ELSEVIER

European Journal of Pharmacology 439 (2002) 149–158

ejp

www.elsevier.com/locate/ejphar

Role of tachykinins in sephadex-induced airway hyperreactivity and inflammation in guinea pigs

Manuela Tramontana*, Paolo Santicioli, Sandro Giuliani, Rose-Marie Catalioto, Alessandro Lecci, Francesca Carini, Carlo Alberto Maggi

Pharmacology Department, Menarini Ricerche S.p.A., Via Rismondo, 12 A, 50131 Florence, Italy

Received 15 October 2001; received in revised form 31 January 2002; accepted 8 February 2002

Abstract

We have studied the effect of selective tachykinin NK_1 and NK_2 receptor antagonists on airway hyperreactivity to acetylcholine and increase of inflammatory cells on bronchoalveolar lavage fluid induced by sephadex beads (20 mg/kg, i.v.) in guinea pigs. Airway hyperreactivity was assessed by measuring the increase of bronchial insufflation pressure to acetylcholine (0.01–30 μmol/kg, i.v.) at 3 h (early phase) and 24 h (late phase) after sephadex administration. An increase in inflammatory cells in bronchoalveolar lavage fluid (eosinophils and macrophages) was detected at 24 h (from $11.6 \cdot 10^6$ to $49.3 \cdot 10^6$ cells) but not at 3 h from sephadex administration. Neurokinin A and substance P levels in bronchoalveolar lavage fluid showed a significant increase at 24 h (from 31.7 ± 11.6 to 561 ± 231 pg/ml and from 5.9 ± 2.6 to 29.3 ± 4.1 pg/ml for neurokinin A and substance P, respectively). At this time point, the tachykinin in bronchoalveolar lavage cellular content was depleted from 232 ± 43 to 21 ± 20 pg/sample and from 56.6 ± 6.7 to 2 ± 2 pg/sample for neurokinin A and substance P, respectively. Capsaicin pretreatment abolished the early but not the late phase of airway hyperreactivity induced by sephadex without modifying bronchoalveolar lavage total cells number and bronchoalveolar lavage levels of neurokinin A and substance P. Administration of the tachykinin NK_2 (nepadutant) and/or the NK_1 receptor antagonist (MEN 11467 or (1*R*,2*S*)-2-*N*[1(*H*)indol-3-yl-carbonyl]-1-*N*[*N*-(*p*-tolylacetyl)-*N*-(methyl)-D-3(2-naphthyl)alanyl}diaminocyclohexane)), 5 min before sephadex, prevented the early phase of airway hyperreactivity to acetylcholine but only nepadutant prevented the late phase. Nepadutant was able to abolish the early phase of airway hyperreactivity if given after sephadex administration and reduced by about 50% the increase of cell number in bronchoalveolar lavage fluid during the late phase, without affecting the levels of neurokinin A and substance P. These findings indicate an involvement of endogenous tachykinins in the genesis of airway hyperreactivity in a guinea-pig model of non-allergic asthma. Early airway hyperreactivity apparently involves release of tachykinins from capsaicin-sensitive afferent nerves acting via tachykinin NK_1/NK_2 receptors. Late airway hyperreactivity involves tachykinins acting via tachykinin NK_2 receptors: inflammatory cells activated/recruited in response to sephadex challenge appear a likely source of tachykinins involved in the late phase of the response.

Keywords: Sephadex; Airway hyperreactivity; (Guinea-pig); Neurokinin A; Substance P; Tachykinin receptor; Nepadutant; MEN 11467; Acetylcholine; Inflammatory cell

1. Introduction

Tachykinins, particularly substance P and neurokinin A, are widely distributed in capsaicin-sensitive sensory nerves in the upper and lower airways of several mammalian species (Maggi, 1995). Tachykinin-containing afferent nerves release mediators from both central and peripheral nerve endings upon stimulation. In the periphery, substance P and neurokinin A released from sensory nerves act via tachykinin NK_1 and NK_2 receptors to trigger a number of biological responses such as mucus secretion (Gashi et al., 1986), microvascular leakage (Lundberg et al., 1983), smooth muscle contraction (Advenier et al., 1997) and inflammatory cell response (Brunelleschi et al., 1992; Lilly et al., 1995; Kudlacz and Knippenberg, 1994) collectively known as neurogenic inflammation (Maggi, 1997; Joos et al., 2001). Some studies indicate a significant role of sensory nerves and neurogenic inflammation in animal models of allergic asthma/airway hyperreactivity (Barnes, 1986; Lundberg and Saria, 1987; Maggi, 1990; Barnes et al., 1991; Advenier et al., 1997).

* Corresponding author. Tel.: +39-0555680360; fax: +39-0555680419.
E-mail address: chimfarm@menarini-ricerche.it (M. Tramontana).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01365-1

Moreover, immunohistochemical studies on human lungs from asthmatic patients showed a presence of substance P-containing nerves (Ollerenshow et al., 1991) and enhanced levels of substance P (Nieber et al., 1992) and neurokinin A (Heaney et al., 1998) have been observed in bronchoalveolar lavage fluid and in sputum (Tomaki et al., 1995). An increase of both tachykinin NK_1 (Adcock et al., 1993) and NK_2 receptors (Bai et al., 1995) was also reported in lungs from asthmatic patients. Moreover, substance P and neurokinin A are able to cause bronchoconstriction if administered in asthmatic but not in normal subjects (Joos et al., 1987; Cheung et al., 1994).

Recently it was shown that the intravenous administration of sephadex beads induces airway hyperreactivity in guinea pig associated with an increase of eosinophils and macrophages in the bronchoalveolar lavage fluid (Conroy and Sirois, 1999). Sephadex G-50 is constituted by water-insoluble beads of 10–40 μm diameter: upon intravenous administration the beads can occlude capillary venules, contributing to the induction of ischemic damage of the airway tissue.

A similar mechanism could be relevant for the pathogenesis of early pulmonary manifestation of nematode infection (*Ascaris lumbricoides*) that, in some phases of biological cycle, reach the lung through systemic circulation. Therefore, it has been hypothesized that sephadex administration mimics this infestation characterized by eosinophilia and increased number of macrophages (Walls and Benson, 1972).

The involvement of tachykinin NK_1, NK_2 and NK_3 receptors in early and late phase of airway hyperreactivity to histamine was demonstrated in guinea pig and murine animal models of allergic asthma (Schuiling et al., 1999; Daoui et al., 2000; Nenan et al., 2001), but not exclusively, since irritants or other non-immunological inflammatory stimuli can induce a tachykinin-mediated bronchoconstriction (Kuwano et al., 1993; Ray et al., 1989; Sakamoto et al., 1992; Thompson et al., 1987).

In the present study, we have investigated the role of tachykinins acting through tachykinin NK_1 and/or NK_2 receptors on airway hyperreactivity and cells infiltration induced by injection of sephadex beads in guinea-pig (Martineau et al., 1995; Maghni et al., 1995, 1996; Conroy and Sirois, 1999).

2. Methods

2.1. Animal surgery

Male Dunkin–Hartley guinea pigs (Charles River, Calco, Italy) weighing 250–300 g, were anaesthetized by i.p. injection of pentobarbital sodium (40 mg/kg) and then a polyethylene catheter (PE50, Clay Adams) was inserted into the left jugular vein, secured in place and exteriorized behind the head through a subcutaneous tunnel. After surgery guinea pigs were allowed to recover for 24 h in individual cages.

Sephadex, G50 superfine, 20 mg/kg was administered intravenously, 24 h following surgery, in a volume of 357 μl/kg and both airway hyperreactivity and the collection of bronchoalveolar lavage fluid were assessed 3 and 24 h after sephadex injection.

The protocols used comply with the European Community guidelines for the use of experimental animals and they have been approved by the Italian government regulations.

2.2. Measurement of airway hyperreactivity

Guinea pigs were anaesthetized with urethane (1.5 g/kg, s.c.). A polyethylene catheter was inserted into the jugular vein for drugs administration. The body temperature was kept constant at 36 °C by a thermoregulating-heating lamp. The animals were mechanically ventilated through a tracheal cannula connected to a ventilation pump (Basile mod. 7025) adjusted at a rate of 60 strokes/min. Guinea pigs were treated with gallamine triethiodide (3.4 μmol/kg, i.v.) as bolus followed by continuous infusion of the same solution at a rate of 300 μl/h during the whole experimental time to prevent spontaneous respiratory movements.

The insufflation pressure was measured by connecting to a side arm of tracheal cannula a pressure transducer (Hewlett Packard 1240) and recorded by a McLab/8s (AD Instruments, Hasting, UK) via Hewlett Packard amplifier (8805D).

Airway hyperreactivity was assessed by i.v. injection, at 3 and 24 h after sephadex beads administration, of increasing doses (0.5 log units) of acetylcholine (0.01–30 μmol/kg) given at 10 min intervals.

Tachykinin NK_1 and NK_2 receptor antagonists were administered 5 min before sephadex administration or 15 min before the dose–response curve to acetylcholine at 3 or 24 h after sephadex.

2.3. Bronchoalveolar lavage and cellular count

Animals that received sephadex or vehicle were anaesthetized with pentobarbital. The lung was lavaged with 50 ml phosphate-buffer saline (PBS, 37 °C, pH 7.4) delivered in 5 ml aliquots. The bronchoalveolar lavage fluid was centrifuged ($400 \times g$ for 10 min). Contaminating red blood cells were lised by hypotonic shock. Total cell number in bronchoalveolar lavage fluid was counted in a Thoma's chamber. Differential cell counts were determined by Giemsa May–Grundvald stain.

2.4. Collection of bronchoalveolar lavage fluid for substance P-like immunoreactivity and neurokinin A-like immunoreactivity determination

After 3 or 24 h following the injection of sephadex beads (20 mg/kg) or its vehicle the animals were anaesthetized by urethane (1.5 g/kg, i.p.), the trachea was cannulated and bronchoalveolar lavage was performed by infusion and

aspiration into the lung of three separate 2 ml aliquots of phosphate-buffer saline (PBS) containing 1 μM DL-thiorphan prewarmed at 37 °C. The recovered bronchoalveolar lavage (4.4 ± 0.06 ml, $n = 80$) was centrifuged to sediment bronchoalveolar lavage cells at 400 × *g* for 10 min, the supernatant was collected, acidified by addition of acetic acid (100 μl/ml), freeze-dried and stored at − 80 °C until assay.

The centrifuged pellet of three samples of the same group were pooled, washed by 5 ml of PBS containing 1 μM DL-thiorphan and centrifuged at 400 × *g* for 10 min. The supernatant was discarded and the washed pellet was homogenized in 2 ml of 2 N acetic acid and kept at 95 °C for 10 min. After centrifugation (20,000 × *g* at 4 °C for 30 min) the homogenate cells supernatant was collected, freeze-dried and stored at − 80 °C until assay.

2.5. Neuropeptides assay

The determination of neurokinin A and substance P levels in bronchoalveolar lavage fluid and in cells homogenate was performed, in assay buffer reconstituted in sample (1 ml for bronchoalveolar lavage and 500 μl for cells homogenate), with commercial Enzyme Immunoassay (EIA) kit according to the manufacturer's instructions.

The assay sensitivity limit was 0.06–0.08 and 0.02–0.04 ng/ml for neurokinin A and substance P, respectively.

2.6. Capsaicin pretreatment

In another series of experiments, the effect of systemic capsaicin pretreatment on airway responsiveness and neuropeptide bronchoalveolar lavage content in guinea-pigs was assessed. Systemic capsaicin pretreatment (50 mg/kg, s.c.) was performed in pentobarbital sodium (40 mg/kg, i.p.)-anaesthetized guinea pigs pretreated with a combined administration of tachykinin NK_1 (MEN 11467, 2 mg/kg, i.p.) and NK_2 (nepadutant 2 mg/kg, i.p.) receptor antagonist 60 min before capsaicin administration in order to prevent capsaicin induced lethality, as described by Patacchini et al. (1999). MEN 11467, nepadutant and capsaicin were dissolved in 2 ml vehicle containing 10% ethanol, 10% Tween 80 and 80% saline. Animals were used 6–7 days after the capsaicin treatment. In a preliminary series of experiments, it was checked that the combined administration of tachykinin NK_1 and NK_2 receptor antagonists did not modify the bronchoconstrictor response induced by [Sar^9]substance P sulfone (0.003 μmol/kg , i.v.), and [βAla^8] neurokinin A (4–10) (0.003 μmol/kg , i.v.), respectively, assessed 7 days from the treatment with antagonists.

2.7. Evaluation of data

The bronchoconstriction was calculated as amplitude (mm Hg) of the response over the basal value.

Experiments were analyzed through one-or two-way (when applicable) analysis of variance ANOVA followed by Fisher's least significant test (LSD) for multiple comparisons.

2.8. Drugs

Sephadex G-50 (superfine 10–40 μm) was obtained from Pharmacia (Uppsala, Sweden). Acetylcholine chloride, gallamine thriethiodide, capsaicin and DL-thiorphan from Sigma (St. Louis, MS, USA). Nepadutant (MEN 11420) or (c{[(β-D-GlcNAc)Asn-Asp-Trp-Phe-Dpr-Leu]c(2β − 5β) was synthetized by conventional solid phase methods at the Chemistry Department in Menarini Ricerche (Florence, Italy) and MEN 11467 or (1*R*,2*S*)-2-*N*[1(*H*)indol-3-yl-carbonyl]-1-*N*[*N*-(*p*-tolylacetyl)-*N*-(methyl)-D-3(2-naphthyl)alanyl}diaminocyclohexane) was synthesized at the Chemistry Department, Menarini Ricerche (Pomezia-Rome, Italy). [Sar^9]substance P-sulfone, [βAla^8]neurokinin A (4–10), EIA kits for neurokinin A-(EIAH-7359) and for substance P (EIAH-7451) were obtained from Peninsula Laboratories (St. Helens, UK), PBS was from Gibco (Grand Island, NY, USA).

3. Results

3.1. Airway hyperreactivity induced by sephadex beads

A significant enhancement of acetylcholine (0.01–30 μmol/kg, i.v.)-induced bronchoconstriction was observed at 3 and 24 h following sephadex injection (Fig. 1A and B). At 3 h from sephadex administration a significant enhancement of bronchoconstriction was observed starting from the dose of 0.3 μmol/kg of acetylcholine up to the highest dose (early phase of airway hyperreactivity) (Fig. 1A, $n = 8–10$, $P < 0.05$). At 24 h from sephadex administration, the bronchial reactivity was enhanced only in response to the highest doses of acetylcholine (late phase of airway hyperreactivity) (3–30 μmol/kg, $n = 8–11$, $P < 0.05$) (Fig. 1B).

Pretreatment with capsaicin (50 mg/kg, s.c., 1 week before) abolished the early phase of airway hyperreactivity induced by sephadex ($n = 7–8$, $P < 0.05$) (Fig. 1C) but did not affect the late phase as compared to the vehicle-pretreated group ($n = 10–14$, $P > 0.05$) (Fig. 1D).

3.2. Effect of nepadutant and MEN 11467 on sephadex-induced airway hyperreactivity to acetylcholine

The selectivity of tachykinin NK_2 and NK_1 receptor antagonist was first checked in control animals against bronchoconstriction induced by acetylcholine (0.01–30 μmol/kg, i.v.), [Sar^9]substance P sulfone (0.003 μmol/kg, i.v), and [βAla^8]neurokinin A-(4–10) (0.003 μmol/kg, i.v.). Nepadutant (1 μmol/kg, i.v.) did not affect the response to [Sar^9]substance P sulfone or acetylcholine, whereas it abolished the [βAla^8]neurokinin A-(4–10)-induced bronchoconstriction (data not shown). On the other hand, MEN 11467 (1 μmol/kg, i.v.), did not affect the response to

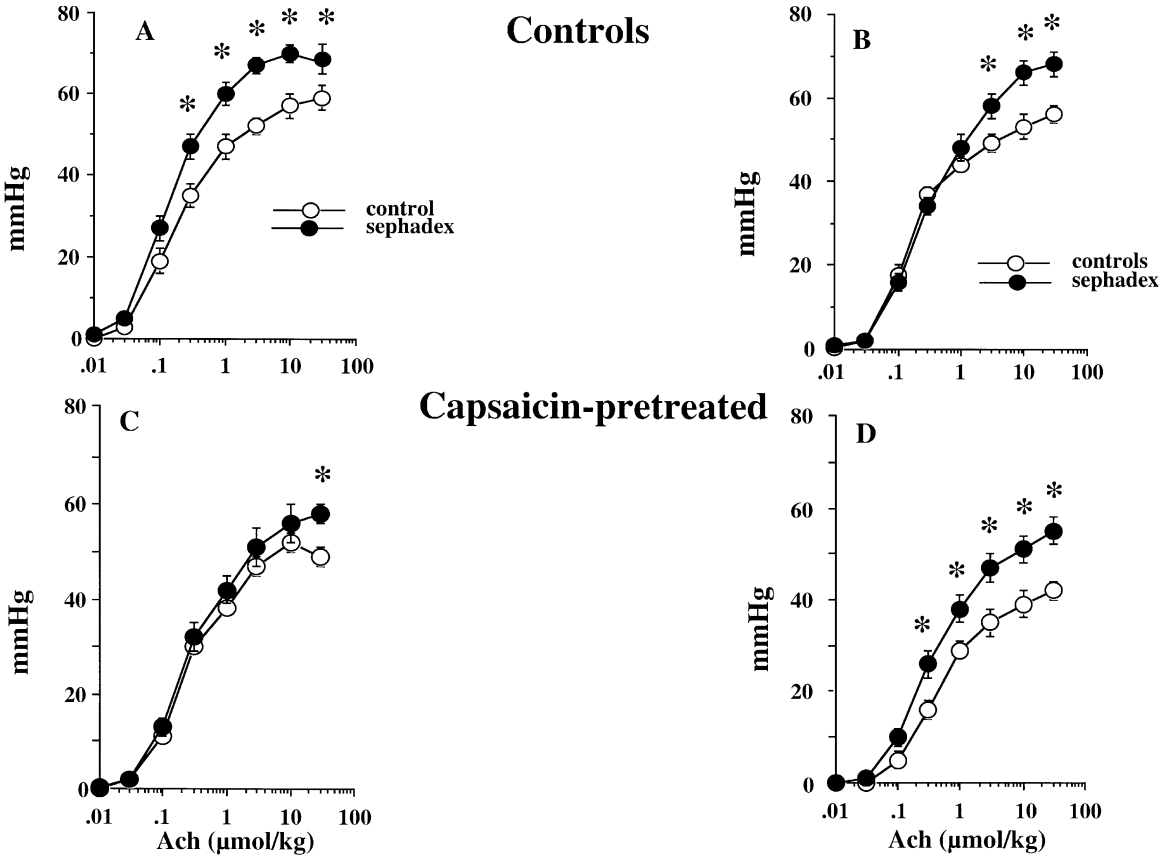

Fig. 1. Dose-dependent increases in pulmonary insufflation pressure, in control (upper panels) and capsaicin-pretreated animals (lower panels), induced by acetylcholine at 3 (panels A and C) and 24 h (panels B and D) from vehicle and sephadex (20 mg/kg, i.v.) administration. Each value is mean ± S.E.M. of 7–14 experiments. * $P<0.05$, significantly different from the respective value of the vehicle group.

[βAla^8]neurokinin A-(4–10) or acetylcholine, whereas it abolished [Sar^9]substance P sulfone-evoked bronchoconstriction (data not shown).

Nepadutant (0.1–1 μmol/kg, i.v., 5 min before sephadex injection) prevented in a dose-dependent manner the early phase of airway hyperreactivity ($n=9–12$, $P<0.05$) (Fig. 2) and, at the dose of 1 μmol/kg, also prevented the late phase of airway hyperreactivity induced by sephadex ($n=8–12$, $P<0.05$) (Fig. 4A and B).

The intravenous administration of the tachykinin NK_1 receptor antagonist, MEN 11467 (0.3–1 μmol/kg, 5 min before sephadex injection) prevented in a dose-dependent manner the early phase of airway hyperreactivity to acetylcholine ($n=8–11$, $P<0.05$) (Figs. 3 and 4C) but did not affect the late phase ($n=9$) (Fig. 4D).

Nepadutant and MEN 11467, at doses which were uneffective if given singularly (0.03 and 0.3 μmol/kg, i.v., respectively) completely prevented the early ($n=9–10$, $P<0.05$) (Fig. 4E) but not the late phase of airway hyperreactivity to acetylcholine ($n=10$) (Fig. 4F).

Nepadutant (1 μmol/kg, i.v.) but not MEN 11467 (1μmol/kg, i.v.) was also able to revert the early phase of airway hyperreactivity if administered 2.45 h after sephadex-injection, i.e., 15 min before the beginning of the dose–response curve to acetylcholine ($n=9$, $P<0.05$) (data not shown).

3.3. Airway cells infiltration induced by sephadex beads

The injection of sephadex beads did not induce a significant increase in the total number of cells in bronchoalveolar lavage fluid at 3 h when compared to the control group ($7.9 \pm 0.7 \cdot 10^6$ and $7.9 \pm 0.4 \cdot 10^6$ cells, $n=6$) (Fig. 5A).

A significant increase in total cell number was observed in bronchoalveolar lavage fluid collected at 24 h from sephadex administration as compared to vehicle-treated animals ($49.3 \pm 5.6 \cdot 10^6$ and $11.6 \pm 1.7 \cdot 10^6$ cells, respectively; $n=6$, $P<0.05$) (Fig. 5B). The increase bronchoalveolar lavage cellularity was due to both eosinophils (from $1.8 \pm 0.4 \times 10^6$ to $21.85 \pm 2.4 \times 10^6$ cells, $n=6$) (Fig. 5D) and macrophages infiltration in the airways (from $12.5 \pm 2 \times 10^6$ to $22.7 \pm 1.2 \times 10^6$ cells, $n=6$) (Fig. 5C).

Nepadutant (1 μmol/kg, i.v., 5 min before sephadex administration) significantly reduced the increase of total cells number found in bronchoalveolar lavage fluid during the late phase by about 56% ($n=6$, $P<0.05$) (Fig. 5B): the

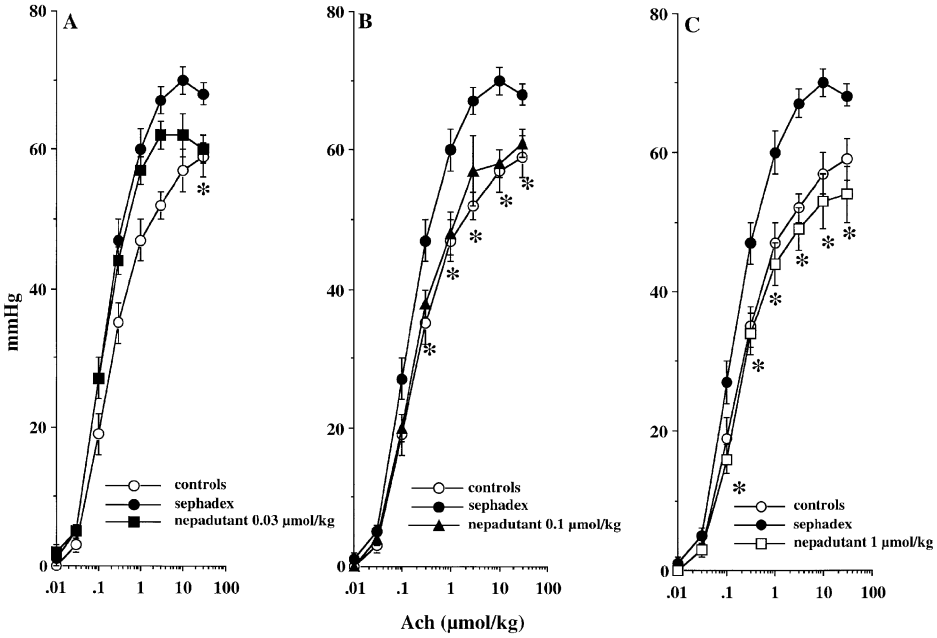

Fig. 2. Inhibitory effect of nepadutant (0.03 – 1 μmol/kg, i.v.) on airway hyperreactivity assessed by intravenous administration of acetylcholine (0.01 – 30 μmol/kg) at 3 h after sephadex administration. Each value is mean ± S.E.M. of 9 – 12 experiments. * $P < 0.05$, significantly different from the respective value of the sephadex-treated group.

effect involved a reduction in both eosinophils ($12.6 \pm 0.4 \times 10^6$ cells, $n = 6$, $P < 0.05$) and macrophages ($9.11 \pm 0.8 \times 10^6$ cells, $n = 6$, $P < 0.05$) (Fig. 5C and D).

Pretreatment with capsaicin did not affect the total cells number increase found in bronchoalveolar lavage fluid induced by sephadex administration (data not shown).

3.4. Neurokinin A-like immunoreactivity and substance P-like immunoreactivity levels in bronchoalveolar lavage fluid

Bronchoalveolar lavage obtained from control guinea-pig at 3 and 24 h from vehicle injection showed low but detectable levels of neurokinin A-like immunoreactivity and

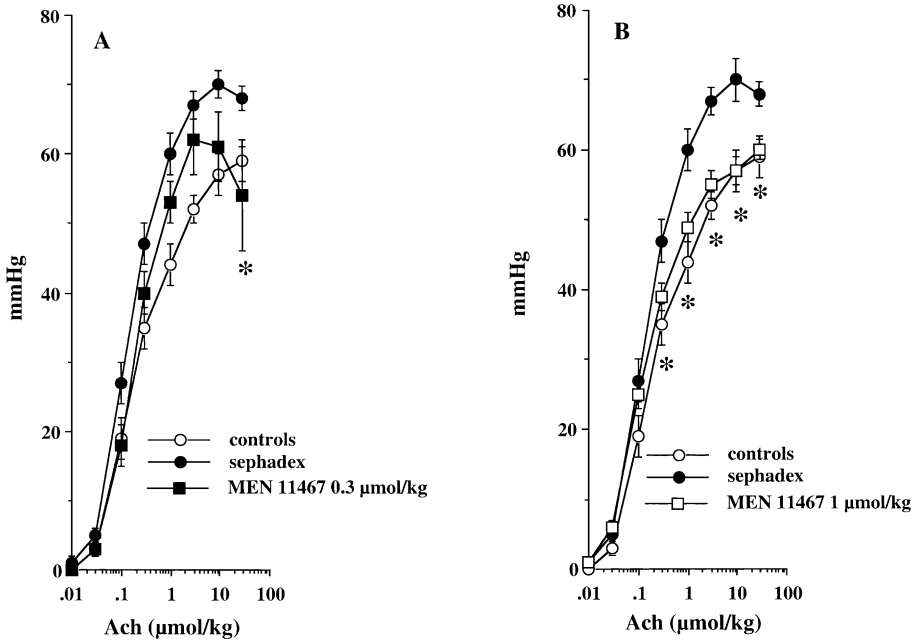

Fig. 3. Inhibitory effect of MEN 11467 (0.3 – 1 μmol/kg, i.v.) on airway hyperreactivity assessed by intravenous administration of acetylcholine (0.01 – 30 μmol/kg) at 3 h after sephadex administration. Each value is mean ± S.E.M. of 8 – 11 experiments. * $P < 0.05$, significantly different from the respective value of the vehicle group.

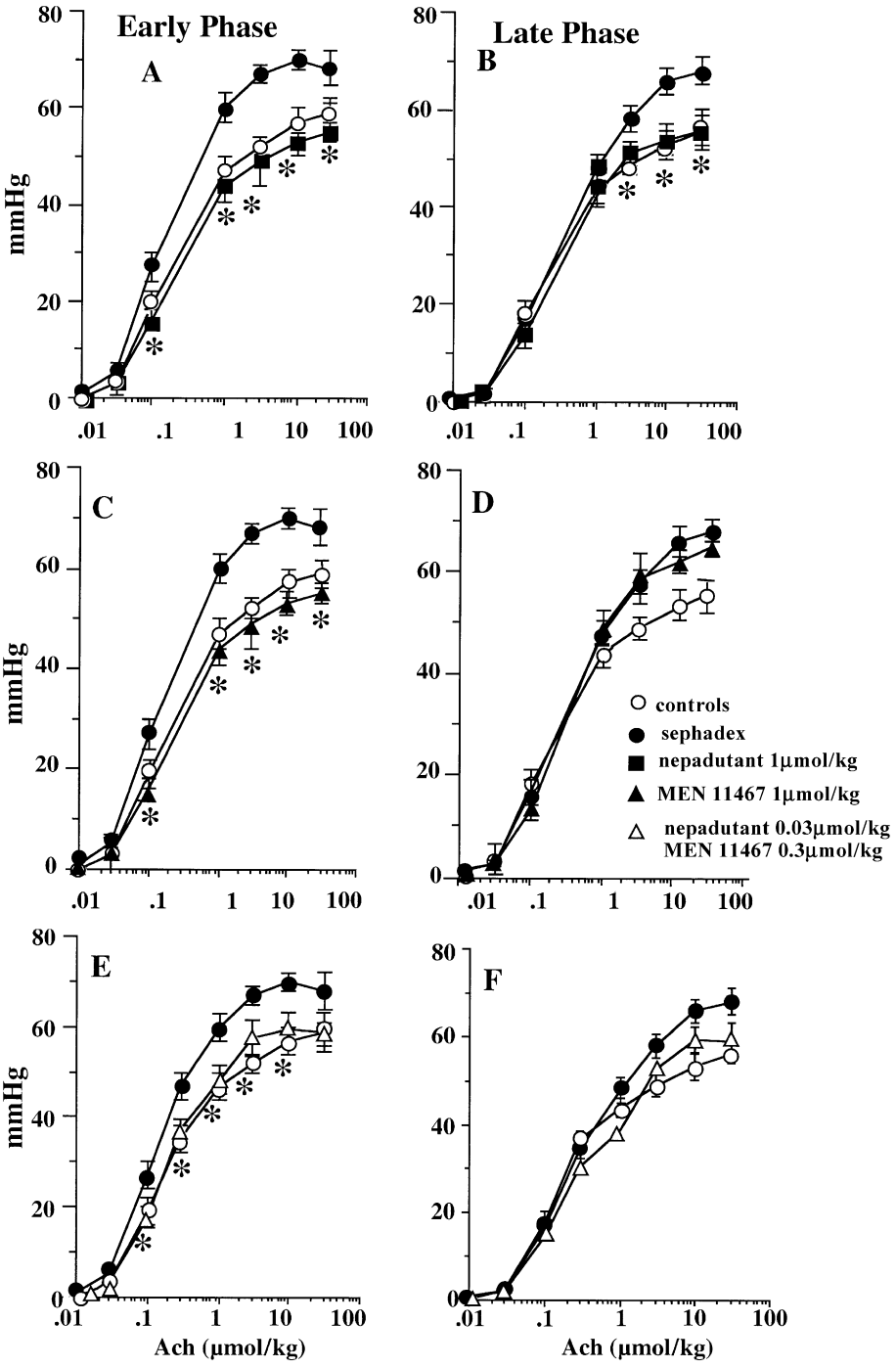

Fig. 4. Inhibitory effect of intravenous administration of nepadutant (1 μmol/kg) (A and B), MEN 11467 (1μmol/kg) (C and D) and nepadutant and MEN 11467 (0.1 and 0.3 μmol/kg, respectively) (E and F) on airway hyperreactivity assessed by intravenous administration of acetylcholine (0.01–30 μmol/kg) at 3 (early phase) and 24 h (late phase) after sephadex administration. Each value is mean ± S.E.M. of 8–12 experiments. * $P < 0.05$ significantly different from the respective value of the sephadex-treated group.

very low levels, near the assay detection limit, of substance P-like immunoreactivity.

As shown in Table 1, the amount of neurokinin A-like immunoreactivity and substance P-like immunoreactivity in bronchoalveolar lavage fluid collected at 3 h from sephadex administration was not significantly different as compared to controls ($n = 10–15$).

After 24 h from sephadex administration, the bronchoalveolar lavage content of neurokinin A-like immunoreactivity and substance P-like immunoreactivity was significantly increased by about 17 and 5 folds, respectively ($n = 10–15$, $P < 0.05$) (Table 1).

Capsaicin pretreatment (50 mg/kg, s.c., 6–7 days before) did not affect the increased bronchoalveolar lavage levels of

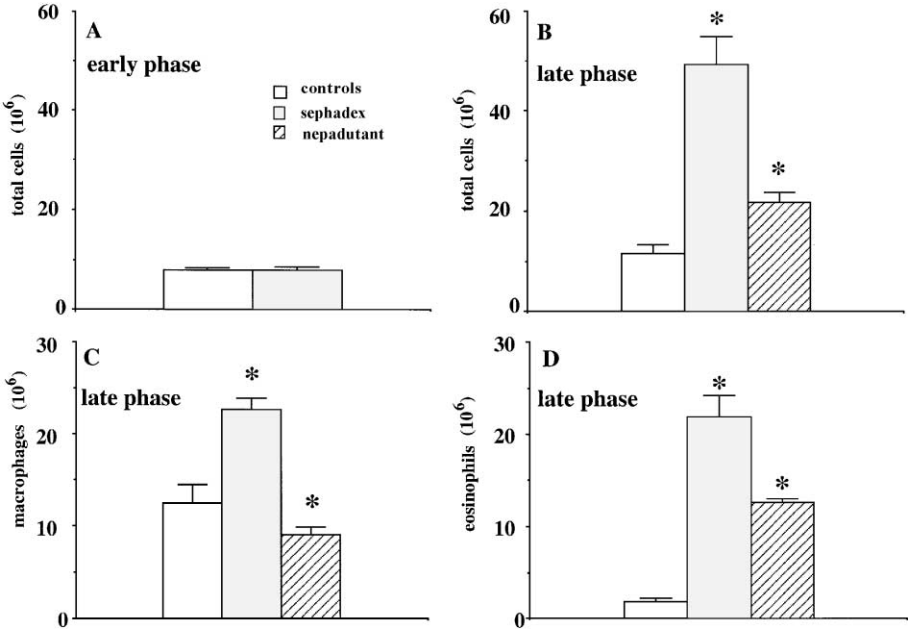

Fig. 5. Effect of sephadex administration on bronchoalveolar lavage total cellular content at 3 (early phase) and 24 h (late phase) after its administration (A and B) and on bronchoalveolar lavage macrophages and eosinophils (C and D) during the late phase. Nepadutant (1 μmol/kg , i.v., 5 min before sephadex) significantly inhibited the sephadex-induced total cells number increase in the late phase (B) and in particular the macrophages (C) and the eosinophils (D). Each column is mean ± S.E.M. of six experiments. * $P<0.05$, significantly different from control group and sephadex-treated group.

both neurokinin A-like immunoreactivity and substance P-like immunoreactivity ($n=6$, $P<0.05$) induced by sephadex administration.

As shown in Table 1, pooled cellular extract from guinea-pig bronchoalveolar lavage showed detectable levels of neurokinin A-like immunoreactivity and substance P-like immunoreactivity. After 3 h from sephadex administration, the amount of neurokinin A-like immunoreactivity and substance P-like immunoreactivity was not significantly different as compared to controls ($n=5$).

On the other hand the cellular content of neurokinin A-like immunoreactivity and substance P-like immunoreactivity in bronchoalveolar lavage fluid was significantly decreased (about 10 and 28 folds, respectively) at 24 h from sephadex administration ($n=5$ for each group, $P<0.05$).

Capsaicin pretreatment (50 mg/kg, s.c., 6–7 days before) did not affect neither the cellular content of substance P-like immunoreactivity and neurokinin A-like immunoreactivity nor the decrease induced by sephadex on both neuropeptides ($n=3$ for each group, $P<0.05$) in guinea pig bron-

Table 1
Neurokinin A-like immunoreactivity (NKA-LI) LI and substance P-like immunoreactivity (SP-LI) level in bronchoalveolar lavage (BAL) fluid and in BAL inflammatory cells in controls and after sephadex (20 mg/kg, i.v.) administration in guinea pig

Bronchoalveolar lavage (BAL) fluid					
	Pretreatment	3 h		24 h	
		Vehicle	Sephadex	Vehicle	Sephadex
NKA-LI (pg/ml)	None	37.2 ± 14.8	38.9 ± 7.8	31.7 ± 11.6	561.7 ± 231.6[a]
	Capsaicin	n.e.	n.e.	41.6 ± 6.6	418 ± 108.7[a]
SP-LI (pg/ml)	None	2.3 ± 0.7	5.9 ± 1.8	5.9 ± 2.6	29.3 ± 4.1[a]
	Capsaicin	n.e	n.e.	1.3 ± 1.3	36.5 ± 5.9[a]
Cellular extract					
	Pretreatment	3 h		24 h	
		Vehicle	Sephadex	Vehicle	Sephadex
NKA-LI (pg/sample)	None	153.4 ± 40	195.4 ± 58	232.4 ± 43.3	21.8 ± 20.3[a]
	Capsaicin	n.e.	n.e.	381 ± 148	23.5 ± 23.5[a]
SP-LI (pg/sample)	None	34.7 ± 4.1	49.2 ± 10.1	56.6 ± 6.7	2 ± 2[a]
	Capsaicin	n.e.	n.e.	46 ± 2	0 ± 0[a]

Capsaicin (50 mg/kg, s.c.) pretreatment was performed 6–7 days before the experiment. Each value is the mean ± S.E.M. of 3–15 experiments; n.e., not evaluated.

[a] $P<0.05$ significantly different from vehicle values.

choalveolar lavage cellular extract collected after 24 h from sephadex pretreatment.

4. Discussion

Sephadex-induced airway hyperreactivity is an animal model of non-allergic asthma used to study the relationship between airway hyperreactivity and eosinophilia (Turner and Martin, 1997). In vivo and in vitro studies were carried out on rats and/or guinea pigs to investigate which inflammatory mediators are involved in the onset of the two phases of airway hyperreactivity and eosinophilia (Takami et al., 1995; Andersson et al., 1996a,b; Williams and Coleman, 1997; Conroy and Sirois, 1999). In the present study, we have used guinea pigs to determine the role of tachykinins, on airway hyperreactivity and bronchoalveolar lavage cells infiltration induced by sephadex. In agreement with Conroy and Sirois (1999), the sephadex induced an increase of airways response to acetylcholine at 3 h (early phase of airway hyperreactivity) and 24 h (late phase of airway hyperreactivity).

To study the role of tachykinin NK_1 and NK_2 receptors during the onset and the development of airway hyperreactivity, we used two potent and selective tachykinin receptor antagonists, nepadutant for tachykinin NK_2 (Catalioto et al., 1998) and MEN 11467 for tachykinin NK_1 receptor (Cirillo et al., 2001). The selectivity of the two antagonists was checked against bronchoconstriction induced by tachykinin NK_1 and NK_2 receptor selective agonists to ensure that, at the highest doses tested in this study, their effect can be reasonably ascribed to a selective blockade of the respective tachykinin receptor in guinea pig airways.

With regards to airway hyperreactivity to acetylcholine, each antagonist alone was able to block the early phase of the response but the same effect was obtained by the coadministration of lower doses of tachykinin NK_1 and NK_2 receptor antagonists. This suggests that endogenous tachykinins via tachykinin NK_1 and NK_2 receptors cooperate in the genesis of this response. Other examples of cooperation between tachykinin NK_1 and NK_2 receptors in determining tachykinergic transmission have been described in the peripheral nervous sistem (Maggi, 2000), however the exact mechanism underlying tachykinergic ‘‘cooperation’’ at postjunctional level in the airways have not been determined yet. Notably nepadutant blocked the early phase of airway hyperreactivity either if administered before or after sephadex administration whereas MEN 11467 was effective only if administered before sephadex. A similar result has been previously reported by Boichot et al. (1995) and Mizuguchi et al. (1996) in models of allergic asthma. MEN 11467 at 1 μmol/kg blocks tachykinin NK_1 receptor mediated bronchoconstriction in guinea pigs as early as at 5 min from its administration and its effect is long lasting (Cirillo et al., 2001): therefore the lack of effect of MEN 11467 on early airway hyperreactivity when administered after sephadex cannot be explained on a pharmacokinetic ground. Since the early phase of airway hyperreactivity to acetylcholine was also completely prevented by capsaicin pretreatment, it could be speculated that sephadex releases both neurokinin A and substance P from sensory nerves located within the airway tissue to induce the early phase of airway hyperreactivity. The effect of neurokinin A, via tachykinin NK_2 receptors, is more persistent whereas the contribution of substance P via tachykinin NK_1 receptors is evident only at earlier times from the challenge and was not longer of importance at 3 h from sephadex administration, possibly because of differential (i.e., faster as compared to neurokinin A) degradation by peptidases (Martling et al., 1987).

On the other hand, the late phase of airway hyperreactivity to acetylcholine was unchanged by capsaicin pretreatment: this phase was also associated to a remarkable increase of bronchoalveolar lavage inflammatory cells (mainly macrophages and eosinophils) and a concomitant marked increase in substance P- and neurokinin A-like immunoreactivity in bronchoalveolar lavage. It is also noteworthy that capsaicin pretreatment, at a dose regimen repeatedly shown effective in depleting tachykinins content from guinea pigs sensory nerves (Martins et al., 1991; Maggi et al., 1995) had not effect at all on bronchoalveolar lavage cell infiltration and bronchoalveolar lavage elevation of tachykinin content at 24 h from sephadex administration. In recent years several groups reported that certain inflammatory cells express the Preprotachykinin I gene and are capable, especially after activation/recruitment, to synthesize and release tachykinins (Maggi, 1997; Joos and Pauwels, 2000) thus providing a non neuronal source of these peptides which may contribute to the overall inflammatory response. Many inflammatory cells (neutrophils, mast cells, macrophages, lymphocytes) can express tachykinin NK_1 receptors, and the blockade of this receptors reduced the number of lymphocytes and granulocytes in the bronchoalveolar lavage in an allergic model (Kaltreider et al., 1997). In the sephadex model, characterized by an increased number of eosinophils and macrophages, it has been shown that the blockade of TNF-α does not reduced eosinophilia (Gater et al., 1996). Since NK_1 receptor stimulation has been linked to TNF-α expression and release from several kinds of inflammatory cells, this type of tachykinin receptor in unlike to be involved in sephadex-induced eosinophilia. In addition to block early phase of airway hyperreactivity, NK_1 receptors could be important in mediating early plasma protein extravasation and neutrophil-induced lung injury, but this possibility deserves further investigation.

Considering the fact that tachykinin content of the cellular component of bronchoalveolar lavage was actually decreased at 24 h from sephadex (despite the consistent increase in bronchoalveolar lavage cells numbers) and that capsaicin pretreatment had not effect either on sephadex-induced bronchoalveolar lavage accumulation of inflamma-

tory cells and elevation of bronchoalveolar lavage fluid tachykinins content, we interpret these data as indication that tachykinins in bronchoalveolar lavage fluid at 24 h from sephadex challenge were likely originating (or secreted) from bronchoalveolar lavage inflammatory cells. Interestingly, the tachykinin NK_2 receptor selective antagonist, nepadutant blocked the late phase of airway hyperreactivity induced by sephadex and concomitantly reduced bronchoalveolar lavage cell infiltration, suggesting a role of tachykinin NK_2 receptor in mediating these responses. Although previous studies have excluded a role of tachykinin NK_2 receptors in eosinophilia induced by IL-5 administration in guinea pigs (Kraneveld et al., 1997), Maghni et al. (2000) found that, following antigen challenge, the blockade of tachykinin NK_2, but not tachykinin NK_1 receptors, reduced the number of bronchoalveolar lavage eosinophils and also decreased the expression of various IL-5 at this level. This suggest that the effect of the blockade of tachykinin NK_2 receptor occurs upstream to IL-5 expression or that eosinophilia can be triggered through different mechanisms, at least one of them involving the activation of tachykinin NK_2 receptor.

Therefore, the effect of nepadutant on the late phase of airway hyperreactivity to sephadex could be attributed to the inhibitory effect on cellular infiltration by blocking tachykinin NK_2 receptors which are activated by neurokinin A released from immune cells.

Tachykinin NK_2 receptor has been found on guinea-pig alveolar macrophages (Brunelleschi et al., 1992) and on human eosinophils in chronic inflammatory disease (Renzi et al., 2000). Measurable levels of tachykinins have been found in both bronchoalveolar lavage fluid (Heaney et al., 1998) and in sputum of patient suffering for asthma or chronic obstructive pulmonary disease (Tomaki et al., 1995).

In conclusion, these data demonstrates a prominent role of tachykinins in both onset and development of the airway hyperreactivity in a guinea pig model of non-allergic asthma. In particular, our study shows that tachykinin NK_2, and at a lesser extent tachykinin NK_1 receptors, are involved in the early phase of airway hyperreactivity while only tachykinin NK_2 receptors are involved in the late phase. Moreover, the present data add strong experimental proof to the concept that immune cells primed/activated at the sites of injury/inflammation can be a relevant source of releasable endogenous tachykinins contributing to development or persistence of the overall pathological process (Maggi, 1997). These data add further weight to a speculation that tachykinin NK_2 receptor antagonists could afford a therapeutical benefit by reducing both the motor and inflammatory components in airway diseases.

Acknowledgements

We would like to thank Dr. Cinzia Gabbrielli for the kind experimental support.

References

Adcock, I.M., Peters, M., Gelder, C., Shirasaki, H., Brown, C.R., Barnes, P.J., 1993. Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids. J. Mol. Endocrinol. 11, 1–7.

Advenier, C., Lagent, V., Boichot, E., 1997. The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough. Eur. Respir. J. 10, 1892–1896.

Andersson, S.E., Hemsen, A., Lundberg, J.M., 1996a. The effect of endothelin receptor blockade on the development of sephadex-induced inflammation in rat lung. Acta Physiol. Scand. 158, 189–193.

Andersson, S.E., Hemsen, A., Zackrisson, C., Lundberg, J.M., 1996b. Release of endothelin-1 into rat airways following sephadex-induced inflammation; modulation by enzyme inhibitors and budesonide. Respiration 63, 111–116.

Bai, T.R., Zhou, D., Weir, T., Walker, B., Hegele, R., Hayashi, S., McKay, K., Bondy, G.P., Fong, T., 1995. Substance P (NK1)-and neurokinin A (NK2)-receptor gene expression in inflammatory disease. Am. J. Physiol. 269, L309–L317.

Barnes, P.J., 1986. Asthma as an axon reflex. Lancet 1, 242–244.

Barnes, P.J., Baraniuk, J.N., Belvisi, M.G., 1991. Neuropeptides in the respiratory tract, Part II. Am. Rev. Respir. Dis. 144, 1391–1399.

Boichot, E.N., Germaine, N., Lagente, V., Advenier, C., 1995. Prevention by the tachykinin NK_2 receptor antagonist SR 48968, of antigen induced airway hyperresponsiveness in sensitized guinea-pigs. Br. J. Pharmacol. 114, 259–261.

Brunelleschi, S., Ceni, E., Fantozzi, R., Maggi, C.A., 1992. Evidence for tachykinin NK-2B-like receptors in guinea-pig alveolar macrophages. Life Sci. 51, PL177–PL178.

Catalioto, R.-M., Criscuoli, M., Cucchi, P., Giachetti, A., Giannotti, D., Giuliani, S., Lecci, A., Lippi, A., Patacchini, R., Quartara, L., Renzetti, A.R., Tramontana, M., Arcamone, F., Maggi, C.A., 1998. MEN 11420 (Nepadutant), a novel glycosylated bicyclic peptide tachykinin NK_2 receptor antagonist. Br. J. Pharmacol. 123, 81–91.

Cheung, D.L., Van Der Veen, H., Den Hartigh, J., Dijkman, J.H., Sterk, P.J., 1994. Effects of inhaled substance P on airway responsiveness to metacholine in asthmatic subject in vivo. J. Appl. Physiol. 77, 1325–1332.

Cirillo, R., Astolfi, M., Conte, B., Lopez, G., Parlani, M., Sacco, G., Terracciano, R., Fincham, C.I., Sisto, A., Evangelista, S., Maggi, C.A., Manzini, S., 2001. Pharmacology of MEN 11467: a potent new selective and orally-effective peptidomimetic tachykinin NK_1 receptor antagonist. Neuropeptides 35, 137–147.

Conroy, D.M., Sirois, P., 1999. Early bronchial hyperresponsiveness following injection of sephadex beads in the guinea-pig: involvement of platelet activating factor and thromboxane A_2. Inflammation 23, 437–448.

Daoui, S., Naline, E., Lagente, V., Emonds-Alt, X., Advenier, C., 2000. Neurokinin B- and specific tachykinin NK_3 receptor agonists-induced airway hyperresponsiveness in the guinea-pig. Br. J. Pharmacol. 130, 49–56.

Gashi, A.A., Borson, D.B., Finkbeiner, W.E., Nadel, J.A., Bausbaum, C.B., 1986. Neuropeptides degranulates serous cells of ferret trachea glands. Am. J. Physiol. 251, C223–C229.

Gater, P.R., Wasserman, M.A., Paciorek, P.M., Renzetti, L.M., 1996. Inhibition of sephadex-induced lung injury in the rat by RO 45-2081, a tumor necrosis factor receptor fusion protein. Am. J. Respir. Cell Mol. Biol. 14, 454–460.

Heaney, L.G., Cross, L.J., McGarvey, L.P., Buchanan, K.D., Ennis, M., Shaw, C., 1998. Neurokinin A is the predominant tachykinin in human bronchoalveolar lavage fluid in normal and asthma. Thorax 53, 357–362.

Joos, G.F., Pauwels, R.A., 2000. Pro-inflammatory effects of substance P: new perspectives for the treatment of airway diseases? Trends Pharmacol. Sci. 21, 131–133.

Joos, G.F., Pauwels, R.A., Van Der Straten, M.E., 1987. The role of neuropeptides as neurotrasmitters of non-adrenergic non-cholinergic nerves in bronchial asthma. Bull. Eur. Physiophatol. Respir. 23, 619–637.

Joos, G.F., De Swert, K.O., Pauwels, R.A., 2001. Airway inflammation and tachykinins: prospects for the development of tachykinin receptor antagonists. Eur. J. Pharmacol. 429, 239–250.

Kaltreider, H.B., Ichikawa, S., Byrd, P.K., Ingram, D.A., Kishiyama, J.L., Sreedharan, S.P., Warnock, M.L., Beck, J.M., Goetzl, E.J., 1997. Upregulation of neuropeptides and neuropeptide receptors in murine model of immune inflammation in lung parenchyma. Am. J. Respir. Cell Mol. Biol. 16, 133–144.

Kraneveld, A.D., Nijkamp, F.P., Van Oosterhout, A.J., 1997. Role for neurokinin-2 receptor in interleukin-5-induced airway hyperresponsiveness but not eosinophilia in guinea-pigs. Am. J. Respir. Crit. Care Med. 156, 367–374.

Kudlacz, E.M., Knippenberg, R.W., 1994. In vitro and in vivo effects of tachykinins on immune cell function in guinea-pig airways. J. Neuroimmunol. 50, 119–125.

Kuwano, K., Boskev, C.H., Paré, P.D., Bai, T.R., Wiggs, B.R., Hogg, J.C., 1993. Small airways dimensions in asthma and chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 148, 1220–1225.

Lilly, C.M., Hall, A.E., Rodger, I.W., Kobzik, L., Haley, K.J., Drazen, J.M., 1995. Substance P-induced histamine release in tracheally perfused guinea-pig lungs. J. Appl. Physiol. 78, 1234–1241.

Lundberg, J.M., Saria, A., 1987. Polypeptide containing neurons in airway smooth muscle. Annu. Rev. Physiol. 49, 557–572.

Lundberg, E., Brodin, J.M., Saria, A., 1983. Effect and distribution of vagal capsaicin-sensitive substance P neurons with special reference to trachea and lung. Acta Physiol. Scand. 119, 243–252.

Maggi, C.A., 1990. Tachykinin receptors in the airways and lung: what should we block? Pharmacol. Res. 22, 527–540.

Maggi, C.A., 1995. The mammalian tachykinin receptors. Gen. Pharmacol. 26, 911–944.

Maggi, C.A., 1997. The effects of tachykinins on inflammatory and immune cells. Regul. Pept. 70, 75–90.

Maggi, C.A., 2000. Principle of tachykinergic co-transmission in peripheral and enteric nervous system. Regul. Pept. 93, 53–64.

Maggi, C.A., Giachetti, A., Dey, R.D., Said, S.I., 1995. Neuropeptides as regulators of airways function: vasoactive intestinal peptide and the tachykinins. Physiol. Rev. 75, 277–322.

Maghni, K., Simard, M.J., Cloutier, S., Arseneault, D., Sirois, P., 1995. Relationship between the development of bronchial hyperresponsiveness and the airway eosinophilia in guinea pigs following injection with sephadex beads. Inflammation Res. 44, S174–S175.

Maghni, K., Simard, M.J., Arseneault, D., Sirois, P., 1996. Kinetics of eosinophilia and eosinophil activation in the development of non allergic bronchial hyperresponsiveness in guinea-pigs injected with sephadex beads. Inflammation 20, 523–535.

Maghni, K., Taha, R., Afif, W., Hamid, Q., Martin, J.C., 2000. Dichotomy between neurokinin receptor actions in modulating allergic airway responses in an animal model of helper T cell type 2 cytokine-associated inflammation. Am. J. Crit. Care Med. 162, 1068–1074.

Martineau, S.Y., Maghni, K., Chakir, M., Plante, G., Sirois, P., 1995. Specific increase of microvascular permeability. Inflammation Res. 44, S168–S169.

Martins, M.A., Shore, S.A., Drazen, J.M., 1991. Capsaicin-induced release of tachykinins: effects of enzyme inhibitors. J. Appl. Physiol. 70, 1950–1956.

Martling, C.R., Theodorsson-Norheim, E., Norheim, I., Lundberg, J.M., 1987. Bronchoconstriction and hypotensive effects in relation to pharmacokinetics of tachykinins in the guinea-pig: evidence for extraneuronal cleavage of neuropeptide K to neurokinin A. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 183–189.

Mizuguchi, M., Fujimura, M., Amemiya, T., Nishi, K., Ohka, T., Matsuda, T., 1996. Involvement of NK_2 receptors rather than tachykinin NK_1 receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs. Br. J. Pharmacol. 117, 443–448.

Nenan, S., Germain, N., Lagent, V., Emonds-Alt, X., Advenier, C., Boichot, E., 2001. Inhibition of inflammatory cell recruitment by the tachykinin NK(3)-receptor antagonist, SR 142801, in a murine model of asthma. Eur. J. Pharmacol. 421, 201–205.

Nieber, K., Baumgarten, C.R., Rathsack, R., Furkert, J., Oeme, P., Kunkel, G., 1992. Substance P and beta-endorfin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. J. Allergy Clin. Immunol. 90, 646–652.

Ollerenshow, S.H., Jarvis, D., Sullivan, C.E., Woolcock, A.J., 1991. SP immunoreactive nerves in airways from asthmatics and nonasthmatics. Eur. Respir. J. 4, 673–682.

Patacchini, R., Bartho, L., Di Giorgio, R., Lénàrd Jr., L., Stanghellini, V., Barbara, G., Lecci, A., Maggi, C.A., 1999. Involvement of endogenous tachykinins and CGRP in the motor responses produced by capsaicin in the guinea-pig common bile duct. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 344–353.

Ray, D.W., Hernandez, C., Leff, A.R., Drazen, J.M., Solway, J., 1989. Tachykinins mediate bronchoconstriction elicited by isocapnic hyperpnea in guinea pig. J. Appl. Physiol. 66, 118–1112.

Renzi, D., Pellegrini, B., Tonelli, F., Surrenti, C., Calabrò, A., 2000. Substance P (neurokinin-1) and neurokinin A (neurokinin-2) receptor gene and protein expression in the healthy and inflammed human intestine. Am. J. Pathol. 157, 1511–1522.

Sakamoto, T., Elwood, W., Barnes, P.J., Chung, K.F., 1992. Pharmacological modulation of inhaled metabisulphite-induced airway microvascular leakage on bronchoconstriction in guinea-pig. Br. J. Pharmacol. 107, 481–488.

Schuiling, M., Zuidhof, A.B., Meurs, H., Zaagsma, J., 1999. Role of tachykinin NK_2 receptor activation in allergen-induced late asthmatic reaction, airway hyperreactivity and airway inflammatory cells influx in conscious, unrestrained guinea-pigs. Br. J. Pharmacol. 127, 1030–1038.

Takami, M., Matsumoto, K., Takata, Y., Furuhama, K., Tsukada, W., 1995. Possible role of thromboxane A_2 in hyperresponsiveness of isolated rat lung tissue in a sephadex-induced eosinophilia model. Int. Arch. Allergy Immunol. 106, 401–409.

Thompson, J.E., Scypinski, L.A., Gordon, T., Sheppard, D., 1987. Tachykinins mediated the acute increase in airway responsiveness caused by soluene diisocyanate in guinea pigs. Am. J. Respir. Crit. Care Med. 136, 43–49.

Tomaki, M., Ichinose, M., Miura, M., Hirayama, Y., Yalauchi, H., Nakayama, N., Scirato, K., 1995. Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis. Am. J. Respir. Crit. Care Med. 151, 613–617.

Turner, D.J., Martin, J.G., 1997. Animal models. In: Barnes, P.J., Grunstein, M.M., Leff, A.R., Woolcock, A.J. (Eds.), Asthma, vol. 1. Lippicot-Raven, NY, pp. 261–274.

Walls, R.S., Benson, P.B., 1972. Mechanism of eosinophilia IX. Induction of Eosinophilia in rats by certain forms of dextran. Proc. Soc. Exp. Biol. Med. 140, 689–693.

Williams, C.M.M., Coleman, J.W., 1997. Cell mobilization and cytokine gene expression in a rat model of non-antigen-induced lung inflammation. Int. Arch. Allergy Immunol. 113, 316–317.