

Gastrin-Releasing Peptide Is Involved in the Establishment of Allergic Rhinitis in Mice

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Objective: Gastrin-releasing peptide (GRP) is a neuropeptide that targets transmembrane-type receptors. Its role in allergic rhinitis (AR) has yet to be investigated. The present study utilized the nasal mucosa of AR model mice to examine GRP and GRP receptor (GRPR) expression levels, localization, and other factors to evaluate their role in AR pathology.

Study Design: In vivo study in an animal model.

Methods: GRP and GRPR expression levels were examined in three different AR models established in BALB/c mice. In addition, a GRPR antagonist (RC-3095) was administered to AR mice to investigate its effect. The distribution of GRPR expression on mast cells in the nasal mucosa with AR was examined. Finally, we investigated the inhibitory effect of RC-3095 on allergy symptoms induced by histamine.

Results: GRP and GRPR were highly expressed in the nasal mucosal epithelium and interstitial tissues surrounding the nasal glands in AR groups according to immunostaining. GRP and GRPR expression as determined by western blotting increased in the nasal mucosa as the degree of nasal sensitization increased. In addition, the average counts of sneezing and nasal rubbing after treatment in the AR + RC-3095 group were significantly lower than those in the AR + nasal saline group. Mast cells often colocalized with GRPR around nasal glands. Moreover, RC-3095 was effective in reducing sneezing induced by histamine.

Conclusion: The GRP-GRPR system is likely to be involved in allergic inflammation. This system may represent a novel therapeutic target for refractory AR.

Key Words: Gastrin-releasing peptide, gastrin-releasing peptide receptor, gastrin-releasing peptide receptor antagonist, allergic rhinitis, model mouse.

Level of Evidence: NA.

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INTRODUCTION

Allergic rhinitis (AR) is an IgE-mediated allergic reaction to allergens, such as pollen and house dust mites, in the nasal mucosa. Chemical mediators, such as histamine and leukotrienes, are released from mast cells in response to the antigen–antibody reaction. Such molecules stimulate the sensory nerve endings and blood vessels of the nasal mucosa, causing paroxysmal sneezing, watery rhinorrhea, and nasal blockage. Irritation of the

trigeminal nerve terminals by histamine in the nasal mucosa causes sneezing by stimulating the sneezing center of the medulla oblongata.

Standard treatments for AR include antihistamines and antileukotriene receptor antagonists, as well as topical nasal steroids used as anti-inflammatories to suppress the localized exaggerated immune response of the nasal mucosa. However, some patients do not respond well to these treatments. For such patients, posterior nasal nerve resection may be performed. The working mechanisms of posterior nasal nerve resection involve the suppression of the secretion of proinflammatory neurotransmitters and inhibition of neurogenic inflammation induced by overactive sensory and parasympathetic nerves.¹ Along the same line, rather than performing surgical resection, it may be possible to medically inhibit proinflammatory neurotransmitters in order to manage resistant AR patients. However, it remains unknown which neurotransmitter(s) should be targeted. It has been reported that nerve repulsion factors such as semaphorin 3A,² neuropeptides such as substance P (SP), and calcitonin gene-related peptide (CGRP) are involved in AR.^{3–7} However, their roles in

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AR have not been thoroughly investigated, and they have not yet been applied in a clinical setting.

Gastrin-releasing peptide (GRP), such as SP and CGRP, is a neuropeptide present in sensory nerves. GRP is involved in neuroendocrine regulation, gastrointestinal secretion, immune response, and cell proliferation. The GRP receptor (GRPR) has been reported to mediate the itch sensation in the spinal cord.⁸ Some studies have shown that the GRP-GRPR system is associated with itching in atopic dermatitis.⁹ Furthermore, GRP was found to mediate asthma attacks in two distinct mouse models of asthma: allergic and air pollution-induced.¹⁰ Baraniuk et al. have already reported the localization and function of GRP and GRPR in the nasal mucosa of hypertrophic rhinitis patients. However, the role of GRP in AR has not been investigated.¹¹

Here, we used three mouse models of AR to examine GRP and GRPR expression and their functional relevance to AR symptoms.

MATERIALS AND METHODS

Mice

Six-week-old female BALB/c mice were purchased from CLEA Japan (Tokyo, Japan). These mice received an ovalbumin (OVA)-free diet at the Laboratory Animal Research Center of Kyorin University. All experiments were approved by the Experiment Animal Ethics Committee of Kyorin University (Tokyo, Japan). All mice were maintained under standard conventional conditions, including standard temperature (24–26°C), humidity (50%–60%), and a 12-hour light–dark cycle.

Induction of AR

BALB/c mice were sensitized using OVA (Sigma-Aldrich, St. Louis, MO) and aluminum hydroxide (Alum) (Sigma-Aldrich). In brief, BALB/c mice were sensitized on days 0, 7, and 14 by intraperitoneal injection (IP) of 25 µg OVA with 1 mg of Alum in 200 µL of phosphate-buffered saline (PBS), as previously described.^{12,13} Control group mice received IP of PBS alone. Allergic group mice were administered intranasal drops (IN) of 20 µL PBS containing 500 µg OVA each day as a continuous AR challenge for 14 consecutive days from day 21 to 34, whereas control group mice were administered IN of 20 µL PBS alone.^{12,13} In order to induce AR in the three mouse models and maintain controls, mice were divided into four groups: group A mice (controls) were administered IN of PBS for 2 weeks after IP of PBS; group B mice (sensitization alone) were administered IN of PBS for 2 weeks after IP of OVA; group C mice (mild AR) were administered IN of PBS for 1 week and OVA for 1 week after IP of OVA; and group D mice (full-blown AR) were administered IN of OVA for 2 weeks after IP of OVA (Fig. 1A). There were six mice in each group.

Evaluation of Allergic Symptoms

On day 34, total counts of sneezing and nasal rubbing during the first 10 minutes after last OVA challenge were counted by investigators in a blinded fashion. The actions of the mice were recorded by video camera.¹⁴

Blood Analysis for Determination of Allergic Reaction

A blood sample was taken from each mouse via cardiac puncture under anesthesia with pentobarbital 24 hours after the final

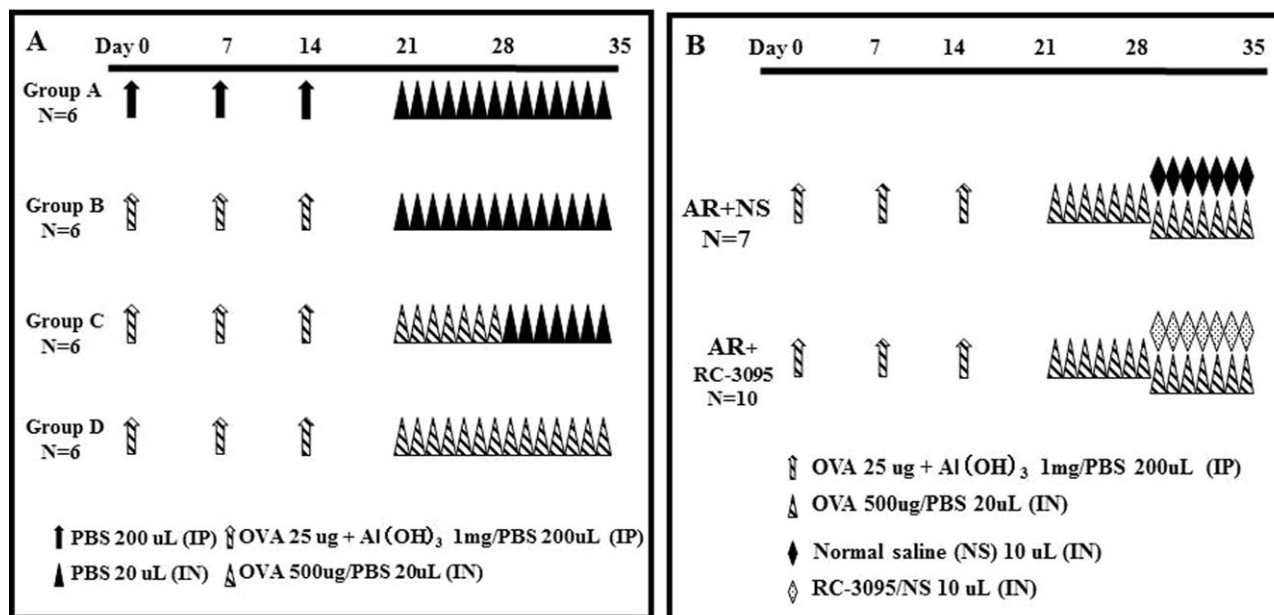


Fig. 1. Experimental protocols used in this study. (A) Protocol for experimental AR model. Allergic mice (group D) were prepared by IP of OVA + Alum on days 0, 7, and 14, followed by daily IN challenge with OVA solution on days 21 through 34. As a control (group A), mice were treated with PBS using the same protocol. Group B was administered IN of PBS for 2 weeks after IP of OVA, and group C mice were administered IN of PBS for 1 week and OVA for 1 week after IP of PBS. Twenty-four hours after the last stimulation, mice were sacrificed to obtain blood and tissue samples. There were six mice in each group. (B) Protocol of intranasal RC-3095 administration in AR mice. Mice were sensitized with OVA on days 0, 7, and 14. RC-3095 (0.3 mg/kg) or NS was administered to the nostrils of sensitized mice at 4 hours prior to OVA intranasal challenge from days 28 to 34. There were seven mice in the AR + NS group and 10 mice in the AR + RC-3095 group. Alum = aluminum hydroxide; AR = allergic rhinitis; IN = intranasal drops; IP = intraperitoneal injection; OVA = ovalbumin; NS = normal saline; PBS = phosphate-buffered saline.

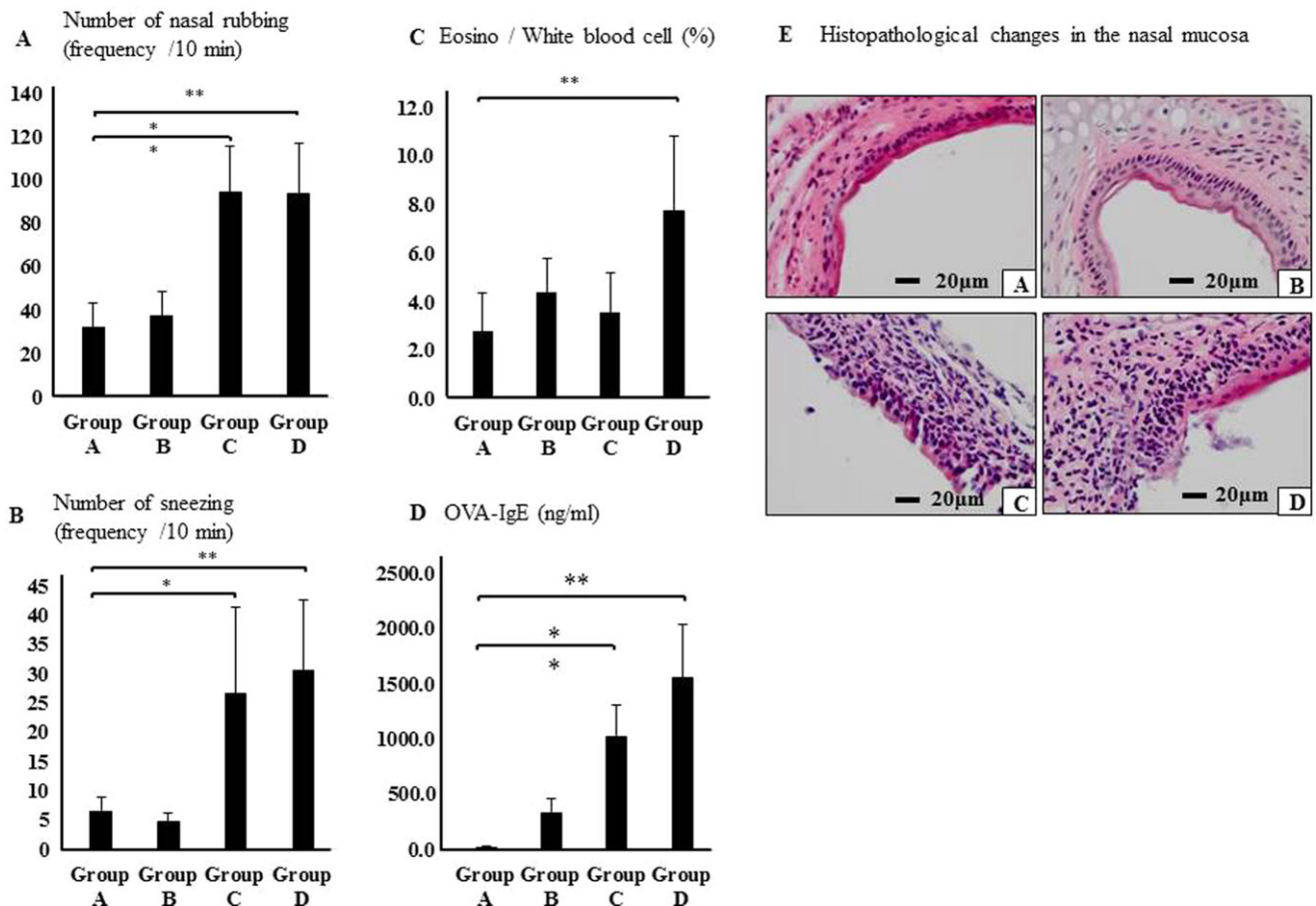


Fig. 2. Allergic symptom scores (A, B), eosinophils (C), serum OVA-IgE levels (D), and histopathological changes in the nasal mucosa (E) in each group. After the last challenge with OVA, instances of nasal rubbing (A) and sneezing (B) were counted for 10 minutes. Percentages of peripheral blood eosinophil and OVA-IgE in serum were elevated in all groups compared with group A. (C) Percentages of peripheral blood eosinophils in all groups. (D) Concentrations of serum OVA-IgE in all groups. $n = 6$ each group. (E) A = group A, B = group B, C = group C, D = group D. * $P < 0.05$; ** $P < 0.01$ versus control group. OVA = ovalbumin. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

allergen challenge. OVA-specific IgE (OVA-IgE) levels in serum samples were evaluated by DS mouse IgE ELISA kit (Dainippon Sumitomo Pharma, Osaka, Japan). The percentage of eosinophils among white blood cells was assayed in whole blood using a Sysmex XT-2000iV automated hematology analyzer (Sysmex Corp., Kobe, Japan).

Histological Analysis of the Nasal Mucosa

After the evaluation of nasal symptoms, mice were euthanized, and nasal mucosa samples were obtained on day 35. The samples were fixed in 4% paraformaldehyde at room temperature (20–25°C) for 4 hours, followed by decalcification in 10% ethylenediaminetetraacetic acid solution at room temperature for 14 days. They were then dehydrated with ethanol and embedded in paraffin blocks. The specimens were cut into 5-μm thick coronal sections and were stained with hematoxylin and eosin (H&E)¹⁵ and toluidine blue (TB).

Immunohistochemistry

Tissue sections were stained using the TSA Fluorescence System (PerkinElmer, Waltham, MA) according to the manufacturer's instructions with minor modifications.¹⁶ Briefly, sections were blocked with TNB buffer (0.1 mol/L Tris-HCl, pH 7.5, 0.5% blocking reagent, and 0.15 mol/L NaCl) containing 5% normal donkey serum. To block endogenous biotin, the sections were further treated with avidin/biotin blocker (Vector Laboratories, Burlingame, CA), and

endogenous peroxidase activity was quenched with 1% H_2O_2 . Primary antibodies, including anti-GRPR antibody ab39883 (1:200; Abcam, Cambridge, UK), anti-GRP (C-20) antibody sc-7788 (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), or anti-tryptase ϵ antibody (S-13) sc-107314 (1:400; Santa Cruz Biotechnology) were applied overnight at 4°C. Slides were washed with TRIS NaCl Tween 20 wash buffer (0.1 mol/L Tris-HCl, pH 7.5, 0.05% Tween-20, and 0.15 mol/L NaCl; Sigma-Aldrich) and incubated with biotin-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or anti-goat IgG (Jackson ImmunoResearch Laboratories), followed by incubation with streptavidin–horseradish peroxidase (HRP) conjugate (Life Technologies, Carlsbad, CA) together with 1 μg/mL 4', 6-diamidino-2-phenylindole. Antigens were detected using tyramide-Cy5 or tyramide-fluorescein isothiocyanate. Sections were mounted in Fluorescence Mounting Medium (Dako, Glostrup, Denmark) and were visualized with an Olympus Fluoview FV1000 laser confocal microscope (Tokyo, Japan). Images were the products of threefold line averaging. Contrast and brightness settings were chosen so that all pixels were within the linear range.

Western Blot Analysis

The skin and cheek muscles were removed from the nose region. Then, nose sections were obtained by cutting coronal sections at the line of the acral part of the nose. Tissue lysates were

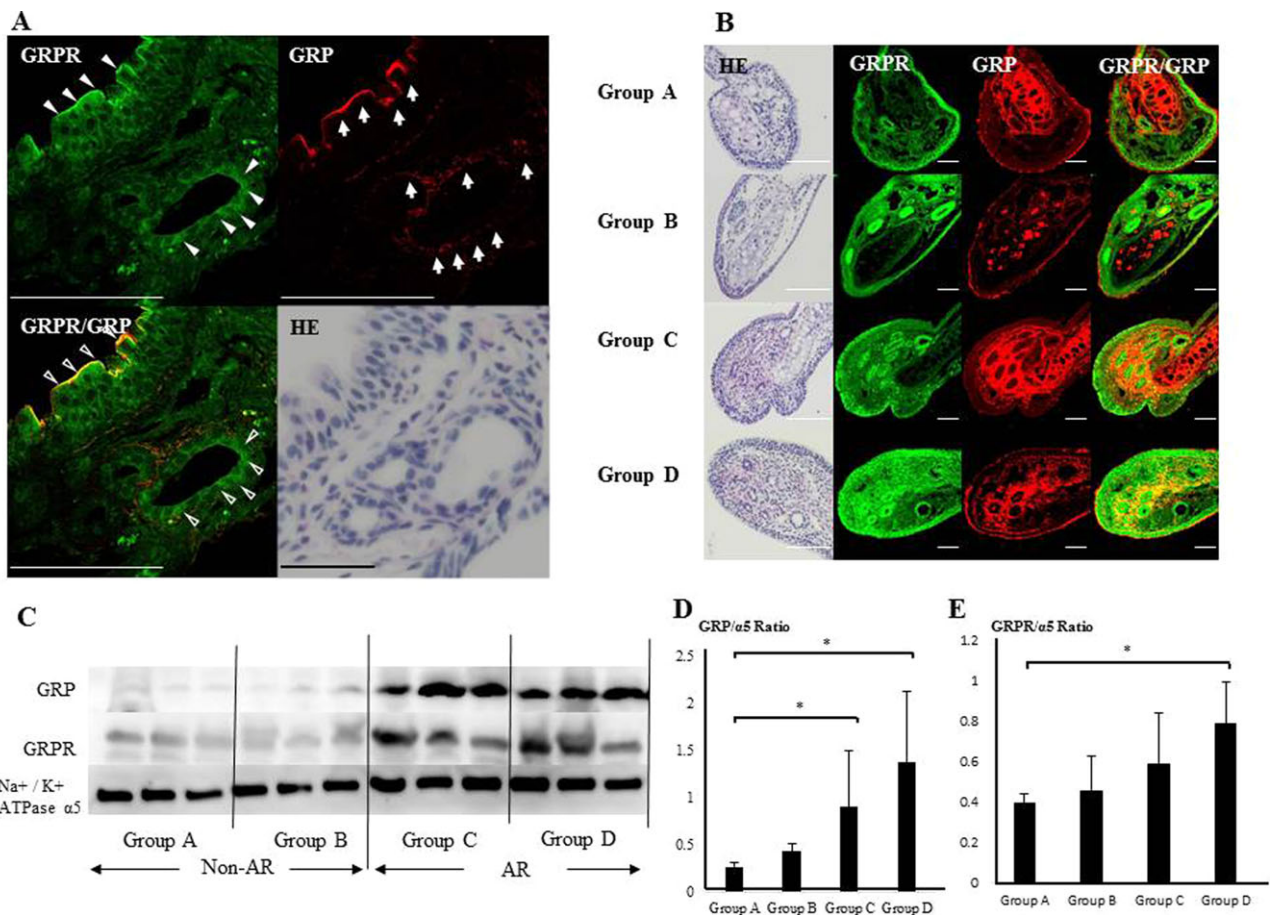


Fig. 3. Localization and expression of GRP and GRPR. (A) Localization of GRP and GRPR in nasal mucosa. H&E staining shows the glands and epithelium in sections from full-blown AR mice. GRP and GRPR immunohistochemistry in the mouse nasal mucosa was carried out using the streptavidin-biotin complex method. A representative photomicrograph (original magnification $\times 100$) of GRP and GRPR immunohistochemistry of nasal mucosa sections from full-blown AR mice. GRP (red) is abundantly expressed in the epithelium and surrounding the nasal glands (white arrow). GRPR (green) is abundantly expressed in the epithelium and submucosal gland (white arrowhead). Arrowheads outlined in white indicate overlay images in the nasal mucosa. Scale bars, 50 μm . (B) GRP and GRPR expression in the nasal mucosa as determined by immunohistochemistry. A representative photomicrograph (original magnification $\times 40$) of GRP and GRPR immunohistochemistry in nasal mucosal sections from all experimental groups. Scale bars, 50 μm . (C) Levels of GRP and GRPR proteins in the nasal mucosa in each experimental group, as determined by western blot analyses. (D) Protein ratios of GRP/ $\alpha 5$ and (E) GRPR/ $\alpha 5$. $n = 6$ for each group. AR = allergic rhinitis; GRP = gastrin-releasing peptide; GRPR = gastrin-releasing peptide receptor; H&E = hematoxylin and eosin. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

prepared in solution A (120-mg tissue sample in 3,000 μL of 1 M Tris-HCl pH 7.4, 150 mM sucrose) containing protease inhibitors (Roche, Mannheim, Germany) and aprotinin (Wako, Tokyo, Japan). Proteins (30 μg) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) or tricine-SDS-PAGE and transferred to a Hybond-P PVDF membrane (GE Healthcare, Little Chalfont, UK). The membrane was incubated overnight at 4°C with antibody against either GRP (1:500; ABBIOTEC, San Diego, CA) or GRPR (1:500; Bioworld Technology, St Louis Park, MN)—or with anti- Na^+ , K^+ -ATPase monoclonal antibody $\alpha 5^{17}$ (loading control) overnight at 4°C. Blots were then probed with HRP-conjugated anti-rabbit or anti-mouse IgG (Jackson ImmunoResearch Laboratories). Blots were photographed using a LAS-4000 (Fujifilm, Tokyo, Japan) chemiluminescence detection device. Protein bands were quantified using ImageQuant TL (GE Healthcare).

GRPR Antagonist (RC-3095) Preparation and Administration

GRPR antagonist (RC-3095; Sigma-Aldrich) was prepared as previously described.^{18,19} The RC-3095 administration

protocol is shown in Figure 1B. Purified RC-3095 was diluted with saline, and 0.3 mg/kg of the solution was intranasally administered to full-blown AR mice 4 hours prior to intranasal OVA administration from day 28 to 34 once per day. Control AR mice were given intranasal normal saline (NS) instead of RC-3095 solution. After the last challenge, total counts of sneezing and nasal rubbing over 10, 20, and 30 minutes were counted by investigators in a blinded fashion. There were seven mice in the AR + NS group and 10 mice in the AR + RC-3095 group.

Histamine Preparation and Administration

We also examined the effects of RC-3095 (0.3 mg/kg) on sneezing and nasal rubbing induced by histamine. Mice were given an intranasal dose of saline or RC-3095 4 hours before the induction of nasal symptoms. After the nasal instillation of 5 μmol histamine diluted with saline (in 10 μL) into the bilateral cavities, the frequencies of sneezing and nasal rubbing were counted over 10, 20, and 30 minutes.

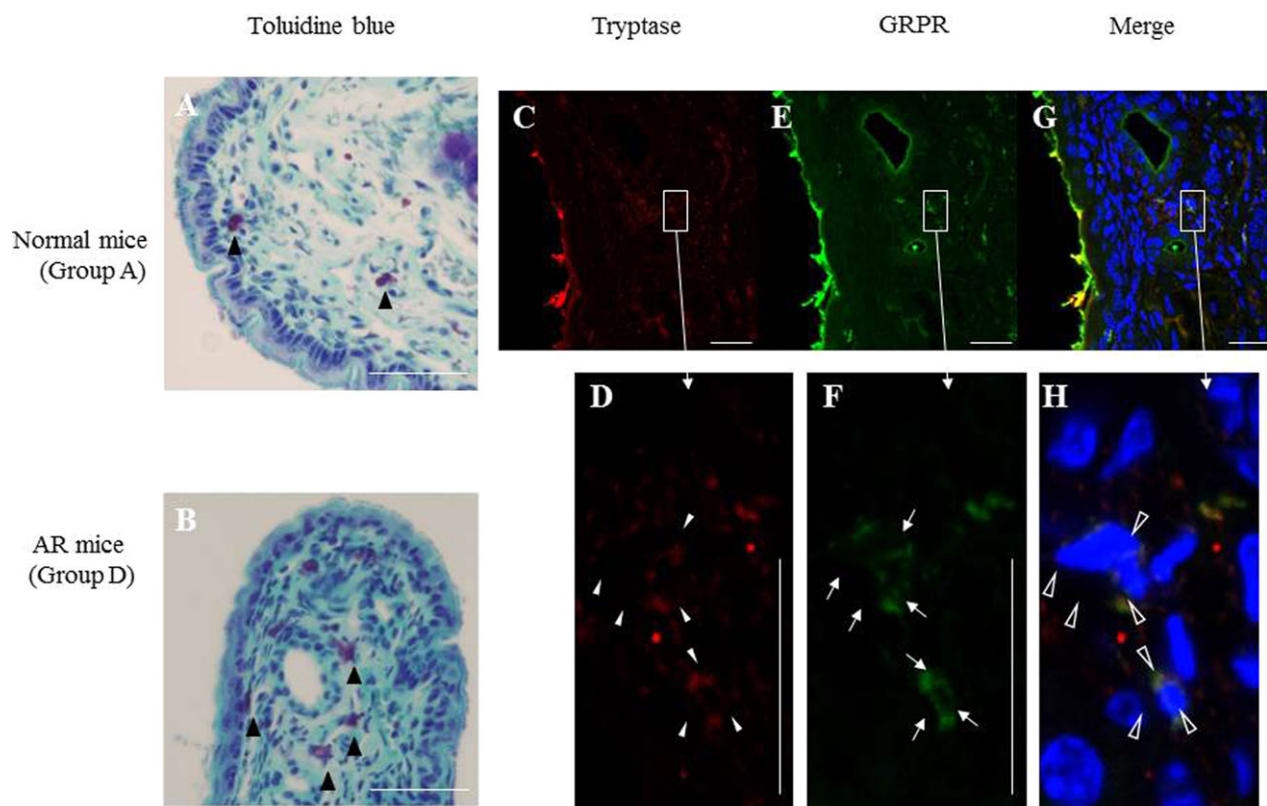


Fig. 4. GRPR expression on mast cells in the nasal mucosa. (A, B) Sections from control mice (group A) and AR mice (group D) were stained with TB. More infiltrating mast cells were found in the nasal mucosa in AR than in control mice. Scale bars, 50 μ m. (C, D, E, F) Immunohistochemical staining was performed with anti-tryptase antibody (white arrow) and anti-GRPR antibody (white arrowhead). Overlay images with DAPI are shown in G and H. GRPR expression was observed on mast cells around nasal glands (arrowheads outlined in white) in the AR mice (group D). AR = allergic rhinitis; GRPR = gastrin-releasing peptide receptor; TB = toluidine blue; DAPI = 4', 6-diamidino-2-phenylindole. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Statistical Analysis

One-way analysis of variance was used to determine the significance of differences between groups. Individual pairs of groups were compared using Scheffé test. The nasal symptom score, percentages of peripheral blood eosinophils, OVA-IgE levels, and ratios of GRP/ α 5 and GRPR/ α 5 in the nasal mucosa were compared between groups. To determine the effect of RC-3095 on allergic symptoms of AR under the stimulation of histidine in the nasal cavity, nasal symptom scores were compared between the AR + NS and AR + RC-3095 groups using Welch *t* test. StatMate IV (ATMS Co., Ltd., Tokyo, Japan) was used for statistical analysis. Statistical significance was defined as $P < 0.05$.

RESULTS

Nasal Symptoms, Serum OVA-Specific IgE, and Eosinophils in AR Model Mice

Allergic symptoms such as sneezing and nasal rubbing were observed for 10 minutes after the last intranasal administration of OVA. Both the mild (group C) and full-blown AR (group D) mice showed significantly more instances of sneezing and nasal rubbing than the control (group A) mice. Systemic sensitization only (group B) mice had a tendency to exhibit more nasal rubbing and less sneezing than group A mice, although this was not

statistically significant (Figs. 2A and 2B). Consistent with these symptoms, percentages of peripheral blood eosinophils and serum OVA-IgE levels were elevated in all groups. A significant difference was observed in the percentage of peripheral blood eosinophils in the full-blown AR and control groups (Fig. 2C), and AR mice (groups C and D) exhibited a significant increase in OVA-IgE levels (Fig. 2D).

Histopathological Changes in the Nasal Mucosa

Multilayered epithelia, edema in the lamina propria, irregularity of the epithelial surface, and prominent accumulation of eosinophils were observed in the nasal mucosa of AR mice² (Fig. 2E). According to immunohistochemistry (IHC) of the nasal mucosa, GRP localized to the epithelia and interstitial tissue surrounding the nasal glands, whereas GRPR localized to the submucosal gland and nasal mucosal epithelia, even in the control mice (Fig. 3A). Stronger and more widespread expression of GRP in the interstitial tissue was observed in groups C and D compared to that in groups A or B (Fig. 3B). In addition, interstitial expression of GRPR and overlapping localization of GRP and GRPR were noted in group D and, to a lesser extent, in group C.

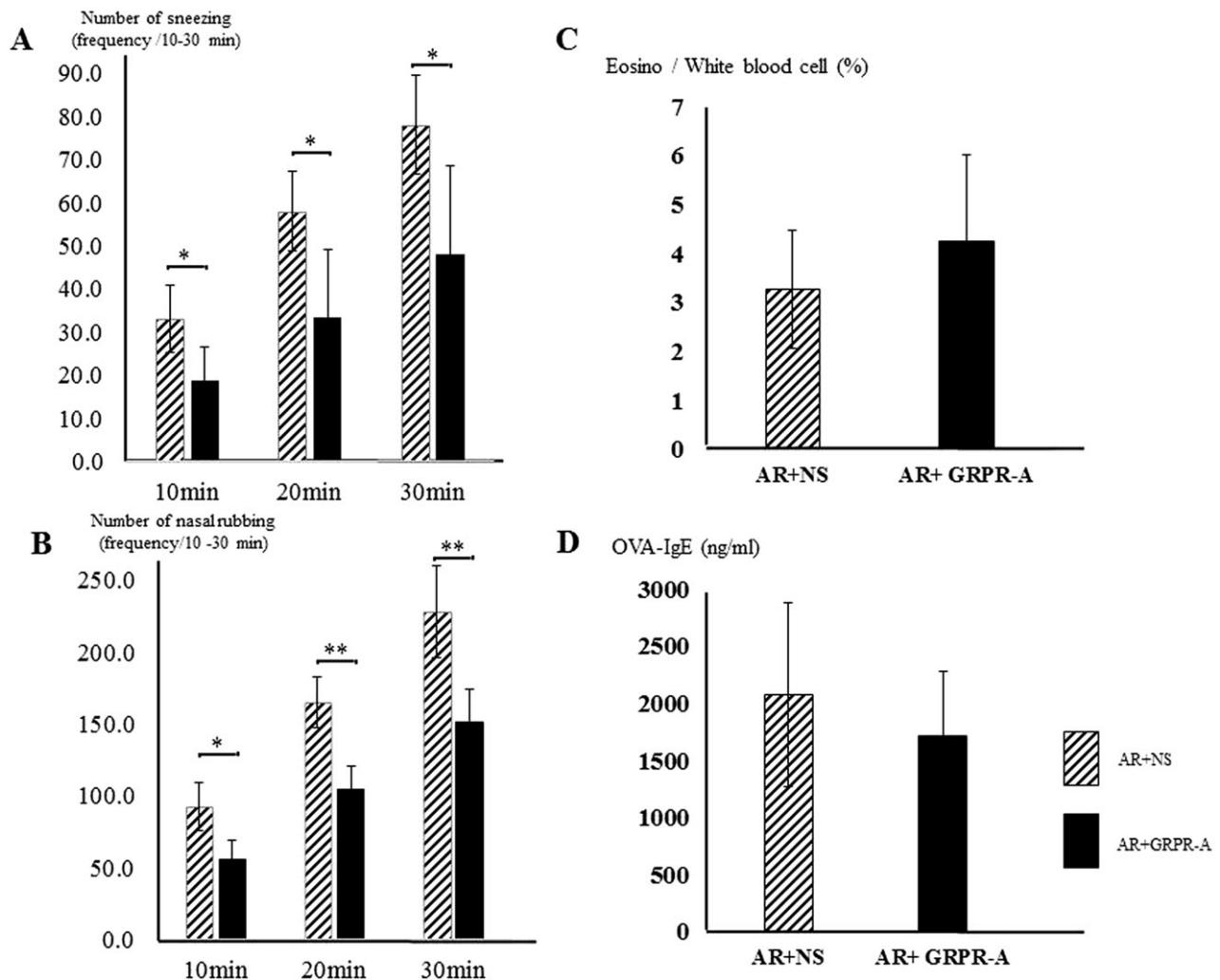


Fig. 5. Effects of RC-3095 on OVA-induced allergic reaction. AAR mice were prepared as in Fig. 1B with RC-3095 or NS treatment performed on days 28 through 34. (A, B) Sneezing was counted for 30 minutes after the last challenge. (C, D) Percentages of peripheral blood eosinophils and levels serum of OVA-IgE in AR + RC-3095 and AR + NS groups. Differences were not statistically significant. * $P < 0.01$, ** $P < 0.001$ versus AR + NS group. AR = allergic reaction; NS = normal saline; OVA = ovalbumin.

Protein Expression Levels of GRP and GRPR in AR Mice

As shown in Figure 3C, GRP and GRPR expression increased in all treatment groups compared with levels in the control group. Differences in GRP expression between the control and AR groups (Fig. 3D) and between the control and full-blown AR group (Fig. 3E) were statistically significant. These results support the aforementioned IHC findings that AR mice (i.e., groups C and D) showed upregulation of GRP and GRPR in the nasal mucosa.

Expression of GRPR on Mast Cells in the Nasal Mucosa

The distribution of GRPR expression on mast cells in the nasal mucosa was examined by IHC and TB staining. Sequential serial sections were stained with TB (Fig. 4A and 4B), anti-tryptase antibody (Fig. 4C and 4D), and anti-GRPR antibody (Fig. 4E and 4F). There was an

increased number of mast cells in the nasal mucosa of AR mice compared to that in control mice. Mast cells were localized around nasal glands. As shown in Figure 4G and 4H, immunoreactivity for tryptase, a marker of mast cells, often colocalized with that for GRPR.

Effect of RC-3095 Administration on Nasal Symptoms, Peripheral Blood Eosinophils, and Serum OVA-IgE Levels in AR Mice

To determine the functional significance of the GRP-GRPR axis, a GRPR antagonist, RC-3095, was administered to full-blown AR mice. Average counts of sneezing and nasal rubbing 10 to 30 minutes after treatment in the AR + RC-3095 group were significantly lower than those in the AR + NS group (Fig. 5A and B). These results suggest that RC-3095 treatment improves AR nasal symptoms in this model. We examined the percentages of peripheral blood eosinophils and serum levels of OVA-IgE in mice to evaluate the effects of RC-3095 on AR. RC-3095 nasal administration had no

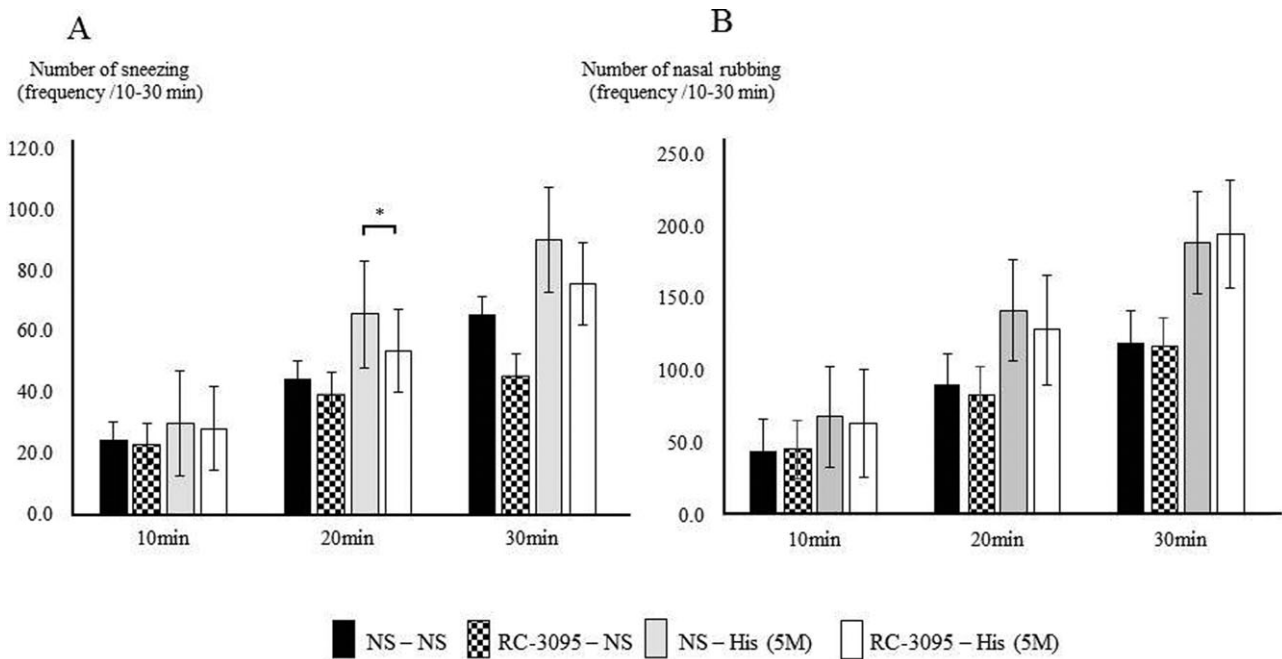


Fig. 6. Effect of RC-3095 on histamine-induced allergic reaction. RC-3095 significantly reduced sneezing induced by histamine for a 20-minute period but had no significant effect on nasal rubbing. $n = 6$ in each group, $*P < 0.05$ versus control group.

significant effect on either the percentage of peripheral blood eosinophils or the levels of OVA-IgE (Fig. 5C and 5D).

Effects of RC-3095 on Histamine-Induced Nasal Symptoms in Mice

Figure 6 shows the incidences of sneezing and nasal rubbing over the 10-, 20-, and 30-minute periods after the intranasal administration of histamine. RC-3095 at a dose of 0.3 mg/kg or saline was administered 4 hours before the induction of nasal symptoms by histamine (5 μ mol/site). As shown in Figure 6A, RC-3095 significantly inhibited sneezing induced by histamine in the 20-minute period following administration.

DISCUSSION

We investigated the role of GRP/GRPR IgE-dependent nasal allergy symptoms in a mouse model of AR at three different sensitization levels. We discovered that GRP and GRPR expression in the mucous membrane increased as allergic inflammation of the mucous membrane increased. Furthermore, GRPR was expressed periglandularly and in mast cells. Administering a GRPR antagonist improved rhinitis symptoms in mice.

The nasal mucosa is innervated by sympathetic nerves, which control the blood vessels; parasympathetic nerves, which regulate the nasal glands; and sensory nerves.²⁰ The sensory nerve of the nasal mucosa belongs to the trigeminal nerve, with axons projecting from the spinal trigeminal nucleus of the brain stem. Nasal mucosal hypersensitivity is worsened by neuropeptide release from sensory trigeminal nerves in a retrograde manner following stimulation of the nasal mucosa by the central nervous system.²¹ This causes neurogenic inflammation manifested as vascular dilatation

and increased permeability, as well as accelerated gland secretion. Numerous studies have examined the roles of SP and CGRP in AR.³⁻⁷ However, neuropeptides other than SP and CGRP remain unexplored. Thus, we focused on GRP, a sensory neuropeptide whose role in AR has not been examined. GRP binds to its G protein-coupled receptor and transmits signals in the central and peripheral nervous systems.²² Its receptor, GRPR, is expressed by various cell types beyond nervous system tissues.

Baraniuk et al. examined the surgically excised mucosa of the inferior nasal concha from 14 patients with hypertrophic rhinitis whose chief complaint was nasal congestion.¹¹ They found the following: 1) GRPR localizes to the submucosal gland and mucosal epithelium; 2) GRP is involved in external secretion; 3) GRPR is not involved in vascular permeability because it is not located within blood vessels; 4) although GRP coexists with other neuropeptides, its correlation with other peptides is unknown; and 5) GRP may regulate the submucosal glandular tissue, and the acceleration of lactoferrin secretion by serous cells has been observed in nasal mucosal cultures following stimulation with GRP.

Allergic reactions are exaggerated immune responses to stimulation from external inhalants and can be viewed as an exaggerated biological defense. If the aforementioned GRP-induced increased mucosal secretion is a part of the defense system, overactivation of the GRP system may be involved in allergic reactions. Thus, we investigated the role of GRP in IgE-mediated immediate allergic reactions in a mouse model of AR. In accordance with the report of Baraniuk et al., which studied the human nasal mucosa, GRP was locally observed in the mucosal epithelium and interstitial area, and GRPR was observed in the mucosal epithelium and glandular tissue.¹¹ In addition, using IHC and WB analyses, we found a positive correlation between allergic inflammation of the nasal mucosa and the expression of GRP and GRPR.¹¹

Andoh et al. demonstrated the existence of mast cells expressing GRPR in the skin and suggested that itching symptoms are caused by the degranulation of GRPR-positive mast cells. In addition, they reported that GRPR antagonists suppress the degranulation of mast cells.²³ We also detected that GRPR expression on mast cells in the nasal mucosa of full-blown AR mice. According to a public domain microarray database search using the BaseSpace Correlation Engine (Illumina), *GRPR* mRNA is highly expressed in human B cells, neutrophils, monocytes, and T cells; therefore, we speculate that not only mast cells but also other immune cells may be involved in the GRP/GRPR system. However, the direct effects of GRP on these cells should be explored further. The localization of GRP/GRPR in the nasal mucosa implies that GRP from sensory nerve terminals may bind to GRPR on glands and mast cells to stimulate serous secretion and mast cell degranulation, respectively. As a matter of fact, bombesin, a GRPR agonist, reportedly induces human nasal mucous and serous cell secretion in vivo.²⁴

Zhou et al. reported that GRP worsens both OVA-induced airway hyperreactivity/eosinophilic inflammation and ozone-induced airway hyperreactivity/neutrophilic inflammation.¹⁰ In both asthma models, GRP blockade abrogates airway hyperreactivity and decreases the numbers of macrophages and granulocytes in bronchoalveolar lavage (BAL) fluid, as well as reducing BAL fluid levels of cytokines implicated in asthma, including those derived from Th1 (IL-2, TNF α) and Th2 (IL-4, IL-5, IL-13) cells.¹⁰ Interestingly, it also has been reported that a GRPR antagonist resulted in a greater anti-inflammatory effect than corticosteroids.²⁵ Our findings of reduced instances of sneezing and nasal rubbing following the administration of a GRPR antagonist in AR mice were consistent with these reports. Furthermore, RC-3095 was especially effective at suppressing the sneezing induced by histamine in the 20-minute period following administration; however, it was not strong. This suggests that RC-3095 does not suppress the mechanism underlying the symptoms caused by histamine (free histamine, or histamine that was directly administered to mice); instead, it suggests that RC-3095 acts on either histamine release by mast cells/basophils or a reaction upstream of this release.

Taken together, the blockade of GRPR may lead to better control of a wide range of inflammatory symptoms caused by type I hypersensitivity reactions. We speculate that GRPR antagonists should reduce the secretion of cytokines from mast cells, which will be the subject of future investigations. Nonetheless, to our knowledge this is the first study to demonstrate the efficacy of a GRPR antagonist in the treatment of AR.

Because it is likely difficult for a GRP antagonist to completely block nasal symptoms, a combination with antagonists of other neuropeptides or antihistamine may be more effective against AR. Because this study suggests an interaction between histamine and GRP, it may be necessary to consider the interplay between GRP and histamine, which is released by degranulation.

CONCLUSION

In summary, this study suggests that GRP contributes to AR symptoms in two ways: First, GRP from

sensory nerve terminals binds to GRPR on glands and stimulates glandular secretion. Second, GRP binds to GRPR on mast cells to stimulate degranulation. Our findings indicate that the GRP-GRPR system is likely to be involved in allergic inflammation. This system may therefore represent a novel therapeutic target for refractory AR.

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