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The tachykinin NK_1 receptor is crucial for the development of non-atopic airway inflammation and hyperresponsiveness

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Abstract

Mast cell activation, bronchoconstriction, inflammation and airway hyperreactivity are prominent features of non-atopic hypersensitivity reactions in mouse airways. We studied the role of tachykinin receptors in mice that were skin-sensitized with dinitrofluorobenzene (or vehicle) and challenged intranasally with dinitrobenzene sulfonic acid. Tachykinin NK_1 receptor blockade, by treatment with the antagonist RP67580, or absence of the tachykinin NK_1 receptor resulted in a strong reduction in the accumulation of neutrophils in the bronchoalveolar lavage fluid, and in the development of tracheal hyperreactivity in mice 48 h after challenge. In contrast, treatment with the tachykinin NK_2 receptor antagonist SR48968 did not affect the dinitrofluorobenzene-induced hypersensitivity reaction. We have previously shown that mast cells play a crucial role in the development of non-atopic asthma. However, we did not observe an inhibitory effect of the tachykinin receptor antagonists or the genetic absence of tachykinin NK_1 receptors on mast cell protease release. In conclusion, distal from mast cell activation, the tachykinin NK_1 receptor is crucial for the infiltration of pulmonary neutrophils and the development of tracheal hyperreactivity in non-atopic asthma.

Keywords: Airway; Substance P; Inflammation; Neutrophil; Mast cell

1. Introduction

Asthma and chronic obstructive pulmonary diseases are among the world's most prevalent airway diseases. Asthma patients can roughly be divided in two groups, atopic and non-atopic. Atopic asthma refers to the genetic predisposition of individuals expressing immunoglobulin E (IgE) specific for certain allergens. In non-atopic patients no allergen-specific IgE can be detected in the blood. A recent review of epidemiological reports by Pearce et al. (2000) suggests that total serum IgE levels are not elevated in half of the asthma patients studied.

Non-atopic asthma is an increasing problem in the developed world. Low molecular weight substances (< 5000 Da) are the most common agents causing occupational asthma without producing specific IgE (Beckett, 2000). Sensitization and local challenge with the low molecular weight compound dinitrofluorobenzene has been shown to induce acute bronchoconstriction, mast cell activation, accumulation on inflammatory cells, airway hyperreactivity and increased vascular permeability in the mouse airways (Kraneveld et al., 2002). Sensitization is not associated with an increase in hapten-specific IgE. Therefore, this murine model is useful to study mechanisms of non-atopic asthma. Buckley and Nijkamp (1994b) showed that dinitrofluorobenzene-induced tracheal hyperreactivity and cellular infiltration were inhibited by capsaicin-induced depletion of excitatory non-adrenergic non-cholinergic (NANC) neuropeptides. From this study, it was hypothesized that excitatory NANC nerves play a role in the pathogenesis of non-atopic asthma.

Excitatory-NANC pathway innervate the airways of human and other mammalian species (Joos and Pauwels, 2000). The excitatory NANC nerves can be activated by various stimuli, that affect the chemosensitive C-fiber afferents in the airways and lead to the local release of neuropeptides (Advenier et al., 1999). Tachykinins and calcitonin gene-

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related peptide (CGRP) are the predominant excitatory NANC neuropeptides in the airways (Solway and Leff, 1991). The tachykinins, substance P and neurokinin A, have various proinflammatory effects that could contribute to changes observed in asthmatic airways such as smooth muscle contraction, vasodilatation, an increase in vascular permeability and infiltration and stimulation of inflammatory cells such as mononuclear cells, neutrophils and mast cells (Joos and Pauwels, 2000). Two receptor subtypes, the tachykinin NK_1 and the tachykinin NK_2 receptor, mediate the biological actions of tachykinins in the airways. The preferred ligand for these receptors are substance P and neurokinin A, respectively (Frossard and Advenier, 1991; Regoli et al., 1994). Tachykinin effects on immune cells can also be non-receptor mediated. Substance P can cause degranulation of mast cells through direct activation of G proteins in the inner surface of the plasma membrane (Mousli et al., 1990).

It is appreciated that mast cells play a critical role in immediate hypersensitivity reactions, involving IgE. However, mast cells also play a prominent role in non-atopic hypersensitivity reactions (Kraneveld et al., 2002; Ramirez-Romero et al., in press). Firstly, mast cell degranulation is observed in dinitrofluorobenzene-sensitized mice directly after dinitrobenzene sulfonic acid challenge (Kraneveld et al., 1997). Furthermore, non-IgE hypersensitivity responses such as neutrophil infiltration and tracheal hyperreactivity are absent in WBB6F1-W/W^v and Sl/Sl^d mast cell-deficient mice. In addition, mast cell reconstitution in these mice restored pulmonary hypersensitivity responses (Kraneveld et al., 2002). As mast cells are in close proximity to excitatory NANC-nerves, the mast cell is thought to be an important mediator cell in neuroimmune interactions (Blennerhassett et al., 1992; Suzuki et al., 1999).

In the present study, we investigated the role of tachykinin receptors in the non-atopic hypersensitivity reactions in the mouse airways leading to cellular accumulation and tracheal hyperreactivity in mice. Furthermore, mast cell activation was assessed early after hapten challenge. The involvement of tachykinin receptors in mast cell activation and the development of a pulmonary cellular influx and airway hyperresponsiveness was studied by using specific tachykinin NK_1 and NK_2 receptor antagonists and tachykinin NK_1 receptor knockout mice.

2. Materials and methods

2.1. Animals

Male BALB/c mice were obtained from Charles River, Someren, the Netherlands. Tachykinin NK_1 receptor knockout mice (back-crossed to BALB/c) were developed and bred by Dr. N. Gerard, Harvard, Boston, USA (Bozic et al., 1996). All mice used were 6–8 weeks of age. The experiments were conducted in accordance with the Animal Care Committee of the Utrecht University (Utrecht, The Netherlands).

2.2. Sensitization and experimental procedure

Mice were skin-sensitized on day 0 and 1 with dinitrofluorobenzene (50 μl 0.5%) or vehicle (acetone/olive oil, 4:1). On day 5, the animals were intranasally challenged with dinitrobenzene sulfonic acid (50 μl 0.6% in phosphate-buffered saline (PBS)). Dinitrofluorobenzene- or vehicle-sensitized mice were intravenously (i.v.) injected with the tachykinin NK_1 receptor antagonist RP67580 (20 mg/kg) or the tachykinin NK_2 receptor antagonist SR48968 (25 mg/kg) at indicated times after sensitization. As a control group, dinitrofluorobenzene and vehicle sensitized mice were i.v. injected with RP65681 (20 mg/kg), the inactive enantiomer of RP67580 or saline in the SR48968 study. The concentration SR48968 used in this study showed to be effective since this tachykinin NK_1 receptor antagonist was capable of significantly inhibiting ear swelling in tachykinin NK_1 receptor knockout mice. Earswelling was caused by intradermal application of neurokinin A (100 pmol/site) (saline/saline: 94 ± 22 μm; neurokinin A/saline: 218 ± 22 μm, $n = 4$; $P < 0.05$. saline/SR48968: 105 ± 13 μm; neurokinin A/SR48968: 134 ± 16 μm, $n = 6$; ns). Previous studies have shown that the dose of RP67580 used in this study is effective in inhibiting tachykinin NK_1 receptor-mediated responses in the mouse (Kraneveld et al., 1995; van Houwelingen et al., 1999). The tachykinin NK_1 receptor antagonist and its inactive enantiomer were administered i.v. using four treatment regimens:

(I) 10 min before and 1 h after the challenge;
(II) 46 and 47 h after the challenge;
(III) 10 min before and 1, 24, 46 and 47 h after the challenge;
(IV) 10 min before challenge.

The tachykinin NK_2 receptor antagonist, or saline as its control, was administered according to regimens III and IV.

2.3. Tracheal reactivity in vitro

Mice were sacrificed with an overdose of pentobarbitone 48 h after intranasal dinitrobenzene sulfonic acid challenge. The trachea was resected in toto and connective tissue was carefully removed using a binocular microscope as described earlier (Buckley and Nijkamp, 1994a). A nine ring length of trachea (taken from just below the larynx) was then transferred to a 10-ml organ bath containing a modified oxygenated Krebs solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 25 mM $NaHCO_3$, 1 mM $NaHPO_4$ and 11.1 mM glucose), aerated with 95% O_2: 5% CO_2, 37 °C. Measurements were expressed as changes in milligram (mg) force. An optimal preload, determined to be 1 g, was placed on the tissue at the beginning of the experiment. The trachea was allowed to equilibrate for at least 1 h. During this period, the bath fluid was refreshed every 15 min. At the end of the equilibrium phase, tracheal contractile reactivity was mea-

sured by recording cumulative concentration–response curves to carbachol (10^{-8} to 10^{-4} M).

2.4. Leukocyte accumulation in bronchoalveolar lavage fluid

Bronchoalveolar lavages were taken from vehicle and dinitrofluorobenzene-sensitized mice 48 h after the challenge. After sacrificing the animals, the trachea was carefully intubated and the catheter was secured with ligatures. The chest cavity was exposed for expansion. Saline (37 °C) was slowly injected via the catheter into the lung and withdrawn in 4 × 1 ml aliquots. The aliquots were pooled and maintained at 4 °C. The lavage fluids were centrifuged (1500 rpm, 10 min, 4 °C) to isolate the bronchoalveolar lavage cells. The cell pellet was resuspended in 150 μl PBS. Total cells were counted using a haemocytometer and expressed as cells/lung. The bronchoalveolar lavage cell preparations were analyzed morphologically after centrifugation on microscopic slides. Air dried preparations were fixed and stained with hematoxylin and eosin to ascertain the leukocyte populations. Results are expressed as neurtrophils/lung in the airway lumen.

2.5. Mast cell activation in vivo

Blood samples of dinitrofluorobenzene- and vehicle-sensitized mice were taken 30 min after intranasal dinitrobenzene sulfonic acid challenge. Blood samples were collected and after centrifugation, sera were stored at −70 °C until use. Levels of mouse mast cell protease 1 (mMCP-1), a selective marker for mast cell degranulation, were measured using a commercially available enzyme-linked immunosorbent assay (ELISA). Results were expressed as ng mMCP-1 per ml serum.

2.6. Materials

Dinitrofluorobenzene and olive oil were purchased from Sigma, St. Louis, USA. The selective tachykinin NK_1 receptor antagonist RP67580 and the inactive enantiomer RP65681 were generous gifts from Rhône-Poulenc Rorer, Dr. C. Garrett in France. The selective tachykinin NK_2 receptor antagonist SR48968 was a generous gift from Dr. X. Emonds-Alt (Sanofi Research, France). Carbachol was purchased from Onderlinge Farmaceutische Groothandel, Utrecht, The Netherlands. Sodium pentobarbitone was obtained from Sanofi, Maassluis, The Netherlands. The mMCP-1 ELISA was from Moredun Scientific, Midlothian, UK. Maxisorp surface 96-well plates were purchased from Nunc Immuno plate, Roskilde, Denmark. The force displacement transducer was purchased from Harvard Bioscience, Boston, MA, USA and the two-channel recorder (Servogar type SE-120) from Plato, Diemen, The Netherlands.

2.7. Statistical analysis

Tracheal hyperreactivity data are expressed as mean and standard error of the mean (S.E.M.). EC_{50} and E_{max} values for the carbachol-induced tracheal contractions were calculated by nonlinear least-squares regression analysis of the measured contractions versus carbachol concentration using the sigmoid concentration–response relationship. The data were analyzed by performing a two-way analysis of variance (ANOVA). Data on the cellular accumulation were studied by a distribution free Kruskal–Wallis ANOVA. mMCP-1 data were analyzed by using a one-tailed unpaired *t*-test. Probability values of $P < 0.05$ were considered significantly different. Analyses were performed by using Graphpad Prism (version 2.01, San Diego, USA).

3. Results

3.1. Effect of tachykinin receptor antagonists on dinitrofluorobenzene-induced tracheal hyperreactivity

Intranasal hapten application in dinitrofluorobenzene-sensitized mice resulted in the development of a tracheal

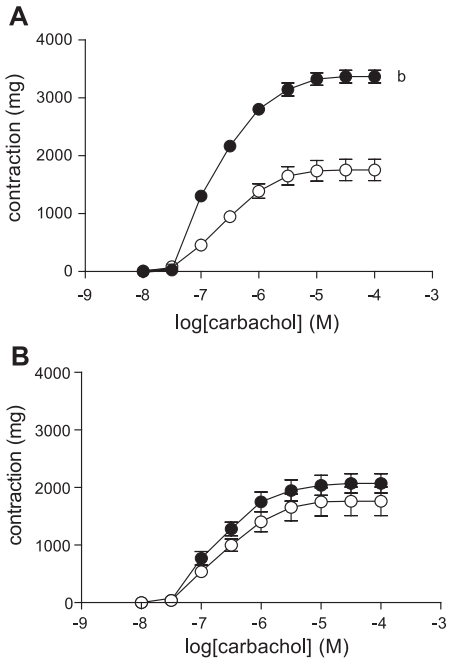

Fig. 1. Effect of tachykinin NK_1 receptor blockade on the development of tracheal hyperreactivity to carbachol 48 h after intranasal dinitrobenzene sulfonic acid challenge in dinitrofluorobenzene-sensitized BALB/c mice. Concentration–response curves were measured in dinitrofluorobenzene-(closed circles) or vehicle-(open circles) sensitized mice treated i.v. with 20 mg/kg RP65681 (control, A) or RP67580 (tachykinin NK_1 receptor antagonist, B) at 10 min before, 1, 24, 46 and 47 h after challenge. Results are expressed as mean ± S.E.M. ($n = 6$). Significant differences ($P < 0.01$) between curves are denoted by ([b]).

hyperreactivity to carbachol at 48 h (Figs. 1A and 2A). In previous studies, we have shown that neuropeptide depletion prevented the development of dinitrofluorobenzene-induced tracheal hyperreactivity (Buckley and Nijkamp, 1994a). We now further focussed on the role of tachykinin NK_1 and NK_2 receptors in the development of this hyperreactivity. Mice were i.v. injected with the tachykinin NK_1 receptor antagonist RP67580 or the tachykinin NK_2 receptor antagonist SR48968 at indicated times after the sensitization. RP67580 had no effect on the dinitrofluorobenzene-induced tracheal reactivity when administered 10 min before and 1 h after the challenge regimen I or at 46 and 47 h after the challenge regimen II (Table 1). Previously, it was demonstrated that it is important to have a sustained concentration of the antagonist present to inhibit the tachykinin NK_1 receptor in vivo (Santoni et al., 1999). After pretreatment with the tachykinin NK_1 receptor antagonist RP67580 10 min before and 1 h and 24, 46 and 47 h after the challenge regimen III, the development of tracheal hyperreactivity was abolished in dinitrofluorobenzene-sensitized animals (Fig. 1B, Table 1). Pretreatment with the inactive enantiomer RP65681 according to all treatment regimens did not affect the tracheal hyperreactivity observed in dinitrofluorobenzene-sensitized mice 48 h after dinitrobenzene sulfonic acid challenge (Table 1). In vehicle-sensitized animals, treatment with RP67580 or RP65681 did not affect basal tracheal reactivity (Fig. 1, Table 1). Pretreatment with the tachykinin NK_2 receptor antagonist SR48968, using regimen III, did not influence the tracheal hyperreactivity (Fig. 2B, Table 1) suggesting that only the tachykinin NK_1 receptor played a role in the induction of non-atopic airway hyperreactivity.

Table 1
Effect of tachykinin NK_1 or tachykinin NK_2 receptor inhibition on the development of tracheal hyperreactivity

Sensitization	Treatment	Regimes	E_{max} (mg)	pD_2
Vehicle	RP65681	I	1593 ± 98	6.9 ± 0.0
Dinitrofluorobenzene		I	2334 ± 196[b]	6.8 ± 0.0[a]
Vehicle	RP67580	I	1384 ± 100	6.9 ± 0.0
Dinitrofluorobenzene		I	2372 ± 167[b]	6.8 ± 0.1
Vehicle	RP65681	II	1736 ± 67	6.8 ± 0.1
Dinitrofluorobenzene		II	2544 ± 368[b]	6.8 ± 0.1
Vehicle	RP67580	II	1533 ± 221	6.7 ± 0.1
Dinitrofluorobenzene		II	2484 ± 173	6.8 ± 0.1
Vehicle	RP65681	III	1755 ± 185	6.6 ± 0.0
Dinitrofluorobenzene		III	3368 ± 110[b]	6.9 ± 0.1
Vehicle	RP67580	III	1760 ± 247	6.7 ± 0.0
Dinitrofluorobenzene		III	2070 ± 166	6.8 ± 0.1
Vehicle	saline	III	1696 ± 157	6.9 ± 0.1
Dinitrofluorobenzene		III	3012 ± 202[b]	6.7 ± 0.0[a]
Vehicle	SR48968	III	1872 ± 359	6.7 ± 0.1
Dinitrofluorobenzene		III	3292 ± 298[b]	6.8 ± 0.1

E_{max} and EC_{50} values are derived from concentration–response curves to carbachol (10^{-8}–10^{-4} M).
Mice were skin-sensitized with dinitrofluorobenzene and challenged intranasally with dinitrobenzene sulfonic acid. Mice were i.v. injected with either RP67580, the inactive enantiomer RP65681, SR48968 or saline following the indicated treatment regimes (see Materials and methods). Results are expressed as mean ± S.E.M. ($n=6$). Significant differences are denoted by ([a]) or ([b]) for $P<0.05$, $P<0.01$ between the vehicle-sensitized and the dinitrofluorobenzene-sensitized group, respectively.

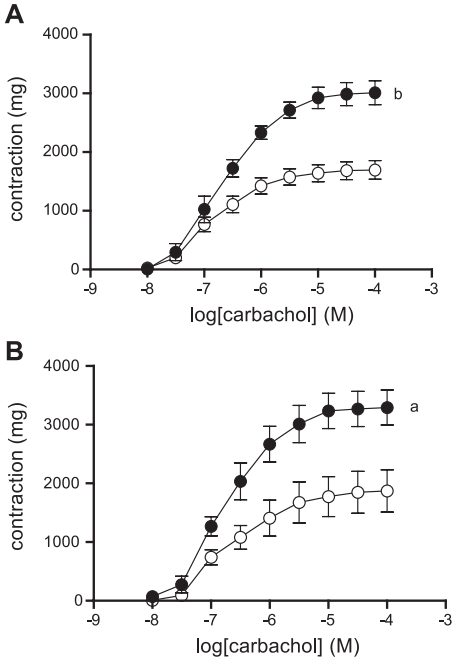

Fig. 2. Effect of tachykinin NK_2 receptor blockade on the development of tracheal hyperreactivity to carbachol 48 h after intranasal dinitrobenzene sulfonic acid challenge in dinitrofluorobenzene-sensitized BALB/c mice. Concentration–response curves were measured in dinitrofluorobenzene-(closed circles) or vehicle-(open circles) sensitized mice treated i.v. with saline (control, A) or with 25 mg/kg SR48968 (neurokinin receptor antagonist, B) at 10 min before, 1, 24, 46 and 47 h after challenge. Results are expressed as mean ± S.E.M. ($n=6$). Significant differences between the vehicle-sensitized and the dinitrofluorobenzene-sensitized group are denoted by ([a]) or ([b]) for $P<0.05$ or $P<0.01$, respectively.

3.2. Dinitrofluorobenzene did not induce tracheal hyperreactivity in tachykinin NK_1 receptor knockout mice

To further confirm the role for the tachykinin NK_1 receptor, we studied the development of hyperreactivity in the airways of tachykinin NK_1 receptor knockout mice. After sensitisation with dinitrofluorobenzene, animals were challenged with dinitrobenzene sulfonic acid and tracheal responses were measured 48 h later. In contrast to control BALB/c mice, in the tachykinin NK_1 receptor knockout animals, no hapten-induced development of tracheal hyperreactivity was observed in dinitrofluorobenzene-sensitized mice compared to vehicle-sensitized animals (E_{max}: Con 1720 mg ± 113 mg, dinitrofluorobenzene 1762 mg ± 118 mg. $n=6$; ns. Fig. 3). Moreover, vehicle-sensitized and hapten-challenged tachykinin NK_1 receptor knockout mice demonstrated a similar tracheal reactivity response upon stimulation with carbachol as vehicle-sensitized

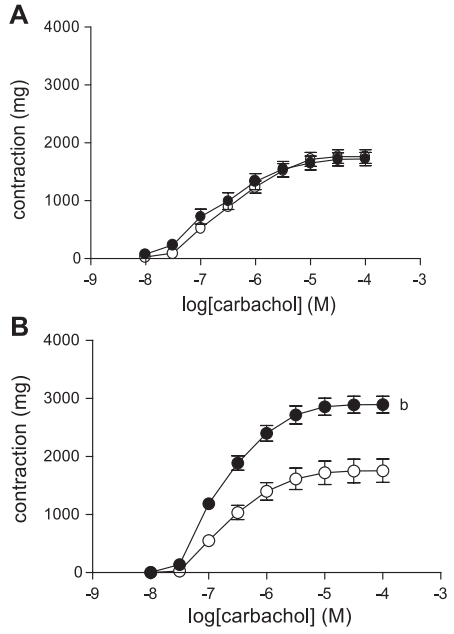

Fig. 3. Effect of tachykinin NK_1 receptor deficiency on tracheal hyperreactivity to carbachol 48 h after intranasal dinitrobenzene sulfonic acid challenge in dinitrofluorobenzene-sensitized mice. Tachykinin NK_1 receptor knock-out mice (A) and control BALB/c mice (B) were dinitrofluorobenzene (closed circles)- or vehicle (open circles)-sensitized and dinitrobenzene sulfonic acid-challenged. Results are expressed as mean ± S.E.M. ($n = 6$). Significant differences between the vehicle-sensitized and the dinitrofluorobenzene-sensitized group are denoted by (^{b}) for $P < 0.01$, respectively.

BALB/c mice (E_{max}: BALB/c mice 1752 mg ± 179 mg, tachykinin NK_1 receptor knockout mice 1720 mg ± 113 mg; $n = 6$; ns).

3.3. The role of tachykinin receptors in dinitrofluorobenzene-induced leukocyte accumulation in bronchial alveolar lavage fluid

An increase in neutrophil accumulation was found in dinitrofluorobenzene-sensitized mice 48 h after dinitrobenzene sulfonic acid challenge (Fig. 4) compared to vehicle-sensitized mice. A separate group of mice were injected with the tachykinin NK_1 or NK_2 receptor antagonist 10 min before and 1, 24, 46 and 47 h after dinitrobenzene sulfonic acid challenge regimen III. The tachykinin NK_1 receptor antagonist, RP67580, significantly inhibited the accumulation of neutrophils in the airway lumen of dinitrofluorobenzene-sensitized mice (Fig. 4A). In contrast, treatment with the tachykinin NK_2 receptor antagonist SR48968 showed no significant decrease in neutrophilic accumulation associated with the pulmonary hypersensitivity response (Fig. 4B). In tachykinin NK_1 receptor knockout mice, no significant differences in the accumulation of neutrophils were found comparing dinitrofluorobenzene- and vehicle-sensitized animals at 48 h after intranasal dinitrobenzene sulfonic acid challenge (Fig. 4C).

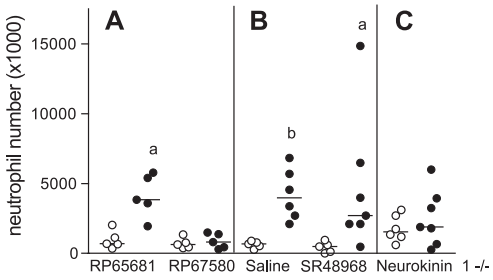

Fig. 4. Effect of the tachykinin NK_1 or NK_2 inhibition or deficiency of the tachykinin NK_1 receptor on neutrophil accumulation in bronchoalveolar fluid. BALB/c mice were dinitrofluorobenzene- or vehicle-sensitized, dinitrobenzene sulfonic acid challenged and: (A) treated with RP67580 or the inactive enantiomer RP65681 at 10 min before and 1, 24, 46 and 47 h after challenge; (B) treated with SR48968 or the inactive control at 10 min before and 1, 24, 46 and 47 h after challenge; (C) Tachykinin NK_1 receptor knockout mice were dinitrofluorobenzene- or vehicle-sensitized and dinitrobenzene sulfonic acid challenged. Open symbols represent vehicle-sensitized and closed symbols dinitrofluorobenzene-sensitized mice. Results are expressed as mean number of neutrophils/lung ± S.E.M. ($n = 6$). Significant differences between the vehicle-sensitized and the dinitrofluorobenzene-sensitized group are denoted by (^{a}) or (^{b}) for $P < 0.05$ or $P < 0.01$, respectively.

3.4. Tachykinin receptors are not involved in dinitrofluorobenzene-induced mast cell activation in vivo

mMCP-1 levels in serum were measured to monitor mast cell activation. Previously, it was demonstrated that in mice, the dinitrofluorobenzene/dinitrobenzene sulfonic acid-induced hypersensitivity reaction was associated with rapid mast cell activation as assessed by elevated serum levels of mMCP-1 at 30 min after challenge. Indeed, in our study, serum mMCP-1 levels were increased 30 min after dinitro-

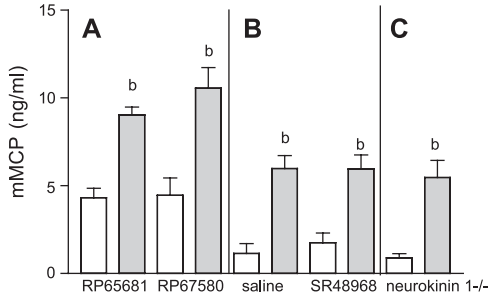

Fig. 5. Tachykinin NK_1 or NK_2 receptor inhibition or deficiency of the tachykinin NK_1 receptor had no effect on mast cell activation induced by intranasal hapten challenge of dinitrofluorobenzene-sensitized mice. BALB/c mice were vehicle- or dinitrofluorobenzene-sensitized, dinitrobenzene sulfonic acid challenged and: (A) treated with RP67580 or the inactive enantiomer RP65681 10 min before challenge; (B) treated with SR48968 or the inactive control 10 min before challenge. (C) Tachykinin NK_1 receptor knockout mice were dinitrofluorobenzene- or vehicle-sensitized and dinitrobenzene sulfonic acid challenged. mMCP-1 levels in serum were measured 30 min after challenge. Open bars represent vehicle-sensitized and closed bars dinitrofluorobenzene-sensitized mice. Results are expressed as mean ± S.E.M. ($n = 6$). Significant differences between the vehicle-sensitized and the dinitrofluorobenzene-sensitized group are denoted by (^{b}) for $P < 0.01$, respectively.

benzene sulfonic acid challenge in dinitrofluorobenzene-sensitized animals when compared to vehicle-sensitized mice (Fig. 5). Neither the tachykinin NK_1 receptor antagonist RP67580 nor the tachykinin NK_2 receptor antagonist SR48968 were able to block early mast cell activation (Fig. 5). Similar mast cell activation was found in dinitrofluorobenzene-sensitized tachykinin NK_1 receptor knockout mice compared to dinitrofluorobenzene-sensitized BALB/c mice 30 min after hapten challenge (Fig. 5).

4. Discussion

The present study provides evidence supporting the involvement of the tachykinin NK_1 receptor in the pathogenesis of non-atopic asthma. To investigate the exact role of the tachykinin NK_1 receptor, we induced non-atopic asthma in mice by dinitrofluorobenzene skin-sensitization and intranasal dinitrobenzene sulfonic acid challenge. Despite the existing evidence describing the involvement of excitatory NANC neuropeptides in non-atopic airway inflammation (Buckley and Nijkamp, 1994a; Kraneveld et al., 2000), it remained unclear which tachykinin receptors are implicated in the development of neutrophil accumulation and tracheal hyperreactivity. The present study shows that neutrophil accumulation and the development of tracheal hyperreactivity, associated with dinitrofluorobenzene-induced hypersensitivity reactions in the mouse, were inhibited by the selective tachykinin NK_1 receptor antagonist RP67580. The tachykinin NK_2 receptor antagonist SR48968 did not affect the non-atopic neutrophil accumulation and tracheal hyperreactivity. The role for the tachykinin NK_1 receptor was further confirmed in tachykinin NK_1 receptor knockout mice. Therefore, it can be concluded that the tachykinin NK_1 receptor, but not the tachykinin NK_2 receptor, plays an important role in the induction of neutrophilic accumulation and tracheal hyperreactivity in the mouse lung.

Using a tachykinin NK_1 receptor antagonist and tachykinin NK_1 receptor knockout mice, the present study shows that the antigen-induced infiltration of neutrophils is dependent on the presence of tachykinin NK_1 receptors. These results are in agreement with the study performed by Bozic et al. (Bozic et al., 1996), in which they demonstrated that neutrophil accumulation was not found in tachykinin NK_1 receptor knockout mice undergoing an immune-complex reaction. Moreover, other studies have shown that substance P or specific tachykinin NK_1 receptor agonists injected locally in skin or airways were able to induce neutrophil infiltration (Iwamoto et al., 1993; Perretti et al., 1993; Saban et al., 1997; Tomoe et al., 1992). The potent inhibitory effects of tachykinin NK_1 receptor antagonists on substance P induced neutrophil infiltration point to the important role of the tachykinin NK_1 receptor. The local microenvironment in the tissue seems to be important, because injection of substance P in healthy rat and mouse skin did not induce neutrophil infiltration (Pinter et al., 1999), but in inflamed skin, the tachykinin NK_1 receptor was involved in mediating neutrophil accumulation (Cao et al., in press). A possible mechanism of action of substance P in inducing infiltration of neutrophils could be the upregulation of the expression of intracellular adhesion molecule (ICAM-1). Previously, we demonstrated a prominent role for ICAM-1 in the cellular infiltration and the development of tracheal hyperreactivity in dinitrofluorobenzene-sensitized and dinitrobenzene sulfonic acid challenged mice (Bloemen et al., 1996). Nakagawa et al. (1995) have shown that substance P is able to upregulate the expression of ICAM-1 on human endothelial cells. Besides the substance P-induced infiltration of neutrophils, it has been shown that substance P activates neutrophils, an effect that could be mediated by the tachykinin NK_1 receptor. Stimulation of human polymorphonuclear cells by substance P leads to superoxide anion production, interleukin-8 or myeloperoxydase release (Payan et al., 1984). This mediator release in turn could lead to the development of tracheal hyperreactivity.

Besides the neutrophil, the mast cell has also been shown to be an essential immune cell in the development of non-atopic pulmonary hypersensitivity reactions (Kraneveld et al., 2002). Mast cells and macrophages lining the mucosal layer of the respiratory tract have been found in the close vicinity of substance P- and CGRP-immunoreactive nerves (Blennerhassett et al., 1992; Kraneveld et al., 2000; Suzuki et al., 1999). Recently, we demonstrated that mast cell-derived tumor necrosis factor alpha (TNF-α) can prime sensory nerves (Van Houwelingen et al., 2002). We hypothesize that the mast cell mediator TNF-α primes NANC nerves to release their tachykinins after second contact with the antigen. The release of tachykinins, in turn, will activate tachykinin NK_1 receptors, possibly on endothelial cells, inducing vasodilatation, vascular leakage and upregulation of adhesion molecules leading to the infiltration of neutrophils. This neutrophilic infiltration could possibly induce in vivo airway responsiveness and in vitro tracheal hyperreactivity.

Activation of mast cells by substance P has been reported to be tachykinin NK_1, tachykinin NK_2 receptor mediated as well as non-receptor mediated, e.g. via direct activation of G-proteins (Lorenz et al., 1998; Maggi, 1997). Tachykinin receptor involvement in mediator release from mast cells may be dependent on agonist concentration, mast cell origin and environmental factors. In our study, neither the tachykinin NK_1 or NK_2 receptor blockade nor deficiency of the tachykinin NK_1 receptor could inhibit mast cell activation as measured by mMCP-1. This suggests that mast cells are activated by non-receptor mediated mechanisms or by other stimuli.

In conclusion, our study points to a role for excitatory NANC nerves in the development of non-atopic asthma. The tachykinin NK_1 receptor is crucial for the accumulation of neutrophils and the development of tracheal hyper-

reactivity, which occurs together or following mast cell activation.

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