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Effects of tachykinins and capsaicin on the mechanical and electrical activity of the guinea-pig isolated trachea

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1 The effects of tachykinins and capsaicin were studied by means of intracellular membrane potential and isometric tension recordings in the isolated trachea of the guinea-pig.

2 The basal membrane potential averaged −51 mV, and most preparations demonstrated spontaneous slow waves. Tetraethylammonium (TEA), a potassium channel blocker (8×10^{-3} M), depolarized the membrane potential to −44 mV and induced a rhythmic activity.

3 In control solution, substance P (10^{-8}–10^{-6} M), [Nle^{10}]-neurokinin A(4–10) (10^{-8}–10^{-6} M) and capsaicin (10^{-7}–10^{-6} M) induced concentration-dependent depolarizations which were statistically significant at the highest concentration tested (depolarization by 10^{-6} M: 8, 11 and 16 mV for the NK_1 agonist, the NK_2 agonist and capsaicin, respectively).

4 In the presence of TEA (8×10^{-3} M), the three substances induced depolarizations which were statistically significant at the highest concentration tested for substance P (10^{-6} M) and at 10^{-7} and 10^{-6} M for both [Nle^{10}]-neurokinin A(4–10) and capsaicin (depolarization by 10^{-6} M: 11, 17 and 10 mV for substance P, [Nle^{10}]neurokinin A(4–10) and capsaicin, respectively).

5 In the presence or absence of tetraethylammonium, [MePhe^7]-neurokinin B (10^{-8}–10^{-6} M) did not induce any significant changes in membrane potential.

6 The depolarizing effects of substance P (10^{-6} M) and [Nle^{10}]-neurokinin A(4–10) (10^{-6} M) were blocked only by the specific antagonists for NK_1 and NK_2 receptors, SR 140333 (10^{-7} M) and SR 48968 (10^{-7} M), respectively. The effects of capsaicin (10^{-6} M) were partially inhibited by each antagonist and fully blocked by their combination.

7 Substance P (10^{-9} to 10^{-4} M), [Nle^{10}]-neurokinin A(4–10) (10^{-10} to 10^{-5} M), [MePhe^7]-neurokinin B and capsaicin (10^{-7} to 10^{-5} M) evoked concentration-dependent contractions.

8 The contractions to substance P were significantly inhibited by SR 140333 (10^{-8} to 10^{-6} M) but unaffected by SR 48968 (10^{-8} to 10^{-6} M). Furthermore, the response to [Nle^{10}]-neurokinin A(4–10) was significantly inhibited by SR 48968 and unaffected by SR 140333 at the same concentrations. Although SR 48968 (10^{-7} M) alone did not influence the effects of substance P, it potentiated the inhibitory effect of SR 140333 (10^{-7} M). A similar synergetic effect of these two compounds was observed in the inhibition of the contractile response to [Nle^{10}]-neurokinin A(4–10).

9 Neither SR 140333 (10^{-7} M) nor SR 48968 (10^{-7} M) alone influenced the contractions to [MePhe^7]-neurokinin B and capsaicin. However, the combination of the two antagonists abolished the contractions to either peptide.

10 These results demonstrate that the stimulation of both NK_1 and NK_2 tachykinin-receptors induced contraction and depolarization of the guinea-pig tracheal smooth muscle and that both receptors were stimulated during the endogenous release of tachykinins by capsaicin. There was no evidence for a major role of NK_3 receptors in the contractile and electrical activity of the guinea-pig isolated trachea.

Keywords: Tachykinins; capsaicin; tracheal smooth muscle; electrophysiology; neurokinin receptor subtypes; subtance P; neurokinin-A; neurokinin-B

Introduction

In the airways, sensory nerve endings, identified as C-fibres, release neuropeptides such as calcitonin gene-related peptide and tachykinins (substance P, neurokinin A and neurokinin B) in response to physiological stimuli but also to inhaled irritant substances (Lundberg & Saria, 1983; Barnes *et al*., 1991a,b). Tachykinins are involved in neurogenic inflammatory disorders and asthma as they cause bronchoconstriction, bronchial vasodilatation, microvascular leakage, inflammatory cell mobilization and activation, and mucous hypersecretion (Barnes *et al*., 1991a,b; Solway & Leff, 1991; Ichinose *et al*., 1992; Manzini, 1994). They bind to specific cell-surface receptors namely NK_1, NK_2 and NK_3, characterized by their preferential activation by endogenous substance P, neurokinin A and neurokinin B, respectively (Regoli *et al*., 1994; Maggi, 1995). Determination of the involvement of each receptor subtype has been possible since the discovery of potent, selective and metabolically-stable antagonists of the NK_2 and NK_1 receptors such as SR 48968 and SR 140333 (Emonds-Alt *et al*., 1992; 1993).

Smooth muscle contraction to neurohumoral substances could involve pharmacomechanical and/or electromechanical coupling (Bolton, 1979). Indeed, acetylcholine histamine (Ahmed *et al*., 1984) and leukotrienes (McCraig & Rodger, 1988) depolarize the membrane of tracheal smooth muscle cells and, in some instances, changes in membrane potential and mechanical responses caused by receptor activation could be correlated. However, the effects of tachykinins on the electrical activity of airway smooth muscle cells are not known at present.

The purpose of this work was to study the contractile responses and the changes in membrane potential of the guinea-pig tracheal smooth muscle cells caused by natural and synthetic tachykinins (substance P, [Nle^{10}]-neurokinin A(4–10) and [MePhe^7]-neurokinin B) and capsaicin, a drug known to

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release endogenous tachykinins from the sensory C-fibre endings (Lundberg & Saria, 1983). To determine the receptors involved in these responses, potent and specific tachykinin receptor antagonists, SR 48968 and SR 140333, were studied.

Methods

Male Hartley guinea-pigs (250–350 g, Charles River, France) were anaesthetized with an overdose of pentobarbitone (25 mg kg^{-1}, intraperitoneally) and the trachea was excised from the animal and cleaned of adhering fat and connective tissue.

Electrophysiological experiments

One segment of the tracheal ring (about 5 mm in width) was isolated and cut open along the longitudinal axis, the epithelial layer was preserved as intact as possible. The strip was pinned horizontally (mucosal surface upward) to the bottom of an organ bath (0.5 ml), continuously superfused at a constant flow rate of 2 ml min^{-1} with a modified Krebs Ringer-bicarbonate solution (37°C, pH 7.4 and gassed with 95% O_2-5% CO_2 gas mixture). Transmembrane potentials were recorded with glass microelectrodes filled with KCl (3 M) with a tip resistance of 50–90 MΩ. The microelectrode was mounted on a sliding micromanipulator (Leitz, St Gallen, Switzerland). The potential recorded was amplified by means of a recording preamplifier (WPI, Intra 767, New Haven CT) with capacitance neutralization. The signal was monitored on an oscilloscope (3091 Nicolet, Madison WI) and continuously recorded on paper (Gould, Valley View, Ohio, U.S.A.) and on a video recorder (TEAC, XR310, Tokyo, Japan); the latter allowed replay for further analysis. Impalements were not accepted as valid unless they were signalled by a sudden change in voltage and were maintained until the membrane potential had stabilized.

Preparations were left to equilibrate for 30 min (at least). Agonists were added in a cumulative manner. If the microelectrode was dislodged during the experimental procedure, impalements were renewed and the next concentration of drug applied. Only one concentration-response curve and only one agonist were studied on a given strip of tissue. Antagonists of the NK_1 and NK_2 tachykinin receptors, SR 140333 (10^{-7} M) and SR 48968 (10^{-7} M), respectively, were incubated for at least 30 min before the addition of the various agonists.

Organ bath experiments

The guinea-pig isolated tracheae were cut into rings and suspended in organ baths filled with modified Krebs-Ringer bicarbonate solution. The epithelial layer was maintained as intact as possible. Tissues were connected to a force transducer (Gould) and changes in tension were recorded on a polygraph (Gould). Rings were stretched step by step until optimal and reproducible contraction to KCl (40 mM) was achieved (basal tension, approximately 2.0 g). A reference contraction was then produced with a concentration of KCl (60 mM) which gave the maximum contraction to the depolarizing solution. After repeated rinses, rings were subjected to a 45 min period of equilibration. Cumulative concentration-response curves for substance P, [Nle^{10}]neurokininA(4–10), [$MePhe^7$]neurokinin B and capsaicin were then performed. Only one concentration-response curve and only one antagonist were studied on a given strip of tissue. Each antagonist-treated preparation had its own time-matched, vehicle-treated control preparation obtained from the same animal. Antagonists of the NK_1 and NK_2 tachykinin receptors, SR 140333 and SR 48968, respectively, were incubated for at least 30 min before the addition of the various antagonists.

In order to palliate an eventual degradation of tachykinins, electrophysiological and organ bath experiments were carried out in the presence of the neutral endopeptidase inhibitor, phosphoramidon (10^{-5} M) and the angiotensin converting enzyme inhibitor, captopril (10^{-6} M). These inhibitors did not alter the resting membrane potential of the tissue (data not shown).

Drugs and solutions

Composition of the modified Krebs-Ringer bicarbonate solution (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, EDTA 0.026 and glucose 11.1 Captopril, capsaicin, phosphoramidon, substance P, tetraethylammonium chloride (Sigma, La Verpillière, France); [Nle^{10}]neurokinin A(4–10), [$MePhe^7$]-neurokinin B (Novabiochem, Paris, France); SR 48968 ((**S**)-N-methyl-N[4-(4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl-butyl]benzamide), SR140333 ((**S**)-1-(2-[3,4-dichlorophenyl)-1(3-isopropoxyphenylacetyl)piperidin-3yl]ethyl)-4phenyl1azo,iabicyclo[2.2.2]octane chloride) (Sanofi Recherche, Montpellier, France). Substance P, [$MePhe^7$]-neurokinin B, captopril and phosphoramidon were dissolved in distilled water and were then diluted in Krebs solution. Solutions of [Nle^{10}]neurokinin A(4–10), capsaicin, SR48968 and SR 140333 were prepared in 0.4% of ethanol, then dissolved in distilled water and diluted in Krebs solution.

Statistical analysis of the results

Data are shown as mean ± s.e.mean. For organ bath experiments, *n* indicates the number of animals from which tissue was taken. Statistical evaluation was performed by a two-way analysis of variance (treatment × concentration) with repeated measure on the two factors. When a significant interaction was observed ($P<0.05$), a complementary analysis was undertaken (Newman-Kuel's test) to identify differences between groups. EC_{50} calculation was performed by a linear regression within the two half log concentrations surrounding the 50% value. Apparent antagonist dissociation constants were determined according to the equation: $K_B = [Ant]/(\text{concentration-ratio} - 1)$ where [Ant] is the concentration of the antagonist and concentration-ratio is the EC_{50} in the presence of the antagonist divided by the EC_{50} of the agonist in the absence of the antagonist. These results were then expressed as the negative logarithm of the K_B (i.e. $-\log(K_B) = pK_B$). pA_2 values were calculated according to Tallarida's method; the slope of the Schild plot was constrained to unity (Tallarida *et al*., 1979). Study of interactions between two antagonists were performed by a three-way analysis of variance (antagonist 1 × antagonist 2 × agonist) with repeated measures on the factor agonist.

For electrophysiological experiments, *n* indicates the number of cells in which membrane potential was recorded. When only one concentration of agonist was studied, unpaired Student's *t* test was performed to determine the difference with control ($P<0.05$). When more than one concentration of agonist was studied, a one-way analysis of variance was performed. When a significant interaction was observed ($P<0.05$), a complementary analysis was undertaken (Newman-Keul's test).

Results

Contractile studies

Effect of NK_1 stimulation In guinea-pig isolated trachea, substance P (10^{-9} M to 10^{-5} M) induced a contractile response (Figure 1). The concentration-response curve was significantly shifted to the right in a concentration-dependent manner by SR 140333 (10^{-8}, 10^{-7} and 10^{-6} M). The maximal response to substance P was not affected by the NK_1 receptor antagonist (Figure 1a). The slope of the Schild plot was significantly different from unity (0.57 ± 0.23). Apparent pA_2 value calculated with the slope of the Schild plot constrained to unity was 7.54 ± 0.20. The NK_2 receptor antagonist SR

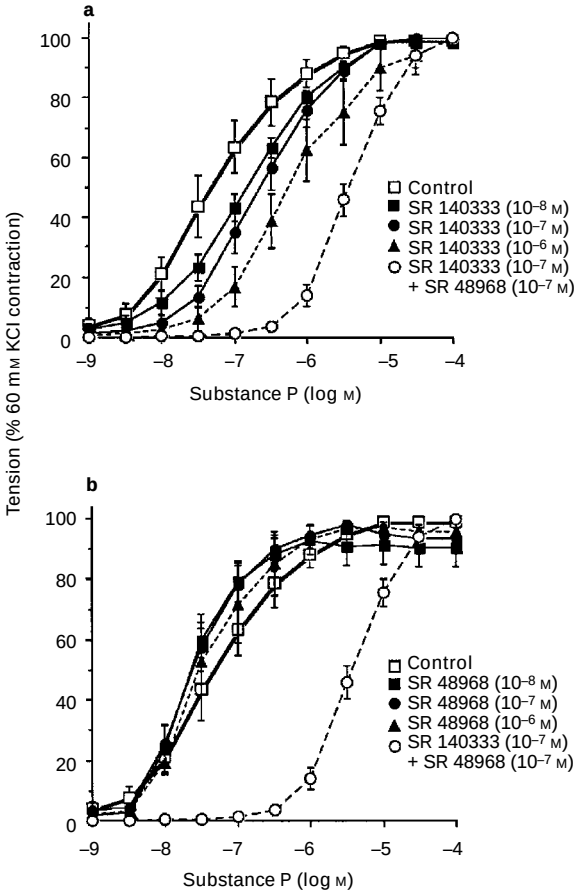

Figure 1 Cumulative concentration-response curves to substance P in guinea-pig isolated trachea (presence of captopril: 10^{-6} M and phosphoramidon: 10^{-5} M). (a) Effect of SR 140333 (10^{-8}, 10^{-7} and 10^{-6} M). A concentration-dependent statistically significant inhibition was observed at the three concentrations studied (two-way analysis of variance, $P<0.05$). (b) Effect of SR 48968 (10^{-8}, 10^{-7} and 10^{-6} M). In both (a) and (b) the effect of the combination of SR 140333 (10^{-7} M) plus SR 48968 (10^{-7} M) is indicated. A statistically significant interaction was observed when the combination of the two compounds was compared to either inhibitor individually at the same concentration (three-way analysis of variance: $P<0.05$): Data are presented as means and vertical lines show s.e.mean ($n=6$).

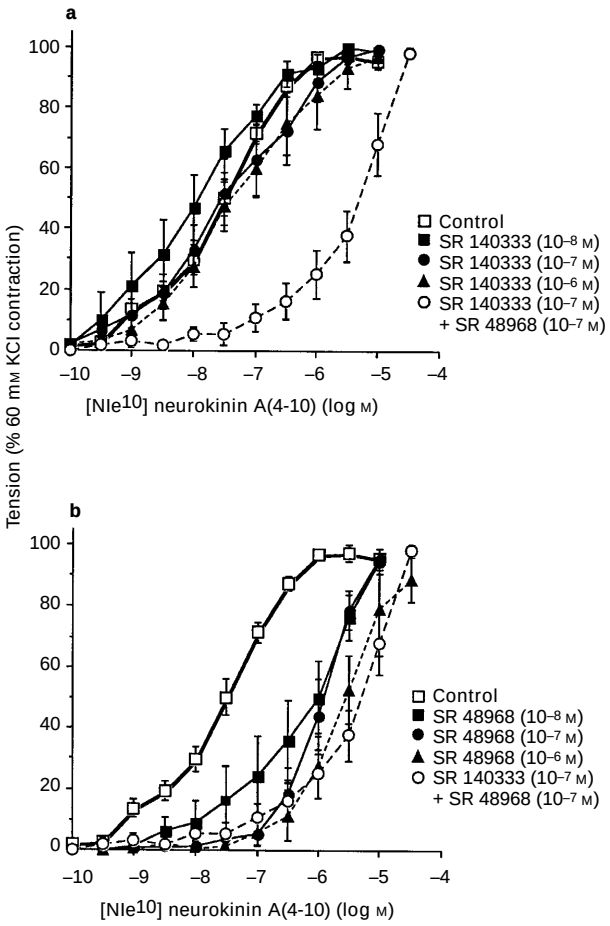

Figure 2 Cumulative concentration-response curves to [Nle^{10}]-neurokinin A(4–10) in guinea-pig isolated trachea (presence of captopril: 10^{-6} M and phosphoramidon: 10^{-5} M). (a) Effect of SR 140333 (10^{-8}, 10^{-7} and 10^{-6} M). (b) Effect of SR 48968 (10^{-8}, 10^{-7} and 10^{-6} M). A concentration-dependent statistically significant inhibition was observed at the three concentrations studied (two-way analysis of variance, $P<0.05$). In both (a) and (b): the effect of the combination of SR 140333 (10^{-7} M) plus SR 48968 (10^{-7} M) is indicated. A statistically significant interaction was observed when the combination of the two compounds was compared to either inhibitor individually at the same concentration (three-way analysis of variance; $P<0.05$). Data are presented as means and vertical lines show s.e.mean ($n=6$).

48968, up to 10^{-6} M, did not influence the contractile response to substance P (Figure 1b). However, in presence of SR 48968 (10^{-7} M) the effect of SR 140333 (10^{-7} M) was significantly potentiated (pK_B of SR 140333 alone 7.61 ± 0.12; pK_B of the combination of SR 140333 + SR 48968: 8.93 ± 0.21, $n=6$), (Figure 1).

Effect of NK_2 stimulation [Nle^{10}]-neurokinin A(4–10) (10^{-10} M to 10^{-5} M) caused smooth muscle contractions which were significantly and concentration-dependently inhibited by SR 48968 (10^{-8}, 10^{-7} and 10^{-6} M) (Figure 2b). The maximal response to [Nle^{10}]-neurokinin A(4–10) was not affected by the NK_2 receptor antagonist. The slope of the Schild plot was significantly different from unity (0.38 ± 0.26). Apparent pA_2 value calculated with the slope of the Schild plot constrained to unity was 8.49 ± 0.24. The NK_1 receptor antagonist SR 140333, up to 10^{-6} M, did not influence the contractile response to substance P (Figure 2a). However in the presence of SR 140333 (10^{-7} M) the effect of SR 48968 (10^{-7} M) was significantly potentiated (pK_B of SR 48968 alone: 8.49 ± 0.23; pK_B of the combination of SR 140333 + SR 48968: 9.15 ± 0.22, $n=6$) (Figure 2).

Effect of NK_3 stimulation [MePhe^7]-neurokinin B (10^{-8} M to 10^{-5} M) caused smooth muscle contractions which were not significantly influenced either by SR 140333 (10^{-7} M) or by SR 48968 (10^{-7} M) but which were fully abolished by the combination of the two antagonists (Figure 3a).

Effect of capsaicin Similarly, capsaicin (10^{-8} M to 10^{-5} M) caused smooth muscle contractions which were not influenced by SR 48968 (10^{-7} M) or SR 140333 (10^{-7} M) individually but abolished by the combination of both NK_1 and NK_2 receptor antagonists (Figure 3b).

Electrophysiological studies

Effects of substance P, [Nle^{10}] neurokinin A(4–10), [MePhe^7] neurokinin B and capsaicin in control solution In control solution, the smooth muscle membrane potential of guinea-pig isolated trachea averaged -51 ± 1 mV ($n=59$). Most of the smooth muscle cells exhibited oscillations of the membrane potential (slow wave) which varied according to the preparation (Figure 4). In preparations presenting slow waves, the mean amplitude and frequency was 10 mV and 60 cycles min^{-1}.

Substance P (10^{-8}, 10^{-7}, 10^{-6} M), [Nle^{10}]-neurokinin A(4–10), (10^{-8}, 10^{-7}, 10^{-6} M) and capsaicin (10^{-7} and 10^{-6} M)

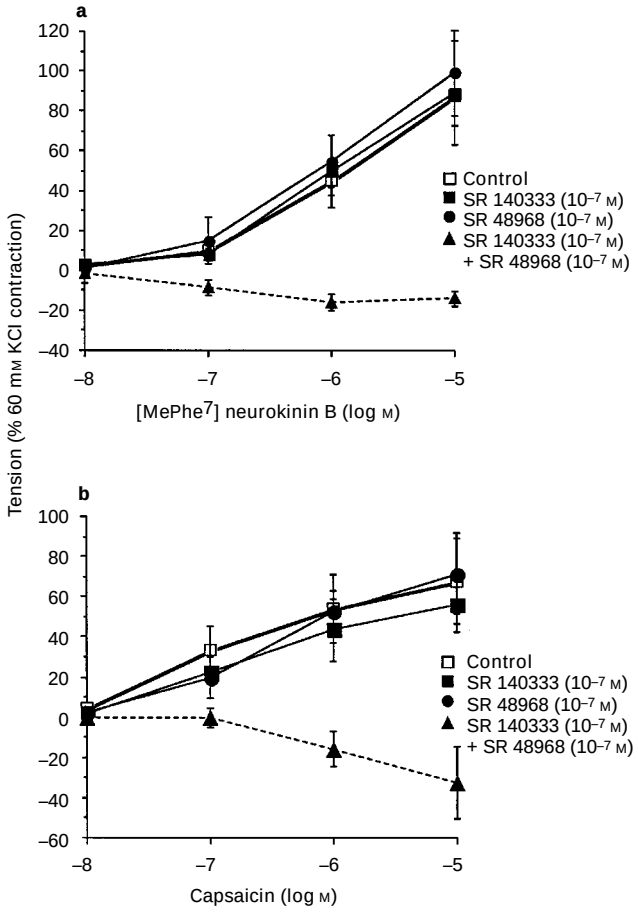

Figure 3 Cumulative concentration-response curves to [MePhe^7]-neurokinin B (a) and capsaicin (b) in guinea-pig isolated trachea (presence of captopril: 10^{-6} M and phosphoramidon: 10^{-5} M). Effects of SR 140333 (10^{-7} M) SR 48968 (10^{-7}M) and the combination of SR 140333+SR 48968. In both cases, the combination of SR 140333 (10^{-7} M)+SR 48968 (10^{-7} M) produced a statistically significant inhibition when compared to control (two-way analysis of variance, $P<0.05$) and a statistically significant interaction was observed when the combination of the two compounds was compared to either inhibitior individually (three-way analysis of variance; $P<0.05$). Data are presented as means and vertical lines show s.e.mean ($n=4$).

induced concentration-dependent depolarizations which were statistically significant at the highest concentration tested (depolarization at 10^{-6} M: 8, 11 and 16 mV, respectively, for NK_1, NK_2 agonist and for capsaicin) (Figures 4 and 5). These three compounds reduced the amplitude of the slow waves as the cells became depolarized and the slow wave activity ultimately ceased.

[MePhe^7]-neurokinin B (10^{-8}–10^{-6} M) did not induce any significant changes in membrane potential or any consistent changes in the electrical activity (Figures 4 and 5).

Effects of substance P, [Nle^{10}] neurokinin A(4–10), [MePhe^7] neurokinin B and capsaicin in the presence of tetraethylammonium (8×10^{-3} M) The potassium channel blocker tetraethylammonium (8×10^{-3} M) depolarized the membrane to -44 ± 1 mV ($n=40$) and in the presence of this inhibitor every single cell studied presented rhythmic electrical activity. Substance P, [Nle^{10}]-neurokinin A(4–10) and capsaicin induced concentration-dependent depolarizations, which were significant at the concentration of 10^{-6} M for substance P and at 10^{-7} M and 10^{-6} M for [Nle^{10}]-neurokinin A(4–10) and capsaicin (Figures 6 and 7). These drugs decreased the amplitude concentration-dependently and tended to increase the frequency of the electrical events

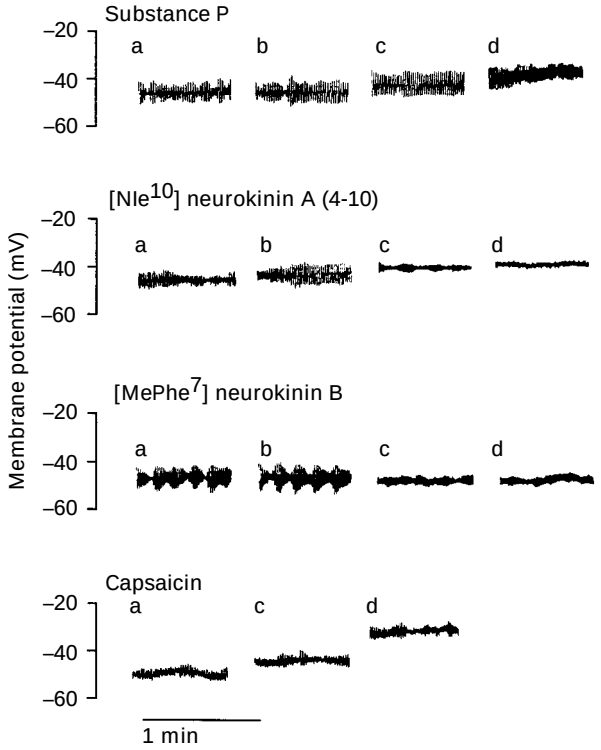

Figure 4 Original tracings showing the effects of substance P, [Nle^{10}]-neurokinin A(4–10), [MePhe^7]-neurokinin B and capsaicin on the intracellular electrical activity of guinea-pig tracheal smooth muscle cells (presence of captopril: 10^{-6} M and phosphoramidon: 10^{-5} M). Control activity (a) and effects of drugs: (b) 10^{-8} M; (c) 10^{-7} M and (d) 10^{-6} M. Tracings shown were obtained during the impalement of a single cell (from a to d). Substance P, [Nle^{10}]-neurokinin A(4–10) and capsaicin caused depolarizations and an increase in slow wave frequency. The highest concentration (10^{-6} M) of these compounds tended to abolish the electrical activity. [MePhe^7]-neurokinin B did not cause any statistically significant changes in the membrane potential.

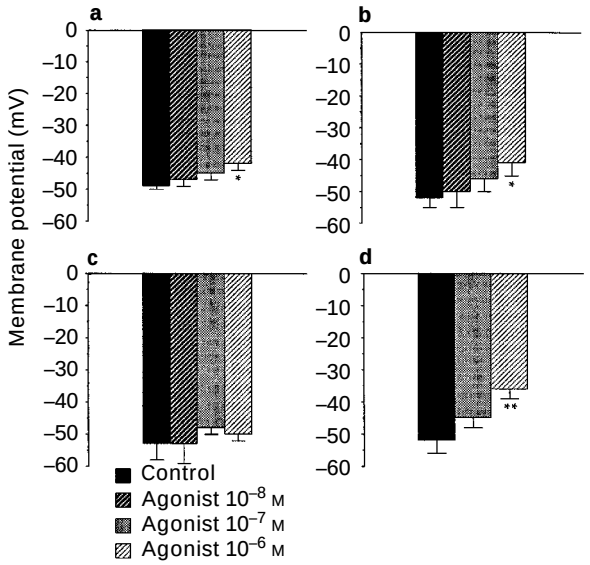

Figure 5 Effects of tachykinins and capsaicin on membrane potential of guinea-pig tracheal smooth muscle cells (presence of captopril: 10^{-6} M and phosphoramidon: 10^{-5} M). (a) Control and in presence of substance P (10^{-8}, 10^{-7} and 10^{-6} M). (b) Control and in presence of [Nle^{10}]-neurokinin A(4–10) (10^{-8}, 10^{-7} and 10^{-6} M). (c) Control and in presence of [MePhe^7]-neurokinin B (10^{-8}, 10^{-7} and 10^{-6} M). (d) Control and in presence of capsaicin (10^{-7} and 10^{-6} M). Data shown are means±s.e.mean ($n=4$ to 28 cells). Asterisks indicate a statistically significant difference from control values (one-way analysis of variance: $^*P<0.05$; $^{**}P<0.01$).

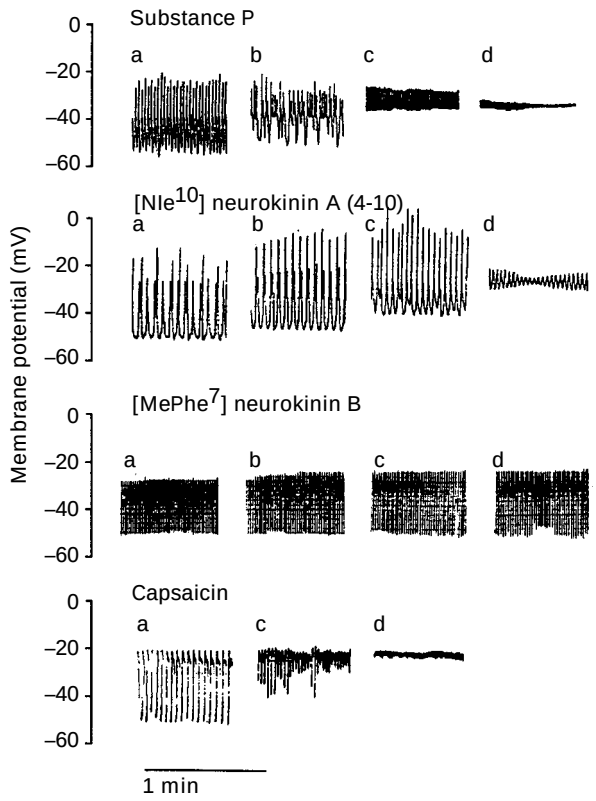

Figure 6 Original tracings showing the effects of substance P, [Nle^{10}]-neurokinin A(4–10), [MePhe^{7}]-neurokinin B and capsaicin on the intracellular electrical activity of guinea-pig tracheal smooth muscle cells in tetraethylammonium-containing solution (8×10^{-3} M) in presence of captopril (10^{-6} M) and phosphoramidon (10^{-5} M). Control activity (a) and effects of drugs: (b) 10^{-8} M; (c) 10^{-7} M and (d) 10^{-6} M. Tracings shown were obtained during the impalement of a single cell (from a to d). Substance P, [Nle^{10}]-neurokinin A(4–10) and capsaicin caused depolarizations and an increase in slow wave frequency. The highest concentration (10^{-6} M) of these compounds tended to abolish the electrical activity. [MePhe^{7}]-neurokinin B did not cause any statistically significant changes in the membrane potential.

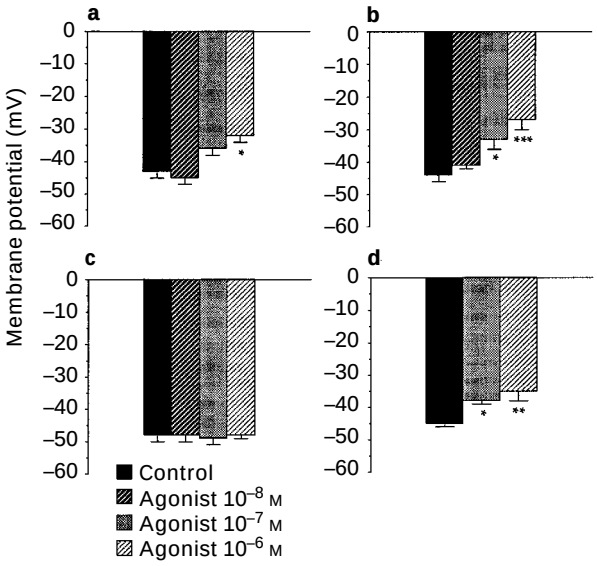

Figure 7 Effects of tachykinins and capsaicin on membrane potential of guinea-pig tracheal smooth muscle cells in tetraethylammonium-containing solution (8×10^{-3} M) in presence of captopril (10^{-6} M) and phosphoramidon (10^{-5} M). (a) Control and in presence of substance P (10^{-8}, 10^{-7} and 10^{-6} M). (b) Control and in presence of [Nle^{10}]-neurokinin A(4–10) (10^{-8}, 10^{-7} and 10^{-6} M). (c) Control and in presence of [MePhe^{7}]-neurokinin B (10^{-8}, 10^{-7} and 10^{-6} M). (d) Control and in presence of capsaicin (10^{-7} and 10^{-6} M). Data shown are means ± s.e.mean ($n=3$ to 17 cells). Asterisks indicate a statistically significant difference from control values (one-way analysis of variance: $*P<0.05$; $**P<0.01$, $***P<0.001$).

which ultimately disappeared when the cell became depolarized (Figure 6).

[MePhe^{7}]-neurokinin B (10^{-8}–10^{-6} M) did not induce significant changes in membrane potential or any consistent changes in the electrical activity (Figures 6 and 7).

Effects of the tachykinin receptor selective antagonists As impalements were more sustained in the presence of tetraethylammonium and as the changes in membrane potential induced by the four substances studied were similar in the presence or absence of tetraethylammonium, the effects of the various antagonists were studied in the presence of this potassium channel blocker.

Neither the antagonists SR 140333 (10^{-7} M) nor SR 48968 (10^{-7} M) alone or in combination affected the membrane potential of the smooth muscle cells of guinea-pig isolated trachea treated with tetraethylammonium (8×10^{-3} M) (Table 1). The depolarizing effect of substance P (10^{-6} M) was blocked by SR 140333 (10^{-7} M) but was unaffected by SR 48968 (10^{-7} M). Conversely, SR 48968 (10^{-7} M) fully blocked the depolarizing effects of [Nle^{10}]-neurokinin A(4–10) (10^{-8} and 10^{-7} M) and partially inhibited the depolarization induced by the highest concentration of the NK_2 receptor agonist (10^{-6} M), while SR 140333 (10^{-7} M) did not significantly influence the response. The effects of capsaicin (10^{-6} M) were partially inhibited by each antagonist. When both tachykinin receptor blockers were present, the tachykinins and capsaicin-induced depolarizations were abolished (Table 2).

Table 1 Effects of tachykinin antagonists on the membrane potential of guinea-pig isolated tracheal smooth muscle cells in tetraethylammonium (8×10^{-3} M)-containing solution in presence of captopril (10^{-6} M) and phosphoramidon (10^{-5}M)

	n	*Membrane potential* (mV)
Control	35	−43 ± 1
SR 14033 (10^{-7} M)	31	−44 ± 1
SR 48968 (10^{-7} M)	65	−44 ± 1
SR 140333 + SR 48968	18	−42 ± 1

Data are shown as mean ± s.e.mean; *n* indicates the number of cells which were impaled.

Discussion

This study shows that the natural NK_1 agonist, substance P, the selective, synthetic NK_2 receptor agonist, [Nle^{10}]-neurokinin A(4–10), administered exogenously and tachykinins endogenously released by capsaicin, induce contractions and depolarize the smooth muscle membrane of the guinea-pig isolated trachea. The selective NK_3 receptor agonist, [MePhe^{7}]-neurokinin B, was a weaker agonist and did not influence the membrane potential. This study confirms that substance P and neurokinin A are potent contractile agents in guinea-pig airway via stimulation of both NK_1 and NK_2 receptors (Regoli, 1987; Maggi *et al*., 1991), whereas neurokinin B and other NK_3 agonists did not (or only weakly) contract guinea-pig airway smooth muscle (Ireland *et al*., 1991; Maggi *et al*., 1991; Ellis *et al*., 1993; Yuan *et al*., 1994). The membrane-depolarizing effect of the NK_1 and NK_2 receptor agonists and the moderate changes which both agents produced on the slow wave activity

Table 2 Effects of SR 140333 (10^{-7} M), SR 48968 (10^{-7} M) and their combination on tachykinin- and capsaicin- (10^{-6} M) induced changes in membrane potential (mV) of guinea-pig tracheal smooth muscle cells

	SR 140333		*SR 48968*		*SR 140333 + SR48968*	
	Control	*+ Agonist*	*Control*	*+ Agonist*	*Control*	*+ Agonist*
Substance P	-44 ± 7	-41 ± 3	-43 ± 2	-30 ± 8 (**)	-44 ± 2	-41 ± 4
[Nle^{10}] NKA (4–10)	-46 ± 2	-33 ± 2 (***)	-45 ± 2	-39 ± 2	-43 ± 4	-37 ± 2
Capsaicin	-42 ± 3	-35 ± 2	-42 ± 2	-35 ± 5	-41 ± 4	-40 ± 2

Data are shown as mean ± s.e.mean ($n = 4$ to 24 cells). The physiological salt solution contained tetraethylammonium (8×10^{-3} M) and the experiments were performed in presence of captopril (10^{-6} M) and phosphoramidon (10^{-5} M). Asterisks indicate a statistically significant difference from respective control values (unpaired t test: $^{**}P < 0.01$; $^{***}P < 0.001$).

are similar to the effects of acetylcholine and histamine obtained in the same tissue (Ahmed *et al*., 1984). It has recently been demonstrated that neurokinin A induces rapid and sustained depolarization in single smooth muscle cells isolated from guinea-pig trachea (Nakajima *et al*., 1995). In addition to the sustained inhibition of K^+ current, the involvement of a Ca^{2+}-dependent Cl^- conductance was demonstrated and could explain the depolarizing effect observed in the presence of the potassium channel blocker tetraethylammonium in our study. This mechanism has also been implicated, in the same tissue, for the depolarizing effect of acetylcholine and histamine (Janssen & Sims, 1992; 1993). Depolarization should cause the opening of voltage-dependent Ca^{2+} channels which may be involved in the mechanism of tachykinin-induced airway smooth muscle contraction. Tachykinins transiently increase the frequency of the slow waves which are Ca^{2+}-dependent and are associated with Ca^{2+} influx (Small, 1982; Foster *et al*., 1983; 1984; McCaig, 1986). Our experimental protocol does not allow us to determine whether calcium influx occurs through voltage-dependent calcium channels or non-selective cation channels. However, in the case of acetylcholine and histamine, which produced similar electrical events, the involvement of voltage-operated Ca^{2+} channels seems to be limited (Janssen & Sims, 1992; 1993). Our study was performed in the presence of the epithelial layer. It has recently been demonstrated that activation of an epithelial NK_1 receptor subtype induces the release of nitric oxide which relaxes the guinea-pig isolated trachea (Figini *et al*., 1996). Furthermore, in this tissue, endogenously released or exogenously applied nitric oxide activates large conductance calcium-activated potassuim channels (Ellis & Conanan, 1994). Thus, in this study which was performed in the absence of a nitric oxide synthetase inhibitor, the mechanical and electrophysiological effects of substance P may have been underestimated.

The contractile and depolarizing effects of the NK_1 agonist were specifically inhibited by the NK_1 receptor antagonist SR 140333 and unaffected by the NK_2 receptor antagonist SR 48968. Conversely, the effects of the NK_2 receptor agonist [Nle^{10}]-neurokinin A(4–10) were specifically inhibited by the NK_2 receptor antagonist and unaffected by the NK_1 receptor antagonist. Similar results have been obtained by Charette *et al*. (1994) in the same preparation. This indicates that both NK_1 and NK_2 receptors are present in the guinea-pig isolated tracheal smooth muscle (McKee, 1993) and that stimulation of these receptors produced changes in both membrane potential and contractions. The apparent pA_2 value found in this study for the NK_2 receptor antagoinst SR 48968 is similar to the values which have been previously published for the guinea-pig and hamster trachea or for the rabbit urinary bladder (Advenier *et al*., 1992; Charette *et al*., 1994; Maggi *et al*., 1994), but one order of magnitude lower than the values found in preparations such as the rabbit pulmonary artery (contractile response) or the human bronchus (Advenier *et al*., 1992; Emonds-Alt *et al*., 1992). However, the apparent pA_2 value found in this study for the NK_1 receptor antagonist SR 140333 is two to three order of magnitude lower than the pD'_2 obtained for this compound in tissues such as the rabbit pulmonary artery (endothelium-dependent relaxation), guinea-pig ileum or the human bronchus (Emonds-Alt *et al*., 1993; Croci *et al*., 1995; Naline *et al*., 1996). In the guinea-pig trachea, NK_1 receptor antagonists are considerably more effective ($\geqslant 2$ order of magnitude) when tested against a NK_1 specific receptor agonist such as [Sar^9, Met(O$_2$)^{11}]-substance P than against substance P itself (Charette *et al*., 1994). Furthermore, in this work and in the study of Charette *et al*. (1994) a marked synergism was observed between the NK_1 and the NK_2 receptor antagonists. The simplest explanation of these data would be that substance P, behaving as a non-selective agonist, interacts with another neurokinin receptor subpopulation such as the NK_2 receptor subtype. However, this explanation is probably too simplistic as, firstly, SR 48968, even at high concentrations, does not individually influence the contractile response to substance P. Secondly, the inhibition by SR 48968 of the contractile response induced by the specific agonist [Nle^{10}]-neurokinin A(4–10) was also potentiated by a concentration of SR 140333 which is totally inactive at the NK_2 receptor. These data suggest some form of cross-talk between the NK_1 and NK_2 receptors in the guinea-pig tracheal smooth muscle cells. This study does not allow us to elucidate this phenomenon.

Capsaicin interacts with receptors present at the sensory C-fibre endings (Lundberg & Saria, 1983; Bevan & Szolcsanyi, 1990) to release neuropeptides, including tachykinins (Maggi & Meli, 1988). In the smooth muscle cells of guinea-pig isolated trachea, capsaicin induced contractions and changes in the membrane potential. These electrical events were similar to those evoked by NK_1- and NK_2-specific agonists when used alone. Each specific antagonist (SR 140333 and SR 48968) partially inhibited the electrical response although the contractile response was not affected. The combination of the two antagonists fully abolish the electrical and mechanical responses. This confirms that capsaicin releases endogenous tachykinins which interact with both the NK_1 and the NK_2 receptor populations (Bertrand *et al*., 1993; Charette *et al*., 1994; Ellis & Undem, 1994; Mitchell *et al*., 1995; Robineau *et al*., 1995). However, a preferential activation of NK_2 receptors by endogenous tachykinins released by capsaicin has been demonstrated (Ellis & Undem, 1994; Mitchell *et al*., 1995). In our study, SR 48968 alone did not affect the contractile response, although a partial inhibition of the depoarlization was observed. Experimental conditions such as whether or not peptidase inhibitors are present, the use of rings versus strips, performing cumulative concentration-response curves versus single dose applications or submitting the tissue to a previous exposure to KCl may explain these discrepancies. In the presence of both inhibitors, capsaicin consistently induced a relaxation of the spontaneous basal tone of the guinea-pig isolated tracheal rings. This phenomenon was not associated with a hyperpolarization of the cell membrane. Its origin is unexplained at present but has been observed by other investigators (Mitchell *et al*., 1995). The tachykinin NK_3 selective receptor agonist [MePhe^7]-neurokinin B induced contractile responses, but no changes in membrane potential. The effect of the NK_3 receptor agonist could be explained by a pharmacomechanical coupling in the activation of an NK_3 receptor located on the smooth muscle cells (Bolton, 1979). However this

hypothesis is unlikely as there are very few or no NK_3 receptors in the guinea-pig airway smooth muscle (Maggi *et al*., 1991; Ellis *et al*., 1993; Yuan *et al*., 1994) and the contraction to [MePhe^7]-neurokinin B is inhibited by the combination of NK_1 and NK_2 receptor antagonists. Indeed, SR 48968 and SR 140333 are virtually inactive at the NK_3 receptor subtype (Advenier *et al*., 1992; Emonds-Alt *et al*., 1993; Maggi *et al*., 1993; Oury-Donat *et al*., 1995). Although the contractile effects of [MePhe^7]-neurokinin B was similar to the response provoked by capsaicin, the activation of an NK_3 prejunctional receptor leading to the release of neurokinins (in this respect mimicking the effect of capsaicin) is also unlikely because the NK_3 receptor agonist did not produce the same level of depolarization as capsaicin, suggesting that [MePhe^7]-neurokinin B did not release endogenous tachykinins. One possible explanation for our results is that [MePhe^7]-neurokinin B exhibits partial agonism at the NK_1 and NK_2 receptors (Regoli *et al*., 1994).

In conclusion, our study demonstrates that both NK_1 and NK_2 tachykinin receptors induced depolarization and contraction of guinea-pig tracheal smooth muscle cells and that both receptors are stimulated during endogenous release of tachykinins by capsaicin.

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References

ADVENIER, C., ROUISSI, N., NGUYEN, Q.T., EMONDS-ALT, X., BRELIERE, J.-C., NELIAT, G., NALINE, E. & REGOLI, D. (1992). Neurokinin A (NK2) receptor revisited with SR 48968, a potent non-peptide antagonist. *Biochem. Biophys. Res. Commun*., **184,** 1418 – 1424.

AHMED, F., FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1984). Some features of the spasmogenic actions of acetylcholine and histamine in guinea-pig isolated trachealis. *Br. J. Pharmacol*., **83,** 227 – 233.

BARNES, P.J., BARANIUK, J.N. & BELVISI, M.G. (1991a). Neuropeptides in the respiratory tract. *Am. Rev. Respir. Dis*., **144,** 1187 – 1198.

BARNES, P.J., BARANIUK, J.N. & BELVISI, M.G. (1991b). Neuropeptides in the respiratory tract. *Am. Rev. Respir. Dis*., **144,** 1391 – 1399.

BERTRAND, C., NADEL, J.A., GRAFT, P.A. & GEPPETTI, P. (1993). Capsaicin increases airflow resistance in guinea-pigs in vivo by activating both NK_2 and NK_1 tachykinin receptors. *Am. Rev. Respir. Dis*., **148,** 909 – 914.

BEVAN, S. & SZOLCSANYI, J. (1990). Sensory neuron-specific actions of capsaicin: mechanisms and applications. *Trends Pharmacol. Sci*., **11,** 330 – 333.

BOLTON, T.B. (1979). Mechanisms of action of tranismitters and other substances in smooth muscle. *Physiol. Rev*., **59,** 606 – 718.

CHARETTE, L., FOULON, D., RODGER, I.W. & JONES, T.R. (1994). Neurokinin (NK2) receptors mediate nonadrenergic noncholinergic responses to electrical stimulation and resiniferatoxin in guinea-pig trachea. *Can. J. Physiol. Pharmacol*., **72,** 182 – 188.

CROCI, T., EMONDS-ALT, X., LE FUR, G. & MANARA, L. (1995). In vitro characterization of the non-peptide tachykinin NK1 and NK2 antagonists, SR 140333 and SR 48968 in different rat and guinea-pig intestinal segments. *Life Sci*., **56,** 267 – 275.

ELLIS, J.L. & CONANAN, N.D. (1994). Effects of potassium channel blockers on relaxations to a nitric oxide donor and to nonadrenergic nerve stimulation in guinea-pig trachea. *J. Pharmacol. Exp. Ther*., **271,** 782 – 786.

ELLIS, J.L. & UNDEM, B.J. (1994). Inhibition by capsazepine of resiniferatoxin- and capsaicin-induced contractions of guinea-pig trachea. *J. Pharmacol. Exp. Ther*., **268,** 85 – 89.

ELLIS, J.L., UNDEM, B.J., KAYS, J.S., GHANEKAR, S.V., BARTHLOW, H.G. & BUCKNER, C.K. (1993). Pharmacological examination of receptors mediating contractile responses to tachykinins in airways isolated from human, guinea-pig and hamster. *J. Pharmacol. Exp. Ther*., **267,** 95 – 101.

EMONDS-ALT, X., DOUTREMEPUICH, J.-D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.-P., PROIETTO, V., VAN BROECK, D., SOUBRIE, P., LE FUR, G. & BRELIERE, J.-C. (1993). In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist. *Eur. J. Pharmacol*., **250,** 403 – 413.

EMONDS-ALT, X., VILAIN, P., GOULAOUIC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., BRELIERE, J.-C. & LE FUR, G. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci*., **50,** PL101 – PL106.

FIGINI, M., EMANUELLI, C., BERTRAND, C., JAVDAN, P. & GEPPETTI, P. (1996). Evidence that tachykinins relax the guinea-pig trachea via nitric oxide release by stimulation of a septide-insensitive NK_1 receptor. *Br. J. Pharmacol*., **117,** 1270 – 1276.

FOSTER, R.W., OKPALUGO, B.I. & SMALL, R.C. (1984). Antagonism of and other actions of verapamil in guinea-pig isolated trachealis. *Br. J. Pharmacol*., **81,** 499 – 507.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983). Evidence that the spasmogenic action of tetraethylammonium in guinea-pig trachealis is both direct and dependent on the cellular influx of Ca^{2+} ion. *Br. J. Pharmacol*., **79,** 255 – 263.

ICHINOSE, M., NAKAJIMA, N., TAKAHASHI, T., YAMAUCH, H., INOUE, H. & TAKISHIMA, T. (1992). Protection against bradykinin-induced bronchoconstriction in asthmatic patients by neurokinin receptor antagonist. *Lancet*, **340,** 1248 – 1251.

IRELAND, S.J., BAILEY, F., COOK, A., HAGAN, R.M., JORDAN, C.C. & STEPHENS-SMITH, M.L. (1991). Receptors mediating tachykinin-induced contractile response in guinea-pig trachea. *Br. J. Pharmacol*., **103,** 1463 – 1469.

JANSSEN, L.J. & SIMS, S.M. (1992). Acetylcholine activates non-selective cation and chloride conductance in canine and guinea-pig tracheal myocytes. *J. Physiol*., **453,** 197 – 218.

JANSSEN, L.J. & SIMS, S.M. (1993). Histamine activates Cl^- and K^+ currents in guinea-pig tracheal myocytes: convergence with muscarinic signalling pathway. *J. Physiol*., **465,** 661 – 677.

LUNDBERG, J.M. & SARIA, A. (1983). Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature*, **302,** 251 – 253.

MAGGI, C.A. (1995). The mammalian tachykinin receptors. *Gen. Pharmacol*., **26,** 911 – 944.

MAGGI, C.A., ASTOLFI, M., GIULANI, S., GOSO, C., MANZINI, S., MEINI, S., PATACCHINI, R., PAVONE, V., PEDONE, C., QUARTARA, L., RENZETTI, A.R. & GIACHETTI, A. (1994). Men 10,627 a novel polycyclic peptide antagonist of tachykinin NK_2 receptors. *J. Pharmacol. Exp. Ther*., **271,** 1489 – 1500.

MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmacol*., **19,** 1 – 43.

MAGGI, C.A., PATACCHINI, R., GIULANI, S. & GIACHETTI, A. (1993). In vivo and in vitro pharmacology of SR 48968 a non peptide tachykinin NK2 receptor antagonist. *Eur. J. Pharmacol*., **234,** 83 – 90.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & SANTICIOLI, P. (1991). Tachykinin receptors and non-cholinergic bronchoconstriction in the guinea-pig isolated bronchi. *Am. Rev. Respir. Dis*., **144,** 363 – 367.

MANZINI, S. (1994). Antagonists of NK_2 tachykinin receptors as a potential new class of antibronchospastic agents. *Gen. Pharmacol*., **25,** 1 – 6.

MCCAIG, D.J. (1986). Electrophysiology of neuroeffector transmission in the isolated, innervated trachea of the guinea-pig. *Br. J. Pharmacol*., **89,** 793 – 801.

MCCAIG, D.J. & RODGER, I.W. (1988). Effects of leukotriene D_4 on the mechanical and electrical properties of guinea-pig isolated trachealis. *Br. J. Pharmacol*., **94,** 729 – 736.

MCKEE, K.T. (1993). Identification of both NK_1 and NK_2 receptors in guinea-pig airways. *Br. J. Pharmacol.*, **110,** 693 – 700.

MITCHELL, R.W., NDUKWU, I., HERRNREITER, A., UZENDOSKI, K., GITTER, B., SOLWAY, J. & LEFF, A.R. (1995). Differential tachykinin receptor subtype activation in capsaicin and KCl contractions of guinea-pig trachealis. *Am. J. Physiol.*, **269,** L837 – L842.

NALINE, E., MOLIMARD, M., REGOLI, D., EMONDS-ALT, X., BELLAMY, J.-F. & ADVENIER, C. (1996). Evidence for functional tachykinin NK1 receptors in human isolated small bronchi. *Am. J. Physiol.*, **271,** L763 – L767.

NAKAJIMA, T., HAZAMA, H., HAMADA, E., OMATA, M. & KURACHI, Y. (1995). Ionic basis of neurokinin-A-induced depolarization in single smooth muscle cells isolated from guinea-pig trachea. *Pflüger Arch. – Eur. J. Physiol.*, **430,** 552 – 562.

OURY-DONAT, F., CARAYON, O., THURNEYESSEN, O., PAILHON, V., EMONDS-ALT, X., SOUBRIE, P. & LE FUR, G. (1995). Functional characterization of the nonpeptide neurokinin3 (NK3) receptor antagonist, SR142801 on the human NK3 receptor expression in chinese hamster ovary cells. *J. Pharmacol. Exp. Ther.*, **274,** 148 – 154.

REGOLI, D. (1987). Peptide receptors in the airways. *Am. Rev. Respir. Dis.*, **136,** S35 – S39.

REGOLI, D., BOUDON, A. & FAUCHERE, J.L. (1994). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46,** 551 – 559.

ROBINEAU, P., LONCHAMPT, M., KUCHARCZYK, N., KRAUSE, J.E., REGOLI, D., FAUCHERE, J.L., PROST, J.F. & CANET, E. (1995). In vitro and in vivo pharmacology of S 16474, a novel dual tachykinin NK_1 and NK_2 receptor antagonist. *Eur. J. Pharmacol.*, **294,** 677 – 684.

SMALL, R.C. (1982). Electrical slow waves and tone of guinea-pig isolated trachealis muscle: effects of drugs and temperature changes. *Br. J. Pharmacol.*, **77,** 45 – 54.

SOLWAY, J. & LEFF, A.R. (1991). Sensory neuropeptides and airway function. *J. Appl. Physiol.*, **71,** 2077 – 2087.

TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). PA_2 and receptor differentiation: A statistical analysis of competitive antagonism. *Life Sci.*, **25,** 637 – 654.

YUAN, L., BURCHER, E. & NAIL, B.S. (1994). Use of selective agonists and antagonists to characterize tachykinin receptors mediating airway responsiveness in anaesthetized guinea-pigs. *Pulm. Pharmacol.*, **7,** 169 – 178.

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