

Role of tachykinin NK₁ and NK₂ receptors in allergen-induced early and late asthmatic reactions, airway hyperresponsiveness, and airway inflammation in conscious, unrestrained guinea pigs

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Summary

Using a guinea pig model of allergic asthma, we investigated the effects of the inhaled, highly selective nonpeptide tachykinin NK₁ and NK₂ receptor antagonists SR 140333 and SR 48968, respectively, on allergen-induced early (EAR) and late (LAR) asthmatic reactions, airway hyperreactivity (AHR) after these reactions, and infiltration of inflammatory cells in the airways. Both SR 140333 (100 nM, 3 min) and SR 48968 (100 nM, 3 min) had no effect on the severity of the EAR, while the NK₂ receptor antagonist SR 48968, but not the NK₁ receptor antagonist SR 140333, caused significant inhibition of the LAR. SR 140333 significantly reduced the allergen-induced AHR to histamine, both after the EAR and the LAR. By contrast, SR 48968 did not affect the AHR after the EAR, but significantly attenuated the AHR after the LAR. Bronchoalveolar lavage studies performed after the LAR indicated that SR 140333 caused significant inhibition of allergen-induced infiltration of eosinophils, neutrophils and lymphocytes, while SR 48968 attenuated the infiltration of neutrophils and lymphocytes, but not of eosinophils. Both NK receptor antagonists tended to reduce the accumulation of ciliated epithelial cells in the airways. These results indicate that NK₁ and NK₂ receptors are importantly, but differentially, involved in the development of allergen-induced airways obstruction, AHR and infiltration of inflammatory cells in the airways. Therefore, both NK₁ and NK₂ receptor antagonists, or dual NK₁ and NK₂ antagonists, could be useful in the treatment of allergic asthma.

Keywords: airway hyperresponsiveness, airway inflammation, allergic asthma, guinea pigs, histamine, NK₁ receptor, NK₂ receptor, SR 140333, SR 48968, tachykinins

Introduction

Tachykinins, such as substance P (SP) and neurokinin A (NKA), which are released upon sensory nerve stimulation in the airways, have been shown to elicit several airway responses, collectively referred to as 'neurogenic inflammation' [1]. These responses include bronchoconstriction, microvascular leakage, vasodilation, mucus secretion, and recruitment and activation of inflammatory cells, and are mediated predominantly by tachykinin NK₁ and NK₂ receptors [1–3]. NK₁ receptors, which are activated preferentially by SP, are mainly involved in microvascular

leakage, vasodilation, mucus secretion, and inflammatory cell responses, whereas NK₂ receptors, activated preferentially by NKA, have been shown to be mainly involved in bronchoconstriction [1–4]. Nevertheless, NK₂ receptors may also contribute to microvascular leakage [5,6] and activation of mast cells [7] and alveolar macrophages [8].

Since sensory nerve stimulation may be induced by allergic mediators and responses of neurogenic inflammation are important features of allergic asthma [1–3], mechanisms underlying neurogenic inflammation may be involved in the pathophysiology of this disease. In asthmatic patients, some indirect evidence has been obtained to support this hypothesis. Thus, enhanced levels of SP were observed in the bronchoalveolar lavage (BAL) fluid [9] and induced sputum [10] of allergic asthmatics, while BAL SP levels

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were further enhanced after allergen challenge of these patients [9]. In addition, immunohistochemistry revealed evidence of an increase in sprouting of SP-containing nerves in asthma [11], whereas an increase in mRNA expression of NK₁ and NK₂ receptors has been observed in lung tissue from asthmatics as well [12,13]. As a possible consequence, both SP and NKA were shown to cause enhanced bronchoconstriction in asthmatic patients [14,15]. Moreover, Cheung *et al.* [16] demonstrated that SP induced airway hyperresponsiveness (AHR) to methacholine in patients with mild asthma, indicating that NK₁ receptor stimulation may be important in the development of AHR, presumably by its potent inflammatory effects.

Direct evidence for the involvement of NK₁ and NK₂ receptors in allergic airway disease, using specific and selective tachykinin NK receptor antagonists, has thus far not been obtained. Therefore, in a guinea pig model of allergic asthma [17,18], we investigated the effects of the inhaled, highly selective nonpeptide tachykinin NK₁ and NK₂ receptor antagonists SR 140333 and SR 48968, respectively, on allergen-induced early asthmatic reactions (EAR) and late asthmatic reactions (LAR), AHR after both reactions, and infiltration of inflammatory cells in the airways.

Methods

Specified pathogen-free Dunkin Hartley guinea pigs (Harlan, Heathfield, UK) were IgE-sensitized to ovalbumin (OA) as described previously [19]. Three weeks after sensitization, the guinea pigs were permanently instrumented with a fluid-filled intrapleural balloon-catheter, enabling prolonged computer-assisted on-line measurement of pleural pressure (P_{pl}) changes under conscious and unrestrained conditions [17]. We have previously demonstrated that changes in P_{pl} are linearly correlated to changes in airway resistance in this model, and hence can be used as a sensitive parameter for allergic and nonallergic bronchoconstriction [17].

After the animals had been habituated to the provocation procedure (1 week after surgery), experimental OA and histamine provocations were performed by inhalation of

aerosolized solutions with step-wise increasing concentrations of the stimuli, until P_{pl} was increased by more than 100% above baseline. The provocation concentration of histamine causing a 100% increase in P_{pl} (PC_{100}) was used as a measure of airway reactivity.

On two different occasions separated by a 1-week interval, OA-sensitized guinea pigs inhaled either vehicle (3 min) or a selective dose of either SR 140333 (100 nM, 3 min) or SR 48968 (100 nM, 3 min) at 30 min before as well as at 5.5 h after OA challenge (i.e. between the EAR and the LAR). Vehicle and SR 140333 or SR 48968 inhalations were alternated using a random crossover design. In a control group, vehicle was inhaled both in the first and in the second week at the time points indicated above. To determine treatment effects on the EAR and LAR, P_{pl} changes were measured continuously during the whole procedure. Magnitudes of EAR and LAR were expressed as the area under the P_{pl} time-response curve (AUC) between 0 and 5 h after allergen provocation for the EAR, and between 8 and 23 h after provocation for the LAR. P_{pl} was expressed as percentage change from baseline and AUC was calculated by trapezoid integration over discrete (5 min) time periods. Inherent to the research question, only dual-responding animals (74%) were included in the study. Histamine PC_{100} values were assessed 24 h before and at 5 h (after EAR) and 23 h (after LAR) after OA provocation. Finally, BAL was performed at 25 h after the second allergen provocation, as described previously [18]. In a control group, OA-sensitized animals were lavaged 25 h after inhalation of saline instead of OA.

SR 140333 and SR 48968 were kindly provided by Sanofi Recherche (Montpellier, France). For the inhalation studies, SR 140333 was dissolved in saline; SR 48968 was dissolved in saline containing 0.001% (w/v) ethanol.

Results

Compared with control inhalations with vehicle performed in the same animals, the inhaled NK₁ receptor antagonist SR 140333 had no significant effect on both the EAR and the

Table 1. The effects of inhalations of vehicle and the selective tachykinin NK₁ and NK₂ receptor antagonists SR 140333 and SR 48968, respectively, on allergen-induced EAR and LAR and histamine PC_{100} values 24 h before and at 5 h (after EAR) and 23 h (after LAR) after allergen provocation of OA-sensitized guinea pigs

Treatment	AUC (% × 5 min)		PC ₁₀₀ histamine (μg/mL)		
	EAR	LAR	Prechallenge	5 h post-OA	23 h post-OA
Vehicle	2799 ± 374	9362 ± 2626	102.0 ± 8.9	43.6 ± 5.4***	59.2 ± 8.9*
SR 140333	3082 ± 397	9061 ± 2516	97.7 ± 10.1	56.6 ± 6.7***†	88.1 ± 9.5†
Vehicle	3583 ± 561	8681 ± 1670	99.2 ± 15.2	35.6 ± 11.6**	60.4 ± 14.7**
SR 48968	3511 ± 853	5191 ± 1702†	107.6 ± 16.6	39.1 ± 8.1***	83.1 ± 17.2*†

Results are expressed as means ± SEM of 5–9 animals. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with prechallenge; † $P < 0.05$ compared with vehicle.

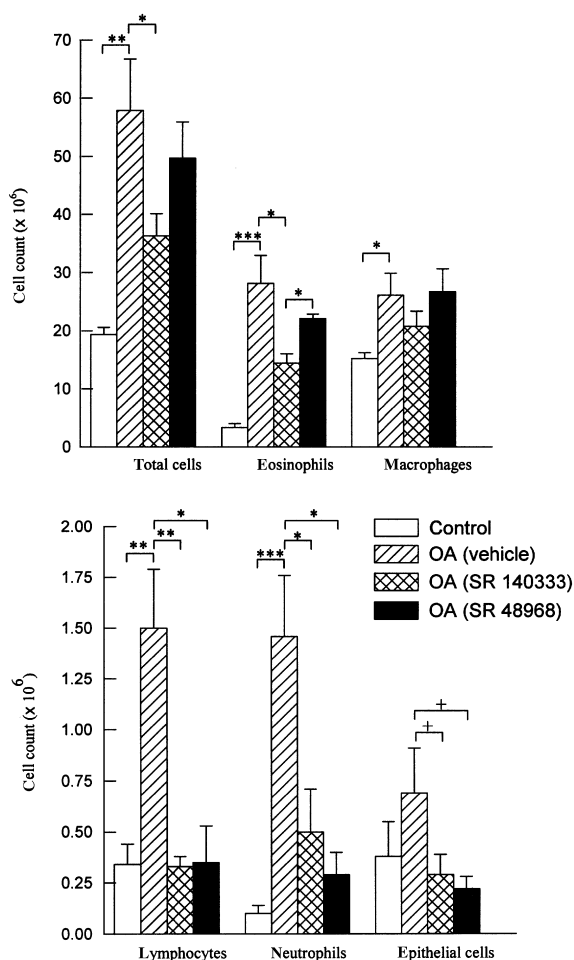


Fig. 1. Bronchoalveolar lavage cell counts at 25 h after inhalation of saline (control, □) or OA. The OA-challenged animals received vehicle (3 min) at 30 min before and 5.5 h after OA challenge during week 1, and either vehicle (3 min, ▨), SR 140333 (100 nM, 3 min, ▩), or SR 48968 (100 nM, 3 min, ■) at 30 min and 5.5 h after OA challenge during week 2. Data represent mean values $\pm$ SEM of 5–7 animals. * P < 0.05, ** P < 0.01, *** P < 0.001, + P = 0.1.

LAR (Table 1). Similarly, the NK₂ receptor antagonist SR 48698 did not affect the EAR; however, the LAR was significantly reduced by $44.2\% \pm 16.4\%$ (P < 0.05) as compared with vehicle (Table 1). Neither SR 140333 nor SR 48968 had a significant effect on basal P_{pl} . In addition, comparable early and late reactions were found after two subsequent allergen challenges, when the animals were treated with vehicle at both occasions (not shown).

Inhalations of vehicle, SR 140333, and SR 48968 had no significant effects on the basal histamine PC_{100} before allergen challenge (not shown). In vehicle-treated animals, OA provocation caused a marked AHR to histamine after both the EAR and the LAR (Table 1). Inhalation of SR 140333 at 30 min before and 5.5 h after allergen challenge by the same animals significantly attenuated the

OA-induced AHR, both at 5 h after the EAR, and at 23 h after the LAR, the responsiveness to histamine after the LAR being not significantly different from the prechallenge value (Table 1). In contrast to SR 140333, SR 48968 did not affect the AHR to histamine after the EAR; however, the AHR after the LAR was significantly reduced by the NK₂ receptor antagonist (Table 1). No changes in allergen-induced AHR were observed when animals were treated with vehicle at two subsequent allergen challenges (not shown).

Compared with a control challenge with saline, OA provocation caused a significant increase in the numbers of eosinophils, macrophages, lymphocytes, neutrophils, and total cells in the BAL from animals that were treated with vehicle at 30 min before and 5.5 h after the second allergen challenge (Fig. 1). In the animals treated with SR 140333 at these time points, the total cell number as well as the numbers of infiltrated eosinophils, lymphocytes, and neutrophils were significantly reduced. Moreover, the number of ciliated epithelial cells tended to be decreased in these animals (Fig. 1). In the SR 48968-treated guinea pigs, no significant change was observed in the number of infiltrated eosinophils. The numbers of lymphocytes and neutrophils in the BAL were significantly reduced, however, while, similar to the SR 140333 treatment, there was a tendency to a reduced number of ciliated cells (Fig. 1).

Discussion

Using a guinea pig model of allergic asthma, the present investigation demonstrates that NK₁ and NK₂ receptors are importantly, but differentially, involved in the development of allergen-induced airways obstruction, AHR, and airway inflammation.

With respect to allergen-induced EAR and LAR, it was demonstrated that both reactions were unaffected by the selective NK₁ receptor antagonist SR 140333, whereas the selective NK₂ antagonist SR 48968 had no effect on the EAR, but caused significant attenuation of the LAR. The absence of effect of both antagonists on the EAR is in line with previous observations in capsaicinized guinea pigs, indicating that reduced tachykinin levels in the airways did not prevent the acute allergen-induced bronchoconstriction [20,21].

The mechanism of inhibition of the LAR by the NK₂ receptor antagonist is presently unclear. First, enhanced synthesis and release of tachykinins during the LAR [22] could contribute to the severity of this reaction via enhanced NK₂ receptor-mediated bronchoconstriction. In addition, airway tachykinin levels could be increased as a consequence of reduced degradation by neutral endopeptidase due to inflammation-induced epithelial damage [23], which may be inhibited by SR 48968.

Inhibition of epithelial damage by SR 48968, as indicated by the reduced number of ciliated cells in the BAL after the

LAR, could also be involved in the attenuation of the development of AHR after this reaction. It is widely accepted that epithelial damage and subsequent AHR may be induced by the release of eosinophil-derived cytotoxic mediators after allergen challenge [24,25]. The infiltration of eosinophils into the airways, however, appeared not to be inhibited by SR 48968. By contrast, we demonstrated that SR 48968 did inhibit the influx of lymphocytes and neutrophils, which might both be involved in the activation of eosinophils [26,27]. Alternatively, neutrophils have the capacity to generate enhanced levels of superoxide anions (O₂⁻) in patients with asthma, which correlates with the AHR to methacholine in these patients [28]. Since allergen challenge in both patients [29] and guinea pigs [30] causes enhanced iNOS expression during the LAR, the reaction product between iNOS-derived NO and O₂⁻, peroxynitrite (ONOO⁻), could be involved in allergen-induced epithelial damage and subsequent AHR after the LAR [31]. Since NO production is deficient after the EAR [30], this might also explain why the NK₂ receptor antagonist was ineffective in attenuating the AHR after this reaction. In this respect, it is also important to note that tachykinins cause enhanced production of O₂⁻ in guinea pig alveolar macrophages, mainly by activation of NK₂ receptors [32,33].

In contrast to SR 48968, SR 140333 was effective in attenuating the AHR after both the EAR and the LAR. The inhibition of allergen-induced AHR after the LAR by SR 140333 appears to oppose previous observations by Boichot *et al.* [34], who found that SR 48968, but not SR 140333, inhibited allergen-induced AHR at 48 h after challenge. These apparently contrasting results might be explained by differences in the time points of AHR measurements (48 h after allergen challenge vs 24 h after challenge in our study), the route of drug administration (systemically vs locally in our study), and the provoking agent used (methacholine vs histamine in our study). With respect to the latter point, it is important to note that methacholine-induced bronchoconstriction results from direct stimulation of airway smooth muscle, whereas histamine-induced bronchoconstriction is partially mediated by stimulation of afferent vagal nerve endings, leading to additional reflex bronchoconstriction, which may be enhanced after allergen challenge [35]. Because tachykinins, predominantly via stimulation of NK₁ receptors, may facilitate cholinergic neurotransmission in guinea pig airways [36], the enhanced cholinergic contribution to histamine-induced bronchoconstriction may involve exaggerated NK₁ receptor stimulation, which is inhibited by SR 140333.

Another mechanism that could be involved is protection against allergen-induced increased accessibility of nerve afferents, which may result from epithelial damage induced by cytotoxic mediators derived from inflammatory cells, as indicated above. In addition to inhibition of infiltration of

lymphocytes and neutrophils in the airways, as also observed with SR 48968, SR 140333 inhibited the allergen-induced recruitment of eosinophils. Since we have previously shown that a pronounced infiltration of eosinophils, as well as activation of these cells, is already present after the EAR and sustains during the LAR [17,37], inhibition of these events by SR 140333 could contribute to the reduced AHR after both the EAR and the LAR. Our results are in line with observations that SP, by stimulating NK₁ receptors, may cause venular adherence [38], recruitment [8], and activation [39] of eosinophils in the airways.

Finally, as airway oedema may contribute to allergen-induced AHR because of thickening of the airway wall [40], inhibition of tachykinin-induced microvascular leakage after allergen challenge by both NK₁ [41] and NK₂ [5,6] receptor antagonists may be important.

Considering the beneficial effects of NK₁ and NK₂ receptor antagonists on the allergen-induced asthmatic reactions, AHR after these reactions, and airway inflammation, these antagonists may be very useful in the treatment of allergic asthma. Since NK₁ and NK₂ receptors are differentially involved in the processes mentioned above, especially combined treatment with both NK₁ and NK₂ receptor antagonists or treatment with a dual NK₁/NK₂ receptor antagonist could be favourable.

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