Mini Review

Involvement of kinins and tachykinins in the development of nasal hyperresponsiveness in a guinea pig pollinosis model

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Although it has been suggested that kinins and tachykinins may be involved in the pathogenesis of allergic rhinitis, inhibitors of these mediators have not yet to be developed for use as therapeutic drugs. Therefore, this study examined whether kinins and tachykinins are involved in the induction of nasal symptoms in a guinea pig pollinosis model.

Sensitized guinea pigs were challenged by forced inhalation of pollen once a week. Sneezing and nasal blockage were observed after pollen challenges. Nasal hyperresponsiveness to an intranasal application of leukotriene (LT) D₄ was assessed 2 days after an antigen challenge.

Neither bradykinin receptor (B₁ and B₂) antagonists nor tachykinin receptor (NK₁ and NK₂) antagonists inhibited sneezing and nasal blockage. In contrast, these antagonists significantly suppressed the development of nasal hyperresponsiveness. An especially strong inhibition was noted for the B₂ receptor and the NK₂ receptor antagonists. Furthermore, exogenous intranasal instillation of kinins (bradykinin and des-Arg¹⁰-kallidin) and tachykinins (neurokinin A and substance P) produced nasal hyperresponsiveness that was similar to the antigen-antibody reaction. On the other hand, immediately after a pollen challenge, an increase was noted in the amount of bradykinin in the nasal cavity lavage fluid, followed by elevation of tachykinin levels.

These results indicate that kinins and tachykinins mediate the development of nasal hyperresponsiveness by preferentially activating B₂ and NK₂ receptors. Therefore, these receptor antagonists may prove effective in the treatment of allergic rhinitis.

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Introduction
Allergic rhinitis is a typical allergic inflammatory disease, with symptoms that include sneezing, nasal secretion and nasal blockage. As compared to healthy individuals, patients with allergic rhinitis not only have an increased nasal responsiveness to specific allergens but also to chemical or physical stimuli. This increase in nasal reactivity to stimuli that occurs after allergen provocation may be similar to the reaction that is provoked by...
increased sensitivity to histamine\(^1\).

Pharmacological analyses have indicated that histamine plays an important role in the induction of sneezing and rhinorrhea, and that nasal blockage can be partly relieved by antagonists against arachidonic acid metabolites, cysteinyl leukotrienes and thromboxane A\(_2\). However, the mechanisms underlying the development of nasal hyperresponsiveness have yet to be clearly defined.

In order to elucidate the induction mechanisms of this disease, we have developed a Japanese cedar pollen-induced allergic rhinitis model in guinea pigs that simulates the disease symptoms noted in humans\(^2,3\). In the model, a pollen challenge induced immediate sneezing and biphasic nasal blockage\(^2,3\). More interestingly, the nasal blockage response of sensitized animals to intranasal instillation of histamine\(^4\) and leukotriene (LT) D\(_4\)\(^5\) appears to be enhanced in proportion to the number of challenges actually performed. However, the actual mechanisms underlying the development of this nasal hyperresponsiveness have yet to be clearly elucidated. This mini-review focuses on the mechanisms of nasal hyperresponsiveness by kinins and tachykinins.

Roles of kinins in allergic rhinitis

Kinins are well known pro-inflammatory peptides that mediate vascular and pain responses. Two distinct kinin receptor subtypes have been identified and characterized: B\(_1\) and B\(_2\) receptors\(^6\). The B\(_2\) receptor is constitutively expressed, and mediates the action of bradykinin and kallidin (lysyl-bradykinin)\(^7\). On the other hand, the B\(_1\) receptor is expressed at a low level in normal tissues but induced by inflammatory stimuli such as interleukin-1 \(\beta\), and mediates the action of des-Arg\(^9\)-bradykinin and des-Arg\(^10\)-kallidin\(^8\).

Bradykinin and kallidin, are generated in nasal secretions during allergic rhinitis\(^7\). In addition, the intranasal application of these bradykinin B\(_2\) receptor agonists increases nasal airway resistance in individuals with and without rhinitis\(^9\). When assessed at 24 h after an allergen challenge, the bradykinin B\(_2\) receptor antagonist, icatibant, effectively suppressed induction of the nasal hyperresponsiveness to histamine but not the allergen-induced nasal blockage that occurred at 10 min after a challenge in individuals with seasonal allergic rhinitis\(^9\). On the other hand, the bradykinin B\(_1\) receptor agonist, des-Arg\(^10\)-kallidin, did not seem to cause any significant change after intranasal application, even in patients with atopic rhinitis\(^9\). However, it has been demonstrated in animal models that the bradykinin B\(_1\) receptor is engaged during allergic airway inflammation\(^10\). Christiansen et al.\(^11\) reported that patients with allergic rhinitis significantly express more bradykinin B\(_1\) receptor mRNA than normal individuals, which provides further support for the animal study findings.

Based on these previous studies, we decided to further assess whether kinins are involved in the induction of allergic rhinitis symptoms in our guinea pig allergic rhinitis model. We evaluated the effects of the bradykinin B\(_1\) receptor antagonist, icatibant, and the bradykinin B\(_2\) receptor antagonist, des-Arg\(^9\)-[Leu\(^3\)]bradykinin, on both the induction of the allergen-induced sneezing and nasal blockage that are generated early and late after an allergen challenge, and on the development of the nasal hyperresponsiveness to LTD\(_4\). We also evaluated whether exogenous intranasal applications of the bradykinin B\(_1\) receptor agonist, des-Arg\(^10\)kallidin, and the bradykinin B\(_2\) receptor agonist, bradykinin, could induce nasal blockage and cause development of nasal hyperresponsiveness to LTD\(_4\) in sensitized--challenged and in non-sensitized animals. The intranasal application of LTD\(_4\) induced nasal blockage but not sneezing. The exogenous application of kinin agonists also did not induce sneezing in the model.

Fig. 1 Effects of B\(_1\) (Des-Arg\(^9\)-[Leu\(^3\)]bradykinin) and B\(_2\) (icatibant) antagonists on the development of nasal hyperresponsiveness

Des-Arg\(^9\)-[Leu\(^3\)]bradykinin (60 nmol/kg) and/or icatibant (10 nmol/kg) were administered i.v. 5 min before the challenge. \(n = 15-29\).

*** p\(<0.001\) vs. sensitized-nonchallenged (negative control). +,+ and ++++, p\(<0.05\), p\(<0.01\) and p\(<0.001\) vs. control.
Results indicated that neither the B₁ nor B₂ receptor antagonists affected the allergen-induced sneezing and nasal blockage\textsuperscript{13}. In contrast, in agreement with clinical examinations\textsuperscript{9}, the bradykinin B₂ receptor antagonist, icatibant, potently suppressed the development of the nasal hyperresponsiveness to LTD\(_4\) (Fig.1)\textsuperscript{12}. The bradykinin B₁ receptor antagonist, des-Arg\textsuperscript{9}-[Leu\textsuperscript{9}]-bradykinin, also significantly inhibited the induction of the hyperresponsiveness (Fig.1)\textsuperscript{12}. In addition, the intranasal instillation of not only the bradykinin B₂ receptor agonist, bradykinin, but also the bradykinin B₁ receptor agonist, des-Arg\textsuperscript{9}-kallidin, induced increased nasal responsiveness to LTD\(_4\) in the sensitized-challenged guinea pig\textsuperscript{12}. The allergen challenge also immediately induced obvious production of bradykinin, with a peak noted at 10 min (Fig.3)\textsuperscript{12}. These results indicate that both the bradykinin B₁ and B₂ receptor agonists that are most likely endogenously produced by the antigen-antibody reaction are significantly involved in the development of the nasal hyper-responsiveness to LTD\(_4\).

On the other hand, it was disappointing to find that the kinin antagonists had no affect on the antigen-induced sneezing and nasal blockage\textsuperscript{12}. However, the effectiveness of the antagonists on the development of the nasal hyperresponsiveness indicates that the inhibition of the kinin action or generation could be a therapeutic method for allergic rhinitis.

Roles of tachykinins in allergic rhinitis

Tachykinins, such as substance P and neurokinin A, are located in the capsaicin-sensitive sensory nerves of the mammalian respiratory tract\textsuperscript{16}. Excitation of these nerves results in the release of these tachykinins, which produce inflammatory responses in the airway tissues\textsuperscript{16}. Since 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\textsuperscript{16}. While over the last decade it has been suggested that these tachykinin receptor antagonists may have possible application as therapeutic drugs for the treatment of allergic rhinitis\textsuperscript{16}, these antagonists have yet to be developed for clinical use probably because of less effectiveness than existing drugs or limitation of clinical examination.

We have recently had the opportunity to examine new NK\(_1\) and/or NK\(_2\) receptor antagonists, which were developed by our collaborator, Zeria Pharmaceutical Co., Ltd. Tachykinin antagonists can be used to determine whether substance P and/or neurokinin A are involved in the induction of allergic rhinitis symptoms, such as sneezing, biphasic nasal blockage and nasal hyperresponsiveness. In addition, we also evaluated whether exogenous intranasal applications of substance P and neurokinin A could cause development of nasal hyperresponsiveness in response to LTD\(_4\) in sensitized-challenged and non-sensitized animals.
Mechanisms underlying the nasal hyperresponsiveness induced by kinins and tachykinins

In allergic patients, substance P has been found to be released in vivo following a nasal challenge using bradykinin\(^9\). As previously described, pollen challenges can increase the amount of bradykinin in the nasal cavity lavage fluid, with peak levels oc-
currying at 10 min after the challenge (Fig.3)\textsuperscript{12}. These results suggest that bradykinin is immediately generated after such challenges and which leads to a stimulation of the sensory nerve endings that secondarily release substance P and neurokinin A. In our allergic rhinitis model, this mechanism is most probably an axon-reflex mechanism. In our preliminary study (unpublished data), the NK\textsubscript{1} and NK\textsubscript{2} dual antagonist potently suppressed the intranasal bradykinin-induced nasal hyperresponsiveness. These findings suggest that an axon-reflex mechanism does indeed exist within the nasal tissue.

In general, the actions of these peptides on the target organs are recognized to be transient and not persistent. In our model, the nasal hyperresponsiveness persisted for at least 2 days. Conversely, although nasal responsiveness was increased in response to histamine\textsuperscript{4}, we were not able to detect any up-regulation of the H\textsubscript{1} receptors in the nasal mucosa of the sensitized-challenged guinea pigs (Mizutani et al., unpublished data). In addition, nasal hyperresponsiveness was non-specifically recognized in response not only to histamine\textsuperscript{4} and LTDs\textsuperscript{15}, but also to the thromboxane A\textsubscript{2} mimetic U-46619\textsuperscript{20}. Figure 4 presents our proposal about the mechanisms of nasal hyperresponsiveness. As has been reported elsewhere\textsuperscript{21}, we also theorized that another mechanism responsible for the nasal hyperresponsiveness could involve an increased penetration of exogenous molecules across the nasal epithelial layer. In this proposed mechanism, it is possible that substance P, neurokinin A and the kinins are all immediately released after the pollen challenge (Fig.4). However, in order to definitively elucidate this potential mechanism, further research is required.

In contrast to our findings in the sensitized-challenged animals, the non-sensitized guinea pigs did not exhibit any nasal hyperresponsiveness after the intranasal application of either substance P or neurokinin A. 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 for the rat airway inflammation model, tachykinin receptors are up-regulated during latent airway inflammation\textsuperscript{22}. However, our preliminary radio-binding study involving the nasal mucosa did not find any evidence of an upregulation of the NK\textsubscript{1} and NK\textsubscript{2} receptors in the tissue of the sensitized-challenged animals (unpublished data). Nadel\textsuperscript{23} suggested an alternative explanation in which multiple antigen challenges cause down-regulation of the neutral endopeptidase in the nasal mucosa. Therefore, the degradation of the exogenously applied tachykinins in our model might actually be decreased.

In order to re-evaluate the effects of these tachykinin receptor antagonists on allergic rhinitis symptoms, we will need to develop an appropriate experimental model that can express symptoms similar to those that are routinely seen in clinical patients.

**Conclusion**

After a pollen challenge, kinins are released, and substance P and neurokinin A are subsequently released. Ultimately, those
tachykinins are responsible for mediating the development of nasal hyperresponsiveness via preferentially activating the NK₂ receptors. Conversely, a recent clinical trial has revealed that an NK₁ and NK₂ dual antagonist is unable to improve lower airway hyperresponsiveness or lung inflammation in asthmatic patients²⁴. In contrast, our present findings do suggest that both the kinin and the tachykinin antagonists might be applicable for use as therapeutic agents for allergic rhinitis. It is hoped that an experimental model will soon be developed that will accelerate the clinical examination of these antagonists, thereby leading to an optimal treatment for allergic rhinitis.

References


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