. *Am. Rev. Respir. Dis.*, **141**, S151–S156.

GUARD, S. & WATSON, S.P. (1991). Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem. Int.*, **13**, 149–165.

HALL, J.M., CAULFIELD, M.P., WATSON, S.P. & GUARD, S. (1993). Receptor subtypes or species homologues: relevance to drug discovery. *Trends Pharmacol. Sci.*, **14**, 376–383.

HIRAYAMA, Y., LEI, Y.-H., BARNES, P.S. & ROGERS, D.F. (1993). Effects of two novel tachykinin antagonists, FK224 and FK888, on neurogenic airway plasma exudation, bronchoconstriction and systemic hypotension in guinea-pigs *in vivo*. *Br. J. Pharmacol.*, **108**, 844–851.

HOLZER, P. (1991). Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol. Rev.*, **43**, 143–201.

ICHINOSE, M., KATSUMATA, U., KIKUCHI, R., FUKUSHIMA, T., ISHII, M., INOUE, C., SHIRATO, K. & TAKISHIMA, T. (1993). Effect of tachykinin antagonist on chronic bronchitis patients. *Am. Rev. Respir. Dis.*, **147** (Suppl.), A318.

LUNDGREN, J.D., WIEDERMAN, C.J., LOGUN, C., PLUTCHOK, J., KALINER, M. & SHELHAMER, J.H. (1989). Substance P receptor-mediated secretion of respiratory glycoconjugate from feline airways in vitro. *Exp. Lung Res.*, **15**, 17–29.

LUTS, A. & SUNDLER, F. (1989). Peptide-containing nerve fibres in the respiratory tract of the ferret. *Cell Tissue Res.*, **258**, 259–267.

MAGGI, C.A. & MELI, A. (1987). The sensory-efferent function of capsaicin-sensitive sensory neurones. *Gen. Pharmacol.*, **19**, 1–43.

MAGGI, C.A., PATACCHINI, R, QUARTARA, L., ROVERO, P. & SANTICIOLLI, P. (1990). Tachykinin receptors in the guinea-pig isolated bronchi. *Eur. J. Pharmacol.*, **197**, 167–174.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993). Tachykinin receptors and tachykinin receptor antagonists. *J. Auton. Pharmacol.*, **13**, 23–93.

MARTIN, C.A.E., NALINE, E., EDMONDS-ALT, X. & ADVENIER, C. (1992). Influence of (±)-CP-96,345 and SR 48968 on electrical field stimulation of the isolated guinea-pig main bronchus. *Eur. J. Pharmacol.*, **224**, 137–143.

McKNIGHT, A.T., MAGUIRE, J.J., WILLIAMS, B.J., FOSTER, A.C., TRIDGETT, R. & IVERSEN, L.L. (1988). Pharmacological specificity of synthetic peptides as santagonists at tachykinin receptors. *Regul. Pept.*, **22**, 127.

MEINI, S., MAK, J.C.W., ROHDE, J.A.L. & ROGERS, D.F. (1993). Tachykinin control of ferret airways: mucus secretion, bronchoconstriction and receptor mapping. *Neuropeptides*, **24**, 81–89.

MIYAYASU, K., MAK, J.C.W., NISHIKAWA, M. & BARNES, P.J. (1993). Characterisation of guinea-pig pulmonary neurokunin type 1 receptors using a novel antagonist ligand, [^{3}H]FK888. *Mol. Pharmacol.*, **44**, 539–544.

MIZOGUCHI, H. & HICKS, C.R. (1989). Effects of neurokinins on ion transport and sulphated macromolecule release in the isolated ferret trachea. *Exp. Lung. Res.*, **15**, 837–848.

MORIMOTO, H., MURAI, M., MAEDA, Y., YAMAOKA, M., NISHIKAWA, M., KIYOTOH, S. & FUJII, T. (1992). FK224, a novel cyclopeptide substance P antagonist with NK_1 and NK_2 receptor selectivity. *J. Pharmacol. Exp. Ther.*, **262**, 398–402.

MURAI, M., MORIMOTO, H., MAEDA, Y., KIYOTOH, S., NISHIKAWA, M. & FUJII, T. (1992). Effects of FK224, a novel compound NK_1 and NK_2 receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory nerve stimulation in guinea-pigs. *J. Pharmacol. Exp. Ther.*, **262**, 402–408.

NAGAKI, M., ISHIHARA, H., SHIMURA, S., SATOH, M., SASAKI, H. & TAKISHIMA, T. (1991). Tachykinins evoke mucus glycoprotein secretion from feline submucosal glands via NK-2 receptors mediating $[Ca^{++}]_i$ as a second messenger. *Am. Rev. Respir. Dis.*, **143** (suppl.), A620.

OLLERENSHAW, S.L., JARVIS, D., SULLIVAN, C.E. & WOOLCOCK, A.J. (1991). Substance P immunoreactive nerves in airways from asthmatics and non asthmatics. *Eur. Respir. J.*, **4**, 673–682.

PEATFIELD, A.C. & RICHARDSON, P.S. (1983). Evidence for non-cholinergic, non-adrenergic nervous control of mucus secretion into the cat trachea. *J. Physiol.*, **342**, 335–345.

RAMNARINE, S.I., HIRAYAMA, Y., LEE, S., BARNES, P.J. & ROGERS, D.F. (1993). Tachykinin NK_1 receptors mediate neurogenic mucus secretion in ferret trachea *in vitro*. *Am. Rev. Respir. Dis.*, **147** (suppl.), A711.

RAMNARINE, S.I. & ROGERS, D.F. (1994). Non-adrenergic, non-cholinergic neural control of mucus secretion in the airways. *Pulmonary Pharmacol.*, **7**, 19–33.

ROBINSON, N.P., VENNING, L., KYLE, H. & WIDDICOMBE, J.H. (1986). Quantification of secretory cells of the ferret tracheobronchial tree. *J. Anat.*, **145**, 173–188.

ROGERS, D.F., AURSUDKIJ, B. & BARNES, P.J. (1989). Effects of tachykinins on mucus secretion in human bronchi in vitro. *Eur. J. Pharmacol.*, **174**, 283–286.

ROGERS, D.F. & BARNES, P.J. (1989). Opioid inhibition of neurally mediated mucus secretion in human bronchi. *Lancet*, **i**, 930–932.

SHIMURA, S., SASAKI, T., OKAYAMA, H., SASAKI, H. & TAKISHIMA, T. (1987). Effect of substance P on mucus secretion of isolated submucosal gland from feline trachea. *J. Appl. Physiol.*, **63**, 646–653.

TOMAKI, M., ICHINOSE, M., NAKAJIMA, N., MIURA, M., YAMAUCHI, H., INOUE, H. & SHIRATO, K. (1993). Elevated substance P concentration in sputum after hypertonic saline inhalation in asthma and chronic bronchitis patients. *Am. Rev. Respir. Dis.*, **147** (suppl.), A478.

WANG, Z-Y., TUNG, S.R., STRICHARTZ, G.R. & HAKANSON, R. (1994). Investigation of the specificity of FK888 as a tachykinin NK_1 receptor antagonist. *Br. J. Pharmacol.*, **111**, 1342–1346.

WEBBER, S.E. (1989). Receptors mediating the effects of substance P and neurokinin A on mucus secretion and smooth muscle tone of the ferret trachea: potentiation by an enkephalinase inhibitor. *Br. J. Pharmacol.*, **98**, 1197–1206.

WEBBER, S.E., LIM, J.C.S. & WIDDICOMBE, J.G., (1991). The effects of calcitonin gene-related peptide on submucosal gland secretion and epithelial albumin transport in the ferret trachea *in vitro*. *Br. J. Pharmacol.*, **102**, 79–84.

WILLIAMS, B.J., CURTIS, N.R., McKNIGHT, A.T., MAGUIRE, J., FOSTER, A. & TRIDGETT, R. (1988). Development of NK-2 selective antagonists. *Regul. Pept.*, **22**, 189.

(Received January 17, 1994
Revised July 20, 1994
Accepted July 29, 1994)