Neurokinin B- and specific tachykinin NK$_3$ receptor agonists-induced airway hyperresponsiveness in the guinea-pig

[1]Samira Daoui, [1]Emmanuel Naline, [2]Vincent Lagente, [3]Xavier Emonds-Alt & *,[1]Charles Advenier

[1]Laboratoire de Pharmacologie, Faculté de Médecine Paris-Ouest, 15 Rue de l'Ecole de Médecine, F75006 Paris , France; [2]Inserm U456, Laboratoire de Pharmacologie, Faculté de Pharmacie, Rennes, France and [3]Sanofi Recherche, Montpellier, France

1 The aim of this study was to determine whether neurokinin B (NKB) or specific agonists of tachykinin NK$_3$ receptors, [MePhe^7]NKB and senktide, were able to induce airway hyperresponsiveness in guinea-pigs. The effects of these compounds were compared to those of substance P (SP), neurokinin A (NKA) and the preferential tachykinin NK$_1$ ([Sar^9, Met(0$_2$)^{11}]SP) or NK$_2$ ([βAla^8]NKA (4-10)) receptor agonists.

2 In guinea-pigs pretreated with phosphoramidon (10^{-4} M aerosol for 10 min) and salbutamol (8.7×10^{-3} M for 10 min), all tachykinins administrated by aerosol (3×10^{-7} to 10^{-4} M) induced airway hyperresponsiveness 24 h later, displayed by an exaggerated response to the bronchoconstrictor effect of acetylcholine (i.v.). The rank order of potency was: [βAla^8]NKA (4-10) > NKA = NKB = senktide = [MePhe^7]NKB = [Sar^9,Met(0$_2$)^{11}]SP > SP.

3 Airway hyperresponsiveness induced by [MePhe^7]NKB was prevented by the tachykinin NK$_3$ (SR 142801) and NK$_2$ (SR 48968) receptor antagonists.

4 Bronchoconstriction induced by tachykinins administered by aerosol was also determined. SP, NKA, NKB and the tachykinin NK$_1$ and NK$_2$ receptor agonist induced bronchoconstriction. The rank order of potency was: NKA = [βAla^8]NKA (4-10) > NKB = SP = [Sar^9,Met(0$_2$)^{11}]SP. Under similar conditions, and for concentrations which induce airway hyperresponsiveness, senktide and [MePhe^7]NKB failed to induce bronchoconstriction.

5 It is concluded that tachykinin NK$_3$-receptor stimulation can induce airway hyperresponsiveness and that this effect is not related to the ability of tachykinins to induce bronchoconstriction.
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Introduction

Tachykinins, a group of neuropeptides including substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are the transmitters of sensory neurones which, in the lung, innervate all compartments of the airway wall from the trachea down to the bronchioles (Baluk & McDonald 1998; Ellis & Undem 1994; Lundberg 1996; Lundberg & Saria 1987). The activation of C-fibre afferent nerves in airways leads to a local release of tachykinins that are responsible for several biological effects: bronchospasm, increase in microvascular permeability, vasodilatation, stimulation of glandular secretions, facilitation of cholinergic neurotransmission, recruitment and activation of inflammatory cells. Sensory nerves also mediate respiratory defence reflexes, such as coughing and sneezing (Ellis & Undem, 1994; Maggi et al., 1993; Widdicombe, 1995).

The biological actions of tachykinins are mediated *via* three types of receptors, denoted tachykinin NK$_1$, NK$_2$ and NK$_3$ which have the highest affinity for SP, NKA and NKB, respectively. This receptor classification has been established from receptor-binding and functional studies using selective agonists or antagonists for tachykinin receptors (Regoli et al., 1994). It has now been recognized that the expression of tachykinin NK$_3$ receptor is confined mainly to the central and peripheral nervous system, whilst tachykinins NK$_1$ and NK$_2$ receptors are expressed both in the nervous system and in target organs, including airways (Baluk et al., 1996; Guard & Watson, 1991; Maggi, 1993; Myers & Undem, 1993).

Several experimental reports using different animal species have suggested that tachykinins are involved in airway hyperresponsiveness, an enhanced bronchoconstrictor response to many different stimuli and a key feature of asthma. Airway hyperresponsiveness is associated with inflammation in the airways and relates closely to the severity of asthma, the frequency of symptoms, and the need for treatment (Barnes, 1989; Boushey et al., 1980; O'Byrne, 1988). Indeed, exposure to a single aerosol of SP elicited 24 h later airway hyperresponsiveness to exogenous bronchoconstrictor agents in guinea-pigs (Boichot et al., 1993). Similar data were observed in asthmatic patients (Cheung et al., 1994). NKA also enhanced methacholine response up to 4 weeks in monkeys (Tamura et al., 1989). Conversely, chronic treatment with high doses (i.p.) of capsaicin which depletes tachykinins from sensory nerves, or single pretreatments with the tachykinin NK$_1$ [CP 96345, SR 140333], NK$_2$ [SR 48968, MEN 10,627] or NK$_1$ + NK$_2$ [MDL 105212; FK 224] receptor antagonists have been reported to prevent airway hyperresponsiveness in various experimental models in guinea-pigs, mice or monkeys (see reviews Advenier et al., 1997; Kraneveld et al., 1997; Spina et al., 1998).

We have recently demonstrated that the NK$_3$ receptor antagonist, SR 142801 (Osanetant) (Emonds-Alt et al., 1995) was also able to inhibit in guinea-pig airway hyperresponsiveness induced by substance P (Daoui et al., 1997) or citric acid (Daoui et al., 1998), and to prevent in human isolated bronchi hyperresponsiveness induced by interleukin 1-β or by passive sensitization (Vincent et al., 1999). To our knowledge, no

*Author for correspondence; E-mail: charles.advenier@wanadoo.fr

studies have been performed in order to determine if agonists for NK$_3$ receptors may induce airway hyperresponsiveness as previously reported for substance P and NKA.

The aim of this study was to determine whether NKB or the specific agonists of NK$_3$ receptors, [MePhe^7]NKB or senktide, were able to induce airway hyperresponsiveness in guinea-pigs. We also compared the potency of NKB and tachykinin NK$_3$ agonists with those of SP, NKA and of tachykinin NK$_1$ and NK$_2$ receptor agonists.

Methods

Airway hyperresponsiveness to acetylcholine

Exposure to tachykinins or tachykinin receptor agonists aerosol. Tricoloured unanaesthetized, unrestrained male or female guinea-pigs (300–400 g), were placed in Plexiglas chamber (30 × 25 × 15 cm) and exposed successively to a nebulized aqueous solution of salbutamol (8.7×10^{-3} M, 10 min) or phosphoramidon (10^{-4} M, 10 min) in order to prevent tachykinins- or tachykinin receptor agonist-induced bronchoconstriction and tachykinin metabolism; 5 min later the animals were exposed to a single aerosol of tachykinins or tachykinin receptor agonists at various concentrations (3×10^{-7} to 10^{-4} M) or vehicle solution as control group for 30 min (Figure 1). An ultrasonic nebulizer (Aerodynamic mean mass median particle diameter of 0.5 to 5 μm, NEB99, Devilbiss, Somerset, PA, U.S.A.) was used. Previous studies have shown that phosphoramidon and/or salbutamol when used alone or in combination were not able to induce airway hyperresponsiveness (Girard et al., 1996; Daoui et al., 1997, 1998).

Assessment of the in vivo *bronchopulmonary reactivity.* 24 h after exposure to tachykinins or tachykinin receptor agonists (Figure 1), animals were anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and placed on a heated blanket (Homeothermic blanket system, Havard Apparatus Ltd, Kent, U.K.). A jugular vein was cannulated for injection of acetylcholine. A trachea cannula was inserted and artificial ventilation was maintained by means of a constant volume ventilator (Model 7025, UGO Basile, Comerio-Varese, Italy). Airway inflation pressure was measured using a pressure transducer (P23XL, Viggo-Spectramed, Bilthoven, Netherlands) connected to the tracheal cannula *via* a side-arm and recorded with a recording microdynamometer (Model 7050, UGO Basile, Comerio-Varese, Italy). The tidal volume (approximately 10 ml kg^{-1}) was adjusted to give a base-line inflation pressure of 8–10 cm H$_2$O at the end of the inspiration. After a stabilization period of 10 min, acetylcholine was administered at increasing doses (10, 20, 50, 100, 200 and 500 μg kg^{-1}, i.v.) 5–10 min apart. Bronchopulmonary responses were expressed as per cent response changes *vs* acetylcholine at 500 μg kg^{-1}. Acetylcholine (500 μg kg^{-1}) responses were expressed in cm H$_2$O.

Pretreatment with drugs. Guinea-pigs received a single dose (1 mg kg^{-1}, i.p.) of the NK$_3$ (SR 142801), NK$_2$ (SR 48968) or NK$_1$ (SR 140333) receptor antagonists, or vehicle 45 min before exposure to [MePhe^7]NKB.

Bronchoconstriction

Tricoloured male or female guinea-pigs (300–400 g) were anaesthetized with urethane (1.25 g kg^{-1}, i.p.) and placed on a heated blanket (Havard Apparatus Ltd, Kent, U.K.) which

maintained body temperature at about 37°C. The left jugular vein was cannulated for injection of acetylcholine. A tracheal cannula was inserted and artificial ventilation was maintained by means of a constant volume ventilator. Animals were ventilated with room air at a rate of 60 breath per min and at a tidal volume of approximately 10 ml kg^{-1}. Airway function was assessed by measuring changes in pleural pressure, which can be regarded as an indicator of airway resistance at least in guinea-pigs (Santing et al., 1992). Pleural pressure was determined with a catether fitted with a 16 G needle inserted into the 6th or 7th intercostal space and connected to a pressure transducer (P23XL, Viggo-Spectramed, Bilthoven, Netherlands). The pleural pressure increase was evaluated as the difference between the baseline value and the maximum response (the peak value) after tachykinin or tachykinin receptor agonist aerosol.

After a 30 min resting period, artificial ventilation was stopped, and the tracheal cannula was connected in spontaneously breathing animals directly to the nebulizer. Aerosols of tachykinin or tachykinin receptor agonist solutions (3×10^{-7} to 10^{-4} M) were administered for 2 min and about 0.3 ml of the solution was nebulized per min. After the pleural pressure was returned to baseline or stabilized, artificial ventilation was set running again. Preliminary experiments have shown that responses were reproducible at least three times at 30 min intervals. Also we have demonstrated that no significant changes were produced by 0.9% saline aerosol (0.3 ml min^{-1}) administration. The bronchopulmonary responses were expressed as per cent changes *vs* acetylcholine (500 μg kg^{-1}, i.v.) administered at the end of the experiment, in spontaneously breathing animals.

Statistical analysis of results

Data are expressed as means ± s.e.mean. EC$_{30}$ value is the dose which provokes an increase in airway inflation pressure of 30% of the maximal effect (Girard et al., 1996; Daoui et al., 1997, 1998). Statistical analysis of the results was assessed by a two-way analysis of variance (ANOVA) followed by a Student's t-test for paired or unpaired data. Probability values of $P < 0.05$ were considered significant.

Drugs

The substances used were: urethane (Prolabo, Paris, France); histamine dihydrochloride, phosphoramidon, salbutamol sulphate (Sigma, St Louis, MO, U.S.A.); acetylcholine hydrochloride (PCH, Paris, France); substance P, [Sar^9, Met(O$_2$)^{11}]substance P, neurokinin A, [βAla^8]neurokinin A(4-10) (Bachem, Paris, France); neurokinin B, [MePhe^7]neurokinin B, senktide (Novabiochem, Paris, France); SR 48968 [(S)-N-methyl-N[(4-acetylamino-4-phenylpiperidino-2-(3,4)dichlorophenyl) butyl]benzamide] (saredutant) used as hydrochloride, SR 140333 [(S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidin-3-yl]etyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane, chloride] (chloride of nolpitantium) and SR 142801 [(R)-(N)-(1-(3-(l-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide] (osanetant) used as hydrochloride (Sanofi Recherche, Montpellier, France). All drugs were dissolved in saline, except SR 48968, SR 140333 and SR 142801 which were dissolved in ethanol and then in diluted saline, and neurokinin B and [MePhe^7]neurokinin B which were dissolved in acetic acid (100%) and then diluted in saline. The maximum amount of ethanol injected (20 μl per 100 g body weight) did not modify the respiratory responses to acetylcholine, the development of
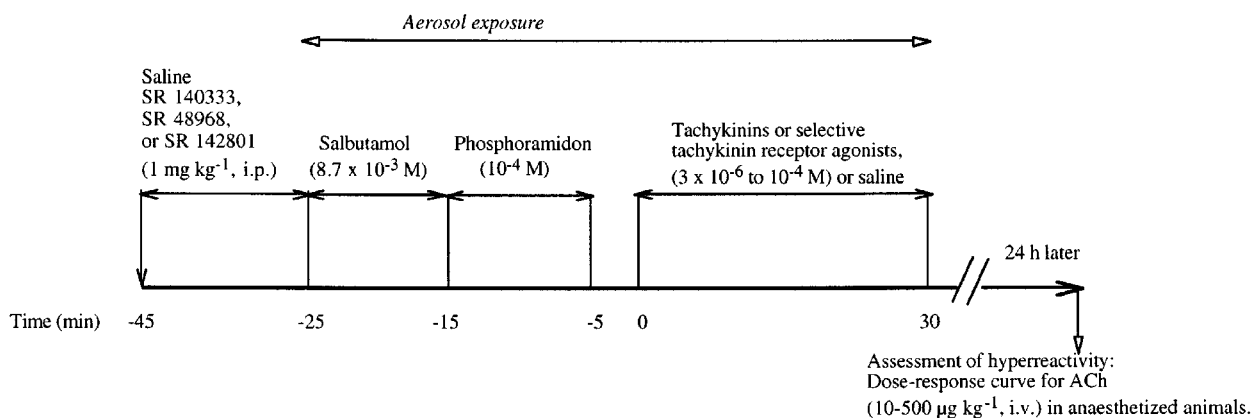
Figure 1 Experimental protocol of induction of airway hyperresponsiveness and measurement of airway inflation pressure in guinea-pigs.
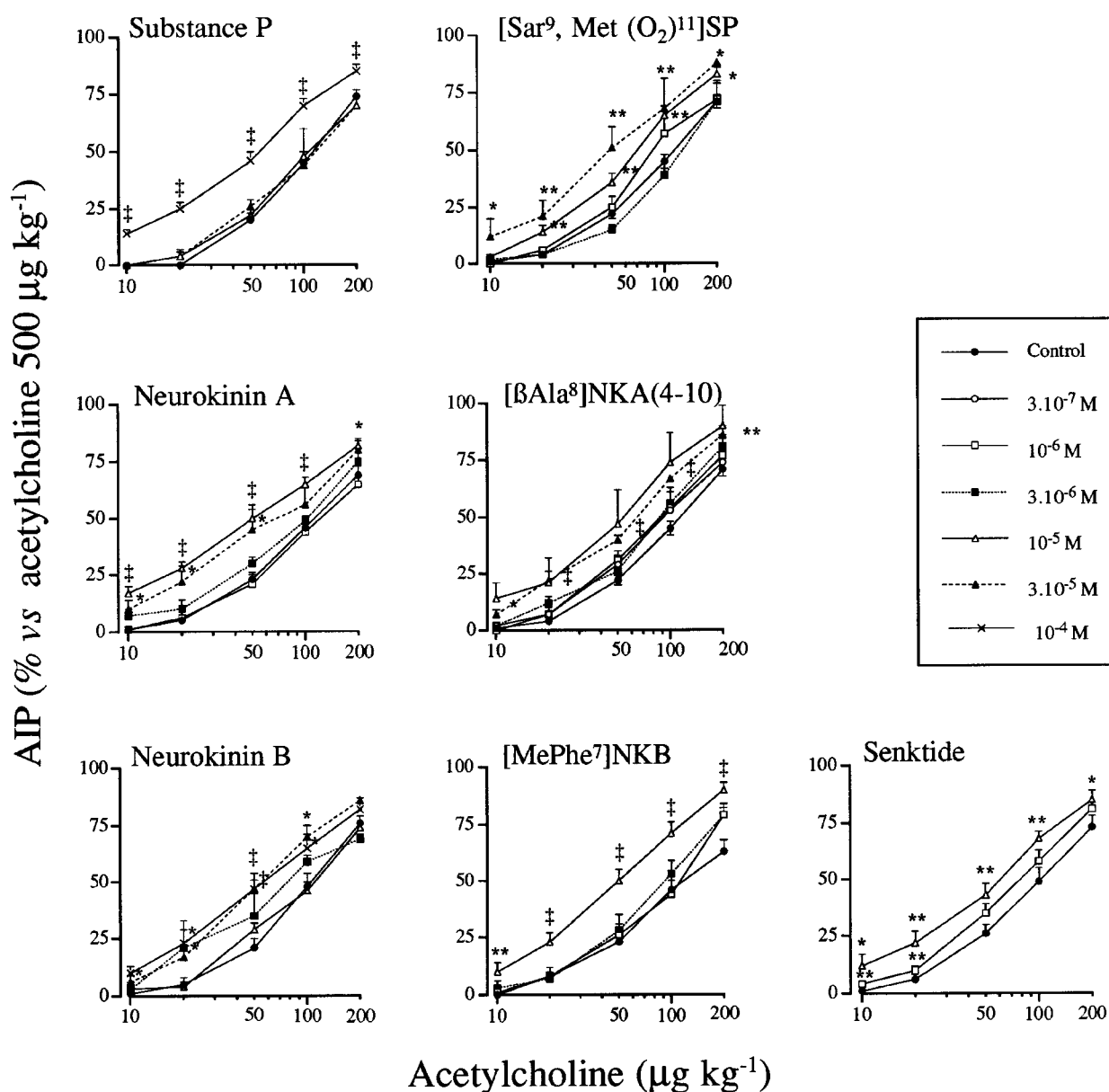

Acetylcholine (µg kg⁻¹)

Figure 2 Cumulative contractile concentration-response curves for acetylcholine $(10-200 \, \mu g \, kg^{-1})$ in anaesthetized guinea-pigs, 24 h after aerosols of salbutamol $(8.7 \times 10^{-3} \, M, 10 \, min)$, phosphoramidon $(10^{-4} \, M, 10 \, min)$ and saline (NaCl 9 ‰, 30 min) or SP, NKA, NKB, [Sar⁹, Met(O₂)¹¹]SP, [βAla⁸]NKA (4-10), [MePhe⁷]NKB and senktide $(3 \times 10^{-7}$ to $10^{-4} \, M, 30 \, min)$. Values are means ± s.e.mean, *n* are reported in Tables 1 and 2. AIP: airway inflation pressure. Significant difference from control shown as: *$P<0.05$ and **$P<0.01$.

airway hyperresponsiveness and bronchoconstriction at the dilution used.

Results

Comparison of the ability of the tachykinins and tachykinin receptor agonists to induce hyperresponsiveness when given by aerosol

Airway hyperresponsiveness developed consistently 24 h after a single tachykinin or tachykinin receptor agonist (3×10^{-7} to 10^{-4} M) exposure in guinea-pigs pretreated with phosphoramidon, as evidenced by a significant leftward shift in dose response curves to acetylcholine-induced broncho-constriction in comparison with matched saline controls (Figure 2). The maximum bronchoconstrictor responses to acetylcholine (E_{max} at 500 μg kg^{-1}) were similar in animals exposed to tachykinins, tachykinin receptor agonists or saline (Tables 1 and 2). ED_{30} values for acetylcholine were significantly lower in animals pretreated with salbutamol, phosphoramidon and exposed to tachykinins or tachykinin receptor agonists than in saline exposed animals control, showing a maximal increase in sensitivity of approximately 2 fold.

Relative potency of agonists

The relative potencies of the tachykinins and their selective analogues were compared using data derived from ED_{30} values (Tables 1 and 2). Significant increases in airway responsiveness were observed from 10^{-6} M for [βAla^8]NKA (4-10), from 10^{-5} M for NKA, NKB, [MePhe^7]NKB, senktide and [Sar^9, Met(O$_2$)^{11}]SP and for 10^{-4} for SP. The rank order of potency was: [βAla^8]NKA (4-10) > NKA = NKB = [MePhe^7]NKB = senktide = [Sar^9, Met(O$_2$)^{11}]SP > SP (Tables 1 and 2).

Effects of SR 140333 (NK$_1$ antagonist), SR 48968 (NK$_2$ antagonist) or SR 142801 (NK$_3$ antagonist) on [MePhe^7]NKB- induced hyperreactivity

Airway hyperresponsiveness to acetylcholine following exposure to [MePhe^7]NKB was abolished by a single dose of the tachykinin NK$_2$ or NK$_3$ receptor antagonists SR 48968 and SR 142801 (1 mg kg^{-1}, i.p.), administered 45 min before [MePhe^7]NKB exposure (Table 3). In contrast after the administration of the selective NK$_1$ antagonist SR 140333 (1 mg kg^{-1}, i.p.), the ED_{30} for acetylcholine did not appear significantly different between control animals and animals pretreated with [MePhe^7]NKB. Only a partial inhibition was observed.

Comparison of the bronchoconstrictor response to tachykinin and selective tachykinin receptor agonists in guinea-pigs in vivo

In control animals that received successive administrations of a 0.9 % NaCl aerolized solution no significant changes in the baseline was observed. The effects of various tachykinins and selective receptor agonists were then examined. Without prior treatment with phosphoramidon (10^{-4} M, aerosol for 10 min), the guinea-pigs did not respond to tachykinin or tachykinin receptor agonist aerosolized solution at concentrations up to 3×10^{-5} M. After pretreatment with phosphoramidon, the guinea-pigs responded to SP, NKA, NKB,

Table 1 Effects of substance P-, neurokinin A- and neurokinin B-aerosol exposure on acetylcholine-induced bronchoconstriction

Broncho-constrictive agents	Aerosol concentration	n	Acetylcholine $ED_{30} \pm$ s.e.mean (μg kg^{-1})	$E_{max} \pm$ s.e.mean (cmH$_2$O)
Control		12	71.3 ± 7.8	31.8 ± 4.6
Substance P				
	10^{-5} M	6	70.7 ± 14.7	25.0 ± 3.6
	3×10^{-5} M	10	68.7 ± 8.7	28.6 ± 1.6
	10^{-4} M	12	$31.8 \pm 4.6^{***}$	38.8 ± 2.0
Neurokinin A				
	10^{-6} M	6	66.7 ± 5.1	31.1 ± 6.5
	3×10^{-6} M	8	50.4 ± 6.3	33.3 ± 3.1
	10^{-5} M	12	$28.8 \pm 4.6^{***}$	34.4 ± 2.2
	3×10^{-5} M	6	$29.3 \pm 10.4^{**}$	32.5 ± 2.1
Neurokinin B				
	3×10^{-6} M	6	54.4 ± 7.4	30.5 ± 21.1
	10^{-5} M	10	$37.5 \pm 4.5^{**}$	24.5 ± 4.2
	3×10^{-5} M	6	$34.7 \pm 5.6^{**}$	32.1 ± 5.3
	10^{-4} M	4	$29.5 \pm 4.8^{***}$	36.9 ± 6.2

Definition of abbreviations: $n =$ number of experiments; $ED_{30} =$ dose of acetylcholine giving 30% increase in airway inflation pressure; $E_{max} =$ increase in airway inflation pressure induced by acetylcholine 500 μg kg^{-1}. Values are mean $\pm$ s.e.mean. Significant differences from control are: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2 Effects of different selective tachykinin receptor agonists on acetylcholine-induced bronchoconstriction

Broncho-constrictive agents	Aerosol concentration	n	Acetylcholine $ED_{30} \pm$ s.e.mean (μg kg^{-1})	$E_{max} \pm$ s.e.mean (cmH$_2$O)
Control		14	71.3 ± 6.7	34.7 ± 3.3
[Sar^9, Met (O$_2$)^{11}]SP				
	10^{-6} M	4	61.5 ± 9.8	30.7 ± 6.2
	3×10^{-6} M	6	91.0 ± 11.9	29.4 ± 4.7
	10^{-5} M	7	$43.1 \pm 4.7^{***}$	32.4 ± 4.1
	3×10^{-5} M	6	$46.6 \pm 5.4^{**}$	29.5 ± 6.1
[βAla^8]NKA (4-10)				
	3×10^{-7} M	6	59.3 ± 7.2	34.1 ± 3.5
	10^{-6} M	6	$50.8 \pm 2.4^*$	32.8 ± 4.6
	3×10^{-6} M	4	55.2 ± 6.7	31.1 ± 3.5
	10^{-5} M	4	$28.9 \pm 10.1^{**}$	30.2 ± 4.6
	3×10^{-5} M	4	$32.8 \pm 4.7^{***}$	30.5 ± 4.0
[MePhe^7] NKB				
	10^{-6} M	6	59.1 ± 7.5	33.0 ± 4.2
	3×10^{-6} M	12	61.7 ± 7.3	30.0 ± 1.1
	10^{-5} M	12	$31.1 \pm 5.0^{***}$	34.8 ± 3.7
Senktide				
	10^{-6} M	10	52.9 ± 6.2	36.2 ± 3.8
	10^{-5} M	6	$29.1 \pm 4.6^{***}$	33.8 ± 6.3

Definition of abbreviations: $n =$ number of experiments; $ED_{30} =$ dose of acetylcholine giving 30% increase in airway inflation pressure; $E_{max} =$ increase in airway inflation pressure induced by acetylcholine 500 μg kg^{-1}. Values are mean $\pm$ s.e.mean. Significant differences from control are: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[Sar^9, Met(O$_2$)^{11}]SP and [βAla^8]NKA (4-10) with an immediate bronchoconstriction. The rank order of potency to induce bronchoconstriction was: [βAla^8]NKA (4-10) = NKA > NKB = SP > [Sar^9, Met(O$_2$)^{11}]SP. Under similar conditions [MePhe^7]NKB and senktide had no effect (Figure 3).

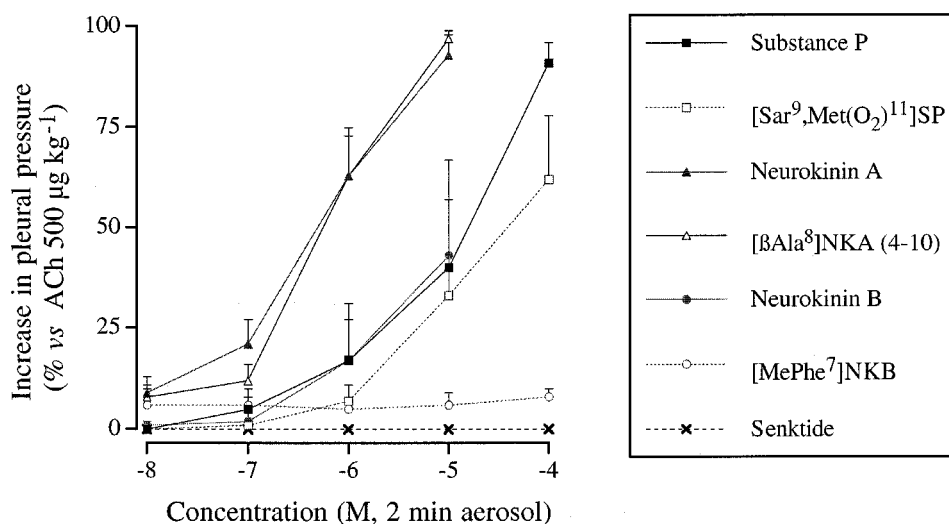**Figure 3** Bronchoconstriction induced by various tachykinins (SP, NKA, NKB) and specific tachykinin receptor agonists [Sar^9, Met(O$_2$)^{11}]SP, [βAla^8]NKA (4-10), [MePhe^7]NKB or senktide (3×10^{-7} to 10^{-4} M, aerosol) in anaesthetized guinea-pigs. Results are expressed as per cent increase of the bronchoconstriction induced by acetylcholine 500 μg kg^{-1} i.v.

Table 3 Influence of the tachykinin receptor agonists, SR 140333, SR 48968 and SR 142801 on [MePhe^7]NKB-induced bronchoconstriction

Broncho-constrictive agents	Dose	n	Acetylcholine $ED_{30} \pm$ s.e.mean (μg kg^{-1})	$E_{max} \pm$ s.e.mean (cm H$_2$O)
Control		14	71.3 ± 6.7	34.7 ± 3.3
[MePhe^7] NKB	10^{-5} M	10	36.2 ± 3.2**	34.1 ± 3.0
[MePhe^7] NKB +				
SR 140333	1 mg kg^{-1} i.p.	7	54.9 ± 9.8	34.9 ± 4.3
SR 48968	1 mg kg^{-1} i.p.	7	86.8 ± 3.5	34.6 ± 2.6
SR 142801	1 mg kg^{-1} i.p.	7	78.7 ± 17.2	34.6 ± 5.0

Definition of abbreviations: n = number of experiments; ED_{30} = dose of acetylcholine that produces 30% increase in airway inflation pressure; E_{max} = increase in airway inflation pressure induced by acetylcholine 500 μg kg^{-1}. Values are mean $\pm$ s.e.mean. Significant differences from control are: **$P < 0.01$.

Discussion

Tachykinin NK$_3$ receptor stimulation induces airway hyperresponsiveness

Tachykinins induce various effects in the bronchopulmonary system and they are recognised to be involved in the pathogenesis of airway hyperresponsiveness in animals models (see introduction and reviews of Advenier *et al.*, 1997; Kraneveld *et al.*, 1997; Spina *et al.*, 1998). Indeed, it has been previously reported that aerosol exposure of SP induces the development of airway hyperresponsiveness in guinea-pigs (Boichot *et al.*, 1993; Daoui *et al.*, 1997). Similar results were obtained in asthmatic patients (Cheung *et al.*, 1994). Moreover, NKA also elicits airway hyperresponsiveness in monkeys (Tamura *et al.*, 1989) but no studies have been conducted to date that evaluate the capacity for NK$_3$ receptor agonists to induce the development of airway hyperresponsiveness.

In the present study, we found that NKB and the tachykinin NK$_3$ receptor agonists, [MePhe^7]NKB (4-10) and senktide are also able to induce airway hyperresponsiveness in guinea-pigs pretreated with phosphoramidon. However, in terms of potency, the selective agonist for tachykinin NK$_2$ receptor [βAla^8]NKA (4-10) appears the most effective, since it significantly elicits a significant leftward shift of the dose-reponse curve to ACh, from the concentration of 10^{-6} M. We also observed that [MePhe^7]NKB and senktide showed a similar effect as NKA, whereas NKB and [Sar^9, Met (O$_2$)^{11}]SP were more effective than SP. The activity of NKB could be mediated through the effect on NK$_1$ and/or NK$_2$ receptors regarding the nonselective activity of this compound on NK$_3$ receptors (Regoli *et al.*, 1994). Nevertheless, the involvement of NK$_3$ receptors in the effects of [MePhe^7]NKB and senktide is strongly suggested by the high selectivity of these agonists on NK$_3$ receptors in several radioligand binding and functional assays (Regoli *et al.*, 1994).

The development of bronchial hyperresponsiveness induced by tachykinins and selective agonists for tachykinin receptors is not related to their bronchoconstrictor effects. Indeed, in this study the guinea-pigs are pretreated by the potent bronchodilator drug, salbutamol, in order to avoid all spasmogenic activities during the aerosol administration of tachykinin and tachykinin selective agonist. Moreover, [MePhe^7]NKB and senktide do not elicit a bronchoconstrictor response by themselves. The dissociation between the bronchoconstrictor activity and the induction of bronchial hyperresponsiveness is also demonstrated by the use of [βAla^8]NKA (4-10) and NKA. Both compounds elicit similar bronchoconstrictor effects (Chan *et al.*, 1994; Yuan *et al.*, 1994 and this study), but [βAla^8]NKA (4-10) induced a more marked leftward shift of the dose-response curve to ACh than NKA. Similar observations and conclusions may be proposed in view of the respective efficacy of [Sar^9, Met(O$_2$)^{11}]SP and SP in the induction of bronchoconstriction and airway hyperresponsiveness.

Tachykinin NK$_2$ and NK$_3$ receptor antagonists prevent NK$_3$-induced airway hyperresponsiveness

The present results showed that airway hyperresponsiveness induced by the selective NK$_3$ receptor agonist [MePhe^7]NKB

was abolished by the tachykinin NK$_3$ receptor antagonist, SR 142801 (osanetant), but also by the tachykinin NK$_2$ receptor antagonist, SR 48968 (saredutant). Similar observations were previously reported on airway hyperresponsiveness induced by SP or citric acid (Daoui et al., 1997, 1998). This later compound has been demonstrated to release endogenous tachykinins from capsaicin sensitive nerve endings (Belmonte et al., 1990; Fox et al., 1995; Geppetti et al., 1991; Steen et al., 1992). In contrast to the inhibitory activity of SR 142801, the effect of SR 48968 and other NK$_2$ receptor antagonists on the development of bronchial hyperresponsiveness have been clearly demonstrated in sensitized and challenged guinea-pigs (Boichot et al., 1995; Kudlacz et al., 1996) or after exposure to toluene diisocyanate (Marek et al., 1996); cold air (Yoshihara et al., 1996); ozone (Masson et al., 1996) or PAF (Perretti & Manzini, 1993) in guinea-pigs.

The tachykinin NK$_1$ receptor antagonist, SR 140333, only elicit a partial inhibition of [MePhe^7]NKB. In previous reports, we observed that SR 140333 did not prevent in guinea-pigs airway hyperresponsiveness induced by SP (Boichot et al. 1996) or by an allergen challenge in sensitized animals (Boichot et al., 1995). The effects of SR 140333 is however debated since Schuiling et al. (1999) reported a preventive effect of this drug in the latter model. These discrepancies could be explained by the different protocols and spasmogenic agents used in both cases.

Hypothesis on the mechanism of action of tachykinin NK$_3$ agonists

To date, it is difficult to provide the exact mechanism and the site of action of NK$_3$ agonists in the development of airway hyperresponsiveness. Indeed, airway hyperresponsiveness is a complex process which involves multiple cell interactions and several mediators. Firstly, it seems that the action of NK$_3$ agonists is mainly due to the NK$_3$ receptor activation pathway. Secondly, this activity may involve neuronal receptors rather than post-junctional effector sites. Thirdly, a cascade of physiopathological process associated with the successive stimulation of tachykinin receptors may also be proposed.

The activity of NKB, [MePhe^7]NKB and senktide involve the NK$_3$ receptor pathway as suggested by the high selectivity of these agonists on this receptor subtype, but also by the inhibitory activity of the selective NK$_3$ antagonist, SR 142801. The compound selectivity has been demonstrated by radioligand binding and well characterized *in vitro* functional assays for tachykinin receptors (Beaujouan et al., 1997; Daoui et al., 1997; Emonds-Alt et al., 1995; Nguyen-Le et al., 1996; Oury-Donat et al., 1995; Patacchini et al., 1995). *In vivo*, the selectivity of SR 142801 is clearly suggested by two assays: in contrast to SR 48968, SR 142801 (1 mg kg^{-1}) did not inhibit bronchoconstriction induced by [Nle^{10}]NKA (4-10) in anaesthetized guinea-pigs (Daoui et al., 1997); and unlike SR 140333, SR 142801 (1 mg kg^{-1}) failed to inhibit the hypotension induced by [Sar^9, Met(O$_2$)^{11}]SP in guinea-pigs and dogs (Emonds-Alt et al., 1993, 1995; Roccon et al., 1996).

A direct priming effect of NK$_3$ receptor stimulation on target cells is unlikely, since a low number of NK$_3$ tachykinin receptor has been identified in lung (Baluk et al., 1996). In agreement with previous studies (Ellis et al., 1993; Killingsworth & Shore, 1995; Maggi et al., 1991), our results demonstrating that [MePhe^7]NKB and senktide do not present bronchoconstrictor activity in the guinea-pig suggest that NK$_3$ receptor stimulation does not lead to the contraction of airway smooth muscle. A

direct participation of NK$_3$ receptors in the impairment of vessels and endothelial cells, leading to microvascular leakage and airway obstruction is also excluded since SR 142801 failed to inhibit tachykinin and capsaicin-induced plasma extravasation which is mainly mediated by NK$_1$ receptors and suppressed by SR 140333 (Inoue et al., 1996). Finally, no effect of tachykinin NK$_3$ receptor stimulation has been demonstrated in mucus or in inflammatory cells (Ellis & Undem, 1994; Maggi et al., 1993; Rogers, 1995).

Tachykinin NK$_3$ receptor may increase neuronal activity and responsiveness of target cells. Several electrophysiological studies have reported that tachykinins elicit an important activity on the control of various neuronal and ganglionic potentials at the periphery and, among tachykinins, neurokinin B and stimulation of tachykinin NK$_3$ receptors seems to play a predominant role. This has been suggested by studies showing that substance P and neurokinin B (but not neurokinin A) induced depolarization of guinea-pig bronchial parasympathetic ganglion neurones, and that neurokinin B was 60-fold more potent and five time more efficient than substance P (Myers & Undem, 1993). Neurokinin B and [Asp^{5,6}, metylPhe^8]SP(5-11) (a selective agonist for NK$_3$ receptors) induced a decrease in membrane resistance (Myers & Undem, 1993). Interestingly, the capsaicin-evoked slow excitatory postsynaptic potential of guinea-pig bronchial and tracheal parasympathetic ganglion neurons (Myers et al., 1996) and the relaxation of the guinea-pig trachea elicited by antidromic stimulation of capsaicin-sensitive vagal afferent nerves were reduced by SR 142801 (Canning et al., 1998). A similar control of neuronal transmission and reflexes by NK$_3$ receptor has also been evidenced in gastrointestinal tract (Johnson et al., 1996, 1998; Mawe, 1995; Zhao et al., 1995). On the basis of these data, however it is not clear whether peripheral neuronal electric activities mediated by tachykinin NK$_3$ receptors may lead to the modulation of airway hyperresponsiveness to ACh. Hence, the importance of the role of tachykinins in nodose ganglia has been recently strengthened in the airway hyperresponsiveness induced in the guinea-pig (Fischer et al., 1996; Weinreich et al., 1997), but until now no evidence for a role of NK$_3$ at this site of action has been demonstrated.

Finally, the mechanism of the prevention of airway hyperresponsiveness induced either by tachykinin NK$_3$ agonists (the present study), SP (Daoui et al., 1997) or citric acid (Daoui et al., 1998) by SR 48968 and SR 142801 is unclear and suggest the involvement of various physiopathological serial process. For example, a costimulation of NK$_3$ + NK$_2$ receptor or NK$_3$ + NK$_1$ receptor has been reported for intestinal motility (Croci et al., 1995). An interrelationship between different tachykinins has been also suggested. Indeed, Schmid et al. (1998) showed that NK$_3$ receptors mediate enhancement of substance P release from rat capsaicin-sensitive spinal cord afferent terminals.

In conclusion, the present study demonstrates the potent ability of neurokinin B and tachykinin receptor agonists [MePhe^7]NKB and senktide to induce airway hyperresponsiveness and the inhibitory effect of the selective NK$_3$ receptor antagonists (SR 142801) on [MePhe^7]NKB-induced hyperresponsiveness in guinea-pigs. For concentrations that induced airway hyperresponsiveness, senktide and [MePhe^7]NKB failed to induce bronchoconstriction. Our data suggest that NK$_3$ receptor stimulation can induce airway hyperresponsiveness and this effect is not related to the ability of tachykinins to induce bronchoconstriction.

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