Clin Exp All 2002; **32**:472–479

The involvement of sensory neuropeptides in airway hyper-responsiveness in rabbits sensitized and challenged to *Parietaria judaica*

B. D'Agostino*†, C. Advenier†, R. de Palma‡, L. Gallelli*, G. Marrocco*, G. F. Abbate‡ and F. Rossi*

**Department of Experimental Medicine, Section of Pharmacology, Faculty of Medicine and Surgery, 2nd University of Naples, Naples, Italy, †Laboratoire de Pharmacologie Respiratoire, Faculté de Médecine Paris-Ouest, Paris, France, and ‡Department of Clinical and Experimental Medicine, Faculty of Medicine and Surgery, 2nd University of Naples, Naples, Italy*

Summary

Background C-fibres have received considerable attention in the context of airway hyper-responsiveness (AHR), in fact several lines of evidence suggest that tachykinins might be involved in the pathogenesis of AHR.

Objective The aim of this study was to investigate the role of capsaicin-sensitive sensory C-fibres and tachykinins in rabbits sensitized to the major allergen of *Parietaria judaica* pollen (Par j1).

Methods Airway responsiveness was determined by exposing sensitized rabbits to cumulative concentrations of aerosolized histamine before and after an allergic challenge and after a pre-treatment with either vehicle or capsaicin or tachykinin receptor antagonists. Bronchoalveolar lavage was performed following histamine challenge and total and differential cell counts were performed.

Results In sensitized rabbits, an AHR to inhaled histamine was observed 24 h after a Par j1 challenge. Capsaicin pre-treatment inhibited the AHR achieved 24 h following antigen exposure ($P < 0.01$). Pre-treatment with the tachykinin NK_2 receptor antagonist, SR 48968, significantly reduced the antigen-induced AHR ($P < 0.05$), while pre-treatment with tachykinin NK_1 (SR 140333) and NK_3 (SR 142801) receptor antagonists did not significantly modify it. Bronchoalveolar lavage fluid obtained from vehicle and capsaicin-treated rabbits challenged with Par j1 exhibited no significant differences in total and differential cell counts.

Conclusions *Parietaria judaica*-induced AHR in immunized rabbits was shown to be inhibited by pre-treatment with capsaicin, an effect that is not related to an action on the associated pulmonary infiltration of eosinophils. The involvement of NK_2 receptor stimulation in this phenomenon also suggests that NK_2 receptor antagonists may be useful for investigating mechanisms of bronchopulmonary alterations in asthmatic patients.

Keywords airway hyper-responsiveness, C-fibres, *Parietaria judaica*, rabbits, sensory neuropeptides, tachykinin receptor antagonists

Submitted 5 February 2001; revised 18 July 2001; accepted 23 September 2001

Introduction

Airway hyper-responsiveness (AHR) to bronchoconstriction to spasmogens is a hallmark of asthmatic patients [1, 2]. It has been suggested that different mechanisms could account for the changes in sensitivity and reactivity of the airway to bronchoconstrictor agents, including airway smooth muscle hyperplasia, facilitated excitation of smooth muscle cells, loss of homeostatic bronchodilator mechanisms, loss of epithelial integrity and reduction of luminal diameter secondary to increased volume of airway smooth muscle and mucosal oedema [3].

Correspondence: Bruno D'Agostino, Department of Experimental Medicine, Section of Pharmacology, Faculty of Medicine and Surgery, 2nd University of Naples, Via Costantinopoli 16, 80138 Naples, Italy. E-mail: bruno.dagostino@unina2.it

Human airways are innervated by populations of afferent nerves, including myelinated Aδ- and unmyelinated C-fibres. Tachykinins, a group of neuropeptides, such as substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), are localized in capsaicin-sensitive sensory C-fibres, which are found beneath and within the epithelium, around blood vessels and submucosal glands and within the bronchial smooth muscle layer [4–8]. C-fibres have received considerable attention in the context of AHR because, among the different inflammatory mediators involved, several lines of evidence suggest that tachykinins might be involved in the pathogenesis of AHR [9,10]. In fact, C-fibres can be stimulated by a wide variety of agents, generating local axon reflex responses and release of tachykinins, which leads to a series of events, referred to as 'neurogenic inflammation', including vasodilatation, microvascular leakage, stimulation of glandular secretion, facilitation of cholinergic transmission, recruitment and activation of some types of inflammatory cells, reflex cough and bronchoconstriction [11].

© 2002 Blackwell Science Ltd

As several allergic mediators may induce sensory nerve stimulation, the mechanisms underlying neurogenic inflammation are cardinal features of allergic asthma and may be involved in the pathophysiology of this disease [7,10–12]. This hypothesis is supported by various observations in asthmatic patients. In fact, it has been claimed that substance P-containing nerves are more abundant in lungs obtained at autopsy from asthmatics compared with healthy individuals [13,14], albeit conflicting results are reported [15]. Furthermore, enhanced levels of SP have been observed in bronchoalveolar lavage (BAL) fluid [16] and in induced sputum [17] of asthmatic patients. Finally, in asthmatic patients, exposure to substance P enhanced maximal airway narrowing to metacholine 24 h later [18].

The development of animal models of airway hyper-reactivity is a common approach to investigate the possible mechanisms involved in the pathogenesis of this phenomenon [9]. Thus, to investigate the role of the capsaicin-sensitive sensory C-fibres and the involvement of tachykinins in allergic airway diseases, we have evaluated in the present study the effect of capsaicin and of tachykinin NK_1, NK_2 and NK_3 receptor antagonists pre-treatments on the development of AHR in rabbits sensitized intraperitoneally and challenged with Par j1 [19], the major allergen of *Parietaria judaica* pollen [20], the most active among pollens distributed around the Mediterranean area [21].

Methods

Animals

New Zealand White (NZW) rabbits, germ-free (Harlan, Italy) and of either sex, were used throughout the study. Animals were housed at constant temperature ($21 \pm 1\,^{\circ}C$), relative humidity ($55 \pm 5\%$), under a regular light/dark schedule (light 07.00 to 19.00). Food and water were freely available. The experimental procedures were in accord with the Italian Legislative Decree 116/92.

Allergen extraction

Briefly, allergenic extract was prepared by incubation of 20 g of *Parietaria judaica* pollen (Allergon, Angelhom, Sweden) in a 0.125-M Na bicarbonate buffer and dyalized for 1 week to exclude low molecular weight. Par j1, the major allergen of *Parietaria judaica* pollen was obtained as previously reported [22].

Immunization protocol

Rabbits were injected intraperitoneally (i.p.) with Par j1 (1 mg) in aluminium hydroxide moist gel (1 mg) and 0.9% sterile saline (0.25 mL) (sensitized rabbits) or with 0.9% sterile saline (0.25 mL) plus aluminium hydroxide moist gel (1 mg) only (unsensitized rabbits). Twenty-eight days later, each animal was boosted with the allergen under similar conditions.

Assessment of cutaneous sensitization

Three to five days after the second injection of allergen and/or adjuvant and saline, the animals underwent direct skin testing to determine their sensitivity to antigen. Par j1 solutions of 10-fold dilutions up to 1 : 100000 were administered intradermally (i.d.) to the shaved dorsolateral skin of the rabbit (0.05 mL per site). The degree of weal estimated after 15 min and indurations indicating obvious signs of an acute inflammatory response were considered positive.

Immunoblotting experiments

Par j1 extract in Tris buffer containing 2% sodium dodecyl sulphate (SDS) was boiled for 5 min, and an electrophoretic run was done on a linear 15% polyacrylamide gel. Resolved proteins were transferred to nitrocellulose paper, which was then incubated with serum from normal rabbits or rabbits immunized with Par j1 extract. The signal was developed with a biotinylated serum anti-rabbit IgG and streptavidin peroxidase with diamino benzidine (Sigma, Milan, Italy) as substrate.

Pulmonary function measurement

For the measurement of pulmonary function, rabbits were premedicated with an intraperitoneal injection of diazepam (5 mg/mL, 5 mL/kg) and subsequently administered Hypnorm® (0.4 mL/kg; a mixture of fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/mL, intramuscularly (i.m.)). This regimen produces neuroleptoanalgesia and is recommended for recovery procedures in laboratory rabbits [23]. Neuroleptoanalgesia was maintained throughout the course of the experiment by the administration of 0.2–0.3 mL Hypnorm® i.m. approximately every 30 min. The animals were placed in the supine position on a padded animal board and intubated with a cuffed endotracheal tube (3.0 mm internal diameter, Mallinckrodt Laboratories, Athlone, Ireland). The cuff was then inflated and the tube connected to a heated (37.5 °C) Fleisch pneumotachograph (size 00).

Flow was measured by using a Validyne differential pressure transducer (Model MP 45–14–871, Validyne Engineering Corp., Northridge, CA, USA). Pleural pressure was estimated by placing a polyethylene catheter with an attached latex balloon in the lower third of the oesophagus, where it remained throughout the experiment, to obtain the maximum expiratory pressure. Transpulmonary pressure, the difference between atmospheric and pleural pressure, was recorded by a second Validyne differential pressure transducer (Model MP 45–24–871) connected between the outflow of the endotracheal tube and the oesophageal balloon. The flow was integrated to give a continuous reading of tidal volume. Total airway resistance (R_L) and dynamic compliance (C_{dyn}) values were calculated by an on-line respiratory analyser (PMS version 8.4, Mumed Ltd, London, UK).

Capsaicin pre-treatment

After the assessment of sensitization, rabbits were separated in two groups: one group treated with capsaicin (total dose of 80 mg/kg, subcutaneously (s.c.)), administered over a 3-day period (5 mg/kg s.c. on day 1, 50 mg/kg s.c. on day 2 and 25 mg/kg s.c. on day 3; and the other group treated with vehicle (10% ethanol, 10% Tween 80 and 80% 0.9% saline) with the same administration procedure. The volume of injection was 1 mL/kg. Lung function experiments were begun 3 days after the final capsaicin administration.

Experimental protocol (Fig. 1)

On day 1, after measurement of baseline lung function, the anaesthetized rabbits were exposed to an aerosol of sterile saline for 2 min and lung function parameters were recorded. Airway

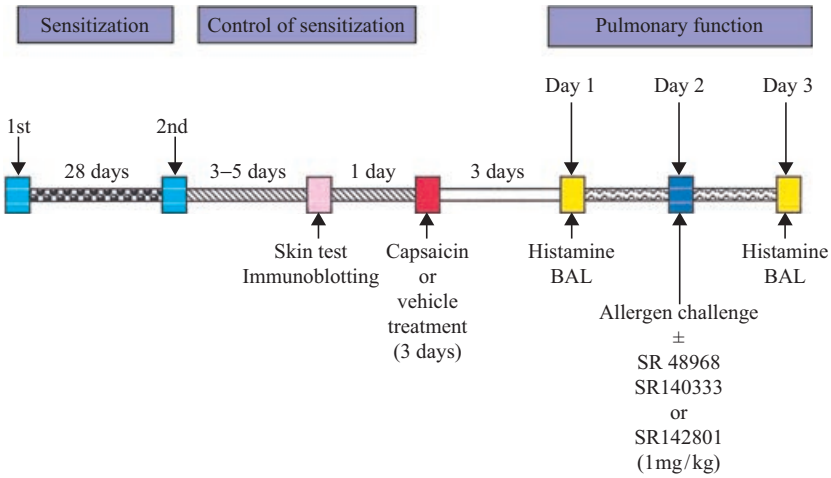

Fig. 1. Experimental protocol of induction of airway hyper-responsiveness in rabbits sensitized and challenged with Par j1.

responsiveness was then determined by exposing animals to cumulative concentrations of aerosolized histamine (1.25 to 80 mg/mL; 2 min per concentration), dissolved in saline, administered directly to the lungs via an endotracheal tube. Following each 2-min aerosol of histamine, animals were disconnected from the nebuliser and attached to the Fleisch tube. The following 10 breaths were recorded and the mean values of R_L and C_{dyn} were calculated.

On day 2, after measurement of baseline lung function, antigen challenge was performed in rabbits with or without capsaicin pre-treatment before and after tachykinin receptor antagonists injection (1 mg/kg intravenously (i.v.), 30 min before allergen exposure). Each antigen challenge consisted of a 4-min aerosol of saline followed by five consecutive aerosols of 4 min duration (total of 20 min) of Par j1 (500 μg/mL), after which respiratory function was recorded as described above.

On day 3, airway responsiveness to histamine was determined as on day 1.

Aerosols of saline and histamine were generated by an ultrasonic nebuliser (De Vilbiss Health Care UK Ltd, Heston, Middlesex, UK). The provocation concentrations (PC) of histamine that produced a 50% increase in airway resistance (R_L) (PC_{50}) or a 35% fall in dynamic compliance (C_{dyn}) (PC_{35}) were determined for each animal by linear interpolation and used as indices of airway responsiveness.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed immediately after histamine challenge on days 1 and 3. The airways were lavaged by use of a polyethylene catheter inserted into the lung via the endotracheal tube; 5 mL of 0.9% sterile saline were injected into the lung and then immediately aspirated with 50% recovery of BAL fluid. Total cell counts were determined under light microscopy using an improved Neubauer haemocytometer. For differential cell counts, 25 μL aliquots were used for centrifugation (Shandon cytospin 2, Shandon Southern Instruments, Sewickley, PA, USA) and the cells were stained with Lendrum's stain (haematoxylin and chromotrope 2R) to facilitate the discrimination of eosinophils. A total of 200 cells were counted differentially and classified as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria. Total and differential cell counts are expressed as the number of cells per millilitre of fluid recovered.

Drugs and chemicals

The drugs and chemicals used were: histamine diphosphate (Carlo Erba Reagent, Milan, Italy); diazepam (Roussel Pharma S.p.A., Milan, Italy); Hypnorm (Janssen Pharmaceutical Ltd, Grove, Oxfordshire, UK); aluminium hydroxide (PIERCE, Rockford, IL, USA); biotinylated serum antirabbit IgG, streptavidin peroxidase, diamino benzidine, SDS, capsaicin (8-methyl-N-vanillyl-6-nonenamide (Sigma, Milan, Italy); SR 48968 ((S)-N-methyl-N-[4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl] benzamide) SR 140333 ((S)-1-(2-[3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo [2,2,2]octane chloride), SR 142801 ((R)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methyl acetamide) (RBI, Milan, Italy).

All solutions were prepared in saline.

Statistical analysis

Bartlett's test for homogeneity of variances was used on all data to determine whether parametric or non-parametric statistics were to be applied. For the lung function studies, statistical analysis was performed on $\log_{10}$ transformed data (PC_{50} and PC_{35}) in order to normalize the distribution of the data and to allow the application of parametric statistics. The paired *t*-test was used for the histamine lung function data before and after allergen challenge.

Bronchoalveolar lavage cell data were analysed using the Kruskall-Wallis one-way analysis of variance. Results were considered significant if $P < 0.05$.

Results

Baseline lung function

Baseline absolute values of R_L or C_{dyn} were not significantly different between the third and first days. Furthermore, no significant difference was observed on the third experimental day between sensitized animals with or without capsaicin pre-treatment (Table 1).

Table 1. Baseline lung function prior to and 24 h following (a) saline challenge (b) antigen challenge in immunized rabbits pretreated with vehicle or capsaicin

	R_L (cm H_2O/l/s)		C_{dyn} (ml/cm H_2O)	
	Pre	24 h Post	Pre	24 h Post
(a) Saline challenge				
Vehicle ($n=5$)	32.3 ± 2.7	33.9 ± 2.8	4.1 ± 0.5	4.0 ± 0.6
Capsaicin ($n=5$)	31.7 ± 2.4	32.5 ± 2.6	3.9 ± 0.7	3.8 ± 0.5
(b) Antigen challenge				
Vehicle ($n=5$)	33.7 ± 2.9	34.7 ± 2.3	4.3 ± 0.7	3.9 ± 0.5
Capsaicin ($n=5$)	34.5 ± 2.4	35.8 ± 2.9	4.0 ± 0.4	3.7 ± 0.4

Values represent mean ± SEM for the number of animals shown in parentheses. R_L = airway resistance. C_{dyn} = dynamic compliance.

Acute antigen-induced bronchoconstriction

In sensitized rabbits, aerosol of antigen caused an acute bronchoconstriction, with an approximately threefold greater increase in R_L and decrease in C_{dyn} in respect to an aerosol of saline solution. This antigen-induced acute bronchoconstriction was unaffected by capsaicin pre-treatment (Fig. 2).

Airway responsiveness to histamine

Effect of antigen challenge Antigen challenge exposure resulted 24 h later in an increase of airway responsiveness to inhaled histamine. In fact, antigen challenge increased the airway responsiveness to inhaled histamine about fourfold ($P < 0.01$) when compared with saline challenge in sensitized rabbits (Fig. 3).

Effect of capsaicin In sensitized rabbits pre-treated with capsaicin, the airway responsiveness to inhaled histamine 24 h after antigen challenge was not significantly different from the pre-challenge values; therefore, capsaicin abolished the AHR induced by antigen ($P < 0.01$) (Fig. 3).

Effect of tachykinin receptor antagonists Pre-treatment with SR 48968, a tachykinin NK_2 receptor antagonist, 1 mg/kg i.v. (30 min before antigen challenge on the second experimental day), did not significantly modify the R_L increase induced by antigen challenge (data not shown), but significantly reduced the antigen-induced AHR to inhaled histamine in terms of both R_L PC_{50} and C_{dyn} PC_{35} (Fig. 4).

Pre-treatment with SR 140333, an NK_1 receptor antagonist (1 mg/kg, i.v. 30 min before exposure to allergen), did not significantly modify the antigen-induced bronchoconstriction and only slightly and not significantly attenuated the antigen-induced AHR to inhaled histamine 24 h following allergen challenge (Fig. 4).

Finally, the antigen-induced bronchoconstriction and AHR to inhaled histamine 24 h following allergen challenge were unaffected by SR 142801, a tachykinin NK_3 receptor antagonist (1 mg/kg i.v., 30 min before exposure to allergen) (Fig. 4).

Analysis of bronchoalveolar lavage (BAL) fluid

The volume of fluid recovered from BAL was not significantly different in sensitized rabbits with or without capsaicin pre-treatment before or after antigen challenge (2.0–3.0 mL, 40 to 60% recovery, data not shown). Prior to saline or Par j1 challenge, total cell counts were not significantly different in rabbits administered either vehicle or capsaicin (Table 2a). In rabbits challenged with saline there was no change in total cells, mononuclear cells or eosinophils, whilst a statistically significant increase was observed in neutrophil numbers 24 h after saline challenge (Table 2a). Total cells recovered in BAL fluid 24 h after antigen challenge were statistically significantly elevated in both treatment groups. Neutrophil numbers were significantly elevated after antigen challenge in both treatment groups (Table 2b). There was a significant increase in the number of eosinophils observed in BAL fluid in both groups 24 h following antigen challenge, whilst mononuclear cell numbers were not altered following antigen exposure in either treatment group (Table 2b).

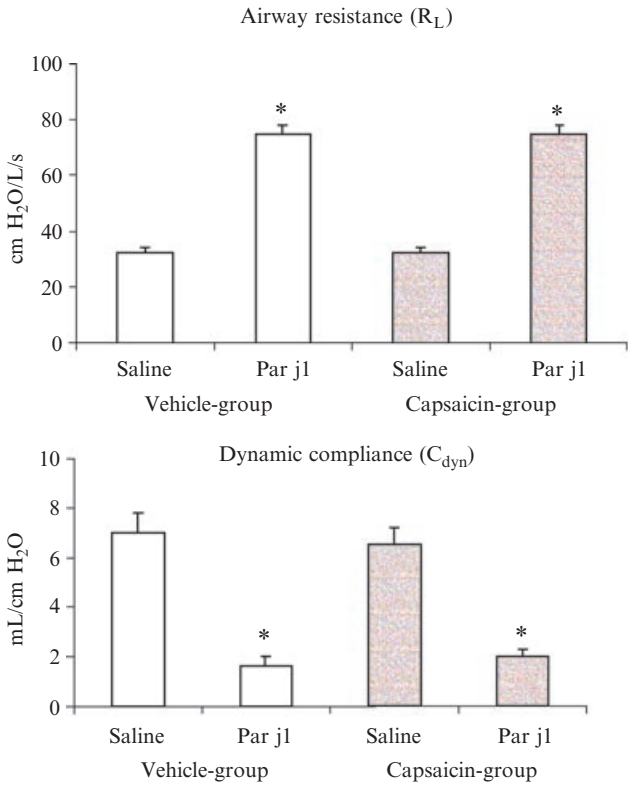

Fig. 2. Effect of Par j1 or saline challenge (aerosol exposure 500 μg/mL) on airway resistance and dynamic compliance in sensitized rabbits ($n=5$) with capsaicin (solid columns) or vehicle (open columns) treatment. *$P < 0.01$ compared with saline treated group.

Discussion

Pollen of *Parietaria judaica* is known to be one of the main causes of allergic diseases in the Mediterranean area [21]. In the current study Par j1, the major allergen of *Parietaria judaica*, induced AHR to inhaled histamine, in Par j1 immunized rabbits, 24 h after Par j1 challenge. Both rabbit and human lungs are close in that they both receive a relatively sparse innervation by sensory nerves [24] and are poorly responsive to the contractile actions of capsaicin [25–28]. In the present study, capsaicin pre-treatment alone had no effect on basal lung function, and basal responsiveness of the airways to inhaled

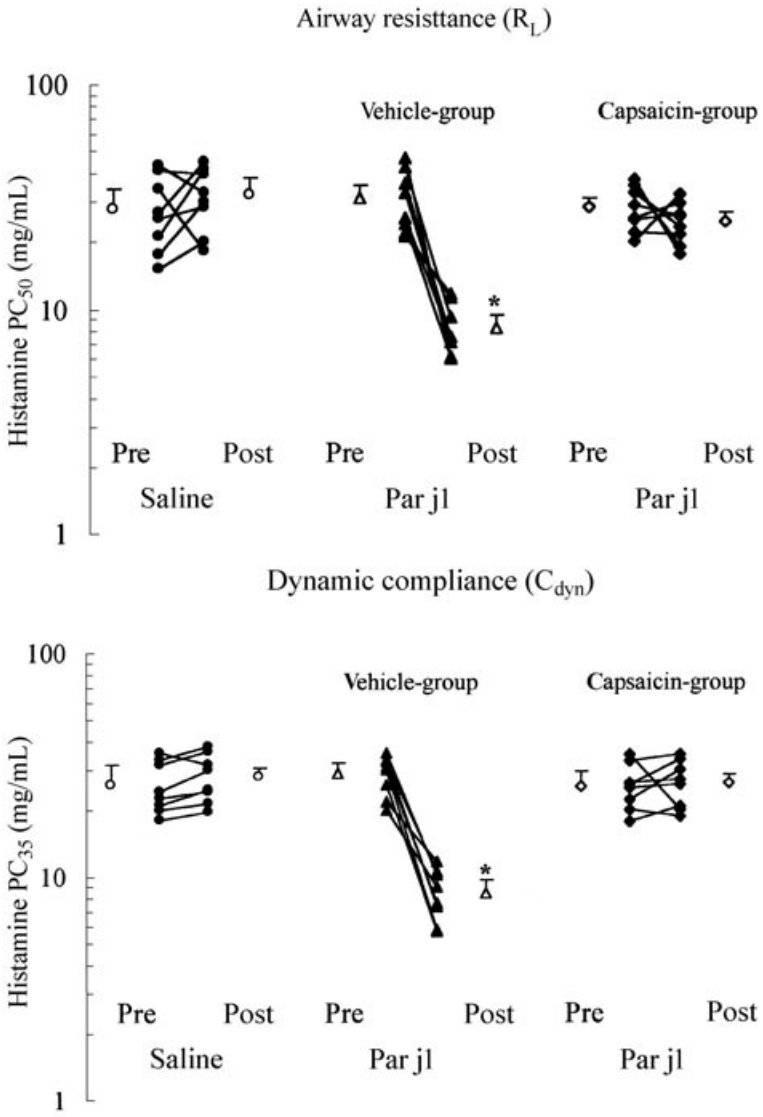

Fig. 3. Airway responsiveness to histamine 24 h before (pre) and 24 h after (post) saline or Par j1 challenge (500 μg/mL) in sensitized rabbits with vehicle or with capsaicin treatment. Results are expressed as provocation concentration (PC) of histamine that produced a 50% increase in airway resistance (R_L) (PC_{50}) or a 35% fall in dynamic compliance (C_{dyn}) (PC_{35}). Closed symbols represent individual animal data; open symbols represent means ± SEM ($n = 5$). *$P < 0.01$ Par j1 vehicle-group vs. saline and Par j1 capsaicin-group.

histamine was unaltered by capsaicin. Moreover, the immediate bronchoconstriction induced by inhaled antigen in immunized rabbits was not affected by capsaicin pre-treatment prior to antigen challenge, suggesting a lack of involvement of sensory neurones in this response. In contrast, capsaicin pre-treatment inhibited the enhanced responsiveness to inhaled histamine achieved 24 h following antigen exposure, a finding supported by previous studies that mentioned a protective effect of capsaicin against AHR in various experimental conditions in rabbits [29] and in guinea-pigs [30].

These observations suggest a role for capsaicin-sensitive sensory nerves in the development of AHR following *Parietaria judaica* exposure.

Stimulation of airway sensory C-fibre nerves elicits a release of sensory neuropeptides into the airway wall. Among the different inflammatory mediators involved, several lines of evidence suggest that tachykinins, such as substance P and neurokinin A, might be involved in the pathogenesis of AHR [31,32].

In particular, Fisher et al. (33) documented an increase of tissue NKA and SP in guinea pig airways, 24 h after the challenge with allergen. On the other hand, a number of observations suggest that NK_2 receptor-mediated activation of alveolar macrophages may be involved in allergen-induced AHR (34). Moreover, as part of the histamine-induced bronchoconstriction occurs through stimulation of vagal afferents (35), allergen-induced changes in excitatory non-adrenergic non-cholinergic (eNANC) neural function could also be involved in the AHR to inhaled histamine. It is remarkable that the accessibility of these afferents for stimuli may be increased after allergen change, as a consequence of epithelial damage caused by cytotoxic mediators derived from infiltrated inflammatory cells (36)

We have shown that SR 48968, like the other tachykinin receptor antagonists, did not modify the acute bronchoconstriction induced by allergen inhalation. This is in agreement with the observation of Maghni [37] who have observed that SR 48968, as well as CP 99994 (a tachykinin NK_1 receptor antagonist), was unable to inhibit the early bronchoconstriction induced by allergen challenge in Brown Norway rats sensitized to ovalbumin, and with Schuiling [38,39], who observed the same effect in sensitized and challenged guinea-pigs. These authors reported, however, that SR 48968 was able to reduce the ovalbumin-induced late airway response following allergen exposure. In the present study, we have shown that SR 48968

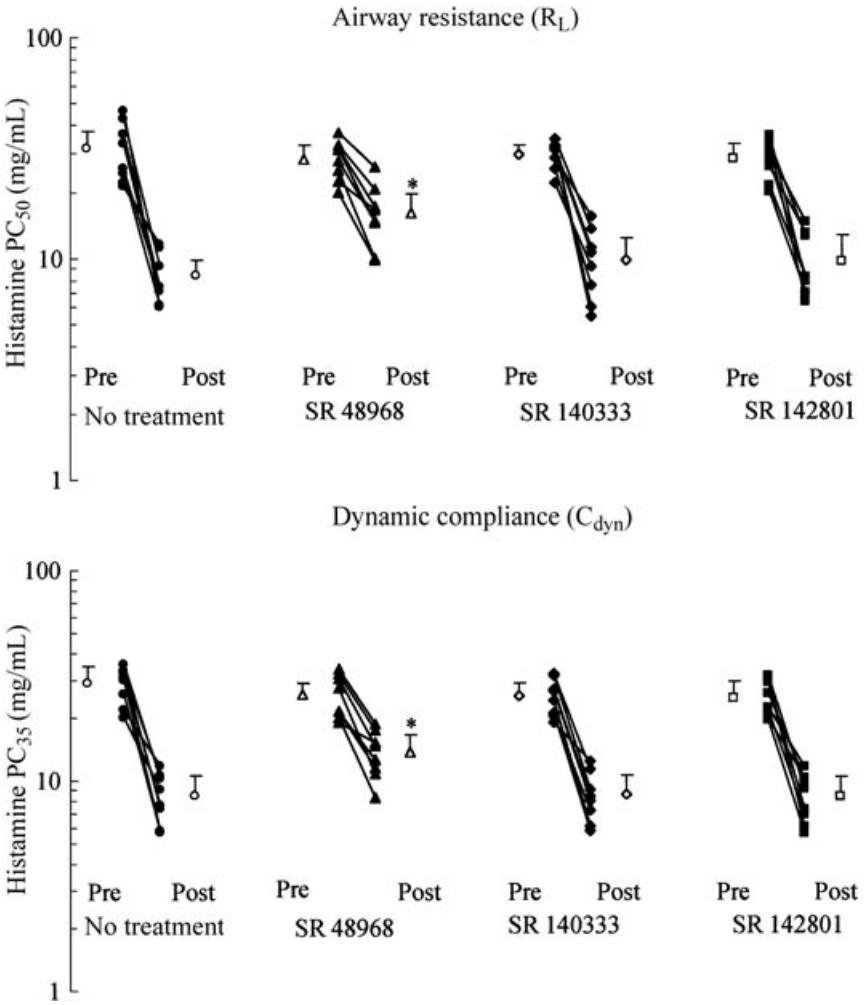

Fig. 4. Airway responsiveness to histamine 24 h before (pre) and 24 h after (post) Par j1 challenge (500 μg/mL) without (no treatment) or with SR 48968 (1 mg/kg; i.v.), SR 140333 (1 mg/kg; i.v.) or SR 142801 (1 mg/kg; i.v.) in rabbits. Results are expressed as provocation concentration (PC) of histamine that produced a 50% increase in airway resistance (R_L) (PC_{50}) or a 35% fall in dynamic compliance (C_{dyn}) (PC_{35}). Closed symbols represent single values for animals; open symbols represent means ± SEM ($n = 5$). *$P < 0.05$ compared with antigen control.

Table 2. Total and differential cell numbers recovered from BAL fluid, prior to and 24 h following saline (a) or Par j1 (b) challenge in immunized rabbits pre-treated with capsaicin or its vehicle

	$\times 10^4$ cells/mL			
	Total	Neutrophils	Eosinophils	Mononuclear cells
(*a*) *Saline exposure*				
Vehicle				
Pre ($n = 5$)	21.6 ± 2.4	0.29 ± 0.04	0.02 ± 0.02	20.2 ± 3.5
24 h ($n = 5$)	22.8 ± 3	0.99 ± 0.03*	0.01 ± 0.01	22.5 ± 3.9
Capsaicin				
Pre ($n = 5$)	23.1 ± 3.1	0.21 ± 0.03	0.03 ± 0.02	23.2 ± 4.1
24 h ($n = 5$)	22.3 ± 4.1	0.97 ± 0.04*	0.02 ± 0.02	26.4 ± 4.5
(*b*) *Antigen challenge*				
Vehicle				
Pre ($n = 5$)	22.3 ± 3.3	0.31 ± 0.03	0.01 ± 0.01	21.3 ± 3.7
24 h ($n = 5$)	40.1 ± 4.1*	1.39 ± 0.04*	1.04 ± 0.2*	23.7 ± 4.1
Capsaicin				
Pre ($n = 5$)	24.1 ± 3.5	0.34 ± 0.04	0.02 ± 0.02	25.6 ± 4.5
24 h ($n = 5$)	41.6 ± 4.3*	1.35 ± 0.03*	1.12 ± 0.1*	28.3 ± 4.2

Values represent mean ± SEM for the number of animals shown in parentheses.*$P < 0.05$ compared with pre-antigen value.

reduced antigen-induced AHR in sensitized rabbits, providing evidence that a NK_2 receptor stimulation is involved in the development of AHR induced by *Parietaria judaica*. Moreover, the finding that pre-treatment with the NK_2 receptor antagonist could reduce the AHR to inhaled histamine is in agreement with previous results obtained in the guinea-pig under various experimental conditions with several tachykinin receptor antagonists: SR 48968 [38, 40–43], FK 224 [44], and MEN 10 627 [45]. However, these results are in contrast to the data obtained by Costello et al. [35], who have shown that SR 140333, but not SR 48968, prevented the alteration of muscarinic presynaptic M_2 receptor activity in sensitized guinea-pigs.

Finally, as the tachykinin NK_1 and NK_3 receptor antagonists, SR 140333 and SR 142801, respectively, failed to reduce the *Parietaria judaica*-induced AHR, NK_1 and NK_3 receptor stimulation appears not to be involved. Conflicting results have been reported concerning the effect of NK_1 and NK_3 receptor antagonists. In guinea-pigs sensitized and challenged to ovalbumin, a preventive effect of SR 140333 against AHR to histamine has been reported by Schuiling [39] but not by Boichot [42]. The effects of SR 142801 do not seem to have been studied in allergic experimental models.

In our study the NK_2 receptor antagonist only reduced the AHR to inhaled histamine induced by *Parietaria judaica*, while

capsaicin pre-treatment completely prevented the development of AHR induced by exposure to antigen in sensitized rabbits. Therefore, it is possible that NK_1 and/or NK_3 receptors may also play a role, but their effects could be masked by a remaining strong effect mediated by NK_2 receptors, or the inhibition of AHR by capsaicin is via some mechanisms other than depletion of tachykinins. In fact, previous studies have shown that the effect of capsaicin was not associated with depletion of tissue levels of SP in the rabbit [25,29].

In bronchoalveolar lavage fluid recovered following antigen challenge, total inflammatory leucocytes were significantly elevated in both capsaicin and control groups. Both neutrophils and eosinophils were significantly increased following antigen in both groups. Neutrophils, however, were also elevated following challenge with saline, indicating the selective nature of the eosinophil recruitment induced by antigen exposure. It appears from these observations that pulmonary eosinophil infiltration induced by antigen does not depend on sensory nerve activation. This agrees with previous findings that eosiniphil accumulation into the lung induced by platelet-activating factor [25] or antigen [29,46] is unaffected by capsaicin pre-treatment. Therefore, in the present study AHR was inhibited by capsaicin in the absence of any effect on the eosinophil infiltration that would further support the idea that cell infiltration and AHR may be dissociated events, as has been supported by others [47–51].

In conclusion, *Parietaria judaica*-induced AHR in immunized rabbits was shown to be inhibited by pre-treatment with capsaicin, an effect that is not related to an action on the associated pulmonary infiltration of eosinophils. Moreover, this study showed the main involvement of NK_2 receptor stimulation in this phenomenon, suggesting that NK_2 receptor antagonists may be useful for investigating mechanisms of bronchopulmonary alterations in asthmatic patients.

References

1 Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. Am Rev Respir Dis 1980; 121:389–413.
2 O'Byrne PM. Allergen-induced airway hyperresponsiveness. J Allergy Clin Immunol 1988; 81:119–27.
3 Sterk PJ, Bel EH. Bronchial hyperresponsiveness: the need for a distinction between hypersensitivity and excessive airway narrowing. Eur Respir J 1989; 2:267–74.
4 Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C. Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res 1984; 235:251–61.
5 Maggi CA. Tachykinin receptors and airway pathophysiology. Eur Respir J 1993; 6:735–42.
6 Maggi CA, Meli A. The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmacol 1988; 19:1–43.
7 Ellis JL, Undem BJ. Pharmacology of nonadrenergic noncholinergic nerves in airway smooth muscle. Pulm Pharmacol 1994; 7:205–23.
8 Lundberg JM. Pharmacology of co-transmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol Rev 1996; 48:113–78.
9 Barnes PJ, Baraniuk JN, Melvisi MG. Neuropeptides in the respiratory tract. (Part II). Am Rev Respir Dis 1991; 144:1391–9.
10 Bertrand C, Geppetti P. Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. Trends Pharmacol Sci 1996; 17:255–9.
11 Advenier C, Lagente V, Boichot E. The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough. Eur Respir J 1997; 10:1892–906.
12 Spina D, Page CP, Morlay J. Sensory neuropeptides and bronchial hyperresponsiveness. In: Said SS. ed. Proinflammatory and Antiinflammatory Peptides. New York: Marcel Dekker, 1997; 112:89–145.
13 Ollerenshaw SL, Jarvis D, Sullivan CE, Woolcock AJ. Substance P immunoreactive nerves in airways from asthmatic and nonasthmatic. Eur Respir J 1991; 4:673–82.
14 Howarth PH, Springall DR, Radington AE et al. Neuropeptide-containing nerves in endobronchial biopsies from asthmatic and nonasthmatic subjects. Am J Respir Cell Mol Biol 1995; 13:288–96.
15 Lilly CM, Bai TR, Shore SA, Hall AE, Drazen JM. Neuropeptide content on lungs from asthmatic and nonasthmatics patients. Am J Respir Crit Care Med 1995; 151:548–53.
16 Nieber K, Baumgarten CR, Rathsack R et al. Substance P and beta-endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. J Allergy Clin Immunol 1992; 90:646–52.
17 Tomaki M, Ichinose M, Miura M et al. Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis. Am J Respir Crit Care Med 1995; 151:613–7.
18 Cheung D, Van Der Veen H, Den Hartig J, Dijkmann JH, Sterk PJ. Effects of inhaled substance P on airway hyperresponsiveness to methacholine in asthmatic subjects *in vivo*. J Appl Physiol 1994; 77:1325–32.
19 Mistrello G, Brenna O, Roncarolo D et al. Monomeric chemically modified allergens: immunologic and physicochemical characterization. Allergy 1996; 51:8–15.
20 Ayuso R, Polo F, Carreira J. Purification of Par j1, the major allergen of *Parietaria judaica* pollen. Molec Immun 1988; 25:49–56.
21 Corbì AL, Carreira J. Identification and characterization of *Parietaria judaica* allergens. Int Arch Allergy Appl Immun 1984; 74:318–23.
22 Corinti S, De Palma R, Fontana A et al. Major histocompatibility complex-independent recognition of a distinctive pollen antigen, most likely a carbohydrate, by human CD8+ αβ T cells. J Exp Med 1997; 186:899–908.
23 Flecknall PA. Laboratory Animal Anaesthesia. An Introduction for Research Workers and Technicians, London: Academic London Press, 1987:89–100.
24 Laitinen LA, Laitinen A, Panula PA, Partanen M, Tervo T. Immunohistochemical demonstration of substance P in the lower respiratory tract of the rabbit but not in man. Thorax 1983; 38:531–6.
25 Spina D, McKenniff MG, Coyle AJ et al. Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbit. Br J Pharmacol 1991; 103:1268–74.
26 Honda I, Kohrogi H, Yamaguchi T, Ando M, Araki S. Enkephalinase inhibitor potentiates substance P- and capsaicin-induced bronchial smooth muscle contractions in humans. Am Rev Respir Dis 1991; 143:1461–18.
27 Chitano P, Di Blasi P, Lucchini RE et al. The effects of toluene diisocynate and of capsaicin on human bronchial smooth muscle *in vitro*. Eur J Pharmacol 1994; 270:167–73.
28 Ellis JL, Sham JSK, Undem BJ. Tachykinin-independent effects of capsaicin on smooth muscle in human isolated bronchi. Am J Respir Crit Care Med 1997; 155:751–5.
29 Herd CM, Gozzard N, Page CP. Capsaicin pretreatment prevents the development of antigen-induced airway hyperresponsiveness in neonatally immunised rabbits. Eur J Pharmacol 1995; 282:111–9.

30 Advenier C, Lagente V. Tachykinins and airway function. Pulm Pharmacol Ther 1998; 11:331–40.
31 Hsiue TR, Garland A, Ray DW et al. Endogenous sensory neuropeptide release enhances non-specific airway responsiveness in guinea pigs. Am Rev Respir Dis 1992; 146:148–53.
32 Boichot E, Lagente V, Paubert Braquet M, Frossard N. Inhaled substance P induces activation of alveolar macrophages and increases airway responses in the guinea-pig. Neuropeptides 1993; 25:307–13.
33 Fisher A, McGregor GP, Saria A, Philippin B, Kummer W. Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation. J Clin Invest 1996; 98:2284–91.
34 Brunelleschi S, Parenti A, Ceni E, Giotti A, Fantozzi R. Enhanced responsiveness of ovalbumin-sensitized guinea-pig alveolar macrophages to tachykinins. Br J Pharmacol 1992; 107:964–9.
35 Costello RW, Fryer AD, Belmonte KE, Jacoby DB. Effects of tachykinin NK_1 receptor antagonists on vagal hyperreactivity and neuronal M_2 muscarinic receptor function in antigen challenged guinea-pigs. Br J Pharmacol 1998; 124:267–76.
36 Sadeghi Hashjin G, Folkerts G, Henricks PA et al. Peroxynitrite induces airway hyperresponsiveness in guinea pig in vitro and in vivo. Am J Respir Crit Care Med 1996; 153:1697–701.
37 Maghni K, Taha R, Afif W, Hamid Q, Martin LG. 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 Respir Crit Care Med 2000; 162:1068–74.
38 Schuiling M, Zhuidhof AB, Meurs H, Zaagsma J. Role of tachykinin in NK_2-receptor activation in the allergen-induced late asthmatic reaction, airway hyperreactivity and airway inflammation cell influx in conscious, unrestrained guinea-pigs. Br J Pharmacol 1999; 127:1030–8.
39 Schuiling M, Zuidhof AB, Zaagsma J, Meurs H. Involvement of tachykin NK1 receptor in the development of allergen-induced airway hyperreactivity and airway inflammation in conscious unrestrained guinea-pigs. Am J Respir Crit Care Med 1999; 159:423–30.
40 Tocker JE, Gertner SB, Welton AF, Seling WM. Vagal stimulation augments pulmonary anaphylaxis in the guinea-pig lung. Am J Respir Crit Care Med 1995; 151:461–9.
41 Boichot E, Germain N, Lagente V, Advenier C. Prevention by the tachykinin NK_2 receptor antagonist, SR 48968, of antigen-induced airway hyperresponsiveness in sensitized guinea-pigs. Br J Pharmacol 1995; 114:259–61.
42 Boichot E, Biyah K, Germain N et al. Involvement of tachykinin NK_1 and NK_2 receptors in substance P-induced microvascular leakage hypersensitivity and airway hyperresponsiveness in guinea-pigs. Eur Respir J 1996; 9:1445–50.
43 Yoshihara S, Geppetti P, Linden A et al. Tachykinins mediate in the potentiation in the antigen-induced bronchoconstrictions by cold air in guinea-pigs. J Allergy Clin Immunol 1996; 97:756–60.
44 Mizuguchi M, Fujimura M, Amemiya T et al. Involvement of NK_2 receptors rather than NK_1 receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs. Br J Pharmacol 1996; 117:443–8.
45 Perretti F, Ballati L, Manzini S, Maggi CA, Evangelista S. Antibronchospastic activity of MEN 10, 627, a novel tachykinin NK_2 receptor antagonist, in guinea–pig airways. Eur J Pharmacol 1995; 273:129–135.
46 Matsuse T, Thomson RJ, Chen XR et al. Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerosolized antigen in guinea pigs. Am Rev Respir Dis 1991; 44:368–72.
47 Boulet LP, Turcotte H, Boutet M, Montminy L, Laviolette M. Influence of natural antigenic exposure on expiratory flows, methacholine responsiveness, and airway inflammation in mild allergic asthma. J Allergy Clin Immunol 1993; 91:883–93.
48 Vianna RMJ, Leduc D, Lefort J et al. Eosinophil recruitment into the mice airway is not sufficient to induce bronchopulmonary hyperresponsiveness. Am Rev Respir Dis 1993; 147:A571.
49 Van Oosterhout AJM, van Ark I, Hofman G, Van der Linde HJ, Fattah D, Nijkamp FP. Role of interleukin-5 and substance P in development of airway hyperreactivity to histamine in guinea-pigs. Eur Respir J 1996; 9:493–9.
50 Kraneveld AD, Nijkamp FP, van Oosterhout AJM. Role for neurokinin-2 receptor in interleukin-5-induced airway hyperresponsiveness but not eosinophilia in guinea-pigs. Am J Respir Crit Care Med 1997; 156:367–74.
51 Kraneveld AD, James DE, de Vries A, Nijkamp FP. Excitatory non-adrenergic-non-cholinergic neuropeptides: key players in asthma. Eur J Pharmacol 2000; 405:113–29.