

Full Paper

Acquired Nasal Hyperresponsiveness Aggravates Antigen-Induced Rhinitis in the Guinea Pig

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Abstract. Whether a state of nasal hyperresponsiveness influences antigen-induced biphasic nasal blockage and sneezing were examined using a guinea pig model of allergic rhinitis. Sensitized animals were challenged with an antigen, Japanese cedar pollen, once every week. Before the 13th challenge, the animals were randomly divided into 2 groups, and then the 13th challenge was performed (Groups A-0 and B-0). The 14th challenge was done on day 2 (Group A-2) and on day 7 (Group B-7) after the 13th challenge, on which nasal hyperresponsiveness was present and absent, respectively. Biphasic nasal blockage and sneezing after the challenge in Group A-2 were more severe than those in Group A-0, while those of Group B-7 were almost the same as those of Group B-0. An anti-histaminic, mepyramine, inhibited sneezing but not the biphasic nasal blockage in Group B-7. A cysteinyl leukotriene (CysLT) antagonist, pranlukast, suppressed the late nasal blockage but not the early blockage and sneezing in Group B-7. In contrast, in Group A-2, mepyramine significantly attenuated not only sneezing but also the early nasal blockage. Pranlukast significantly inhibited both nasal blockage and sneezing in Group A-2. In conclusion, nasal hyperresponsiveness aggravated the antigen-induced nasal responses, to which histamine and CysLTs considerably contributed.

Keywords: hyperresponsiveness, allergic rhinitis, histamine, cysteinyl leukotriene, nasal blockage

Introduction

When patients with allergic rhinitis are exposed to the relevant allergen, over 90% show an immediate response with sneeze, rhinorrhea and nasal blockage; in addition, approximately 50% further develop a late phase reaction with the predominant symptom being nasal blockage (1–3). Because sneezing and watery rhinorrhea in allergic rhinitis could be inhibited by treating patients with antihistaminics, these symptoms are triggered mainly via the stimulation of nasal terminals of the trigeminal nerve by histamine (4). Although the detailed sequence of events leading to the occurrence of allergic nasal blockage are not yet fully elucidated, it has been reported that a cysteinyl leukotriene (CysLT)-receptor antagonist reduced nasal blockage (5, 6).

In addition to the specific antigen-induced nasal

symptoms, the state of nasal hyperresponsiveness to stimuli other than the relevant antigen is one of the characteristic features of patients with allergic rhinitis. In individuals with pollinosis, in whom the antigen exposure period and antigen-free period can be clearly distinguished, nasal hyperresponsiveness is frequently observed during the pollen season, but not during the off-season (7). Furthermore, clinical observations have revealed that allergic rhinitis patients show nasal hyperresponsiveness to histamine and LTD₄ (8–10). Since these chemical mediators have been reported to be involved in the induction of antigen-induced nasal responses (11, 12), it is important to evaluate whether antigen-induced nasal responses are intensified when nasal hyperresponsiveness is being developed. However, due to limitations associated with clinical research, an adequate experimental model is indispensable for analysis of this vital issue.

Recently, we established a Japanese cedar pollen-

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induced reproducible allergic rhinitis model in guinea pigs, which showed biphasic elevations of specific airway resistance (sRaw) (nasal blockage) and immediate sneezing at antigen (the pollen) inhalation challenge (13). Furthermore, in this model, nasal hyperresponsiveness to histamine gradually developed in response to repeated pollen inhalation challenge, and it was considerably short-lived: clearly recognized at 10 h and 2 days after a pollen challenge but disappeared by day 7 (14). In addition, we recently found that the sensitized-challenged animal shows hyperresponsiveness to LTD₄, demonstrating similar appearance and disappearance patterns to the case of histamine (15). The magnitude of the hyperresponsiveness to LTD₄ was greater than that to histamine (14, 15). In general, nasal hyperresponsiveness in patients with seasonal allergic rhinitis disappears within a short period following the elimination of antigen. Thus, our guinea pig experimental model can be useful for analyzing the mechanism of nasal hyperresponsiveness.

The present study investigates how the severity of allergic rhinitis, as indicated by nasal blockage and sneeze, is altered in the presence of nasal hyperresponsiveness in the guinea pig model. In addition, the contributions of histamine and CysLTs to their symptoms was evaluated using a classical histamine H₁-receptor antagonist, mepyramine, and a CysLT-receptor antagonist, pranlukast.

Materials and Methods

Animals

Male, 3-week-old, Hartley guinea pigs weighing 250–300 g were purchased from Japan SLC, Hamamatsu. The animals were housed in a temperature-controlled room at 23 ± 1°C and 60 ± 10% humidity, illuminated from 08:00–20:00 h. They were fed a standard laboratory diet and given water ad libitum. The first sensitization was started 2 weeks after the purchase.

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

Materials

Reagents and their sources were as follows: histamine dihydrochloride and leukotriene (LT) D₄ (Wako Pure Chem., Osaka); pranlukast hemihydrate (donated from the laboratory of Ono Pharm. Co., Ltd., Osaka); mepyramine maleate (Sigma Chem., St. Louis, MO, USA); and lidocaine hydrochloride (Fujisawa Pharm. Co., Ltd., Osaka). The other reagents used were the highest grade of commercial products available.

LTD₄ in physiological saline containing 0.5% ethanol

and histamine in physiological saline were prepared. Pranlukast and mepyramine were suspended in 0.5% methylcellulose.

Japanese cedar (*Cryptomeria japonica*) pollens were harvested by ourselves in Shiga and Gifu prefectures, Japan. Cedar pollen extracts used for the sensitization were prepared as previously described (13). Al(OH)₃ gels were prepared from 0.25 N NaOH and 0.25 N Al₂(SO₄)₃, as previously described (16).

Study design

The protocol followed in this study is shown in Fig. 1. Guinea pigs that had been sensitized with pollen extract plus Al(OH)₃ were repeatedly challenged with the cedar pollen once every week. Before the 13th challenge, the sensitized guinea pigs were randomly divided into two groups, Groups A and B, and nasal responsiveness to intranasal histamine and LTD₄ was estimated 2 (Group A-2) and 7 (Group B-7) days after the 13th challenge in comparison with that of non-sensitized-non-challenged animals. In separate experiments, guinea pigs of Groups A and B were challenged (the 14th challenge) with the antigen 2 (Group A-2) and 7 (Group B-7) days after the 13th challenge, respectively. The 14th antigen-induced biphasic nasal blockage and sneeze in Groups A-2 and B-7 were compared in terms of degree with those associated with the 13th antigen-challenge-induced response (Group A-0 and B-0), and that of non-sensitized-challenged guinea pigs, which were forced to inhale the pollen on the day corresponding to the 13th challenge. In addition, the effects of mepyramine and pranlukast on the 14th antigen-induced biphasic nasal blockage and sneeze were examined using Groups A-2 and B-7.

Sensitization and challenge

As previously described (13), guinea pigs were bilaterally intranasally sensitized by instillation with 3 µL per nostril of cedar pollen extracts adsorbed on Al(OH)₃ gel at 1 µg protein/mg Al(OH)₃/10 µL twice daily for 7 days. Prior to each sensitization, the upper airway mucosal surface was topically anesthetized by subjecting the animal to a 5-min inhalation of a 4% lidocaine hydrochloride mist, which was generated with an ultrasonic nebulizer (NE-U12; Omron, Osaka). This procedure, providing effective sensitization by prolonged retention of the antigen plus Al(OH)₃ in the nasal cavity, was employed based on the research findings that lidocaine reduces the ciliary beat frequency of the guinea pig airway in vitro (17) and that topical anesthetic drugs do not decrease mucosal absorbency (18). Then, the sensitized animal was bilaterally intra-

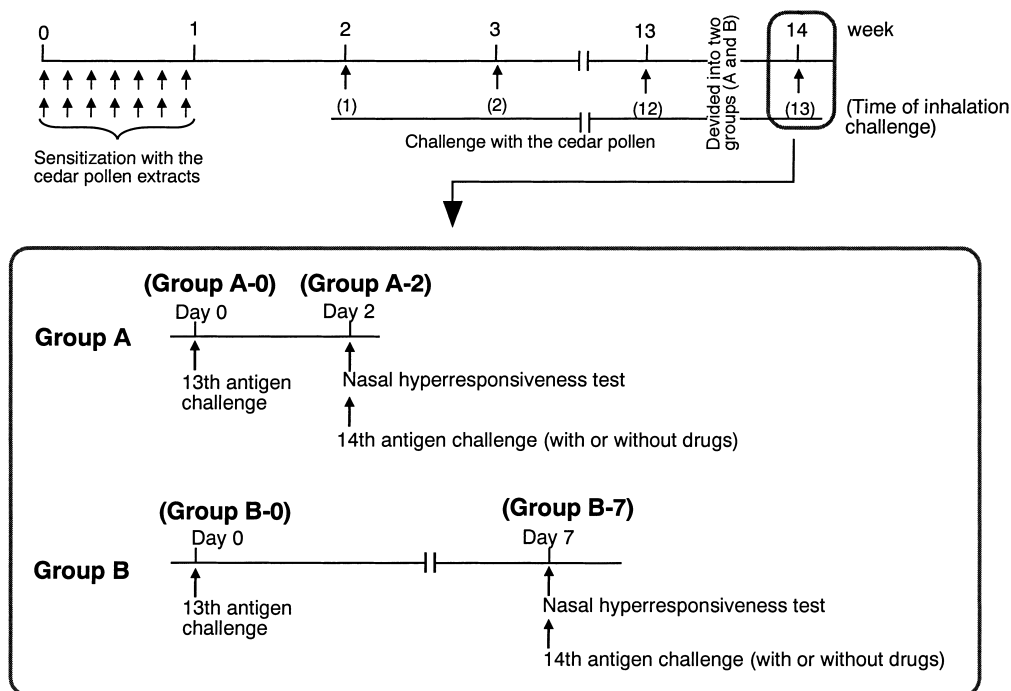


Fig. 1. Experimental protocol in this study. The guinea pigs were divided into two groups (Groups A and B) after the 12th antigen challenge. Seven days after the 12th challenge, both groups were subjected to the 13th challenge (Groups A-0 and B-0). Then the nasal hyperresponsiveness test for histamine and LTD₄ or the 14th challenge was performed 2 (Group A-2) and 7 days (Group B-7) after the 13th challenge.

nasally challenged by quantitative inhalation of the cedar pollen at a dose of 1.8 mg/each nostril by using a hand-made inhalation apparatus (19).

Measurement of sRaw

sRaw was measured by a two-chambered, double-flow plethysmograph system according to the method of Pennock et al. (20). In brief, an animal was placed with its neck extending through the partition of a two-chambered box, and sRaw was measured with the Data analyser Pulmos-I (M.I.P.S., Osaka) after detection of the airflow by sensors attached to both the front and rear chambers. Time-course changes of sRaw were measured after the respective 13th and 14th challenges.

Elevations of sRaw after pollen inhalation in the sensitized guinea pig appeared to reflect total (upper and lower) airway resistance because 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 (19). In addition, although it has been reported that the early bronchoconstrictor response is characterized by rapid and shallow breathing in a guinea pig asthma model (21), the pollen inhalation challenge-induced elevation of sRaw correlates well with the decrease in respiratory frequency and is accompanied

by deep breathing in the present experimental allergic rhinitis model (13). Furthermore, after the pollen inhalation challenge, accumulation of eosinophils in the lung, which is characteristic of allergic bronchial asthma, was completely absent (S. Kohno et al., unpublished data). Thus, it can be concluded that the changes of sRaw induced by antigen challenge in the present manner exclusively reflect the nasal response and not the lower airway response.

Estimation of nasal responsiveness to histamine and LTD₄

On the 2nd day (in Group A-2) and on the 7th day (in Group B-7) after the 13th pollen inhalation challenge, the nasal airway responsiveness to histamine and LTD₄ were measured as previously described (14, 15). In brief, increasing doses of histamine (10^{-4} and 10^{-2} M) or LTD₄ (10^{-8} and 10^{-6} M) ($10 \mu\text{L}/\text{cavity}$) were consecutively applied bilaterally to the nasal cavities at an interval of 20 min. sRaw was measured before the instillation and 10 min after the vehicle and each dose of the agonists. Magnitudes of responsiveness to histamine and LTD₄ were compared with those of non-sensitized-challenged guinea pigs. Because histamine (14) and LTD₄ (15) induced dose-dependent increases of sRaw in the sensitized-challenged animals at

10^{-6} – 10^{-2} and 10^{-12} – 10^{-6} M, respectively, we used 10^{-4} and 10^{-2} M histamine and 10^{-8} and 10^{-6} M LTD₄ in the present study.

As shown in our previous report (15) and the present literature, administration of LTD₄, a well-known potent bronchoconstrictor, into the nasal cavities of the non-sensitized guinea pig produced no elevation of sRaw even at 10^{-6} M. In addition, Narita et al. (22) reported that most Evans blue dye instilled intranasally was found within the nasal cavity. Thus, it can be concluded that the changes in sRaw induced by instillation of histamine and LTD₄ in the present manner entirely reflect the nasal response and not the lower airway response.

Counting of sneezing frequency

In preliminary experiments, we confirmed differences between the respiratory patterns of sneezing and coughing using the two-chambered, double-flow plethysmograph system in guinea pigs. A cough induced by a forced inhalation of stimuli [for example fine mists of capsaicin solution ($50 \mu\text{M}$)] is characterized by a sudden expiration irrespective of inspiration or expiration immediately beforehand. In contrast, sneezing, which was only observed with the cedar pollen inhalation in sensitized animals, is characterized by an explosive expiration just after a deep inspiration. On the basis of this information, we determined sneezing frequency at 0–10 min and 10 min–1 h after the respective 13th (in Group A-0 and B-0) and 14th (in Group A-2 and B-7) pollen inhalation challenges were determined.

Administration of mepyramine and pranlukast

Mepyramine (10 mg/kg, p.o.) and pranlukast (30 mg/kg, p.o.) were administered 1 h before the 14th challenge in Groups A-2 and B-7. It has been reported that pranlukast at this dose specifically inhibits the respective bronchoconstrictive responses induced by CysLTs, but not those by other agonists in guinea pigs *in vivo* (23).

Data analyses

Statistical analyses were performed by one-way analysis of variance (ANOVA). If a significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. Comparison of the 13th and the 14th antigen-induced responses were performed by the paired *t*-test. A probability value (*P*) of less than 0.05 was considered to be statistically significant.

Results

Nasal hyperresponsiveness to histamine and LTD₄

We first evaluated whether nasal hyperresponsiveness to histamine and LTD₄ was present in Groups A-2 and B-7. Consistent with our previous results (14, 15, 24–27), considerable nasal responsiveness to histamine (Fig. 2a) and LTD₄ (Fig. 2c) was evident compared with those of non-sensitized-non-challenged guinea pig at 2 days after the 13th pollen challenge (Group A-2), yet the nasal hyperresponsiveness to histamine (Fig. 2b) and LTD₄ (Fig. 2d) had disappeared by day 7 (Group B-7). On the other hand, when non-sensitized guinea pigs were forced to inhale the pollen, and then nasal responses to histamine and LTD₄ were assessed 2 days after the exposure, no difference in the responses from

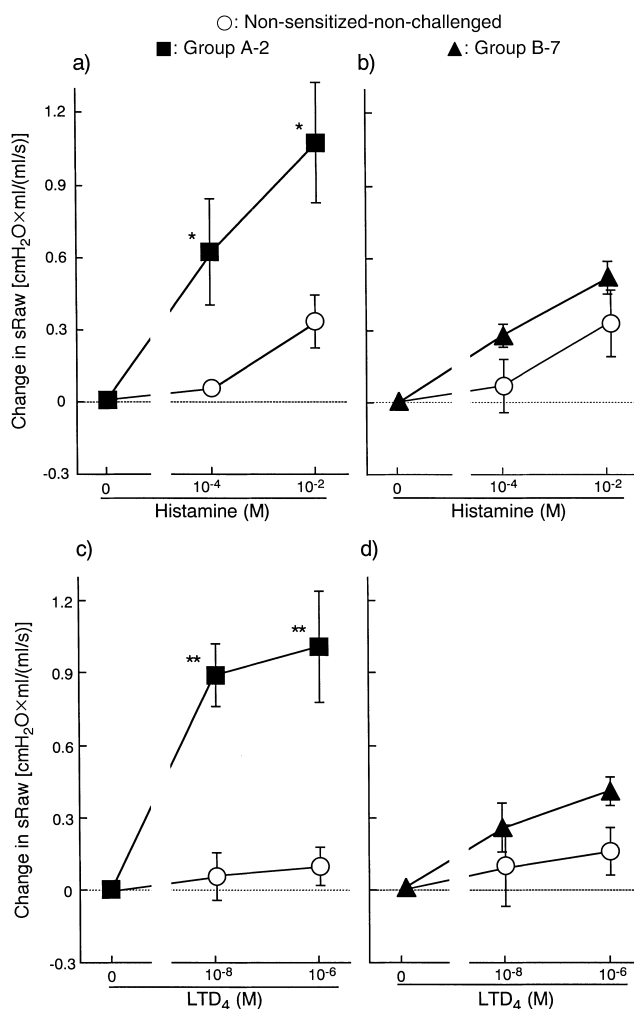


Fig. 2. Nasal hyperresponsiveness to histamine (a and b) and leukotriene (LT) D₄ (c and d); in a and c, Group A-2 and in b and d, Group B-7. Each point represents the mean ± S.E.M. of 7 or 8 animals. Significantly different from the non-sensitized-non-challenged animal (**P* < 0.05, ***P* < 0.01).

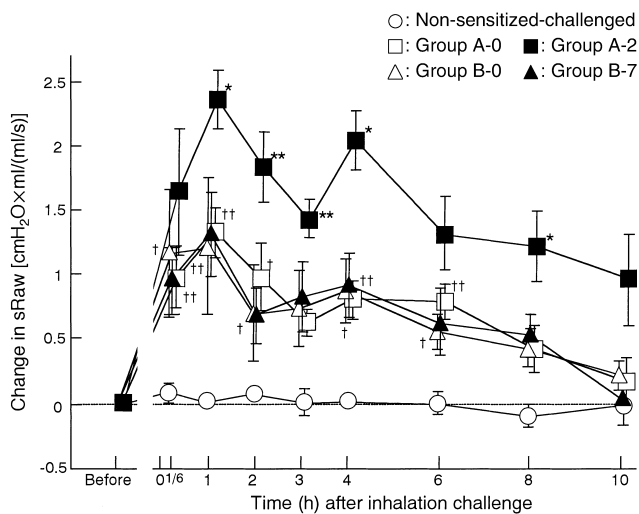


Fig. 3. Time-course changes in specific airway resistance (sRaw) after the 13th (Group A-0 and B-0) and the 14th (Group A-2 and B-7) antigen challenges. Each point represents the mean $\pm$ S.E.M. of 8 animals. Significantly different from the non-sensitized-challenged animal ($^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$) or from Group A-0 ($^*P<0.05$, $^{**}P<0.01$).

non-sensitized-non-challenged animals was observed (14, 15).

Time-course changes of sRaw induced by antigen challenges

As shown in Fig. 3, time-course changes of sRaw following the 13th antigen challenge in Groups A-0 and B-0 were very similar to each other: Biphasic sRaw elevation with peaks at 1 and 4 h, respectively, was induced after the challenge in an almost completely identical fashion as we observed in our previous studies (13, 24–28). This result is a matter of course because sensitized guinea pigs were randomly divided into 2 groups (Groups A-0 and B-0) before the 13th challenge. When the next (14th) challenge was performed 2 days later (Group A-2), both the early and late sRaw elevations were markedly potentiated compared

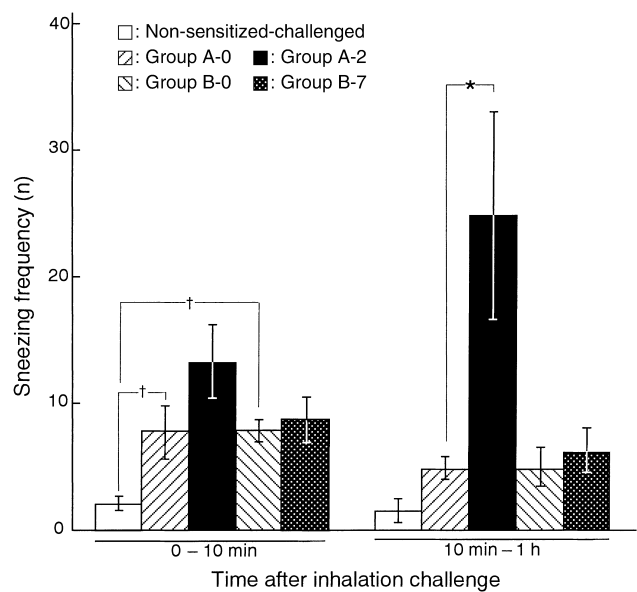


Fig. 4. Time-course changes in sneezing frequency after the 13th (Group A-0 and B-0) and 14th (Group A-2 and B-7) antigen challenges. Each column represents the mean $\pm$ S.E.M. of 8 animals. Significantly different from the non-sensitized-challenged animal ($^{\dagger}P<0.05$) or Group A-0 ($^*P<0.05$).

with responses of Group A-0. However, such potentiation was not observed when the 14th challenge was done 7 days after the 13th challenge (Group B-7) (Fig. 3).

Table 1 shows the area under the response curves (AUCs) for the increase in sRaw at the early (0–3 h) and late (3–10 h) phase in the respective groups. AUCs of both the early and late phase responses at the challenge in Group A-2 were approximately 2 and 2.5 fold greater, respectively, than those in Group A-0. In Group B-7, no increase in AUCs of the early and late sRaw elevations were seen when compared with those in Group B-0.

Time-course changes of sneezing frequency induced by antigen challenges

Sneezing frequencies at 0–10 min and 10 min–1 h

Table 1. Changes in the area under the response curve (AUC) for the increase in specific airway resistance (sRaw) at the early (0–3 h) and late (3–10 h) phases after the 13th and 14th antigen challenges

Group	Time of challenge	Early phase		Late phase	
		AUC	Increase (%)	AUC	Increase (%)
Group A-0	13th	2.89 $\pm$ 0.49	—	3.90 $\pm$ 0.88	—
Group A-2	14th	5.55 $\pm$ 0.60 ^{**}	92	9.68 $\pm$ 1.32 ^{**}	148
Group B-0	13th	2.76 $\pm$ 1.14	—	3.86 $\pm$ 0.58	—
Group B-7	14th	2.37 $\pm$ 0.51	–14	3.52 $\pm$ 1.24	–9

Data are presented as the mean $\pm$ S.E.M. (n = 8). Significantly different from the respective values at the 13th challenge ($^{**}P<0.01$). AUC: area under the response curve for the increase in sRaw.

after the 13th challenge were similarly increased in Groups A-0 and B-0 with significance compared to the negative control group (non-sensitized-challenged) (Fig. 4). When the subsequent 14th challenge was conducted 2 days after the 13th challenge (in Group A-2), a considerably larger number of sneezes were observed particularly at 10 min–1 h in comparison with frequencies of Group A-0 (Fig. 4). On the other hand, no significant difference in the sneezing frequency was observed between Groups B-7 and B-0 (Fig. 4).

Effects of mepyramine and pranlukast on antigen-induced biphasic sRaw elevation in Groups A-2 and B-7

Similar to our previous results (15, 25), neither the early nor late elevation of sRaw induced by the antigen inhalation challenge in Group B-7 was affected by mepyramine, whereas pranlukast potently suppressed the late phase response in Group B-7, while the early response was barely influenced (Fig. 5b). The inhibitory rate of pranlukast on the late response was 76% when calculated from AUCs (Table 2). On the other hand, the early phase response in Group A-2 was significantly suppressed by mepyramine; in addition, the late response also tended to be slightly attenuated by the antihistaminic. Furthermore, pranlukast significantly and markedly inhibited both the early and the late sRaw elevations in Group A-2 (Fig. 5a). As shown in Table 2, AUC at the early phase in Group A-2 was decreased by mepyramine by 31%, and the inhibitory rates of pranlukast on both the early and the late phase were more than 50%.

Effects of mepyramine and pranlukast on antigen-induced sneeze in Groups A-2 and B-7

As in a previous study (25), mepyramine but not pranlukast was found to significantly decrease sneezing frequency at 0–10 min after the challenge by approxi-

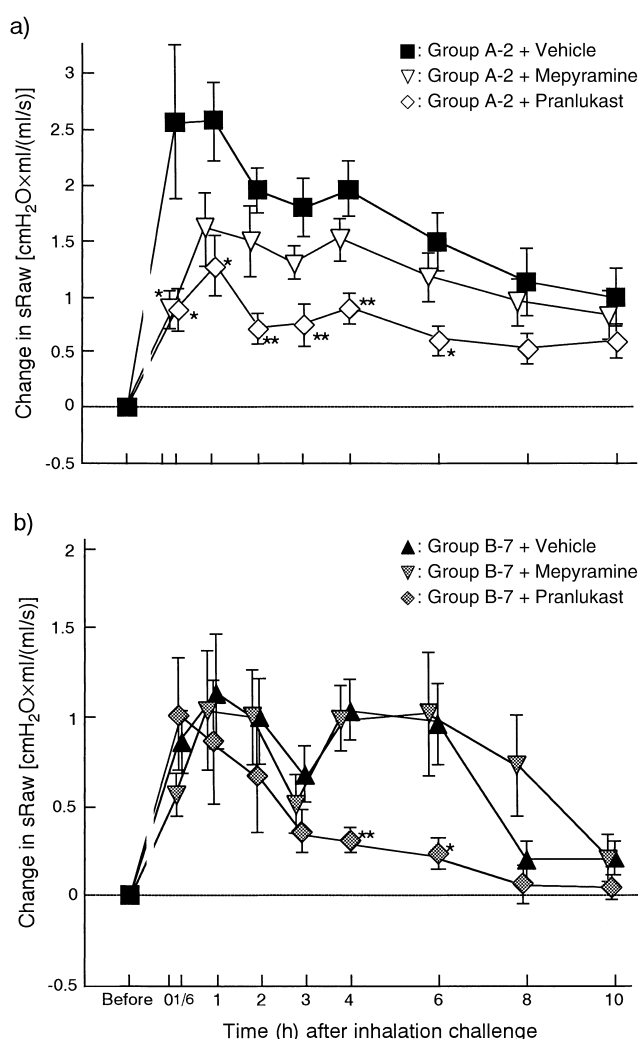


Fig. 5. Effects of mepyramine and pranlukast on the early and late specific airway resistance (sRaw) elevations induced by the 14th antigen challenge in Group A-2 (a) and Group B-7 (b). Mepyramine (10 mg/kg) and pranlukast (30 mg/kg) were administered orally 1 h before the challenge. Each point represents the mean $\pm$ S.E.M. of 10–14 animals. Significantly different from the respective vehicle-treated groups (* P <0.05, ** P <0.01).

Table 2. Effects of mepyramine and pranlukast on the area under the response curve (AUC) for the increase in specific airway resistance (sRaw) at the early (0–3 h) and late (3–10 h) phase in Groups A-2 and B-7

Group	Drug	Early phase		Late phase	
		AUC	Inhibition (%)	AUC	Inhibition (%)
Group A-2	Vehicle	6.50 $\pm$ 0.69	—	10.07 $\pm$ 1.35	—
	Mepyramine	4.98 $\pm$ 0.64*	31	7.97 $\pm$ 1.14	21
	Pranlukast	2.68 $\pm$ 0.46**	59	4.55 $\pm$ 0.77**	55
Group B-7	Vehicle	2.82 $\pm$ 0.43	—	4.46 $\pm$ 0.46	—
	Mepyramine	2.46 $\pm$ 0.49	13	5.23 $\pm$ 1.24	–17
	Pranlukast	2.01 $\pm$ 0.56	29	1.09 $\pm$ 0.39**	76

Mepyramine (10 mg/kg) and pranlukast (30 mg/kg) were administered orally 1 h before the antigen challenge. Data are presented as the mean $\pm$ S.E.M. (n = 10–14). Significantly different from the respective values of vehicle-treated groups (* P <0.05, ** P <0.01). AUC: area under the response curve for the increase in sRaw.

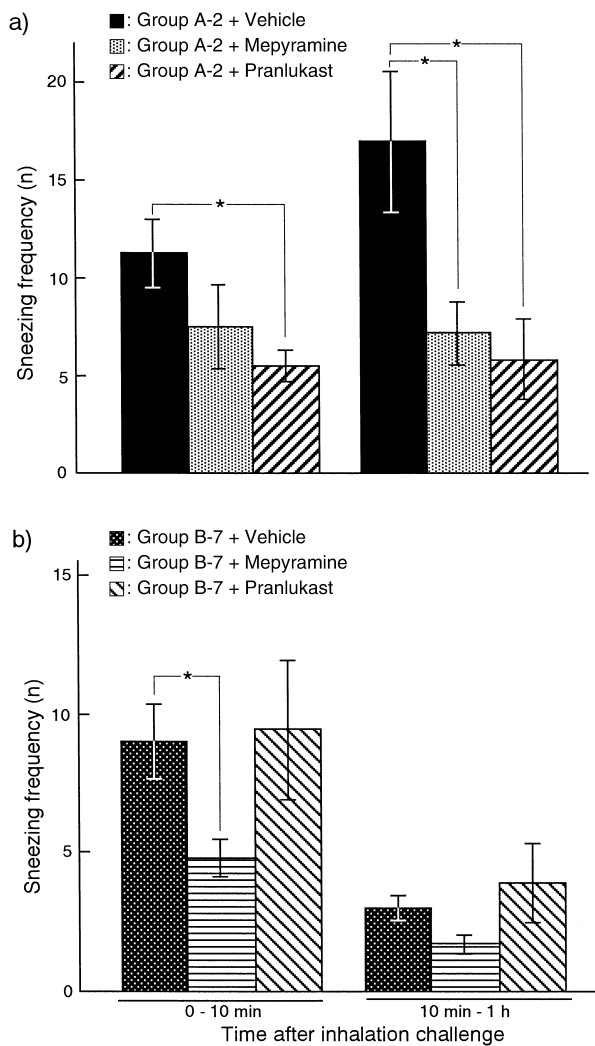


Fig. 6. Effects of mepyramine and pranlukast on the increase of sneezing frequency induced by the 14th antigen challenge in Group A-2 (a) and Group B-7 (b). Mepyramine (10 mg/kg) and pranlukast (30 mg/kg) were administered orally 1 h before the challenge. Each column represents the mean $\pm$ S.E.M. of 10–14 animals. Significantly different from the respective vehicle-treated groups (* P <0.05).

mately 50% in Group B-7. The response at 10 min – 1 h also tended to be suppressed by mepyramine (Fig. 6b). On the other hand, the induction of sneezes within 10 min after the challenge in Group A-2 was suppressed by both mepyramine and pranlukast without and with significance, respectively. In addition, the number of sneezes at 10 min – 1 h was potently and significantly decreased by both mepyramine and pranlukast (Fig. 6a).

Discussion

Consistent with our previous findings (14, 15), hyperresponsiveness to both histamine and LTD₄ were clearly recognized 2 days after the 13th antigen inhala-

tion challenge and then disappeared by the 7th day. When the 14th antigen challenge was performed 2 days after the 13th challenge, the magnitudes of the induced biphasic sRaw elevation and sneeze were more marked than those at the 13th challenge (Group A-2 and A-0). In contrast, the 14th challenge performed 7 days after the previous challenge induced an almost identical degree of nasal response to those at the 13th challenge (Groups B-7 and B-0). These results strongly suggest that the aggravation of the antigen-induced nasal responses is due to the presence of hyperresponsiveness to histamine and LTD₄.

The present results prompted us to investigate whether the respective contribution of histamine and CysLTs to the antigen-induced nasal responses at the 14th challenge in Groups A-2 and B-7 differed from each other. It was found that when nasal hyperresponsiveness was absent, neither the early nor the late phase nasal blockage induced by the antigen was suppressed by mepyramine, while the anti-histaminic potently inhibited the induction of sneeze. In addition, pranlukast produced a strong inhibition of the late phase nasal blockage, yet the early increases of sRaw and sneezing frequency were not attenuated by it. In contrast, when nasal hyperresponsiveness was recognized, early phase nasal blockage was moderately suppressed by mepyramine. Furthermore, pranlukast potently suppressed not only the late but also the early phase nasal blockage. More interestingly, the occurrence of sneeze in the presence of hyperresponsiveness was strongly abolished by pranlukast as well as by mepyramine. These pharmacological experiments indicate that the contribution of chemical mediators to the induction of allergic nasal symptoms was altered when nasal hyperresponsiveness was present.

In several experimental allergic rhinitis models of guinea pigs reported to date (22, 29) including ours (15, 25), antigen-induced late phase nasal blockage was markedly suppressed by pranlukast, while the early phase nasal obstruction was only slightly suppressed or not affected by the CysLT antagonist. In addition, in a review of the literature (22, 25, 29), we found that antigen-induced sneeze was inhibited by an anti-histamine but not by pranlukast. These reported findings almost completely agree with the present effectiveness of the drugs after the disappearance of hyperresponsiveness, suggesting that the influence of nasal hyperresponsiveness to stimuli on antigen-induced nasal symptoms may be substantially excluded in the other models. On the other hand, a double-blind, placebo-controlled trial of a CysLT antagonist, zafirlukast, in patients with acute seasonal (ragweed) allergic rhinitis revealed that the drug significantly suppressed not only

nasal congestion but also sneezing (5). Based on this finding, CysLTs are involved in the induction of sneeze in the clinical setting. These patients must have been repeatedly exposed to antigen in the pollen season, and thus they might have acquired nasal hyperresponsiveness to CysLTs. From these reports, in experimental allergic rhinitis, the features of the antigen-induced nasal symptoms in the presence of the nasal hyperresponsiveness may be more similar to the pathogenesis shown in the clinical setting than those in the absence of it.

We believe that nasal hyperresponsiveness is closely associated with the intensification of the antigen-induced sRaw elevation. The detailed mechanisms of the occurrence of hyperresponsiveness are not clear. However, to date, we have observed the following: 1) Both histamine- (14) and LTD₄- (15) induced increases of sRaw in the sensitized-challenged animal were mainly due to dilatation of nasal blood vessels through H₁ and CysLT₁ receptor activation, respectively. 2) H₁ receptors in the nasal mucosa tissues in the sensitized-challenged animal should not be upregulated compared with those in the non-sensitized animal because amounts of specific binding of radiolabelled mepyramine to the membrane fraction of the nasal mucosa from the sensitized and the non-sensitized animals were not different from each other (N. Mizutani et al., unpublished data). 3) LTD₄-induced sRaw elevation in the sensitized-challenged guinea pig was potently suppressed by a nitric oxide (NO) synthase inhibitor, *N*^ω-nitro-L-arginine methyl ester (15). 4) Nasal responsiveness to an NO donor, sodium nitroprusside, of the sensitized-challenged guinea pig was the same as that of the non-sensitized animal (15). 5) Amount of NO in the nasal cavity lavage fluid after the intranasal application of LTD₄ in the sensitized-challenged guinea pig was higher than that in the non-sensitized animal (15). In light of these results, we are now considering that NO production from the endothelial cells in the nasal blood vessels in response to chemical mediators may be enhanced at the 2nd day after the antigen challenge.

Regarding involvement of inflammatory cells in the induction of nasal hyperresponsiveness, because eosinophils markedly increased in the nasal cavity lavage fluid after challenges in our model (30), we previously evaluated whether eosinophils recruited are true pathogenic component of the nasal hyperresponsiveness (26). Unexpectedly, when the eosinophilia in the nasal cavity was completely suppressed by a treatment of an anti-IL-5 mAb (TRFK-5), the magnitude of the nasal hyperresponsiveness was not affected (26). Thus, it is strongly suggested that eosinophils are not required for the development of the induction. However, other inflam-

matory cells than eosinophils including lymphocytes and neutrophils that are also increased after the challenge (30) might contribute to the induction of the nasal symptom.

On the other hand, in regard to the enhancement of sneezing in the presence of the hyperresponsiveness, the intensified part mainly consisted of CysLT-mediated response. However, mechanisms of CysLT-induced sneeze are unknown. We previously reported that cedar pollen inhalation induced sneezes by 2 or 3 times even in non-sensitized guinea pigs (13), and thus the inhibitory ratio of mepyramine and pranlukast on the allergic sneezing can be calculated to be both more than 70%, indicating that the two drugs inhibited some common pathways leading to the appearance of sneeze. Therefore, CysLTs may produce sneezes synergistically with histamine.

The cellular source of histamine and CysLTs, which were released immediately after the challenge, is likely to be nasal mucosal mast cells. It may be that the amounts of chemical mediators released from the mast cells in response to the 14th antigen challenge are enhanced at the 2nd day but not the 7th day after the 13th antigen challenge. However, when sensitized guinea pigs were chronically exposed to aerosolized antigen, the amount of histamine anaphylactically released from isolated lung fragments was apparently decreased (31). Thus, it is unlikely that potentiation of anaphylactic production and/or release of chemical mediators from mast cells is induced on day 2 but not day 7. Nevertheless, because we found no evidence on cellular source(s) of CysLTs especially during the late phase nasal blockage, they must be clarified.

In conclusion, the existence of nasal hyperresponsiveness to histamine and LTD₄ were substantially responsible for the aggravation of antigen-induced sneezing and biphasic nasal blockage. Furthermore, the relative contributions of histamine and CysLTs to the appearance of allergic nasal symptoms were considerably altered by the presence of hyperresponsiveness. We believe that the pathogenesis of allergic rhinitis when hyperresponsiveness appeared more closely resembles that of the clinical setting than that without it.

Acknowledgments

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