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Tachykinin NK_3 receptor agonists induced microvascular leakage hypersensitivity in the guinea-pig airways

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Abstract

Microvascular leakage hypersensitivity is a main component of neurogenic inflammation and of tachykinin effects.The aim of this study was to examine the ability of neurokinin B and of the tachykinin NK_3 receptor agonists, [MePhe^7]neurokinin B or senktide, to potentiate when given by aerosol the microvascular leakage induced by histamine in guinea-pig airways and to compare their effects to those of tachykinin NK_1 (substance P, [Sar^9,Met(O$_2$)^{11}]substance P) or tachykinin NK_2 (neurokinin A, [βAla^8]neurokinin A (4–10)) receptor agonists. Guinea-pigs were pretreated successively for 10 min with aerolized salbutamol and phosphoramidon; 15 min later, they were exposed for 30 min to an aerosolized solution of tachykinin receptor agonists; 24 h later, the animals were anaesthetized and vascular permeability was quantified by extravasation of Evans blue dye. Neurokinin B, [MePhe^7]neurokinin B and senktide (3×10^{-6}–3×10^{-5} M) induced a potentiation of the effects of histamine on the vascular permeability in the trachea and main bronchi. Compared to other tachykinin NK_1 and NK_2 receptor agonists, the order of potency was: senktide>neurokinin B=[Sar^9,Met(O$_2$)^{11}]substance P=[βAla^8]neurokinin A (4–10)=[MePhe^7]neurokinin B>neurokinin A>substance P. The potentiation by [MePhe^7]neurokinin B of histamine-induced microvascular leakage was abolished by the tachykinin NK_1 receptor antagonist SR140333 ([(*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidin-3-yl]etyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane, chloride]) or the tachykinin NK_3 receptor antagonists SR 142801 ([(*R*)-(*N*)-(1-(3-(l-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide]) and SB 223412 ([(*S*)-(−)-*N*-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide]). In conclusion, these results suggest that tachykinin NK_3 receptors might be involved in the potentiation of histamine-induced increase in microvascular permeability.

Keywords: Tachykinin NK_3 receptor; Airway; Microvascular leakage

1. Introduction

Neurogenic inflammation is a complex process involving vasodilatation, plasma protein extravasation and edema, glandular secretion and immunoinflammatory cell chemotaxis and activation. This process may contribute to airway edema and to airflow obstruction and is considered to be one of the main inflammatory events in airway diseases, such as bronchial asthma or chronic obstructive pulmonary disease. Neurogenic inflammation is the result of the activation of sensory nerve endings and the subsequent production of neuropeptides, namely, tachykinins such as substance P, neurokinin A and neurokinin B (Solway and Leff, 1991; Ellis and Undem, 1994; Lundberg, 1996; Baluk and McDonald, 1998).

The biological actions of tachykinins are mediated through three types of receptors, denoted tachykinin NK_1, NK_2 and NK_3, which have the highest affinity for substance P, neurokinin A and neurokinin B, respectively (Regoli et al., 1994). Substance P, neurokinin A and neurokinin B have been shown to induce microvascular leakage in the guinea-pig airways when injected intravenously (Rogers et al., 1988). Substance P was more potent than neurokinin A or neurokinin B in increasing microvascular permeability, which indicate that tachykinin NK_1 receptors are mainly

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involved in neurogenic inflammation in the central airways of guinea-pigs (Rogers et al., 1988). Moreover, the tachykinin NK_1 receptor antagonists, CP 96345 ([2S,3S)-*cis*-2(diphenylmethyl)-*N*-[2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2.]octane-3-amine) or 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]), have been reported to inhibit or reduce the microvascular leakage induced by exogenous substance P, capsaicin, electrical stimulation of the cervical vagus nerves, bradykinin or antigen challenge in guinea-pig airways (Lei et al., 1992; Bertrand et al., 1993; Qian et al., 1993). The tachykinin NK_2 receptors also partially mediate the neurogenic plasma extravasation in secondary bronchi and intraparenchymal airways of the guinea-pig, namely, in distal airways (Tousignant et al., 1993).

The pathophysiological role of tachykinin NK_3 receptor still remains poorly understood. Many of the functional studies with neurokinin NK_3 receptors agonists or antagonists have been performed on peripheral tissues in vitro but few have investigated neurogenic plasma extravasation in vivo in rodent trachea or/and main bronchi. We have previously demonstrated in guinea-pigs that substance P or citric acid, when given by inhalation, induce 24 h later a potentiation of the effects of histamine on vascular permeability (Daoui et al., 1997; Boichot et al., 1996). Moreover, this effect was abolished by the tachykinin NK_3 receptor antagonist, SR 142801 (osanetant) ([(*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidin-3-yl]etyl}-4-phenyl-1-azoniabicyclo[2.2.2]octane, chloride]) and the tachykinin NK_1-receptor antagonist, SR 140333 (nolpitantium), but not by the tachykinin NK_2 receptor antagonist, SR 48968, (saredutant) ([(*S*)-*N*-methyl-*N*[(4-acetylamino-4-phenylpiperidino-2-(3,4)dichlorophenyl) butyl]benzamide]) (Daoui et al., 1997). These results, obtained with selective antagonists, strongly suggest that tachykinins are involved in addition to their direct effect in the development of airway microvascular leakage hyperresponsiveness in inflammatory conditions, and indicate that NK_3 receptor stimulation may play an important role in this phenomenon.

The aim of this study was to investigate whether the tachykinin NK_3 receptor agonists, such as [$MePhe^7$]neurokinin B, senktide and neurokinin B, were able to potentiate the microvascular leakage induced by histamine in guinea-pig airways. These effects were compared to those of substance P and of the tachykinin NK_1 receptor agonist-[Sar^9,$Met(O_2)^{11}$]substance P- and the natural-neurokinin A- or selective-[βAla^8]neurokinin A (4–10)-NK_2 receptor agonists. We also studied the effects of the specific tachykinin receptor antagonists, SR 140333 (NK_1) (Emonds-Alt et al., 1993), SR 48968 (NK_2) (Emonds-Alt et al., 1992) or SR 142801 (NK_3) (Emonds-Alt et al., 1995) and SB 223412 (NK_3) ([(*S*)-(−)-*N*-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide]) (Sarau et al., 1997), respectively, on the development of this hyperreactivity. Finally, in order to study the relationship between the direct effect of tachykinin on vascular permeability and their ability to potentiate the effects of histamine, we have also determined the immediate and direct effect of the tachykinin receptor agonists ([Sar^9,$Met(O_2)^{11}$]substance P, [βAla^8]neurokinin A (4–10) and [$MePhe^7$]neurokinin B) on the vascular permeability.

2. Materials and methods

2.1. Exposure to tachykinins or tachykinin receptor agonists aerosol

Tricoloured unanesthetized, unrestrained male or female guinea-pigs (300–400 g), were placed in pexiglas chamber (30 × 25 × 15 cm) and exposed successively to nebulised aqueous solution of salbutamol (8.7 mM, 10 min) or phosphoramidon (0.1 mM, 10 min) in order to prevent

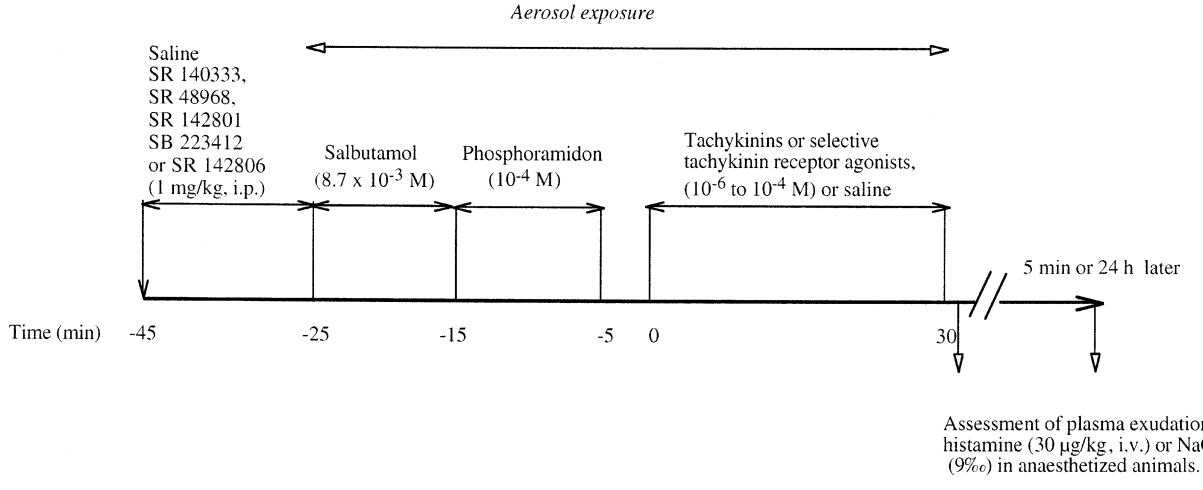

Fig. 1. Experimental protocol of airway microvascular leakage in guinea-pigs.

tachykinin- or tachykinin receptor agonist-induced bronchoconstriction and tachykinin metabolism, respectively; 5 min later the animals were exposed to a single aerosol of tachykinins receptor agonists at various concentrations (3×10^{-7}–1×10^{-4} M) or vehicle solution (NaCl, 9‰) as control group for 30 min (Fig. 1). An ultrasonic nebulizer (aerodynamic mean mass median particule diameter of 0.5 to 5 μm, NEB99, Devilbiss, Somerset, PA, USA) was used.

2.2. Measurement of airway microvascular leakage

Five minutes or 24 h after tachykinins or tachykinin receptor agonists exposure, vascular permeability was quantified by the extravasation of Evans blue dye, which correlates well with extravasation of radiolabelled albumin in the skin and airways (Rogers et al., 1988). Animals were anaesthetized with urethane (1.25 g/kg, i.p.). A jugular vein was cannulated for injection of drugs. After a rest period, Evans blue dye (30 mg/kg, i.v.) was injected followed 1 min later by saline (1 ml/kg, i.v.) or histamine (30 μg/kg, i.v.); 5 min later, the thorax was opened and a blunt-ended 13-gauge needle was passed through a left ventriculotomy into the aorta. The ventricles were cross-clamped and blood was expelled through an incision in the right atrium at 80-mmHg pressure with about 100 ml saline (pH 5.5), in order to remove the intravascular dye from the systemic and pulmonary circulations until the perfusate was clear. The lungs were then removed. The connective tissues, vasculature, and parenchyma were gently scraped away, and the airways were divided into four components: lower part of trachea, main bronchi and proximal (the proximal 3 mm portion) and distal intrapulmonary airways (Rogers et al., 1988). The tissues were blotted dry, placed in preweighed tubes and reweighed, and their dye content was extracted in formamide at 37 °C for 18 h. Dye concentration was quantified by light absorbance at 620 nm (DCP spectrophotometer, Vital, Dieren, Netherlands) and its tissue content (ng dye/mg wet weight tissue) was calculated from a standard curve of dye concentrations in the 0.5–10 μg/ml range.

2.3. Pretreatment with tachykinin receptor antagonists

Guinea-pigs received a single dose (1 mg/kg, i.p.) of the NK_3 (SR 142801, SB 223412), NK_2 (SR 48968) or NK_1 (SR 140333) receptor antagonists, SR 142806 (the inactive

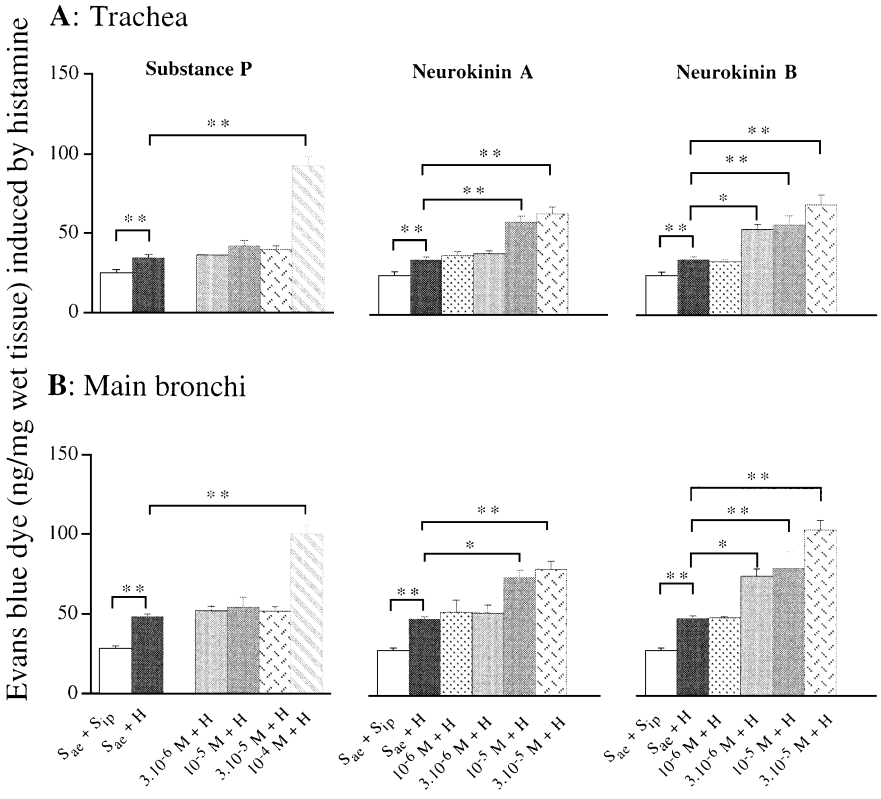

Fig. 2. Histograms illustrating effects of natural tachykinins-substance P, neurokinin A and neurokinin B- (10^{-6}–10^{-4} M, 30 min) on plasma exudation induced by histamine in anesthetized guinea-pigs airways 24 h after aerosols of salbutamol (8.7 mM, 10 min), phosphoramidon (1 mM, 10 min) and saline (NaCl 9‰, 30 min) or substance P, neurokinin A, neurokinin B. Vertical bars represent responses to saline (S_{ip}) or histamine (H) (1 min after Evans blue dye injection) after an exposure 24 h earlier to salbutamol (8.7 mM, 10 min), phosphoramidon (1 mM, 10 min) and saline (NaCl 9‰, 30 min)(S_{ae}) or substance P, neurokinin A, neurokinin B (10^{-6}–10^{-4} M, 30 min, aerosol). Values are means ± S.E.M., $n = 6$–8. Significant difference from control histamine are shown as: * $P < 0.05$ and ** $P < 0.01$.

enantiomer of SR 142801) or vehicle 45 min before exposure to [MePhe^7]NKB.

In another set of experiments, we tested the ability of SR 142801 (1 mg/kg, i.p. 30 min) on substance P- (0.3 μg/kg, i.v.) or histamine (30 μg/kg, i.v.)-induced increase in vascular permeability.

All experiments have been carried out in accordance with the declaration of Helsinki for care and use of laboratory animals.

2.4. Statistical analysis of results

Data are expressed as means ± S.E.M. Statistical analysis of the results was performed using analysis of variance for unpaired data and Bonferroni test. Probability values of less than 0.05 were considered significant.

2.5. Drugs

The following drugs were used: urethane (Prolabo, Paris, France); histamine dihydrochloride, phosphoramidon, salbutamol sulphate (Sigma, St. Louis, MO, USA), 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), CP 96345, 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), SR 142806 (the inactive enantiomer of SR 142801) used as hydrochloride (Sanofi Recherche, Montpellier, France). SB 223412 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide] (SmithKline Beecham, King of Prussia, USA). All drugs were dissolved in saline except SR 48968, SR 140333, SB 223412, SR 142801 and SR 142806, which were dissolved in ethanol, and saline and neurokinin B and [MePhe^7]neurokinin B, which were dissolved in saline (NaCl 9‰). The maximum amount of ethanol injected (20 μl/100 g body weight) did not modify the respiratory responses to histamine and the development of airway microvascular leakage at the dilution used.

3. Results

3.1. Comparison of the ability of the tachykinin receptor agonists to potentiate, when given by aerosol, the histamine-induced extravasation of Evans blue dye in airways

In preliminary experiments, we did not observed any significant differences at any airway level in extravasation of Evans blue dye after sham stimulation with inhaled saline

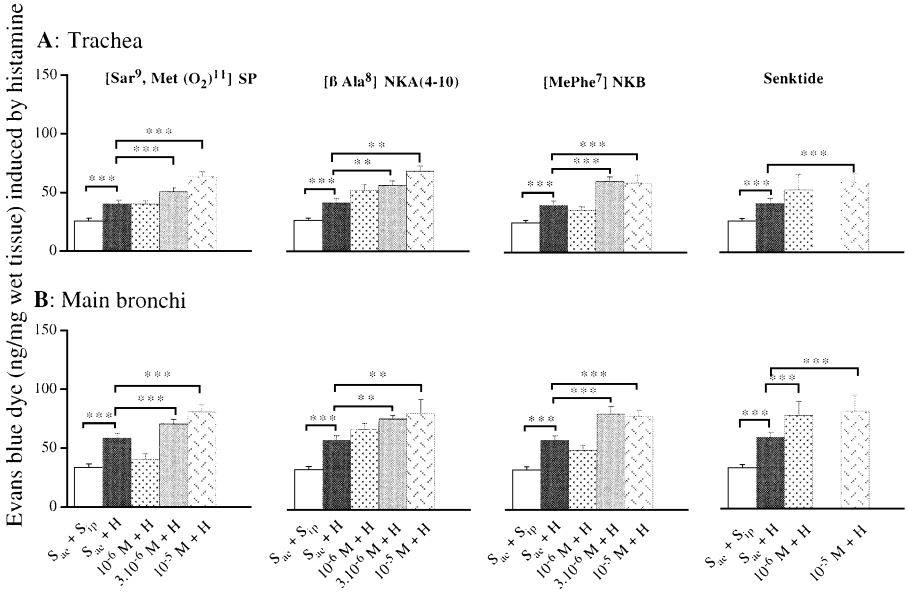

Fig. 3. Histograms illustrating effects of selective tachykinin receptor agonists (10^{-6}–10^{-4} M, 30 min) on plasma exudation induced by histamine in anaesthetized guinea-pigs airways 24 h after aerosols of salbutamol (8.7 mM, 10 min), phosphoramidon (1 mM, 10 min) and saline (NaCl 9‰, 30 min) (S_{ae}) or [Sar^9,Met(O$_2$)^{11}]substance P, [βla^8]neurokinin A(4–10), [MePhe^7]neurokinin B, senktide (3×10^{-7}–10^{-4} M, 30 min). Vertical bars represent responses to saline ((S_{ip})) or histamine (H) (1 min after Evans blue dye injection) after an exposure 24 h earlier to salbutamol (8.7 mM, 10 min), phosphoramidon (0.01 M, 10 min) and saline (NaCl 9‰, 30 min) (S_{ae}) or [Sar^9,Met(O$_2$)^{11}]substance P, [βla^8]neurokinin A (4–10), [MePhe^7]neurokinin B, senktide (10^{-6} to 10^{-5} M, 30 min, aerosol). Values are means ± S.E.M., $n=6$–8. Significant difference from control histamine shown as: $^{**}P<0.01$ and $^{***}P<0.001$.

(9‰) in animals pretreated with saline or ethanol (20 μl/100 g body weight, i.p.). Inhaled tachykinin or selective tachykinin receptor agonists ([Sar^9, Met $(O_2)^{11}$]substance P, substance P, [βAla^8]neurokinin A (4–10), neurokinin A, [MePhe^7]neurokinin B or neurokinin B (10^{-6} to 10^{-4} M), induced 24 h later a significant potentiation in the amount of extravasated dye induced by histamine, compared to control animals in trachea and main bronchi in a dose-related manner (Figs. 2 and 3). The effect of the tachykinins was observed in the trachea and main bronchi but not in distal airways (data not shown). Inspection of the concentration–response curves reveals that the order of potency of the tachykinins and their selective analogues in increasing vascular leakage induced by histamine was: senktide>neurokinin B=[Sar^9,Met$(O_2)^{11}$]substance P=[βAla^8]neurokinin A (4–10)=[MePhe^7]neurokinin B>neurokinin A>substance P (significant increase for 3×10^{-6} M)>NKA (significant increase for 10^{-5} M)>SP (significant increase for 10^{-4} M).

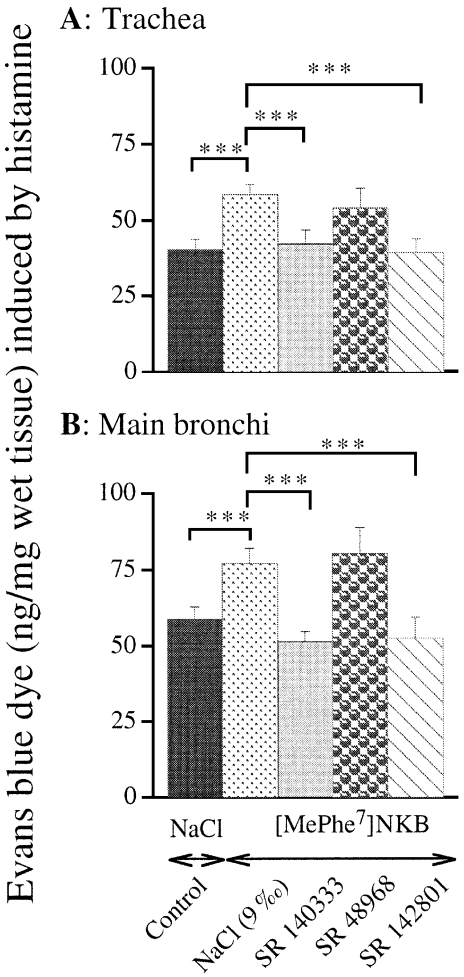

Fig. 4. Effects of the tachykinin NK_1, NK_2 or NK_3 receptor antagonists, SR 140333, SR 48968 or SR 142801 on the potentiation by [MePhe^7]neurokinin B administered by aerosol (10^{-5} M) 24 h earlier of Evans blue dye extravasation induced by histamine (30 μg/kg) in guinea pig trachea and main bronchi. Doses of tachykinin receptor antagonists are: 1 mg/kg, given 45 min before [MePhe^7]neurokinin B aerosol exposure. Values are means ± S.E.M., $n = 5$ per group. Significant difference from control with histamine after [MePhe^7]neurokinin B aerosol exposure are shown as: *** $P < 0.001$.

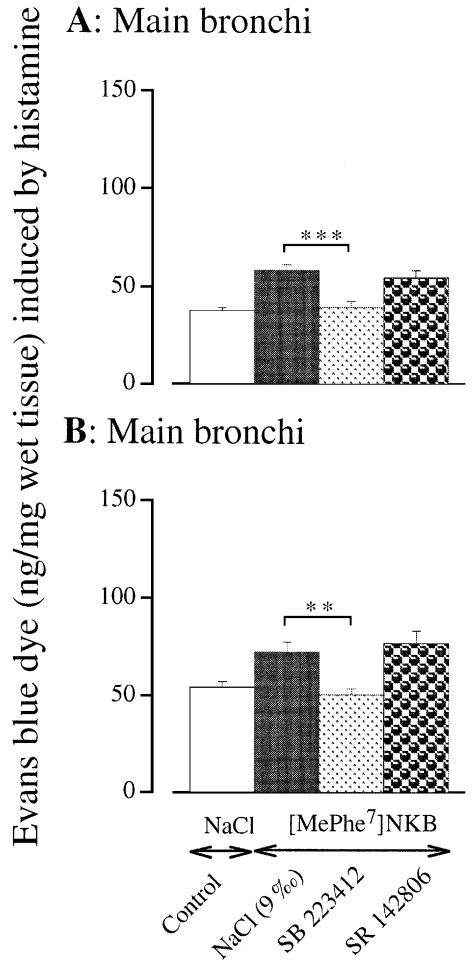

Fig. 5. Effects of the tachykinin NK_3 or the inactive enantiomer of the tachykinin NK_3 receptor antagonists, SB 223412 or SR 142806, respectively, on the potentiation by [MePhe^7]neurokinin B administered by aerosol (10^{-5} M) 24 h earlier of Evans blue dye extravasation induced by histamine (30 μg/kg) in guinea pig trachea and main bronchi. Doses of tachykinin receptor antagonists are: 1 mg/kg, given 45 min before [MePhe^7]neurokinin B aerosol exposure. Values are means ± S.E.M., $n = 5$ per group. Significant difference from control with histamine after [MePhe^7]neurokinin B aerosol exposure are shown as: ** $P < 0.01$, *** $P < 0.001$.

3.2. Effects of SR 140333 (NK_1 antagonist), SR 48968 (NK_2 antagonist), SR 142801 or SB 223412 (NK_3 antagonists), SR 142806 (the inactive enantiomer of SR 142801) on the potentiation by [MePhe^7]neurokinin B of the histamine-induced increase in microvascular leakage

The potentiation by [MePhe^7]neurokinin B of histamine-induced microvascular leakage in trachea and bronchi was abolished by the tachykinin NK_1 and NK_3 receptor antagonists, SR 140333 and SR 142801 (Fig. 4) or SB 223412

(Fig. 5), but was unmodified by the NK_2 receptor antagonist, SR 48968 (Fig. 4) or by the inactive enantiomer of SR 142801, SR 142806 (Fig. 5).

3.3. Immediate effects of tachykinin receptor agonists on vascular permeability

After pretreatment with salbutamol and phosphoramidon, guinea-pigs were exposed to an aerosol of saline or of one of the selective tachykinin receptor agonists (10^{-5} M, 30 min) in order to determine whether an immediate response (5 min) could be identified. Guinea-pigs responded to [Sar^9,$Met(O_2)^{11}$]substance P with an immediate plasma extravasation in trachea and main bronchi, but no effect was observed in proximal and distal airway (data not shown).

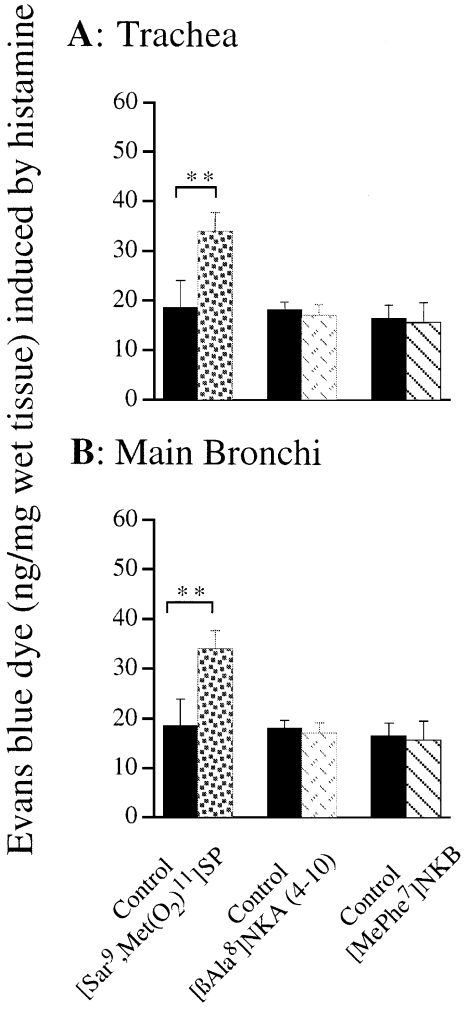

Fig. 6. Histograms illustrating the immediate effects of tachykinin receptor agonists on plasma exudation in guinea-pigs airways. Animals were pretreated with salbutamol, phosphoramidon and exposed to [Sar^9,$Met(O_2)^{11}$]SP, [βla^8]NKA (4–10) or [$MePhe^7$]neurokinin B (10^{-5} M, 30 min). After anaesthesia, Evans blue dye extravasation in airways was determined. Values are means ± S.E.M., $n=5$. Significant difference from control shown as: $**P<0.01$.

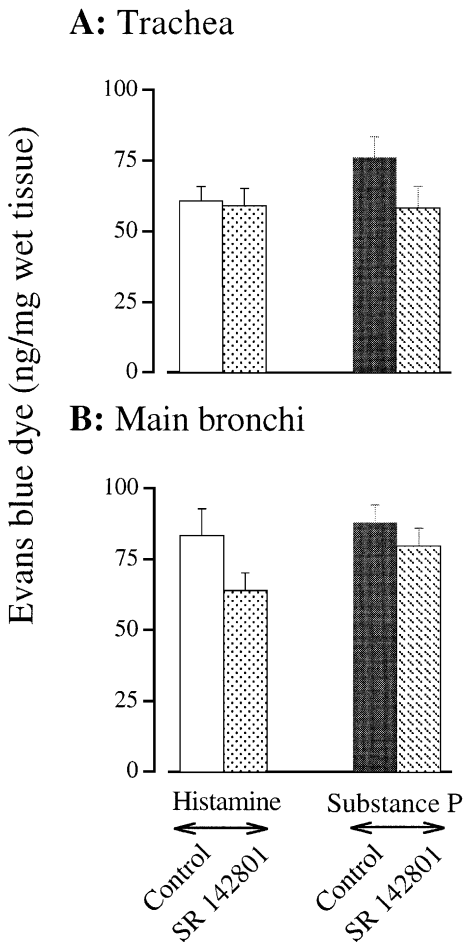

Fig. 7. Histograms illustrating the effects of tachykinin NK_3 receptor antagonist, SR 142801 (1 mg/kg, i.p., 30 min) on vascular permeability induced by histamine (30 μg/kg, i.v.) or substance P (0.3 μg/kg, i.v.) in guinea-pigs airways. Values are means ± S.E.M., $n=5$.

Under similar condition, [βAla^8]neurokinin A (4–10), [$MePh^7$]neurokinin B and senktide had no effect (Fig. 6).

In another set of experiments, we have shown that SR 142801 (1 mg/kg, i.p., 30 min) did not modify vascular permeability induced by histamine (30 μg/kg, i.v.) or substance P (0.3 μg/kg, i.v.) in trachea or main bronchi (Fig. 7).

4. Discussion

Tachykinins are now recognized to be involved in the pathogenesis of airway hyperresponsiveness in various animals experimental models. Indeed, it has been reported that aerosol exposure of substance P induces the development of airway hyperresponsiveness in guinea-pigs, characterized by an increased response to the bronchoconstrictor effect of acetylcholine (Boichot et al., 1993; Daoui et al., 1997). Similar results were obtained in asthmatic patients (Cheung et al., 1994). Neurokinin A elicits airway hyperresponsiveness in monkeys (Tamura et al., 1989) and we have recently shown that neurokinin B and the tachykinin

NK_3 receptor agonists [MePhe^7]neurokinin B and senktide induce airway hyperresponsiveness in guinea-pigs (Daoui et al., 2000).

We have also shown that substance P and citric acid, which induces a release of tachykinins (Geppetti et al., 1991; Fox et al., 1995), when given by aerosol, induce, 24 h later, a potentiation of the effects of histamine on vascular permeability and that this effect was abolished by the tachykinin NK_1 (SR 140333) and NK_3 (SR 142801) receptor antagonists (Daoui et al., 1997, 1998). Moreover, to date, no study has evaluated the capacity for NK_3 receptor agonists to induce the development of airway hypersensitivity to the microvascular leakage induced by histamine.

In the present study, we showed that neurokinin B and the NK_3 receptor agonists, [MePhe^7]neurokinin B or senktide, were able to induce airway hypersensitivity to histamine in guinea-pigs pretreated with phosphoramidon. Furthermore, in terms of potency neurokinin B, senktide and [MePhe^7]neurokinin B appear as effective as [Sar^9,Met$(O_2)^{11}$]substance P and [βAla^8]neurokinin A (4–10), since they elicit a significant potentiation of the effect of histamine from the concentration of 3×10^{-6} M. In our experimental model, [Sar^9,Met$(O_2)^{11}$]substance P appears 30-fold more effective than substance P, whereas [Sar^9,Met$(O_2)^{11}$]-substance P and SP elicit similar potency on rabbit vena cava or urinary bladder (Regoli et al., 1994). Nevertheless, the activity of [Sar^9,Met$(O_2)^{11}$]substance P appeared to be intrinsically more important than substance P (Regoli et al., 1994). Regarding the low selectivity of neurokinin B on NK_3 receptors, the activity of this compound could be mediated through the effect on NK_1 and/or NK_2 receptors (Regoli et al., 1994). However, the involvement of NK_3 receptors in the effects of [MePhe^7]neurokinin B is strongly suggested by the high selectivity of this agonist on NK_3 receptors in several radioligand binding and functional assays (Regoli et al., 1994).

The development of bronchial hypersensitivity to the histamine-induced microvascular leakage induced by tachykinins and selective agonists for tachykinin receptors is not related to their direct effects on vascular permeability. Indeed, we report that, in contrast to [Sar^9,Met$(O_2)^{11}$]substance P, [MePhe^7]neurokinin B and [βAla^8]neurokinin A (4–10) did not elicit microvascular leakage by themselves at concentration threefold higher than that, inducing 24 h later hypersensitivity to histamine.

The present results also showed that airway hypersensitivity to histamine-induced microvascular leakage induced by the selective NK_3 receptor agonist [MePhe^7]neurokinin B was abolished by the tachykinin NK_3 receptor antagonists, SR 142801 (osanetant) or SB 223412 (Sarau et al., 1997), but also by NK_1 receptor antagonist, SR 140333 (nolpitantium). Similar observations were previously reported for the tachykinin NK_1 receptor antagonist in the initiation of hypersensitivity to vascular permeability in the gastrointestinal tract in a model of delayed type of hypersensitivity induced by dinitrofluorobenzene in mice (Kraneveld et al., 1995). Furthermore, SR 140333 and SR 142801 also inhibited airway hypersensitivity to histamine induced by SP or citric acid in guinea-pigs (Daoui et al., 1997, 1998; Boichot et al., 1996). In contrast, the tachykinin NK_2 receptor antagonist SR 48968 (saredutant) or the inactive enantiomer of SR 142801, SR 142806, did not prevent tachykinin-induced hypersensitivity to histamine. SR 48968 and other NK_2 receptor antagonists have been clearly demonstrated to inhibit bronchial hyperresponsiveness to acetylcholine or metacholine in sensitized and challenged guinea-pigs or after exposure to toluene diisocyanate, cold air, ozone or PAF in guinea-pigs (Advenier et al., 1997; Spina et al., 1998).

The inhibition by SR 142801 or SB 223412 of enhanced hypersensitivity to histamine-induced microvascular leakage by [MePhe^7]NKB (this study) or by SP (Daoui et al., 1997) or citric acid (Daoui et al., 1998) is an argument for the involvement of tachykinin NK_3 receptors in this hyperreactivity. Indeed, the selectivity of the compound is clearly demonstrated by radioligand binding and in vitro by functional assays characterized for tachykinin receptors (Emonds-Alt et al., 1995; Oury-Donat et al., 1995; Daoui et al., 1997). In vivo, the selectivity of SR 142801 is clearly suggested by two assays: in contrast to SR 48968, SR 142801 (1 mg/kg) did not inhibit bronchoconstriction induced by [Nle^{10}]neurokinin A (4–10) in anaesthetized guinea-pigs, whereas SR 48968 was effective (Daoui et al., 1997), and unlike SR 140333, SR 142801 (1 mg/kg) failed to inhibit the hypotension induced by [Sar^9,Met$(O_2)^{11}$]substance P in guinea-pigs and dogs (Emonds-Alt et al., 1993, 1995; Roccon et al., 1996). In addition, we have shown that under our experimental conditions, SR 142801 did not modify plasma extravasation induced in airways by substance P or histamine.

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, since a low number of tachykinin NK_3 receptors has been identified in lung (Baluk and McDonald, 1998). Tachykinin NK_3 receptor may increase neuronal activity as shown in guinea-pig bronchial parasympathetic ganglion neurons (Myers and Undem, 1993; Myers et al., 1996; Canning et al., 1998). A similar control of neuronal transmission and reflexes by NK_3 receptor has also been evidenced in gastrointestinal tract (Mawe, 1995; Zhao et al., 1995; Johnson et al., 1996). Another hypothesis could involve the up-regulation of NK_3 receptor on target cells as suggested by such a phenomena on human T cells following inflammatory process (Braun et al., 1999).

Finally, the mechanism of the induction of airway hypersensitivity to histamine induced either by tachykinin NK_3 agonists (the present study), SP (Daoui et al., 1997) or citric

acid (Daoui et al., 1998) and its prevention by SR 140333, SR 142801 or SB 223412 suggest the involvement of various physiopathological serial process. Examples of positive cooperation between tachykinin receptors stimulation have been reported for intestinal motility in the guinea-pig. Indeed, Guard and Watson (1987) noted that the neurogenic contraction produced by the NK_3 receptor-selective agonist senktide in the longitudinal muscle of the ileum was only partially prevented by atropine, whereas the atropine-resistant component could be markedly reduced by desensitization of the tissue to substance P methyl ester. Thus, it was suggested that NK_3 receptors are present on acetylcholine- and/or tachykinin-containing myenteric plexus neurons, whose stimulation leads to release of acetylcholine and tachykinins, respectively; this in turn results in smooth muscle contraction via activation of muscarinic acetylcholine and NK_1 receptors present on interstitial and smooth muscle cells. Similarly, Croci et al. (1995) and Patacchini et al. (1997) showed that senktide-induced contraction of the guinea-pig ileum or common bile duct is prevented by blocking either NK_1, NK_2 and NK_3 receptors or muscarinic receptors. This suggests a sequential activation of excitatory transmission with the presence of prejunctional autoreceptors of the NK_3 type, and postjunctional of the NK_1 or NK_2 type (Maggi et al., 1997a,b; Patacchini et al., 2000). Similarly, it is possible that a mechanism of serial control on nervous and cellular components may be present in the development of bronchial hyperresponsiveness and/or histamine hypersensitivity.

In conclusion, the present study demonstrated the potent ability of neurokinin B and the tachykinin receptor agonist [$MePhe^7$]neurokinin B or senktide to induce airway hypersensitivity to histamine-induced microvascular leakage and the inhibitory effect of selective NK_3 receptor antagonist (SR 142801 or SB 223412) on [$MePhe^7$]neurokinin B-induced hypersensitivity in guinea-pigs. For concentrations that induced airway hypersensitivity, [$MePhe^7$]NKB failed to induce microvascular leakage in airways. Our data suggest that airway hyperresponsiveness to histamine is not related to the ability of tachykinins to induce plasma extravasation.

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