ELSEVIER

European Journal of Pharmacology 341 (1998) 211–223

ejp

Tachykinin NK_2 receptors predominantly mediate tachykinin-induced contractions in ovine trachea

Ann M. Reynolds *, Paul Reynolds, Mark Holmes, Raffaele Scicchitano

Lung Research Laboratory, Hanson Centre for Cancer Research, Royal Adelaide Hospital, North Terrace, Adelaide, SA 5000, Australia

Received 14 October 1997; accepted 21 October 1997

Abstract

In vitro studies were conducted to characterize the contractile effects of tachykinins in normal ovine trachea with a view in the future to compare tachykinin contractile responses in allergic tissue. Tracheal smooth muscle strips were prepared for in vitro studies of isometric contraction in response to cumulative addition of carbachol, acetylcholine, histamine, neuropeptide gamma, substance P, neurokinin A, neurokinin B, [Sar^9, $Met(O_2)^{11}$]substance P, [Nle^{10}]neurokinin A-(4–10), and [Succinyl-Asp^6, Me-Phe^8]substance P-(6–11) (senktide). The rank order of potency was neuropeptide gamma > carbachol > neurokinin A ≥ [Nle^{10}]neurokinin A-(4–10) > acetylcholine ≥ histamine. Phosphoramidon enhanced the contractile response to neurokinin A and substance P, but not to neuropeptide gamma, [Sar^9, $Met(O_2)^{11}$]substance P or senktide. Repeated cumulative concentration responses for acetylcholine, substance P, neurokinin A, [Sar^9, $Met(O_2)^{11}$]substance P and histamine were also conducted to test for tachyphylaxis. No tachyphylaxis to acetylcholine, substance P, or neurokinin A was observed, however, [Sar^9, $Met(O_2)^{11}$]substance P and histamine did exhibit tachyphylaxis. Atropine had no effect on tracheal contractions to neurokinin A and substance P, while [Sar^9, $Met(O_2)^{11}$]substance P contractions were atropine sensitive. Pyrilamine did not affect substance P-induced tracheal smooth muscle contractions, indicating that the response to substance P was not mediated by histamine release. These results show that, in vitro, natural tachykinins induce tracheal smooth muscle contraction predominantly by a direct effect mediated by tachykinin NK_2 receptors, and a small tachykinin NK_1 receptor mediated cholinergic mechanism. © 1998 Elsevier Science B.V.

Keywords: Airway, sheep; Smooth muscle; Neuropeptide; Neutral endopeptidase; Tachykinin receptor agonist, specific

1. Introduction

Tachykinins are a group of neuropeptides found in sensory nerves. Five tachykinins have been identified in mammalian neural tissue, substance P, neurokinin A and neurokinin B, and the amino terminally extended forms of neurokinin A, neuropeptide K and neuropeptide-gamma (Helke et al., 1990). Tachykinins mediate their biological effects by activating specific receptors, termed tachykinin NK_1, NK_2 and NK_3. The endogenous tachykinins, substance P, neurokinin A and neurokinin B exhibit preferential binding to the tachykinin NK_1, NK_2 and NK_3 receptors, respectively.

Neurokinin A and substance P have been detected in the lung of many species including man (Lundberg et al., 1984). Nerve fibres containing substance P have been observed within and beneath the airway epithelium, around blood vessels and submucosal glands, within the bronchial smooth muscle layer, and around tracheobronchial ganglia. They have also been detected throughout the tracheobronchial tree: trachea, bronchi, bronchioles and more distal airways to the alveoli. The physiological activity of endogenous tachykinins is modulated by enzymatic degradation, principally by neutral endopeptidase (EC 3.4.24.11) (Martling, 1987).

A number of animal species have been used as experimental models of asthma. The sheep in vivo model of antigen-induced bronchoconstriction is well characterized and shows many similarities to human asthma (Abraham et al., 1983). Using this experimental asthma model we have obtained evidence for the involvement of endogenously released tachykinins in the acute bronchoconstrictor response to inhaled *Ascaris suum* (Rice et al., 1995a). Moreover, our in vivo results in non-allergic sheep (Rice et al., 1995b) and those of others (Corcoran and Haigh, 1992)

* Corresponding author. Tel.: +61-8-82225302; fax: +61-8-82223134.

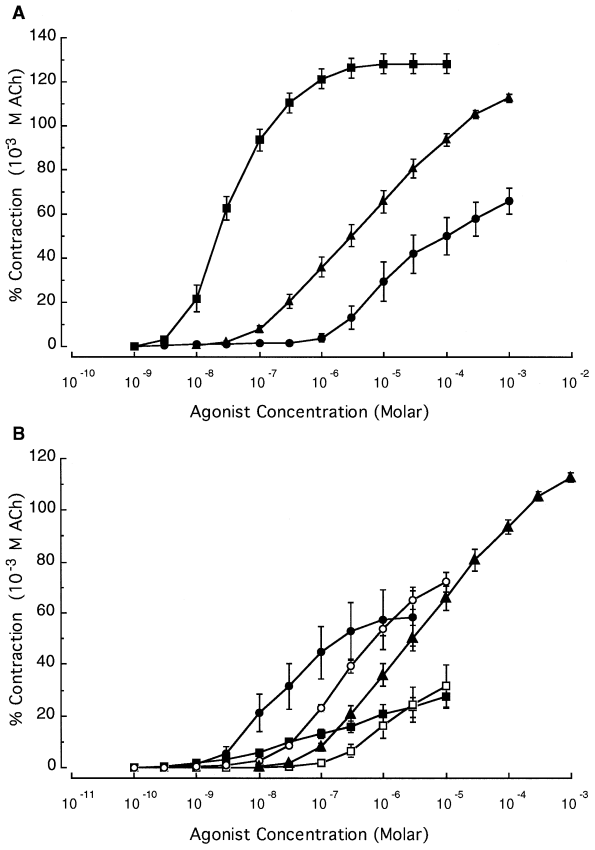

Fig. 1. (A) Mean cumulative concentration–response curves for carbachol (■), acetylcholine (▲), and histamine (●) on ovine isolated tracheal smooth muscle. Mean responses are from 6 animals and are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M. (B) Mean cumulative concentration–response curves for neurokinin A (○), neuropeptide gamma (●), substance P (■), neurokinin B (□), and acetylcholine (▲) on ovine isolated tracheal smooth muscle. Mean responses are from 6 animals and are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M.

suggested that in these animals tachykinin airway responses were mediated by tachykinin NK_1 receptors and involved a cholinergic mechanism. These effects, however, are in contrast to human studies where tachykinin-induced bronchoconstriction appears to be principally mediated via tachykinin NK_2 receptors, with a small cholinergic component (Joos et al., 1987; Crimi et al., 1990; Cheung et al., 1992).

Therefore, in order to pursue our studies on the role of tachykinins in antigen-induced bronchoconstriction, it was necessary to undertake more extensive well-controlled, in vitro, studies to elucidate the mechanisms of action of tachykinins on ovine airway smooth muscle. Although the contractile actions of tachykinins, in vitro, have been studied extensively in many species, there has been no work reported on isolated sheep airways. Firstly, we examined the in vitro contractile response of normal ovine tracheal smooth muscle to the tachykinin peptides: substance P, neuropeptide gamma, neurokinin A, neurokinin B, tachykinin NK_1-specific agonist [Sar^9,$Met(O_2)^{11}$]substance P, NK_2 receptor agonist [Nle^{10}]neurokinin A-(4–10), NK_3 agonist [Succinyl-Asp^6, Me-Phe^8]substance P-(6–11) (senktide), as well as acetylcholine, carbamylcholine chloride (carbachol), and histamine. Secondly, we determined whether substance P, neurokinin A and [Sar^9,$Met(O_2)^{11}$]substance P act directly on tracheal smooth muscle or through endogenous acetylcholine release or indirectly through other mediators such as histamine.

2. Methods and materials

2.1. General

Adult female merino sheep were killed using a captive bolt device (Supercash Mark-II, England). Tissues from four to six animals were used in each experimental group. Their lungs were excised *en bloc* and placed in ice-cold oxygenated buffered Krebs–Henseleit solution of the following composition (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$ and 11 glucose (BDH AnalaR®, Merck, Kilsyth) (pH 7.4) for

Table 1
Mean maximal effect, geometric mean EC_{50} and apparent affinities for various agonists in ovine tracheal smooth muscle

Agonist	*n*	E_{max} [a]	EC_{50} (M)	Mean pD_2 (± S.E.M.)
Carbachol	6	128.1 ± 4.5	3.23×10^{-8} (2.52×10^{-8}, 4.14×10^{-8})	7.49 ± 0.04
Acetylcholine	6	112.4 ± 2.0	4.93×10^{-6} (2.06×10^{-6}, 1.18×10^{-5})	5.31 ± 0.15
Histamine	6	65.6 ± 5.9	1.18×10^{-5} (4.55×10^{-6}, 3.05×10^{-5})	4.93 ± 0.16
Neuropeptide gamma	6	58.4 ± 11.7	1.19×10^{-8} (6.45×10^{-9}, 2.21×10^{-8})	7.92 ± 0.10
Neurokinin A	6	72.0 ± 3.8	2.16×10^{-7} (1.32×10^{-7}, 3.53×10^{-7})	6.67 ± 0.08
[Nle^{10}]neurokinin A-(4–10)	6	45.3 ± 5.7	5.45×10^{-7} (2.96×10^{-7}, 1.01×10^{-6})	6.26 ± 0.10
Substance P	6	27.4 ± 4.0	NC	NC
Neurokinin B	6	31.4 ± 8.4	NC	NC

[a] Mean E_{max} ± S.E.M. were expressed as a percentage of the response to 10^{-3} M acetylcholine.
n = number of sheep, pD_2 values were calculated as $-\log EC_{50}$. Geometric mean EC_{50} values and 95% confidence limits (are shown in parenthesis). NC, not calculated as agonist contractile response at 10^{-5} M still linear.

Table 2
Geometric mean EC_{25} [a] values for tachykinins in ovine tracheal smooth muscle

Agonist	*n*	EC_{25} (M)	95% confidence limits
Neuropeptide gamma	6	2.67×10^{-8} [b]	5.32×10^{-9}, 1.34×10^{-7}
Neurokinin A	6	1.16×10^{-7} [b]	9.10×10^{-8}, 1.47×10^{-7}
Substance P	6	2.48×10^{-6} [b]	5.87×10^{-7}, 1.05×10^{-5}
Neurokinin B	6	3.32×10^{-6} [c]	1.05×10^{-6}, 1.05×10^{-5}

[a]The concentration of agonist at which a contractile response equal to 25% of that induced by 10^{-3} M acetylcholine is attained (see Section 2). Values are geometric mean EC_{25} values and 95% confidence limits.
[b]Significantly different from each other (unpaired *t*-test), $P < 0.05$.
[c]Not significantly different from substance P EC_{25} (unpaired *t*-test), $P < 0.83$.
n = number of sheep.

transportation to the laboratory. Experiments were either commenced within 1.5 h of excision or the tissue was stored in the oxygenated salt solution at 4°C for subsequent use within 36 h. Preliminary studies revealed no change in contractility to acetylcholine or carbachol after this period of time.

Strips of tracheal smooth muscle (3–5 × 20–25 mm) were dissected from the posterior portion of the trachea above the origin of the right upper lobe. The tracheal smooth muscle was mounted on steel hooks, the lower hook was attached to a fixed support rod and the upper hook to a Grass FT03 force-displacement transducer (Grass Instruments, Qunicy, MA). The latter was mounted on a rack and pinion clamp so that resting muscle length and therefore resting tension could be optimized (Mitchell et al., 1989).

The tissues were suspended under tension (1.5–2.0 g) in 20 ml siliconized, water-jacketed, glass organ baths (Harvard Apparatus, South Natick, MA) in Krebs–Henseleit solution continuously bubbled with 95% O_2: 5% CO_2 and maintained at 38°C. The tracheal smooth muscle segments were allowed to equilibrate in the water baths for 90 min prior to the addition of agonists. During the equilibration period the bath solution was changed every 20 min and resting load adjusted to maintain optimal tension throughout the equilibration period. Changes in tracheal smooth muscle tension were measured isometri-

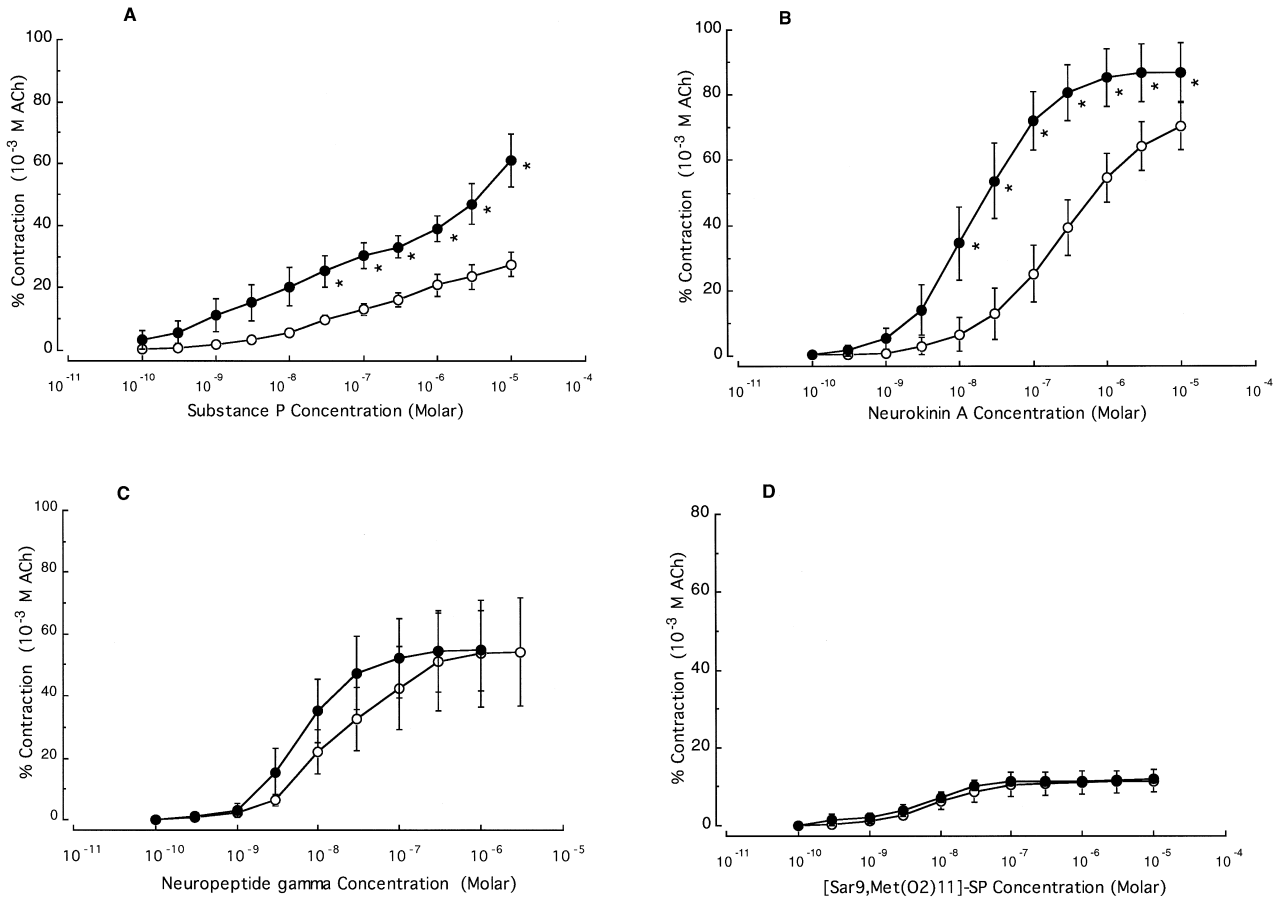

Fig. 2. Cumulative concentration–response curves for substance P (A, $n = 6$); neurokinin A (B, $n = 5$); neuropeptide gamma (C, $n = 4$); and [Sar^9, $Met(O_2)^{11}$]substance P (D, $n = 4$) in the absence (○) and presence (●) of phosphoramidon 10^{-5} M. Mean responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M. * Denotes a statistically significant ($P < 0.05$, paired *t*-test) difference between tissues treated with and without phosphoramidon.

cally and continuously recorded on polygraph (Neotrace 600 ZEF, Neomedix Systems, Sydney). Whenever possible paired tissue samples were studied in duplicate.

2.2. Effects of agonists

This series of experiments was designed to determine the relative potency and efficacy of tachykinins, acetylcholine, carbachol and histamine to contract normal ovine tracheal smooth muscle. At the end of the equilibration period, when a stable baseline tension was achieved, a reference contractile response to 10^{-3} M acetylcholine was obtained. Once this contraction had reached a plateau, the tissues were washed every 20 min until baseline tone was re-established (usually 60–90 min). Cumulative concentration–response curves were obtained for acetylcholine, carbachol, histamine, the tachykinin peptides: substance P, neuropeptide gamma, neurokinin A, neurokinin B, and the tachykinin NK_1, NK_2 and NK_3 receptor-specific agonists; [Sar^9, $Met(O_2)^{11}$]substance P, [Nle^{10}]neurokinin A-(4–10) and senktide, respectively. This was achieved by the stepwise addition of increasing concentrations of agonist to the organ bath (in logarithmic increments) once the response to the preceding concentration had reached a plateau.

2.3. Effect of neutral endopeptidase inhibition

In this series of experiments, following the reference acetylcholine contraction, the neutral endopeptidase inhibitor, phosphoramidon (10^{-5} M, final bath concentration) (Hudgin et al., 1981), was added to the organ bath of one of the paired tissues, the other receiving no treatment. After a 30 min incubation period, cumulative concentration–response curves were obtained for substance P, neuropeptide gamma, neurokinin A, [Sar^9, $Met(O_2)^{11}$]substance P and senktide.

2.4. Effect of atropine, and pyrilamine on substance P, [Sar^9, $Met(O_2)^{11}$]substance P and neurokinin A contractility

To establish whether tachykinin-induced smooth muscle contraction was by a direct effect, by the liberation of histamine or via a cholinergic mechanism, we compared substance P, neurokinin A and [Sar^9, $Met(O_2)^{11}$]substance P contractility in the presence and absence of atropine (10^{-6} M), and substance P induced contraction in the presence of the histamine H_1-receptor antagonist, pyrilamine (10^{-6} M). In addition, the effectiveness of the atropine and pyrilamine blockade was confirmed by obtaining cumulative concentration–response curves for acetylcholine and histamine, respectively in the presence and absence of these inhibitors.

In this series of experiments two successive cumulative concentration–response curves were obtained for each agonist (substance P, neurokinin A, [Sar^9, $Met(O_2)^{11}$]substance P, acetylcholine and histamine). This enabled each tissue to act as its own control and provided an opportunity to detect the possible confounding effect of tachyphylaxis for each agonist under investigation. Following the first cumulative concentration–response curve the tissues were washed until stable baseline tone was re-established (90–120 min). At this point, one of the paired tissues received the antagonist (atropine or pyrilamine), the other no treatment. Following a 30 min incubation period the second cumulative concentration–response curve was performed.

2.5. Analysis of results

In each tracheal smooth muscle preparation, contractile responses to each agonist concentration studied was expressed as a percentage of the contractile response to acetylcholine (10^{-3} M) except for the tachyphylaxis experiments (see below). A cumulative concentration–response curve was then constructed relating the cumulative concentration of the agonist to the response. Apparent affinities pD_2 values (defined as the negative log of the agonist concentration that caused 50% of maximal effect, $-\log EC_{50}$) were calculated. The maximal effect (E_{max}) was calculated as the maximal tension generated for each agonist, at the highest agonist concentration studied, and expressed as a percentage of that obtained with acetylcholine (10^{-3} M). These values were interpolated from the cumulative concentration–response curve for each experiment. In cases when duplicate tissues were studied a mean

Table 3
Geometric mean EC_{25} [a] and 95% confidence limits for tachykinins in the absence and presence of phosphoramidon (10^{-5} M).

Agonist	*n*	EC_{25} (M)[a] control	EC_{25} (M)[a] phosphoramidon
Substance P	6	2.10×10^{-6} (5.62×10^{-7}, 7.84×10^{-6})	2.17×10^{-8} (1.61×10^{-9}, 2.94×10^{-7})[b]
Neurokinin A	5	1.10×10^{-7} (1.98×10^{-8}, 6.10×10^{-7})	6.97×10^{-9} (1.37×10^{-9}, 3.54×10^{-8})[b]
Neuropeptide gamma	4	2.72×10^{-8} (3.20×10^{-9}, 2.30×10^{-7})	7.35×10^{-9} (9.86×10^{-10}, 5.40×10^{-8})
[Sar^9,$Met(O_2)^{11}$]-substance P	4	NC	NC

[a]The concentration of agonist at which a contractile response equal to 25% of that induced by 10^{-3} M acetylcholine is attained (see Section 2). Values are geometric mean EC_{25} values and 95% confidence limits (shown in parenthesis).
[b]Significantly different from control (paired *t*-test), $P < 0.05$.
NC, not calculated due to low agonist activity at 10^{-5} M in this tissue.

cumulative concentration–response curve was constructed for each experiment and a geometric mean EC_{50} calculated. To determine the rank order of potency of the tachykinins and to detect any significant shift in the cumulative concentration–response curve following neutral endopeptidase inhibition an EC_{25} (defined as the molar concentration of agonist required to induce a 25% contraction of that produced by 10^{-3} M acetylcholine) was calculated. The EC_{25} was an arbitrarily determined response which was on the exponential portion of the cumulative concentration–response curve (Black et al., 1988). Results are expressed as mean ± standard error of the mean (S.E.M.) (n, number of animals). EC_{50} and EC_{25} are given as geometric mean values with 95% confidence limits.

In experiments in which two consecutive cumulative concentration–response curves were obtained, the contractile responses to each concentration of agonist in the first cumulative concentration–response curve were expressed as a percent of the maximal response (E_{max}) in that tracheal smooth muscle strip. Contractile responses in the second cumulative concentration–response curve were expressed as a percentage of the E_{max} of the first cumulative concentration–response curve.

Statistical analyses were performed using two-tailed paired and unpaired Student's t-test. Values were considered significant at $P < 0.05$ level.

2.6. Drugs

Stock solutions of substance P, neurokinin A, neuropeptide gamma, [Sar^9, Met(O$_2$)^{11}]substance P, (Auspep, Melbourne), acetylcholine chloride, carbamylcholine chloride (carbachol), phosphoramidon, atropine sulfate, and pyrilamine (Sigma Chemical, St. Louis, MO), and histamine acid phosphate (BDH AnalaR®, Merck, Kilsyth), were made up in distilled water and aliquots kept at −70°C. Stock solutions of neurokinin B, [Nle^{10}]neurokinin A-(4–10) and senktide (Auspep, Melbourne) were prepared in 40% dimethylsulfoxide (BDH AnalaR®) and stored at −70°C. On each study day, serial dilutions were prepared in Krebs–Henseleit solution and kept on ice for the duration of the experiment. All compounds were added to the bath in volumes not exceeding 0.5% of the total bath volume. Plastic vials and pipette tips were used to store and handle the tachykinin peptides and other agents. Organ baths were coated with Coatasil (Ajax Chemicals, Sydney).

3. Results

3.1. Effects of agonists

In the first series of experiments the contractile responses to several non-peptide agonists and tachykinins were studied. The cumulative concentration–response curves are shown in Fig. 1A–B. Carbachol was the most active non-peptide agonist studied having the highest E_{max} of 128.1 ± 4.5% of the reference contraction to 10^{-3} M acetylcholine, and a pD_2 value of 7.49 ± 0.04 (Table 1).

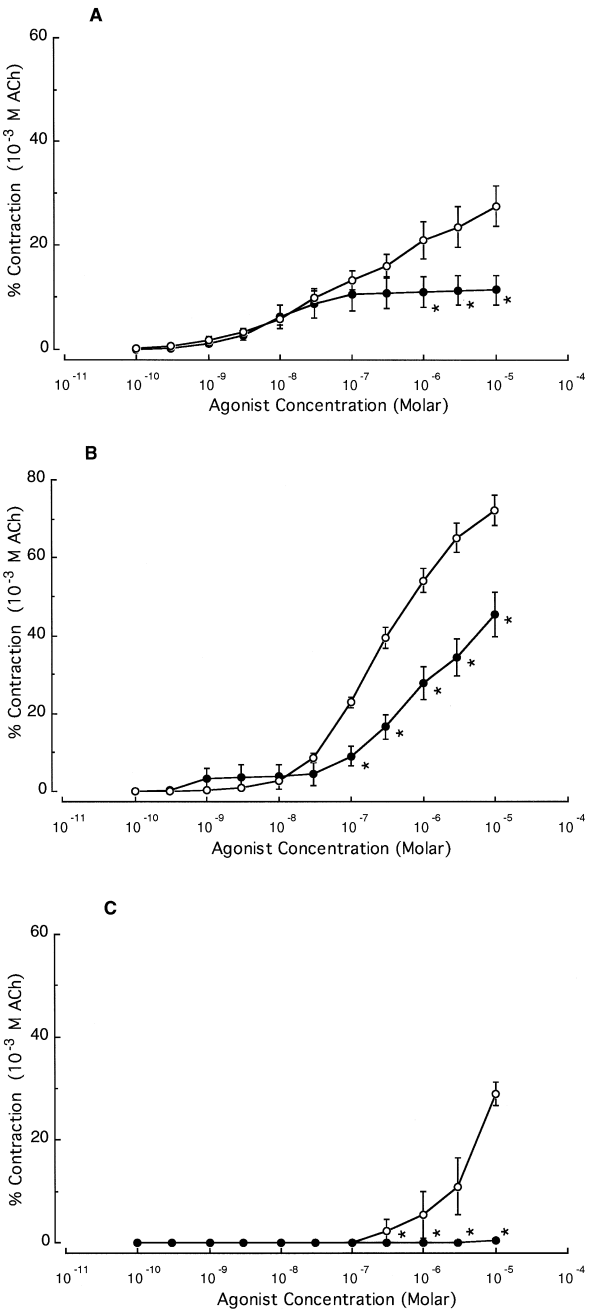

Fig. 3. (A) Effect of the tachykinin NK_1 receptor specific agonist, [Sar^9, Met(O$_2$)^{11}]substance P (●, $n = 4$) and substance P (○, $n = 6$) on isolated tracheal strips. Mean responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. (B) Effect of the tachykinin NK_2 receptor specific agonist, [Nle^{10}]neurokinin A-(4–10) (●, $n = 4$) and neurokinin A (○, $n = 6$) on isolated tracheal strips. Mean responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. (C) Effect of the tachykinin NK_3 receptor specific agonist, senktide (●, $n = 3$) and neurokinin B (○, $n = 6$) on isolated tracheal strips. Mean responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M. * Denotes a statistically significant ($P < 0.05$, unpaired t-test) difference between treatment groups.

Acetylcholine had a higher potency and E_{max} than histamine at the concentrations studied (Fig. 1A, Table 1).

All the tachykinins studied produced concentration dependent contractions in ovine tracheal smooth muscle, however, maximal contractile responses were not always achieved for each tachykinin at the concentrations used. Therefore, EC_{50} and pD_2 values could not be determined for all tachykinins. An EC_{25} value (see Section 2), however, was calculated for each tachykinin and a rank order of potency established (Table 2), this being neuropeptide gamma > neurokinin A > substance P ≥ neurokinin B. Neuropeptide gamma was approximately 10 times more potent than neurokinin A, pD_2 values 7.92 ± 0.10 and 6.67 ± 0.08, respectively ($P < 0.05$, unpaired *t*-test, Table

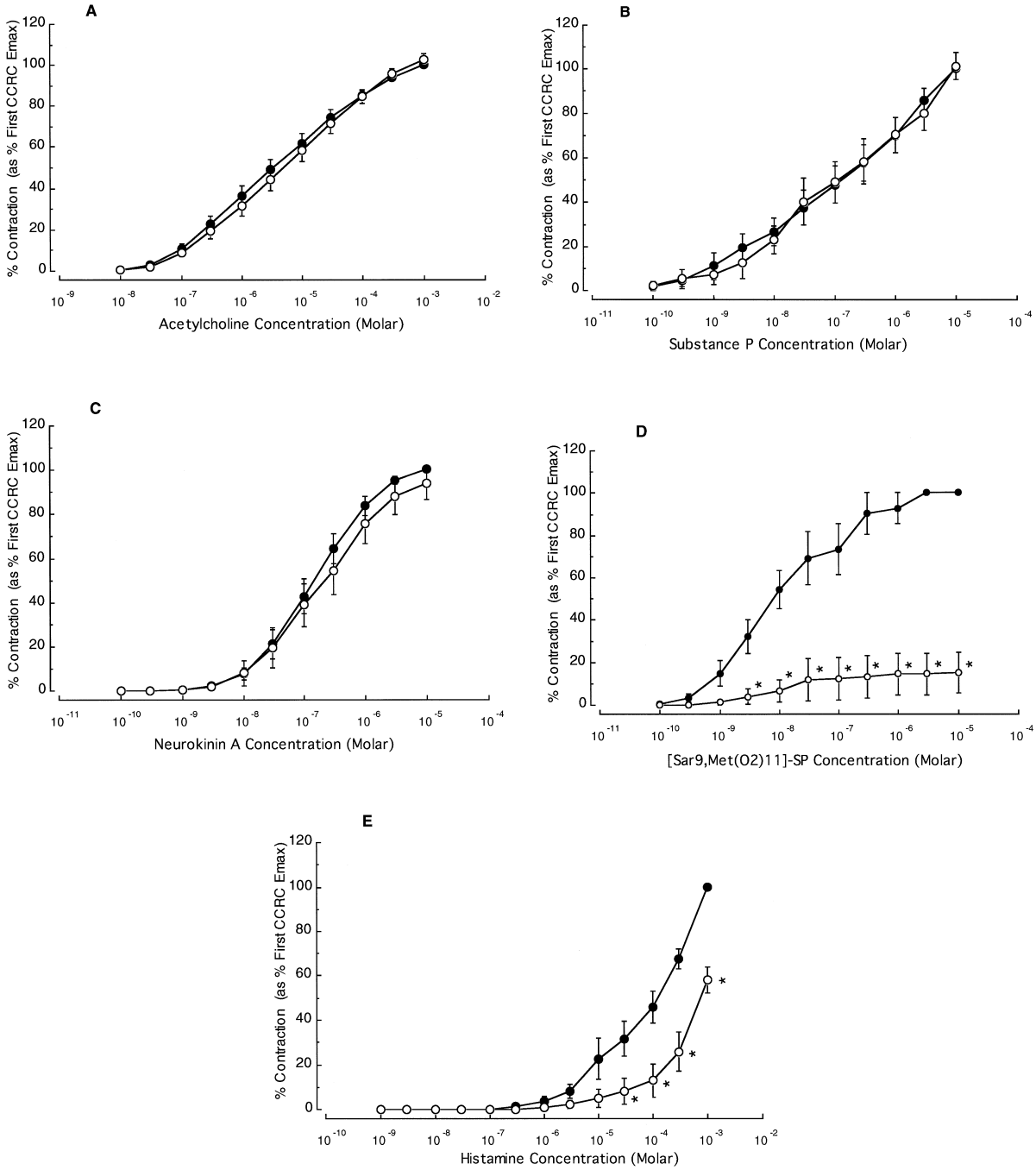

Fig. 4. Mean cumulative concentration–response curves for acetylcholine (A, $n = 6$); substance P (B, $n = 6$); neurokinin A (C, $n = 6$); [Sar^9,$Met(O_2)^{11}$]substance P (D, $n = 4$); and histamine (E, $n = 3$). (●) and (○), first and second cumulative concentration–response curves respectively, in the same tracheal strip. Contractile responses are expressed as a percent of the maximal response in the first cumulative concentration–response curve (First CCRC E_{max}). Vertical bars indicate S.E.M. * Denotes a statistically significant difference ($P < 0.05$, paired *t*-test) from first cumulative concentration–response curves.

Table 4
Mean maximal effect (E_{max}) and mean EC_{50} values for agonists of first and second cumulative concentration–response curves (CCRC) in ovine tracheal smooth muscle strips

Agonist	*n*	E_{max} [a]	EC_{50} [b] (M)	
		second CCRC	first CCRC	second CCRC
Acetylcholine	6	82.5 ± 10.3	3.15×10^{-6} (1.03×10^{-6}, 9.62×10^{-6})	4.57×10^{-6} (1.70×10^{-6}, 1.23×10^{-5})
Substance P	6	101.1 ± 6.1	1.92×10^{-7} (5.14×10^{-8}, 6.78×10^{-7})	1.50×10^{-7} (2.67×10^{-8}, 8.48×10^{-7})
Neurokinin A	6	93.9 ± 7.3	1.39×10^{-7} (5.34×10^{-8}, 3.63×10^{-7})	2.10×10^{-7} (4.63×10^{-8}, 9.48×10^{-7})
Histamine	3	58.0 ± 6.0[c]	7.53×10^{-5} (1.78×10^{-5}, 3.17×10^{-4})	6.86×10^{-4} (2.96×10^{-4}, 1.59×10^{-3}) [c]
[Sar^9, $Met(O_2)^{11}$]substance P	4	15.3 ± 9.4[c]	9.87×10^{-9} (1.70×10^{-9}, 5.73×10^{-8})	NC

[a] Values for maximal contractile response (E_{max}) are expressed as mean ± S.E.M. as a percentage of the maximal contraction achieved in the first cumulative concentration–response curve.
[b] Geometric mean EC_{50} values and 95% confidence limits (in parenthesis) were calculated from each individual experiment.
n, is the number of animals used per group.
[c] $P < 0.05$, significantly different from first cumulative concentration–response curve (paired *t*-test).
NC, not calculated due to low activity at 10^{-5} M.

1). At 3×10^{-6} M, similar efficacies for neuropeptide gamma and neurokinin A were observed, E_{max} 58.4 ± 11.7% acetylcholine and 65.0 ± 3.7% acetylcholine, respectively, (Fig. 1B). Substance P and neurokinin B had similar E_{max} values at 10^{-5} M (Table 1) and potencies as reflected in the calculated EC_{25} values (Table 2).

3.2. Effect of neutral endopeptidase inhibition

Phosphoramidon did not cause any significant change in baseline tension. Neutral endopeptidase inhibition increased the amplitude of contractions to substance P and neurokinin A. Phosphoramidon (10^{-5} M) produced a leftward shift in the cumulative concentration–response curve for substance P, neurokinin A and neuropeptide gamma compared to control tracheal smooth muscle strips (Fig. 2A, B and C, respectively). This leftward shift, however, was only statistically significant for substance P and neurokinin A as reflected in the lower EC_{25} values (Table 3). Phosphoramidon did not change the amplitude of contraction or shift the cumulative concentration–response curve for the tachykinin NK_1 receptor selective agonist, [Sar^9, $Met(O_2)^{11}$]substance P (Fig. 2D), and nor to senktide (results not shown).

3.3. The effect of specific NK_1, NK_2 and NK_3 receptor agonists

The contractile effects of the specific tachykinin NK_1, NK_2 and NK_3 receptor agonists [Sar^9, $Met(O_2)^{11}$]substance P, [Nle^{10}]neurokinin A-(4–10) and senktide are shown in Fig. 3A, B and C, respectively. In each graph contractile responses to the corresponding endogenous tachykinin is also shown. The specific tachykinin NK_1 receptor agonist (Fig. 3A) is relatively inactive in ovine tracheal smooth muscle with an E_{max} at 10^{-5} M of only 11.4 ± 2.8% acetylcholine compared with substance P. The selective tachykinin NK_2 agonist, [Nle^{10}]neurokinin A-(4–10) elicited significant contractions in tracheal smooth muscle strips, however, this again was lower than that seen with neurokinin A (Table 1, Fig. 3B). Senktide was inactive in the tracheal smooth muscle preparation with an E_{max} of 0.5 ± 0.3% acetylcholine compared with neurokinin B (E_{max} 31.4 ± 8.4% acetylcholine, Fig. 3C).

Table 5
Mean maximal effect (E_{max}) and mean EC_{50} values for agonists in the second cumulative concentration–response curve in control and atropine treated tracheal strips

Agonist	*n*	E_{max} [a]		EC_{50} [b] (M)	
		control	atropine	control	atropine
Acetylcholine	6	102.4 ± 3.0	46.0 ± 4.6[c]	4.50×10^{-6} (1.75×10^{-6}, 1.16×10^{-5})	1.15×10^{-3} [c] (7.09×10^{-4}, 1.85×10^{-3})
Substance P	4	95.7 ± 7.6	101.2 ± 5.3	1.20×10^{-7} (6.08×10^{-8}, 2.38×10^{-7})	9.39×10^{-8} (6.21×10^{-9}, 1.42×10^{-6})
Neurokinin A	4	100.1 ± 9.6	100.9 ± 4.2	1.52×10^{-7} (2.52×10^{-8}, 9.2×10^{-7})	1.26×10^{-7} (2.47×10^{-8}, 6.40×10^{-7})

[a] Values for maximal contractile response (E_{max}) for second cumulative concentration–response curve are expressed as mean ± S.E.M. as a percentage of the maximal contraction achieved in the first cumulative concentration–response curve.
[b] Geometric mean EC_{50} values and 95% confidence limits (in parenthesis) were calculated from the mean EC_{50} values of the second cumulative concentration–response curve in each individual experiment.
[c] $P < 0.05$, significantly different between control and atropine treated tissue (paired *t*-test).
n, is the number of animals used per group.

3.4. Effect of atropine and pyrilamine on substance P, [Sar^9, Met(O$_2$)^{11}]substance P and neurokinin A contractility

In order to determine if acetylcholine, substance P, neurokinin A, [Sar^9, Met(O$_2$)^{11}]substance P and histamine demonstrated tachyphylaxis, successive cumulative concentration–response curves were obtained for each agonist separated by 90–100 min. Results for the consecutive cumulative concentration–response curves for acetylcholine, substance P, neurokinin A, [Sar^9, Met(O$_2$)^{11}]substance P and histamine are shown in Fig. 4A–E, respectively. No significant decrease in E_{max} or shift in the cumulative concentration–response curve for acetylcholine, substance P and neurokinin A was seen, compared with the first cumulative concentration–response curve (Fig. 4A–C, Table 4). Substance P, neurokinin A and acetylcholine, did not exhibit tachyphylaxis. In contrast a tachyphylactic response to successive [Sar^9, Met(O$_2$)^{11}]substance P and histamine cumulative concentration–response curves was observed in tracheal smooth muscle (Fig. 4D–E, Table 4).

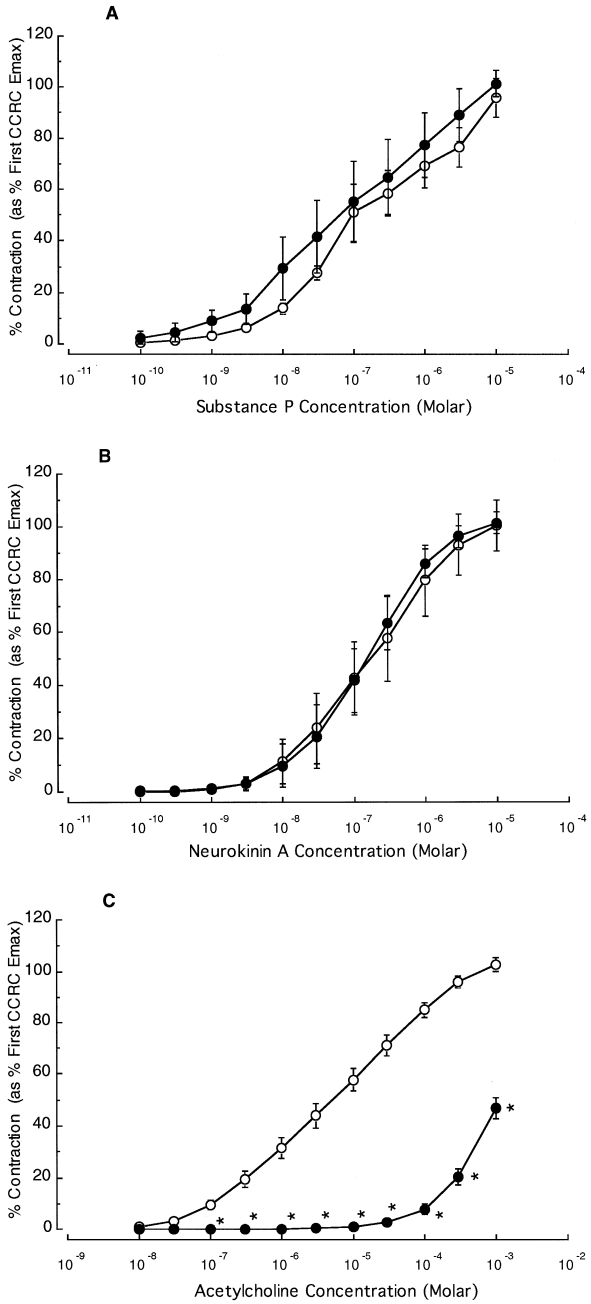

Fig. 5. Mean second cumulative concentration–response curves for (A) substance P ($n = 4$), (B) neurokinin A ($n = 4$), (C) acetylcholine ($n = 6$) in the absence (○) and presence (●) of 10^{-6} M atropine. Contractile responses are expressed as a percent of the maximal response in the first cumulative concentration–response curve (First CCRC E_{max}). Vertical bars indicate S.E.M. * Denotes a statistically significant difference ($P < 0.05$, paired t-test) between control and atropine treated tissue.

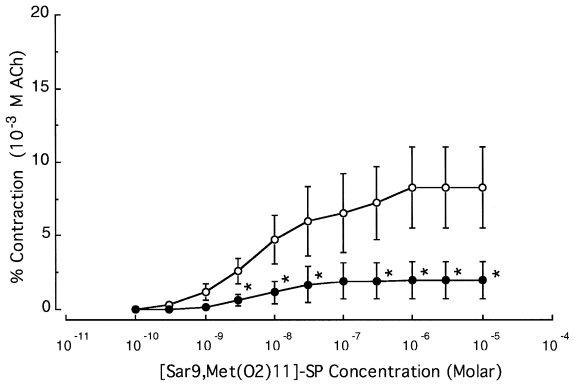

Fig. 6. Effect of the absence (○) and presence (●) of 10^{-6} M atropine on [Sar^9, Met(O$_2$)^{11}]substance P contractions in paired tracheal smooth muscle strips. Points represent mean values from 4 animals. Contractile responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M. * Denotes a statistically significant difference ($P < 0.05$, paired t-test) between control and atropine treated tissue.

In the absence of tachyphylaxis, each tissue's contractile response can be normalized to a standard contraction (i.e., the maximal effect obtained in the first cumulative concentration–response curve) and thus allows for direct comparisons between tissues receiving different treatments. Atropine at 10^{-6} M did not reduce the magnitude of substance P and neurokinin A induced contractions, nor did it affect the potency of these agonists (Table 5, Fig. 5A–B). Adequate atropine blockade was achieved as evidenced by the significant 285-fold rightward shift of the acetylcholine cumulative concentration–response curve in the presence of 10^{-6} M atropine, Table 5, Fig. 5C.

Since consecutive cumulative concentration–response curves for [Sar^9, Met(O$_2$)^{11}]substance P exhibited tachyphylaxis (Fig. 4D) the effect of atropine on [Sar^9, Met(O$_2$)^{11}]substance P contractility was examined in paired tissue samples. In these experiments atropine (10^{-6} M) was added to one of the paired tissues while the other bath received no treatment. The presence of atropine significantly attenuated the contractile response to the tachykinin

NK_1 receptor agonist, [Sar^9, $Met(O_2)^{11}$]substance P (Fig. 6); E_{max} being $8.3 \pm 2.8\%$ and $2.0 \pm 1.3\%$ of 10^{-3} M acetylcholine, in the absence and presence of atropine, respectively ($P < 0.03$).

Pyrilamine, an histamine H_1 receptor antagonist, had no affect on the cumulative concentration–response curve for substance P (Fig. 7A). In order to establish that adequate histamine receptor antagonism was achieved with pyrilamine (10^{-6} M), cumulative concentration–response curves for histamine were performed in paired tissue samples, since consecutive cumulative concentration–response curves for histamine exhibited tachyphylaxis (Fig. 4E). In this series of experiments pyrilamine was added to one of the paired tissues 30 min prior to obtaining the cumulative concentration–response curve for histamine. Fig. 7B documents that 10^{-6} M pyrilamine was sufficient to significantly antagonize histamine contractility in ovine tracheal smooth muscle, causing a significant rightward shift in the cumulative concentration–response curve (Table 4). The additions of atropine and pyrilamine to the organ baths did not cause any significant change in baseline tone.

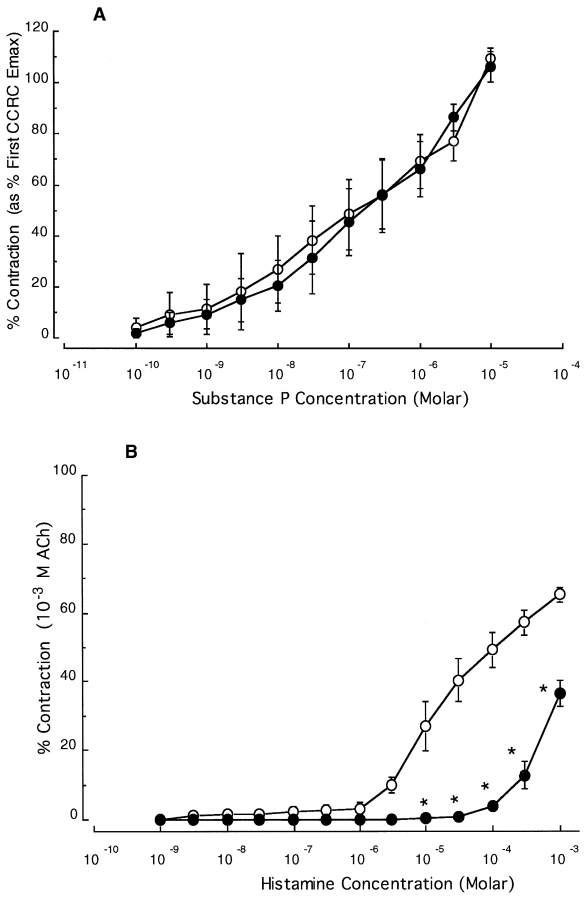

Fig. 7. (A) Mean second cumulative concentration–response curves for substance P ($n = 3$) in the absence (○) and presence (●) of 10^{-6} M pyrilamine. Contractile responses are expressed as a percent of the maximal response in the first cumulative concentration–response curve (First CCRC E_{max}). Vertical bars indicate S.E.M. (B) Effect of the absence (○) and presence (●) of 10^{-6} M pyrilamine on histamine contractions in paired tracheal smooth muscle strips. Points represent mean values from 3 animals. Contractile responses are expressed as a percentage of the reference contraction to 10^{-3} M acetylcholine. Vertical bars indicate S.E.M. * Denotes a statistically significant difference ($P < 0.05$, paired t-test) between control and pyrilamine treated tissue.

4. Discussion

We have demonstrated that, neuropeptide gamma, substance P, neurokinin A and neurokinin B contract ovine tracheal smooth muscle in a concentration-dependent manner. The rank order of potency was neuropeptide gamma > neurokinin A > substance P ≥ neurokinin B. The contractile responses to substance P and neurokinin A were increased by inhibition of neutral endopeptidase. Tachykinin-induced contraction in vitro appears to be predominantly mediated by tachykinin NK_2 receptors, although, tachykinin NK_1 receptors also appear to be involved. Substance P and neurokinin A contractions were not mediated by cholinergic mechanisms. However, a significant cholinergic component was present in [Sar^9, $Met(O_2)^{11}$]substance P induced tracheal smooth muscle contraction. Repeated exposure of tracheal smooth muscle to either acetylcholine, substance P or neurokinin A did not exhibit tachyphylaxis although, tachyphylaxis was observed to [Sar^9, $Met(O_2)^{11}$]substance P and histamine. In the case of substance P, the contractile effect was not mediated indirectly through histamine release (Foreman and Jordan, 1983).

To our knowledge, this study is the first to characterize the contractile effects of neuropeptide gamma, substance P, neurokinin A, and neurokinin B in ovine tracheal smooth muscle. Of the tachykinins studied, neuropeptide gamma was the most potent which has also been found for isolated airways from rabbits (Black et al., 1992), guinea pigs (Warner et al., 1990; Burcher et al., 1991b) and human bronchi (Burcher et al., 1991a; Qian et al., 1994). For neuropeptide gamma, neurokinin A and substance P, (in the absence of phosphoramidon) the rank order of potency parallels that seen in human, rabbit, and guinea pig airways (Advenier et al., 1987; Naline et al., 1989; Black et al., 1990, 1992; Warner et al., 1990; Qian et al., 1994). Furthermore, we show that neuropeptide gamma and neurokinin A are more potent contractile agonists than acetylcholine and histamine.

The rank order of potency for carbachol, acetylcholine and histamine in ovine tracheal smooth muscle is similar to that reported for human and rabbit isolated airway smooth muscle. The pD_2 values for acetylcholine and carbachol reported in this study are similar to those reported by others for sheep (Tomioka et al., 1991; Jackowski et al., 1993) and are more similar to those determined in isolated human airways (Cerrina et al., 1989; Naline et al., 1989; Knight et al., 1990; Qian et al., 1994) than for rabbit and guinea pig airway smooth muscle

(Advenier et al., 1987; Devillier et al., 1988; Goroumaru-Shinkai et al., 1992) (Table 6).

Contractile responses to substance P and neurokinin A were sensitive to the inhibition of endopeptidase by phosphoramidon. Phosphoramidon produced a non-uniform increase in the potency of substance P, neurokinin A and neuropeptide gamma (93-fold, 16-fold and 4-fold leftward shift of the EC_{25}, respectively). However, the change for neuropeptide gamma failed to reach significance.

The reason for the apparent lack of potentiation by phosphoramidon with neuropeptide gamma is unclear. Differences in the magnitude of augmentation following neutral endopeptidase inhibition have been observed in guinea pig and human bronchi, where substance P and neurokinin A enhancement is greater than that for neuropeptide gamma (Naline et al., 1989; Warner et al., 1990; Qian et al., 1994), indicating substance P and neurokinin A are more sensitive to neutral endopeptidase degradation. Furthermore, the potentiating effect of neutral endopeptidase inhibition for a given tachykinin is more pronounced in smaller airways than in tracheal tissue (Black et al., 1990; Goroumaru-Shinkai et al., 1992). Therefore, the lack of potentiation for neuropeptide gamma we observed in tracheal smooth muscle may reflect regional distribution of neutral endopeptidase and the susceptibility of specific tachykinins to neutral endopeptidase degradation. The lack of enhancement of the [Sar^9, $Met(O_2)^{11}$]substance P contractile response by phosphoramidon was not surprising since substance P-analogues substituted in position 9 are relatively insensitive to neutral endopeptidase inactivation (Crimi et al., 1990).

The contractile response to tachykinins in ovine tracheal smooth muscle appears to be predominantly mediated by tachykinin NK_2 receptors as evidenced by the significant contraction observed with the specific tachykinin NK_2 receptor agonist, [Nle^{10}]neurokinin A-(4–10). However, tachykinin NK_1 receptors appear to participate, as the tachykinin NK_1 receptor specific agonist ([Sar^9, $Met(O_2)^{11}$]substance P) did induce some contraction in tracheal smooth muscle. The lack of a contractile effect of senktide indicates tachykinin NK_3 receptors are not functionally present on ovine tracheal smooth muscle. The higher E_{max} for neurokinin A compared to [Nle^{10}]neurokinin A-(4–10) at equimolar concentrations is likely to be due to non-specific binding of neurokinin A to tachykinin NK_1 receptors.

We have shown, in vitro, that substance P and neurokinin A induced contractions are not mediated by a cholinergic mechanism, since their effect remained unchanged in the presence of atropine. An important finding of this present study, is that the contractile response to the highly selective tachykinin NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]substance P was atropine sensitive and exhibited tachyphylaxis. In isolated human, guinea pig, and

Table 6
Comparison of mean pD_2 for various agonists found in ovine, human, guinea pig and rabbit, tracheal[a] and bronchial[b] tissue

Agonist	Mean pD_2				
	present study	human	other sheep	guinea pig	rabbit
Carbachol	7.49[a]	6.26[b,c]	7.59[a,n]	6.86[a,c]	6.99[a,d]
Acetylcholine	5.31[a]	5.53[b,g]	5.37[a,j]	5.89[a,c]	6.43[a,i]
Histamine	4.93[a]	5.07[b,k]	ND	6.05[a,c]	4.99[a,d]
Neurokinin A	6.67[a]	6.99[b,l]	ND	8.18[a,h]	7.33[a,i]
[Nle^{10}]neurokinin A(4–10)	6.26[a]	6.22[b,l]	ND	7.25[a,h]	—
Neuropeptide gamma[p]	8.21[a]	8.35[b,m]	ND	8.74[b,o]	8.80[a,f]
Substance P	NC	4.92[b,l]	ND	6.03[a,h]	5.60[a,e]
Neurokinin B	NC	Inactive[b,l]	ND	6.83[a,h]	—

[a] Tracheal smooth muscle.
[b] Bronchial smooth muscle.
[c] Advenier et al. (1987).
[d] Armour et al. (1985).
[e] Black et al. (1990).
[f] Black et al. (1992).
[g] Cerrina et al. (1989).
[h] Devillier et al. (1988).
[i] Goroumaru-Shinkai et al. (1992).
[j] Jackowski et al. (1993).
[k] Knight et al. (1990).
[l] Naline et al. (1989).
[m] Qian et al. (1994).
[n] Tomioka et al. (1991).
[o] Zeng et al. (1994).
[p] In each case, the pD_2 was determined in the presence of phosphoramidon (10^{-5} M).
ND, not previously determined in this species.
NC, not calculated due to low agonist activity at 10^{-5} M in this tissue.

hamster airways substance P-induced contractions are reported to be via a direct effect, as antihistamines and antimuscarinic agents (atropine) have no effect on the contractile response (Lundberg et al., 1983; Uchida et al., 1987; Maggi et al., 1989). However, in isolated ferret trachea substance P-induced contractions appear to be partially mediated by a cholinergic mechanism (Sekizawa et al., 1987a,b), while in the rabbit there are conflicting data for either a direct or indirect effect of tachykinins on airway smooth muscle (Tanaka and Grunstein, 1984; Armour et al., 1991; Colasurdo et al., 1995). In all those studies substance P which lacks specificity was the agonist used. Our data, using specific tachykinin NK_1 and NK_2 receptor agonists show that the effect of substance P on tracheal smooth muscle can be partitioned into two mechanisms. Firstly, substance P acts via tachykinin NK_1 receptors associated with postganglionic nerves in tracheal smooth muscle, resulting in the release of acetylcholine, and secondly by a direct effect on tracheal smooth muscle tachykinin NK_2 receptors.

It would appear that in in vitro studies the apparent absence or presence of a cholinergic mechanism in species is due to the different methodologies which result in changes to the relative contribution of the tachykinin NK_1 and NK_2 receptor mediated-effects of substance P on tracheal smooth muscle. In the rabbit, Tanaka and Grunstein (1984) used small doses of substance P ($4–8 \times 10^{-8}$ M) on tracheal smooth muscle which preferentially activate prejunctional tachykinin NK_1 receptors on cholinergic nerves rather than the smooth muscle tachykinin NK_2 receptors (Cook et al., 1990) thus accentuating the cholinergic component. Armour et al. (1991), used higher doses of substance P (10^{-5} M) which would tend to mask a small cholinergic effect of substance P in tracheal smooth muscle via prejunctional tachykinin NK_1 receptors because of the overriding predominant effect of substance P on tachykinin NK_2 receptors. Furthermore, in rabbit bronchial smooth muscle the atropine sensitive component disappeared indicating that substance P-induced contractions were mediated by a direct action on both tachykinin NK_1 and NK_2 receptors (Cook et al., 1990). Cholinergic pathways appear to be less important in smaller airways as cholinergic receptor density and sensitivity decrease from proximal to distal airways (Barnes et al., 1983; Armour et al., 1985), while tachykinin receptor sensitivity and density increases from larger to smaller airways (Black et al., 1990). Tachykinin-induced contractions in human bronchial smooth muscle are solely mediated by tachykinin NK_2 receptors (Naline et al., 1989; Advenier et al., 1992) while in the guinea pig tachykinins mediate their contractile effects in tracheal smooth muscle and bronchial smooth muscle via tachykinin NK_1 and NK_2 smooth muscle receptors (Ireland et al., 1991; Maggi et al., 1991).

Our in vitro results demonstrate that the predominant action of substance P and neurokinin A is to induce contraction in tracheal smooth muscle via a direct effect on tachykinin NK_2 receptors. However, in vivo studies in sheep, substance P administered intravenously is a more potent bronchoconstrictor than neurokinin A, and the airway response is cholinergically mediated (Corcoran and Haigh, 1992; Parsons et al., 1992; Rice et al., 1995b). It is postulated that, in vivo, exogenous tachykinins induce bronchoconstriction by the activation of cholinergic nerves resulting in the prejunctional release of acetylcholine from postganglionic nerves, and by the release of mast cell mediators, in addition to any direct contractile effect on airway smooth muscle. In addition, the relative contributions of each of these mechanisms to the overall bronchoconstrictor response appears to differ between species (Joos et al., 1994). From our in vivo sheep studies, tachykinin-induced bronchoconstriction appears to be predominantly mediated by an tachykinin NK_1 receptor cholinergic mechanism given the tachykinin NK_1 receptor antagonist and atropine sensitivity of the observed bronchoconstriction (Rice et al., 1995b). The lack of a direct tachykinin NK_2-mediated bronchoconstrictor effect to substance P in vivo, may simply reflect the relatively small amounts of substance P reaching tachykinin NK_2 smooth muscle receptors, since a significant proportion of both the aerosol and intravascular substance P doses would be inactivated by neutral endopeptidase and angiotensin-converting enzyme (Joos et al., 1994).

The use of two consecutive cumulative concentration–response curves has been suggested as the most valid method for comparing the effect of different treatments on contractile responses. Contractions are normalized to a standard contraction, thus eliminating the confounding effects of variability in the actual magnitude of contraction (Marthan et al., 1987; Black et al., 1989). Using this experimental approach we have demonstrated, in vitro, that substance P and neurokinin A induced airway smooth muscle contractions do not exhibit tachyphylaxis. The absence of tachyphylaxis to substance P and neurokinin A has also been observed in isolated hamster, ferret and guinea pig airways (Sekizawa et al., 1987b; Maggi et al., 1989; Ireland et al., 1991), however, there is conflicting evidence for tachyphylaxis in rabbit airways (Tanaka and Grunstein, 1984; Cook et al., 1990; Armour et al., 1991). This discrepancy once again, is most likely due to the differences in experimental design (see above) which would change the relative contributions of the tachykinin NK_1 cholinergic effect (which does exhibit tachyphylaxis) in tracheal smooth muscle contractions induced by substance P.

Ovine tracheal smooth muscle did exhibit tachyphylaxis to histamine, a phenomenon that has been shown both, in vivo and in vitro, in dogs (Antol et al., 1988), but appears not to occur in isolated human airways (Marthan et al., 1987; Black et al., 1989). In vivo, human subjects have demonstrated tachyphylaxis to inhaled histamine (Manning et al., 1987), however, an absence of tachyphylaxis to histamine inhalation has also been observed (Ruffin et al.,

1981) and has been attributed to corticosteroid use in this group of subjects.

The lack of a tachyphylactic effect to histamine in isolated ovine smooth muscle reported by Eyre (1969) may be explained by the fact that his study simply used single bolus doses of agonist whereas, we conducted formal cumulative concentration–response studies. This latter approach allows for a more detailed assessment of agonist efficacy and potency, without failing to detect tachyphylaxis.

In conclusion, we have demonstrated that airway in vitro responses to tachykinins, acetylcholine and histamine in sheep are similar to those in man, and indeed, more so than those of the guinea pig or rabbit. Given the regional differences in tachykinin contractility observed in other species (Manzini et al., 1989; Black et al., 1990, 1992; Goroumaru-Shinkai et al., 1992), further experiments are required to characterize tachykinin receptor function in smaller ovine airways.

Acknowledgements

Supported by a grant from the Special Purposes Fund, Royal Adelaide Hospital, and a scholarship provided by the Department of Thoracic Medicine, Royal Adelaide Hospital. We wish to thank Associate Professor Carol Armour, Department of Pharmacy, and Dr. Peter Johnson, Department of Pharmacology, University of Sydney for their help.

References

Abraham, W.M., Delehunt, J.C., Yerger, L., Marchette, B., 1983. Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. Am. Rev. Respir. Dis. 128, 839–844.

Advenier, C., Naline, E., Drapeau, G., Regoli, D., 1987. Relative potencies of neurokinins in guinea pig trachea and human bronchus. Eur. J. Pharmacol. 139, 133–137.

Advenier, C., Naline, E., Toty, L., Bakdach, H., Emonds-Alt, X., Vilain, P., Breliere, J.C., Le Fur, G., 1992. 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.

Antol, P.J., Gunst, S.J., Hyatt, R.E., 1988. Histamine tachyphylaxis in canine airways despite prostaglandin synthesis inhibition. J. Appl. Physiol. 65, 1944–1949.

Armour, C.L., Black, J.L., Berend, N., 1985. Histamine and carbachol contractile responses in proximal and distal airways of the rabbit. Clin. Exp. Pharmacol. Physiol. 12, 19–23.

Armour, C.L., Johnson, P.R., Alouan, L.A., Black, J.L., 1991. Substance P-induced contraction of rabbit airways: Mechanism of action. J. Appl. Physiol. 70, 813–817.

Barnes, P.J., Basbaum, C.B., Nadel, J.A., 1983. Autoradiographic localization of autonomic receptors in airway smooth muscle. Marked differences between large and small airways. Am. Rev. Respir. Dis 127, 758–762.

Black, J.L., Johnson, P.R.A., Armour, C.L., 1988. Potentiation of the contractile effects of neuropeptides in human bronchus by an enkephalinase inhibitor. Pulm. Pharmacol. 1, 21–223.

Black, J.L., Marthan, R., Armour, C.L., Johnson, P.R., 1989. Sensitization alters contractile responses and calcium influx in human airway smooth muscle. J. Allergy Clin. Immunol. 84, 440–447.

Black, J., Diment, L., Armour, C., Johnson, P., 1990. Distribution of substance P receptors in rabbit airways, functional and autoradiographic studies. J. Pharmacol. Exp. Ther. 253, 381–385.

Black, J.L., Diment, L.M., Alouan, L.A., Johnson, P.R., Armour, C.L., Badgery-Parker, T., Burcher, E., 1992. Tachykinin receptors in rabbit airways – characterization by functional, autoradiographic and binding studies. Br. J. Pharmacol. 107, 429–436.

Burcher, E., Alouan, L.A., Johnson, P.R., Black, J.L., 1991a. Neuropeptide gamma, the most potent contractile tachykinin in human isolated bronchus, acts via a 'non-classical' NK_2 receptor. Neuropeptides 20, 79–82.

Burcher, E., Mussap, C.J., Geraghty, D.P., McClure-Sharp, J.M., Watkins, D.J., 1991b. Concepts in characterization of tachykinin receptors. Ann. NY Acad. Sci. 632, 123–136.

Cerrina, J., Labat, C., Haye-Legrande, I., Raffestin, B., Benveniste, J., Brink, C., 1989. Human isolated bronchial muscle preparations from asthmatic patients: Effects of indomethacin and contractile agonists. Prostaglandins 37, 457–469.

Cheung, D., Bel, E.H., Den Hartigh, J., Dijkman, J.H., Sterk, P.J., 1992. The effect of an inhaled neutral endopeptidase inhibitor, thiorphan, on airway responses to neurokinin A in normal humans in vivo. Am. Rev. Respir. Dis. 145, 1275–1280.

Colasurdo, G.N., Loader, J.E., Graves, J.P., Larsen, G.L., 1995. SP-induced contraction of airway smooth muscle in normal and allergen-sensitized rabbits: Mechanism of action. J. Appl. Physiol. 78, 428–432.

Cook, J.A., Brunner, S.L., Tanaka, D.T., 1990. Neurokinin receptors mediating substance P-induced contraction in adult rabbit airways. Am. J. Physiol. 258, L99–106.

Corcoran, B.M., Haigh, A.L., 1992. The effect of tachykinins on sheep bronchomotor tone. Exp. Physiol. 77, 471–479.

Crimi, N., Palermo, F., Oliveri, R., Palermo, B., Vancheri, C., Polosa, R., Mistretta, A., 1990. Influence of antihistamine (astemizole) and anticholinergic drugs (ipratropium bromide) on bronchoconstriction induced by substance P. Ann. Allergy 65, 115–120.

Devillier, P., Advenier, C., Drapeau, G., Marsac, J., Regoli, D., 1988. Comparison of the effects of epithelium removal and of an enkephalinase inhibitor on the neurokinin-induced contractions of guinea-pig isolated trachea. Br. J. Pharmacol. 94, 675–684.

Eyre, P., 1969. The pharmacology of sheep tracheobronchial muscle: A relaxant effect of histamine on the isolated bronchi. Br. J. Pharmacol. 36, 409–417.

Foreman, J., Jordan, C., 1983. Histamine release and vascular changes induced by neuropeptides. Agents Act. 13, 105–116.

Goroumaru-Shinkai, M., Yamamoto, R., Funayama, N., Takayanagi, I., 1992. Regional differences of the contractile responses to acetylcholine and neurokinin A in rabbit airway: Heterogeneous distribution of the metabolic enzymes. Gen. Pharmacol. 23, 1057–1061.

Helke, C.J., Krause, J.E., Mantyh, P.W., Couture, R., Bannon, M.J., 1990. Diversity in mammalian tachykinin peptidergic neurons: Multiple peptides, receptors, and regulatory mechanisms. FASEB J. 4, 1606–1615.

Hudgin, R.L., Charleson, S.E., Zimmerman, M., Mumford, R., Wood, P.L., 1981. Enkephalinase: Selective peptide inhibitors. Life Sci. 29, 2593–2601.

Ireland, S.J., Bailey, F., Cook, A., Hagan, R.M., Jordan, C.C., Stephens-Smith, M.L., 1991. Receptors mediating tachykinin-induced contractile responses in guinea-pig trachea. Br. J. Pharmacol. 103, 1463–1469.

Jackowski, J., Chapman, G.A., Abraham, W.M., Ahmed, T., 1993. Ovine tracheal muscle contraction in vitro: Inhibition by calcium channel blockers gallopamil and verapamil. Respiration 60, 27–31.

Joos, G., Pauwels, R., Van der Straeten, M., 1987. Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. Thorax 42, 779–783.

Joos, G.F., Germonpre, P.R., Kips, J.C., Peleman, R.A., Pauwels, R.A., 1994. Sensory neuropeptides and the human lower airways – present state and future directions. Eur. Respir. J. 7, 1161–1171.

Knight, D.A., Adcock, J.A., Phillips, M.J., Thompson, P.J., 1990. The effect of epithelium removal on human bronchial smooth muscle responsiveness to acetylcholine and histamine. Pulm. Pharmacol. 3, 198–202.

Lundberg, J.M., Martling, C.R., Saria, A., 1983. Substance P and capsaicin-induced contraction of human bronchi. Acta Physiol. Scand. 119, 49–53.

Lundberg, J.M., Hokfelt, T., Martling, C.R., Saria, A., Cuello, C., 1984. Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res. 235, 251–261.

Maggi, C.A., Patacchini, R., Rovero, P., Meli, A., 1989. The hamster isolated trachea: A new preparation for studying NK-2 receptors. Eur. J. Pharmacol. 166, 435–440.

Maggi, C.A., Patacchini, R., Quartara, L., Rovero, P., Santicioli, P., 1991. Tachykinin receptors in the guinea-pig isolated bronchi. Eur. J. Pharmacol. 197, 167–174.

Manning, P.J., Jones, G.L., O'Byrne, P.M., 1987. Tachyphylaxis to inhaled histamine in asthmatic subjects. J. Appl. Physiol. 63, 1572–1577.

Manzini, S., Conti, S., Maggi, C.A., Abelli, L., Somma, V., Del Bianco, E., Geppetti, P., 1989. Regional differences in the motor and inflammatory responses to capsaicin in guinea pig airways. Correlation with content and release of substance P-like immunoreactivity. Am. Rev. Respir. Dis. 140, 936–941.

Marthan, R., Armour, C.L., Johnson, P.R., Black, J.L., 1987. The calcium channel agonist BAY K8644 enhances the responsiveness of human airway muscle to KCl and histamine but not to carbachol. Am. Rev. Respir. Dis. 135, 185–189.

Martling, C.R., 1987. Sensory nerves containing tachykinins and CGRP in the lower airways. Functional implications for bronchoconstriction, vasodilatation and protein extravasation. Acta Physiol. Scand. Suppl. 563, 1–57.

Mitchell, R.W., Koenig, S.M., Kelly, E., Stephens, N.L., Leff, A.R., 1989. Ca^{2+}-dependent facilitated shortening in isotonic contraction of trachealis muscle. J. Appl. Physiol. 66, 632–637.

Naline, E., Devillier, P., Drapeau, G., Toty, L., Bakdach, H., Regoli, D., Advenier, C., 1989. Characterization of neurokinin effects and receptor selectivity in human isolated bronchi. Am. Rev. Respir. Dis. 140, 679–686.

Parsons, G.H., Nichol, G.M., Barnes, P.J., Chung, K.F., 1992. Peptide mediator effects on bronchial blood velocity and lung resistance in conscious sheep. J. Appl. Physiol. 72, 1118–1122.

Qian, Y., Advenier, C., Naline, E., Bellamy, J.F., Emonds-Alt, X., 1994. Effects of SR 48968 on the neuropeptide gamma-induced contraction of the human isolated bronchus. Fundam. Clin. Pharmacol. 8, 71–75.

Rice, A., Thornton, A., Holmes, M., Reynolds, P., Scicchitano, R., 1995a. Tachykinins contribute to immediate allergic airways response in sheep. Am. J. Respir. Crit. Care Med. 151, A346.

Rice, A., Thornton, A., Holmes, M., Reynolds, P., Scicchitano, R., 1995b. The effect of infused substance P in normal sheep. Am. J. Respir. Crit. Care Med. 151, A107.

Ruffin, R.E., Alpers, J.H., Crockett, A.J., Hamilton, R., 1981. Repeated histamine inhalation tests in asthmatic patients. J. Allergy Clin. Immunol. 67, 285–289.

Sekizawa, K., Tamaoki, J., Graf, P.D., Basbaum, C.B., Borson, D.B., Nadel, J.A., 1987a. Enkephalinase inhibitor potentiates mammalian tachykinin-induced contraction in ferret trachea. J. Pharmacol. Exp. Ther. 243, 1211–1217.

Sekizawa, K., Tamaoki, J., Nadel, J.A., Borson, D.B., 1987b. Enkephalinase inhibitor potentiates substance P- and electrically induced contraction in ferret trachea. J. Appl. Physiol. 63, 1401–1405.

Tanaka, D.T., Grunstein, M.M., 1984. Mechanisms of substance P-induced contraction of rabbit airway smooth muscle. J. Appl. Physiol. 57, 1551–1557.

Tomioka, K., Jackowski, J.T., Abraham, W.M., 1991. Characterization of sulfidopeptide leukotriene responses in sheep tracheal smooth muscle in vitro. Can. J. Physiol. Pharmacol. 69, 805–811.

Uchida, Y., Nomura, A., Ohtsuka, M., Hasegawa, S., Goto, K., Kimura, S., Sugita, Y., Uchiyama, Y., 1987. Neurokinin A as a potent bronchoconstrictor. Am. Rev. Respir. Dis. 136, 718–721.

Warner, E.A., Krell, R.D., Buckner, C.K., 1990. Pharmacologic studies on the differential influence of inhibitors of neutral endopeptidase on nonadrenergic, noncholinergic contractile responses of the guinea pig isolated hilar bronchus to transmural electrical stimulation and exogenously applied tachykinins. J. Pharmacol. Exp. Ther. 254, 824–830.

Zeng, X.P., Lavielle, S., Burcher, E., 1994. Evidence for tachykinin NK-2 receptors in guinea-pig airways from binding and functional studies, using [^{125}I]-[Lys^5, Tyr(I_2)^7, MeLeu^9, Nle^{10}]-NK A-(4–10). Neuropeptides 26, 1–9.