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Important roles of tachykinins in the development of allergic nasal hyperresponsiveness in guinea-pigs

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Clinical and Experimental Allergy

Summary

Background Although it has been suggested that the use of tachykinin receptor antagonists might prove to be an effective treatment for allergic rhinitis (AR), they are not used clinically. Therefore, we decided to examine the effects of tachykinin receptor antagonists on AR symptoms in an appropriate experimental model.

Objective To evaluate newly developed tachykinin receptor antagonists in a Japanese cedar pollen-induced AR model and to determine their effect on allergen-induced sneezing, nasal blockage, and nasal hyperresponsiveness (NHR).

Methods Sensitized guinea-pigs were challenged by forced inhalation of pollen once every week. Sneezing and nasal blockage were observed after pollen challenges. NHR (nasal blockage) to an intranasal application of leukotriene D₄ was assessed 2 days after an antigen challenge. We also evaluated whether intranasal dosing with a tachykinin causes NHR. NK₁ and NK₂ receptor antagonists were administered before an intranasal treatment with antigen or tachykinin. Amounts of tachykinins present in nasal cavity lavage fluid were measured by an enzyme immunoassay.

Results Although an NK₁ and NK₂ receptor dual antagonist showed no effect on pollen-induced sneezing and biphasic nasal blockage, it did completely suppress the development of NHR. Experiments using specific NK₁ or NK₂ receptor antagonists revealed that NK₂ receptor activation was preferentially involved in the development of hyperresponsiveness. Increases in the levels of substance P (SP) and neurokinin A (NKA) in the nasal tissue were noted 20 min–1 h after the challenge. Intranasal instillation of either SP or NKA-induced NHR, which was almost completely inhibited by NK₂ receptor antagonists and partially inhibited by NK₁ receptor antagonists.

Conclusions SP and NKA, which are released early after the challenge, mediate the development of NHR by preferentially activating NK₂ receptors. Therefore, NK₂ receptor antagonists might prove to be effective treatment of AR.

Keywords allergic rhinitis, hyperresponsiveness, NK₁ receptors, NK₂ receptors, tachykinin

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Introduction

When specific allergens are intranasally applied in patients who have allergic rhinitis (AR), more than 90% of the patients immediately start sneezing and develop rhinorrhoea and nasal obstruction [1]. About 50% of these patients also develop a late-phase nasal blockage [1, 2]. Furthermore, as compared with the responsiveness of healthy individuals, patients with AR experience increased nasal responsiveness to chemical or physical

stimuli other than the specific allergens [3, 4]. The increase in nasal reactivity to stimuli that occurs after allergen provocation may be similar to the reaction that is provoked by an increased sensitivity to histamine [5].

We have established a Japanese cedar pollen-induced AR model in guinea-pigs, in which the animals experience symptoms that are similar to those described above. In the model, guinea-pigs were sensitized by intranasal instillation with pollen extracts, and then intranasally challenged with the pollen once every week. After the initial few

challenges, the subsequent challenges induced increased sneezing and biphasic nasal blockage [6, 7]. Furthermore, it has also been reported that the nasal blockage response of sensitized animals to intranasal instillation of histamine [8] and leukotriene (LT) D₄ [9, 10] appears to be enhanced in proportion to the number of challenges. The nasal hyperresponsiveness (NHR) that is induced persists for at least for 2 days, and then disappears by day 7 [8, 9]. When the pollen challenge was performed on day 2 after the challenge, the pollen-induced sneezing and biphasic nasal blockage were augmented, suggesting that NHR aggravates the antigen-induced responses [11].

Tachykinins, such as substance P (SP) and neurokinin A (NKA), are located in the capsaicin-sensitive sensory nerves of the mammalian respiratory tract [12]. Excitation of these nerves results in the release of tachykinins, which produces inflammatory responses in the airway tissues [13]. Because tachykinins are capable of inducing nasal secretion, vasodilatation and plasma extravasation, evidence suggests that they may be involved in the pathogenesis of upper airway allergy in humans [14–16]. While it has also been suggested for the last decade that tachykinin receptor antagonists might be able to be used as therapeutic drugs for the treatment of AR [17], these tachykinin receptor antagonists have yet to be developed for clinical use. However, in order to re-evaluate the effects of these tachykinin receptor antagonists on AR symptoms, we need to develop an appropriate experimental model that expresses symptoms similar to those seen clinically.

In our guinea-pig model of AR, which uses newly developed NK₁ and/or NK₂ receptor antagonists, we assessed whether SP and NKA are involved in the induction of AR symptoms, such as sneezing, biphasic nasal blockage and NHR. In addition, in sensitized-challenged and non-sensitized animals, we evaluated whether exogenous intranasal applications of SP and NKA can cause development of NHR in response to LTD₄.

Materials and methods

NK₁ and/or NK₂ antagonists used

In this study, we used the newly developed NK₁ and NK₂ receptor dual antagonist, ID034554, a specific NK₁ receptor antagonist, ID045287, and a specific NK₂ receptor antagonist, ID045246. In addition, because other NK₁ and NK₂ receptor-specific antagonists have been widely used in animal studies, we also examined the NK₁ receptor antagonist, LY303870, and the NK₂ receptor antagonist, SR48968. Zeria Pharmaceutical Co. (Tokyo, Japan) was responsible for the development of the new antagonists and also synthesized all of the compounds used in the study.

Measurements of binding affinities of NK₁ and/or NK₂ antagonists to NK₁ and NK₂ receptors

For measurement of binding affinities to NK₁ receptors, Chinese hamster ovary cells (CHO-K1), which stably express human NK₁ receptors, were used. The NK₁ receptor-expressing cells were subcultured at 10⁶ cells/well for 48 h, followed by the addition of [³H]SP (0.5 nM, Amersham Biosciences, Buckinghamshire, UK) and various concentrations of NK₁ and/or NK₂ antagonists. After incubation at 37 °C for 40 min, the cells were washed and then dissolved in 1 M NaOH, followed by the counting of the radioactivity. For assessment of the non-specific binding, an excess concentration of the NK₁ antagonist, L703606 (Sigma-Aldrich, St Louis, MO, USA), was used.

For the study of the NK₂ receptors, a mixture of Cloned Neurokinin Receptor Subtype 2 Human (BioSignal Packard, Montréal, Canada), [³H]SR48968 (0.85 nM, Amersham Biosciences) and NK₁ and/or NK₂ antagonists were incubated at 27 °C for 60 min and then filtrated over glass microfibre filters. Subsequently, radioactivity retained on the filters was counted. For assessment of the non-specific binding, an excess concentration of unlabelled SR48968 was used.

Sensitization and challenge with cedar pollen

Japanese cedar pollen (*Cryptomeria japonica*) was harvested in Gifu and Shiga Prefectures in Japan in 1998. The cedar pollen extract and Al(OH)₃ gel were prepared as previously described [6, 18]. Twice each day for 7 days, male, 4-week-old, Hartley guinea-pigs (Japan SLC, Hamamatsu, Japan) were sensitized by intranasal instillation of the cedar pollen extracts adsorbed on Al(OH)₃ gel (100 µg protein/100 mg Al(OH)₃ per mL) into the bilateral cavities at a volume of 3 µL/nostril [7]. Sensitized animals were intranasally challenged once every week by inhalation of 1.8 mg of the cedar pollen per nostril (3.6 mg/animal) under spontaneous breathing without any anaesthesia using a hand-made inhalation apparatus [6].

The study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Study design

We have previously reported that biphasic nasal blockage was consistently induced during the 4th–13th challenges [7], and that the magnitude of the antigen-induced NHR to LTD₄ exhibited a plateau during the 13th–30th challenges [9]. Therefore, all experiments were conducted during the 13th–20th challenges. In order to evaluate involvement of tachykinins in antigen-induced sneezing and biphasic nasal blockage, and in the development of the antigen-induced NHR, the first experiments examined the pre- and/or post-treatment effects of the NK₁ and NK₂ receptor

dual antagonist ID034554 by injecting the compound before and/or after the 13th, 14th or 15th pollen challenges. We then evaluated the effects of the specific NK₁ and NK₂ receptor antagonists, ID045287 and ID045246, respectively, on the development of antigen-induced NHR at the 17th or 18th challenge. We compared these effects with those observed for the other specific NK₁ and NK₂ receptor antagonists, LY303870 and SR48968. We also assessed the time-course changes for the amount of SP and NKA found in the nasal cavity lavage fluid (NCLF) after the 13th challenge. In the final step, we examined whether intranasal application of SP or NKA could induce NHR in response to LTD₄ in non-sensitized and sensitized animals 7 days after the 20th challenge. The effects of ID045287 and ID045246 on SP- or NKA-induced NHR were also evaluated.

Measurement of specific airway resistance

As an indicator of respiratory function (nasal blockage), the specific airway resistance (sRaw) in conscious guinea-pigs was measured using a two-chambered, double-flow plethysmograph system (Pulmos-I, M.I.P.S., Osaka, Japan), as has been previously described [19].

Elevations of sRaw after pollen inhalation in the sensitized guinea-pig appeared to reflect total (upper and lower) airway resistance, as the guinea-pig functionally respire through the nose and not through the mouth. We have previously demonstrated that our inhalation procedure deposits 99.999% of the pollen in the upper airway [6]. Furthermore, after the pollen inhalation challenge, accumulation of eosinophils in the lung, which is characteristic of allergic bronchial asthma, was not observed in this model [20], even though nasal eosinophilia was markedly induced [21]. Thus, the changes of sRaw induced by antigen challenge in the present model exclusively reflect the nasal response and not the lower airway response.

Antigen-induced sneezing and nasal blockage, and nasal hyperresponsiveness in response to leukotriene D₄

Sneezing frequency was counted within 1 h after a pollen challenge. As an indicator of nasal blockage, changes in sRaw were measured before and 1–10 h after the challenge.

In addition, 2 days after a pollen challenge, NHR in response to LTD₄ was assessed by consecutively instilling 10 µL/nostril of 10⁻⁸ and 10⁻⁶ M of LTD₄ (Cayman Chem., Ann Arbor, MI, USA) into the bilateral nasal cavities at intervals of 20 min [9]. The sRaw value was measured immediately before and 10 min after each set of two LTD₄ instillations. Because LTD₄ induces a dose-dependent increase in the sRaw of the sensitized-challenged animals at 10⁻¹²–10⁻⁶ M [9], we used 10⁻⁸ and 10⁻⁶ M in the

present study. LTD₄ is a well-known and potent bronchoconstrictor. As we have shown in a previous study [9], and as has been reported in the present literature, LTD₄ administration into the nasal cavities of non-sensitized guinea-pig does not elevate the sRaw, even at 10⁻⁶ M. In addition, Narita et al. [22] has reported that almost all of the dye instilled intranasally remains within the nasal cavity 1 h after the instillation. Thus, the LTD₄-induced changes in sRaw entirely reflect the nasal response and are not indicative of a lower airway response.

The NK₁ and NK₂ receptor dual antagonist ID034554 was administered i.p. 0.5 h before and/or 2.5 h after a pollen inhalation challenge. The other specific NK₁ or NK₂ receptor antagonists, ID045287, ID045246, LY303870 and SR48968 were administered i.p. 0.5 h before an antigen challenge.

Substance P and neurokinin A-induced nasal hyperresponsiveness in response to leukotriene D₄

In accordance with a protocol used in our previous study [23], we instilled 10 µL/nostril of SP (10⁻³ M) or NKA (10⁻³ M) (both from Peptide Ins., Osaka, Japan) into the bilateral nasal cavities of non-sensitized and sensitized guinea-pigs. In the sensitized animal, tachykinin was administered on day 7 after the 20th challenge. The acquisition of NHR in response to LTD₄ was evaluated 2 days after exposure to SP or NKA, as described above. ID045287 (10 mg/kg) and ID045246 (3 mg/kg) were administered i.p. 30 min before the intranasal application of SP or NKA.

Measurement of substance P and neurokinin A concentrations in the nasal cavity lavage fluid

The nasal cavities of the sensitized animals were lavaged with saline before and after the 13th pollen inhalation challenge under pentobarbital anaesthesia (30 mg/kg, i.p.), as has been previously described [6]. In brief, warm saline (1 mL at 37 °C) was aspirated from the left nostril under a reduced light pressure produced by an air pump. NCLF was collected from the right nostril into a tube containing 200 µL of a protease inhibitor cocktail (50 µM phosphoramidon, 50 µM captopril, 1250 U/mL aprotinin and 10.25 mM ethylenediaminetetraacetic acid).

SP and NKA in the supernatant of NCLF were partially purified by a C-18 reversed phase column as per the method of Fehder et al. [24]. Amounts of SP and NKA were measured by the enzyme immunoassay (EIA) kits for SP (Cayman Chem.) and NKA (Peninsula Lab., San Carlos, CA, USA). Immunoreactivities of the EIA kit antibody to SP, NKA and NKB were 100%, 2.7% and 0.04%, respectively. Immunoreactivities of the NKA kit antibody to SP, NKA and NKB were 0.05%, 100% and 80%, respectively.

Statistical analyses

Data were statistically evaluated by the one-way analysis of variance test. If a difference appeared to be significant, the individual group difference was determined using Bonferroni's correction for multiple tests. *P*-values <0.05 were considered statistically significant.

Results

Binding affinities of NK₁ and/or NK₂ antagonists to NK₁ and NK₂ receptors

As shown in Table 1, ID045287 showed a high binding affinity for the NK₁ receptors with a *K_i* value of 0.34 nM. This value was comparable to that seen for LY303870, whereas the affinity of ID045287 for the NK₂ receptors

Table 1. Binding affinities of NK₁ and/or NK₂ receptor antagonists to NK₁ and NK₂ receptors

Antagonist	<i>K_i</i> value (nM)	
	NK ₁ receptors	NK ₂ receptors
NK ₁ receptor antagonist		
ID045287	0.34	602
LY303870	0.31	NT
NK ₂ receptor antagonist		
ID045246	602	0.16
SR48968	NT	0.46
NK ₁ and NK ₂ receptor dual antagonist		
ID034554	0.43	0.79

NT, not tested.

was approximately 2000 times lower than that seen for the NK₁ receptors. In contrast, ID045246 exhibited a higher affinity for the NK₂ receptors than SR48968, although the compound had a considerably lower affinity for the NK₁ receptors. On the other hand, ID034554 exhibited high affinities for both the NK₁ and NK₂ receptors (Table 1).

Antagonistic activities of ID034554, ID045287 and ID045246 against various receptors associated with the autonomic and central nervous systems, autacoids and other bioactive substances were comprehensively assessed at 0.1 or 10 µM. However, none of these compounds exhibited any significant binding with regard to the receptors (data not shown).

Effects of an NK₁ and NK₂ dual antagonist, ID034554, on antigen-induced sneezing, biphasic nasal blockage and development of nasal hyperresponsiveness in response to leukotriene D₄

Consistent with our previous studies [7, 20], sneezing was immediately induced within 1 h after the pollen challenge (Fig. 1a), with nasal blockage induced with biphasic peaks at 1 and 4 h after the challenge (Fig. 1b). Administration of ID034554 (10 mg/kg) 0.5 h before and 2.5 h after the challenge did not affect the induction of sneezing or nasal blockage (Fig. 1a and b).

In contrast, pre- and post-treatment with ID034554 almost completely suppressed the development of NHR on day 2 after the challenge (Fig. 2a). Even when ID034554 was given only one time at 0.5 h before the challenge, the development of NHR was completely inhibited (Fig. 2a). However, a single post-treatment with the compound had no effect on the development of NHR

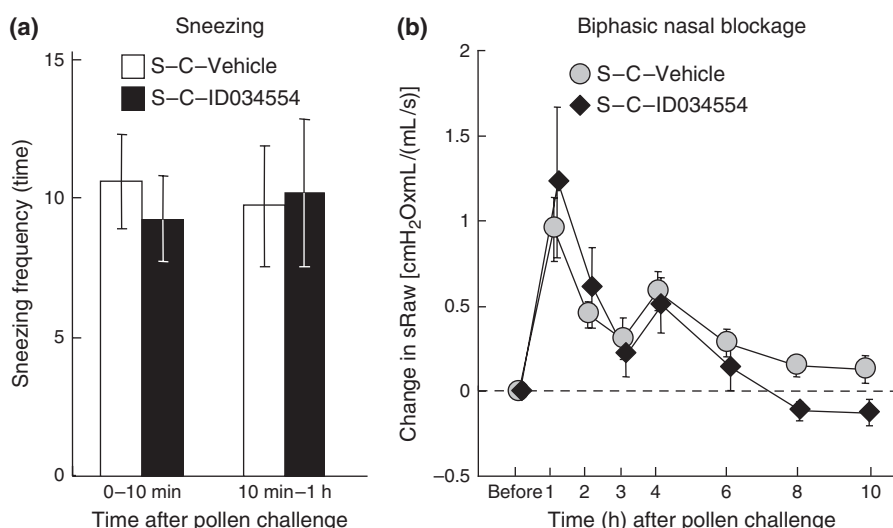


Fig. 1. Effect of a newly developed NK₁ and NK₂ receptor dual antagonist, ID034554, on (a) sneezing and (b) biphasic nasal blockage induced by pollen challenge in sensitized guinea-pigs. Vehicle or ID034554 (10 mg/kg, i.p.) was administered 0.5 h before and 2.5 h after the 13th antigen challenge. After the challenge, sneezing frequency and changes in specific airway resistance (sRaw) were measured 0–1 and 0–10 h, respectively. Each column and point represents the mean ± SE of 10 animals. S-C, sensitized-challenged.

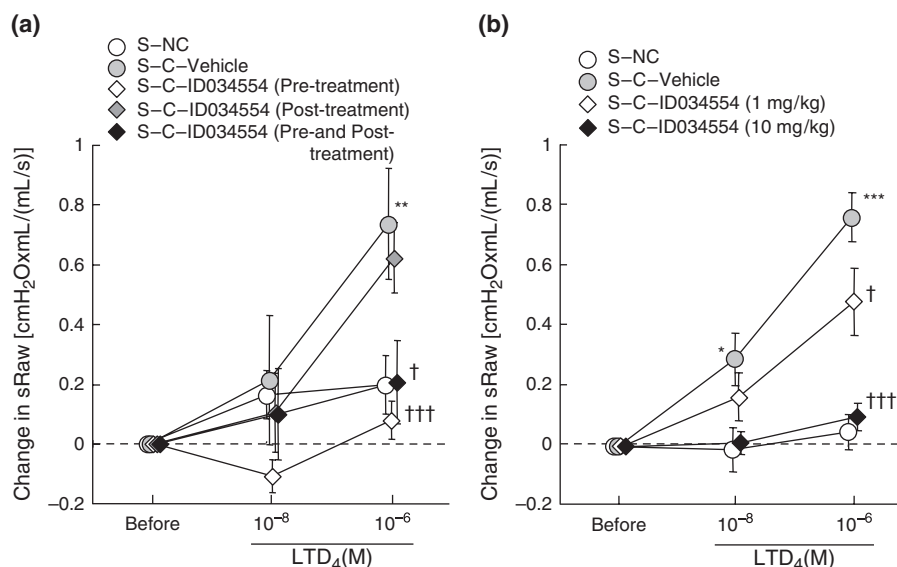


Fig. 2. (a) Pre- and/or post-treatment effect and (b) dose-dependent effect of a newly developed NK₁ and NK₂ receptor dual antagonist, ID034554, on the development of nasal hyperresponsiveness (NHR) induced by the 14th or 15th pollen challenge in sensitized guinea-pigs. (a) Vehicle or ID034554 (10 mg/kg, i.p.) was given once 0.5 h before or 2.5 h after the antigen challenge, or given twice 0.5 h before and 2.5 h after the challenge. (b) Vehicle or ID034554 was administered i.p. one time at 0.5 h before the challenge at 1 and 10 mg/kg, respectively. Nasal responsiveness to leukotriene (LT) D₄ was assessed 2 days after the challenge. Each point represents the mean $\pm$ SE of 8–10 animals. sRaw, specific airway resistance; S-NC, sensitized–non-challenged; S-C, sensitized–challenged. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with the S-NC group. † P < 0.05, †† P < 0.001 compared with the S-C-Vehicle group.

(Fig. 2a). In addition, the pre-treatment effect of ID034554 was dose dependent at 1 and 10 mg/kg (Fig. 2b).

Effects of specific NK₁ or NK₂ antagonists on the development of nasal hyperresponsiveness in response to leukotriene D₄

Specific NK₁ and NK₂ antagonists, ID045287 and ID045246, respectively, were administered 0.5 h before the challenge, and the nasal responsiveness to LTD₄ was assessed on day 2. While ID045287 significantly, albeit it only partially, inhibited the acquisition of NHR at 10 mg/kg, there was no effect noted at 1 mg/kg (Fig. 3a). On the other hand, ID045246 (0.3 and 3 mg/kg) dose dependently suppressed the development of NHR, with an almost complete inhibition at 3 mg/kg (Fig. 3b).

We also tested the specific NK₁ antagonist LY303870 and the NK₂ antagonist SR48968, and found similar results to ID045246, as SR48968 completely suppressed the development of NHR at 1 mg/kg (Fig. 3d). LY303870 also partially suppressed the development of NHR by approximately 50%, with the degree of inhibition at 1 and 3 mg/kg found to be similar to each other (Fig. 3c).

Time-course changes in the substance P and neurokinin A in the nasal cavity lavage fluid after antigen challenge

The SP and NKA levels were significantly increased at both 20 min and 1 h after the challenge, with a tendency

for the SP level to be high even at 3 h. Subsequently, the tachykinin levels in both cases returned to pre-challenge levels (Fig. 4a and b).

Substance P- or neurokinin A-induced nasal hyperresponsiveness in response to leukotriene D₄

We assessed whether NHR could be induced by a single dose of SP or NKA in non-sensitized and sensitized-challenged guinea-pigs. After SP and NKA exposure in the sensitized-challenged animals, NHR was observed on day 2 in response to LTD₄ (Fig. 5b), whereas in the non-sensitized animals, neither SP nor NKA caused NHR (Fig. 5a).

SP- and NKA-induced NHR were significantly suppressed by both ID045287 and ID045246. Inhibition by ID045246 at 3 mg/kg was consistently greater than that seen for ID045287 at 10 mg/kg (Fig. 5c and d).

Discussion

Although the NK₁ and NK₂ receptor dual antagonist administration showed no effect on the pollen challenge-induced sneezing and biphasic nasal blockage, the development of NHR was potently suppressed by the compound. The inhibition was achieved by a one-time i.p. injection of the compound at 0.5 h before the pollen challenge. However, administration of the drug 2.5 h after the challenge had no influence on the NHR. In addition, both

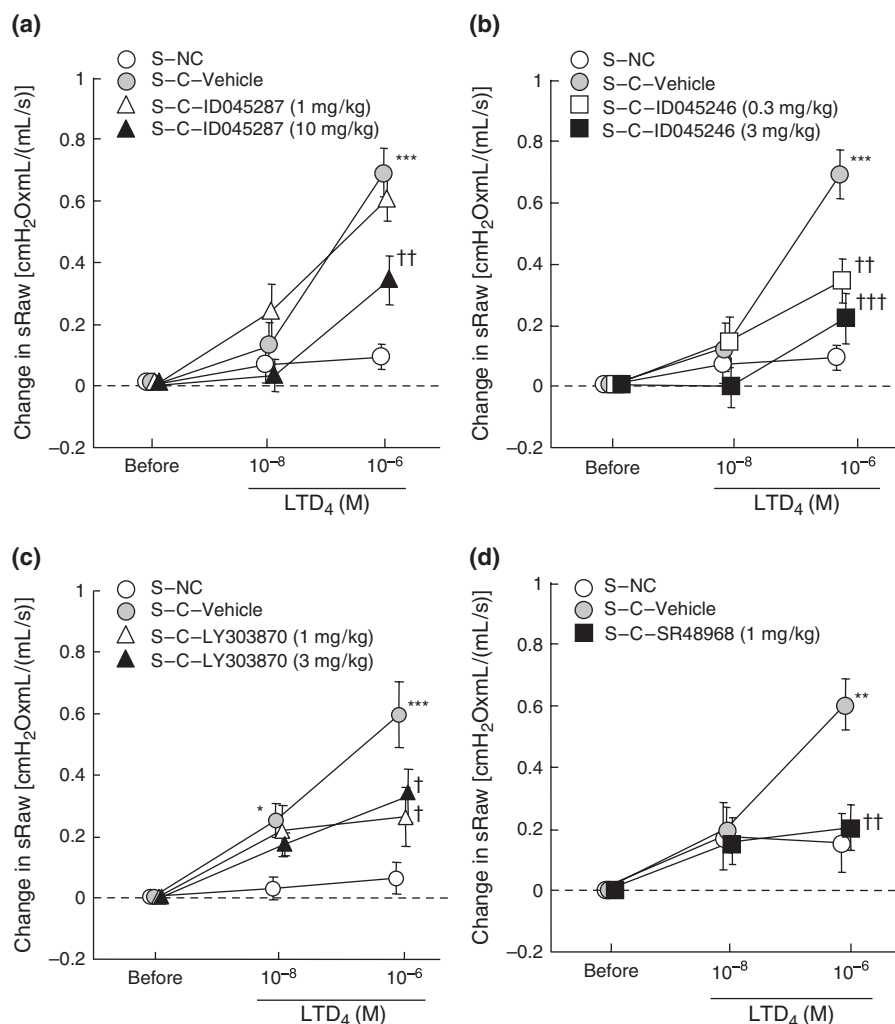


Fig. 3. Effect of the specific NK₁ receptor antagonists (a) ID045287 and (c) LY303870, and the NK₂ receptor antagonists (b) ID045246 and (d) SR48968, on the development of nasal hyperresponsiveness (NHR) induced by the 17th or 18th pollen challenges in sensitized guinea-pigs. Vehicle, ID045287 (1 and 10 mg/kg), ID045246 (0.3 and 3 mg/kg), LY303870 (1 and 3 mg/kg) or SR48968 (1 mg/kg) were administered i.p. 0.5 h before the challenge. Nasal responsiveness to leukotriene (LT) D₄ was assessed 2 days after the challenge. Each point represents the mean $\pm$ SE of 8–16 animals. sRaw, specific airway resistance; S-NC, sensitized–non-challenged; S-C, sensitized–challenged. * P < 0.05, ** P < 0.01, *** P < 0.001 compared with the S-NC group. † P < 0.05, †† P < 0.01, ††† P < 0.001 compared with the S-C–Vehicle group.

SP and NKA concentrations in the NCLF were significantly increased starting from 20 min until 1 h after the challenge. These results strongly suggest that SP and NKA are released and exert their effects within at least 2.5 h after the challenge, thereby leading to the development of NHR.

To examine these results further, we performed pharmacological experiments that used specific antagonists and which revealed that both NK₁ and NK₂ receptor activations were involved in the development of NHR. These experiments showed that specific NK₂ antagonists almost completely suppressed the development of NHR, whereas the NK₁ antagonists did not produce complete inhibition even when there were increases in the doses administered. Therefore, with regard to the development

of NHR, activation of the NK₂ receptor appears to be much more important than NK₁ receptor activation.

Additionally, exogenous instillation of either SP or NKA also induced NHR, which was almost completely inhibited by a NK₂ antagonist and only partially inhibited by a NK₁ antagonist. Although it has been generally accepted that SP and NKA have high affinities for the NK₁ and NK₂ receptors, respectively [25, 26], our study indicates that NHR development is due to these tachykinins preferentially stimulating the NK₂ receptors. Collectively, in cases of both endogenous and exogenous tachykinin-induced NHR, activation of NK₂ receptors may be the preferential mechanism. In asthmatic responses, it has been reported that NK₂ receptor activation plays a major role in IL-5-induced airway hyperresponsiveness in guinea-pigs [27].

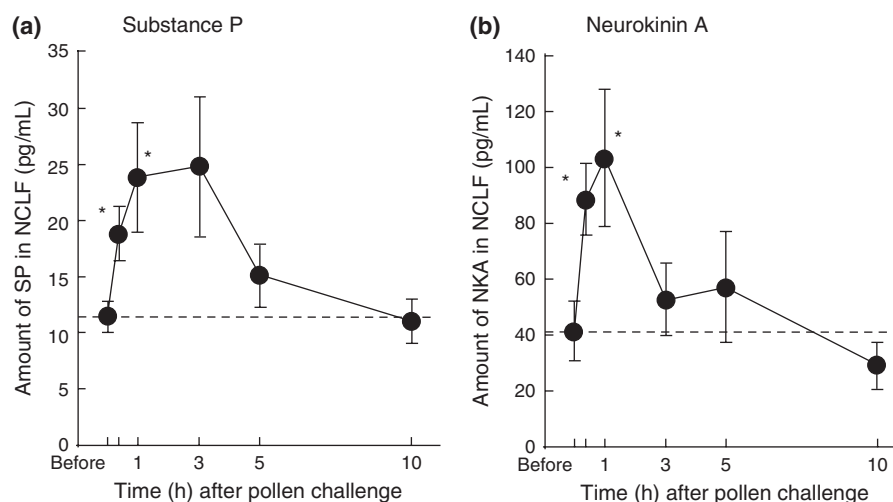


Fig. 4. Time-course changes for the amount of (a) substance P (SP) and (b) neurokinin A (NKA) in the nasal cavity lavage fluid (NCLF) after the 13th pollen challenge in sensitized guinea-pigs. Each point represents the mean $\pm$ SE of 10–13 animals. * $P < 0.05$ as compared with the value before the challenge.

To the best of our knowledge, the present findings are the first to demonstrate the efficacy of tachykinin antagonists on upper airway hyperresponsiveness.

However, we have previously reported that kinins, such as bradykinin (BK) and des-Arg⁹-BK, play important roles in the development of NHR because both the B₁ and B₂ receptor antagonists can effectively suppress the induction of NHR. In addition, we have also found that the intranasal application of either BK or des-Arg⁹-BK caused NHR [23], similar to the results obtained after the SP or NKA treatments in the current study. The involvement of BK in development of NHR has also been reported in patients with AR [28]. SP has been found to be released *in vivo* following a nasal challenge using BK in allergic patients [29]. In our previous study, pollen challenges caused increases in the amounts of BK in the NCLF, with peak levels occurring at 10 min after the challenge [23]. These results suggest that BK is immediately generated after the challenge and stimulates the sensory nerve endings to secondarily release SP and NKA. In our AR model, this mechanism is most probably an axon-reflex mechanism. The NK₁ and NK₂ dual antagonist ID034554 potently suppressed the intranasal BK-induced NHR in our preliminary study (unpublished data). This suggests that an axon-reflex mechanism does indeed exist in the nasal tissue. However, to completely confirm this, we need to further evaluate the detailed relationship between kinins and tachykinins during the NHR acquisition process.

The current experiments were not able to elucidate the mechanisms by which kinins and tachykinins cause NHR. In general, the actions of these peptides on target organs are recognized to be transient and not persistent. In our model, the NHR persisted for at least 2 days. On the other hand, although nasal responsiveness was increased in response to histamine [8], we were not able to detect any

up-regulation of the H₁ receptors in the nasal mucosa of the sensitized-challenged guinea-pigs (N. Mizutani et al., unpublished data). In addition, the NHR was non-specifically recognized in response to not only histamine [8] and LTD₄ [9], but also the thromboxane A₂ mimetic U-46619 [10, 20]. As has been reported elsewhere [30], we also speculated that one of the mechanisms for NHR involves an increased penetration of exogenous molecules across the nasal epithelial layer. SP, NKA and kinins that are released early after the pollen challenge may be involved in this proposed mechanism. However, in order to elucidate this potential mechanism, further research will need to be undertaken.

In contrast to our findings in the sensitized-challenged animals, the non-sensitized guinea-pigs did not exhibit NHR after the intranasal application of either SP or NKA. Based on these findings, we can conclude that multiple challenges with pollen may create a chronic inflammatory condition in the nasal tissues, in which the nasal target cells are easily activated by exogenous tachykinins. As has been reported in a rat airway inflammation model, tachykinin receptors are up-regulated in latent airway inflammation [31]. However, our preliminary radio-binding study involving the nasal mucosa did not show any evidence of an up-regulation of the NK₁ and NK₂ receptors in the tissue of the sensitized-challenged animals (data not shown). Nadel [32] suggested an alternative explanation in which the neutral endopeptidase may be down-regulated in the nasal mucosa by multiple antigen challenges. Therefore, in our model, the degradation of exogenously applied tachykinins might be decreased.

In conclusion, the release of SP and NKA within 2.5 h after the pollen challenge mediates the development of NHR by preferentially activating NK₂ receptors. However, a recent clinical trial has also revealed that an

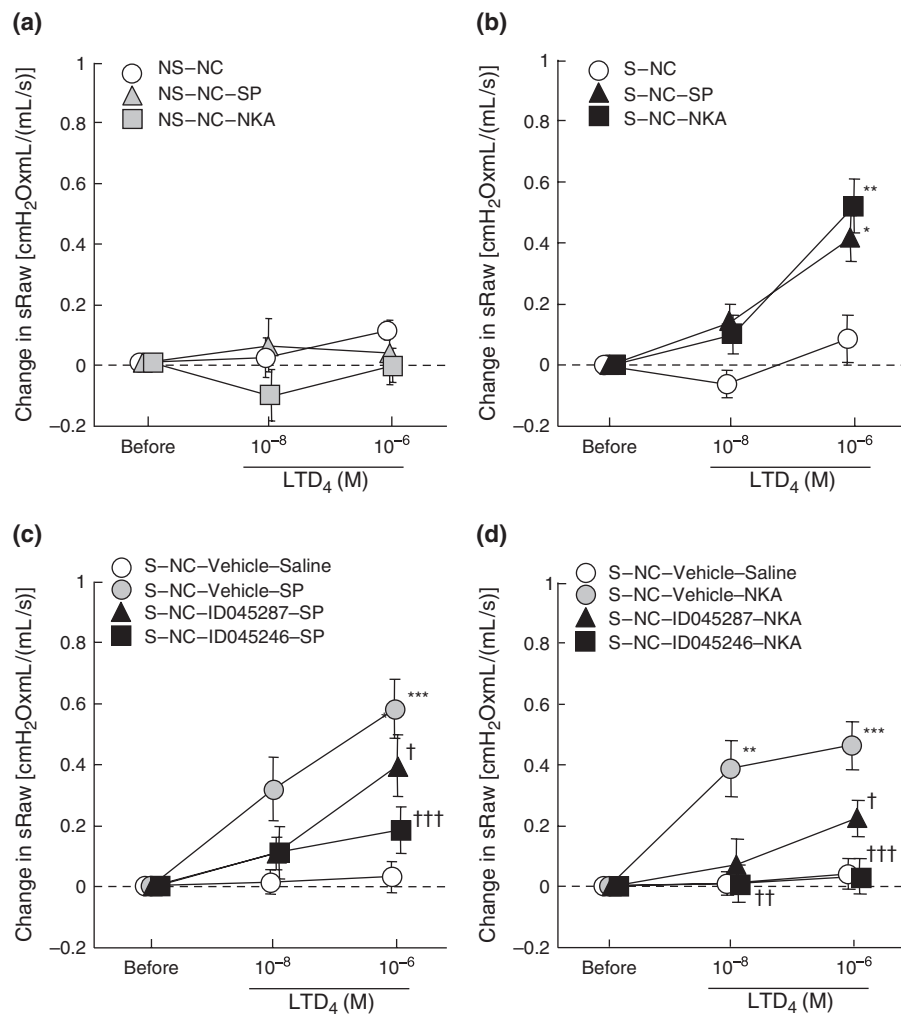


Fig. 5. Substance P (SP)- and neurokinin A (NKA)-induced nasal hyperresponsiveness (NHR) in (a) non-sensitized–non-challenged (NS–NC) and (b) sensitized–non-challenged (S–NC) guinea-pigs, and the effects of newly developed specific NK₁ and NK₂ receptor antagonists, ID045287 and ID045246, respectively, on the induction of NHR by (c) SP and (d) NKA. Saline, SP (10^{-3} M) or NKA (10^{-3} M) were intranasally instilled in NS–NC (a), and S–NC (b) guinea-pigs on day 7 after the 20th pollen challenge. In all cases, the nasal responsiveness to leukotriene (LT) D₄ was assessed on day 2 after the tachykinin treatment. Vehicle, ID045287 (10 mg/kg) or ID045246 (3 mg/kg) were administered i.p. 0.5 h before the intranasal treatment with SP or NKA. Each point represents the mean $\pm$ SE of 8–18 animals. sRaw, specific airway resistance. * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the S–NC–Saline or the S–NC–Vehicle–Saline group. $^{\dagger}P$ < 0.05, $^{\dagger\dagger}P$ < 0.01 and $^{\dagger\dagger\dagger}P$ < 0.001 compared with the S–NC–Vehicle–SP or the S–NC–Vehicle–NKA group.

NK₁ and NK₂ dual antagonist does not improve the lower airway hyperresponsiveness and the lung inflammation in asthmatic patients [33]. Therefore, our present findings suggest that both the NK₁ and NK₂ dual antagonists and the NK₂ receptor antagonists might be applicable for use as therapeutic agents for the upper airway disease AR.

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References

- Iliopoulos O, Proud D, Adkinson NF Jr *et al*. Relationship between the early, late, and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. *J Allergy Clin Immunol* 1990; **86**:851–61.
- Pelikan Z. Late and delayed response of the nasal mucosa to allergen challenge. *Ann Allergy* 1978; **41**:37–47.
- Naclerio RM. Allergic rhinitis. *N Engl J Med* 1991; **325**:860–9.
- Kanthawatana S, Maturim W, Fooanant S, Manorot M, Trakultivakorn M. Evaluation of threshold criteria for the nasal

- histamine challenge test in perennial allergic rhinitis. *Asian Pac J Allergy Immunol* 1997; 15:65–9.
- 5 Walden SM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Naclerio RM. Antigen-provoked increase in histamine reactivity. Observations on mechanisms. *Am Rev Respir Dis* 1991; 144:642–8.
 - 6 Nabe T, Shimizu K, Mizutani N *et al.* A new model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs. *Jpn J Pharmacol* 1997; 75:243–51.
 - 7 Nabe T, Mizutani N, Shimizu K, Takenaka H, Kohno S. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. *Inflamm Res* 1998; 47:369–74.
 - 8 Mizutani N, Nabe T, Sasaki K, Takenaka H, Kohno S. Nasal hyperresponsiveness to histamine induced by repetitive exposures to cedar pollen in guinea pigs. *Eur Respir J* 1999; 14:1368–75.
 - 9 Mizutani N, Nabe T, Imai A, Sakurai S, Takenaka H, Kohno S. Markedly increased nasal blockage by intranasal leukotriene D₄ in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. *Jpn J Pharmacol* 2001; 86:170–82.
 - 10 Tanaka Y, Mizutani N, Fujii M, Nabe T, Kohno S. Different mechanisms between thromboxane A₂- and leukotriene D₄-induced nasal blockage in guinea pigs. *Prostaglandins Other Lipid Mediat* 2006; 80:144–54.
 - 11 Mizutani N, Nabe T, Takenaka H, Kohno S. Acquired nasal hyperresponsiveness aggravates antigen-induced rhinitis in the guinea pig. *J Pharmacol Sci* 2003; 93:437–45.
 - 12 Lundberg JM, Hokfelt T, Martling C, 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.
 - 13 Barnes PJ. Asthma and an axon reflex. *Lancet* 1986; 1:242–5.
 - 14 Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. *J Allergy Clin Immunol* 1990; 86:620–7.
 - 15 Konno A, Numata T, Terada N, Hanazawa T, Nagata H, Motosugi H. Role of substance P in the vascular response of nasal mucosa in nasal allergy. *Ann Otol Rhinol Laryngol* 1996; 105:648–53.
 - 16 Evangelista S, Paoli S, Giachetti A, Manzini S. Involvement of tachykinin NK₁ receptors in plasma protein extravasation induced by tachykinins in the guinea pig upper airways. *Neuropeptides* 1997; 31:65–70.
 - 17 Bertland C, Geppetti P. Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. *Trends Pharmacol Sci* 1996; 17:255–9.
 - 18 Nabe T, Shinoda N, Yamashita K, Yamada M, Yamamura H, Kohno S. Comparative studies on nebulizers for antigen inhalation in experimental asthma. *Allergol Int* 1997; 46:261–7.
 - 19 Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* 1979; 46:399–406.
 - 20 Yamasaki M, Mizutani N, Sasaki K *et al.* Involvement of thromboxane A₂ and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. *Inflamm Res* 2001; 50:466–73.
 - 21 Yamasaki M, Sasaki K, Mizutani N *et al.* Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. *Inflamm Res* 2001; 50:474–82.
 - 22 Narita S, Asakura H, Shirasaki H, Kataura A. Effects of a cysteinyl leukotriene antagonist, ONO-1078 (pranlukast), on total airway resistance after antigen challenge in sensitized guinea pigs. *Inflamm Res* 1997; 46:143–6.
 - 23 Sugahara S, Nabe T, Mizutani N, Takenaka H, Kohno S. Kinins are involved in the development of allergic nasal hyperresponsiveness in guinea pigs. *Eur J Pharmacol* 2003; 476:229–37.
 - 24 Fehder WP, Ho WZ, Campbell DE *et al.* Development and evaluation of a chromatographic procedure for partial purification of substance P with quantification by an enzyme immunoassay. *Clin Diagn Lab Immunol* 1998; 5:303–7.
 - 25 Regoli D, Drapeau G, Dion S, D'Orleans-Juste P. Pharmacological receptors for substance P and neurokinins. *Life Sci* 1987; 40:109–17.
 - 26 Maggi CA, Patacchini R, Rovero P, Giachetti A. Tachykinin receptors and tachykinin receptor antagonists. *J Auton Pharmacol* 1993; 13:23–93.
 - 27 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.
 - 28 Turner P, Dear J, Scadding G, Foreman JC. Role of kinins in seasonal allergic rhinitis: icatibant, a bradykinin B₂ receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen. *J Allergy Clin Immunol* 2001; 107:105–13.
 - 29 Baumgarten CR, O'Connor A, Dokic D, Schultz KD, Kunkel G. Substance P is generated *in vivo* following nasal challenge of allergic individuals with bradykinin. *Clin Exp Allergy* 1997; 27:1322–7.
 - 30 Numata T, Konno A, Terada N *et al.* Time course of changes in absorption of macromolecule through the nasal mucosa after antigen challenge in guinea pig model of allergic rhinitis. *Ann Otol Rhinol Laryngol* 2000; 109:1120–4.
 - 31 Xue J, Zhao H, An Y, Liang A, Zhao C. Expression of substance P receptor mRNA in nasal mucosa of rat in allergic rhinitis model. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 2000; 35:247–50.
 - 32 Nadel JA. Decreased neutral endopeptidases: possible role in inflammatory diseases of airways. *Lung* 1990; 168 (Suppl.): 123–7.
 - 33 Boot JD, de Haas S, Tarasevych S *et al.* Effect of an NK₁/NK₂ receptor antagonist on airway responses and inflammation to allergen in asthma. *Am J Respir Crit Care Med* 2007; 175:450–7.