

Elevated Nasal Mucosal G Protein Levels and Histamine Receptor Affinity in a Guinea Pig Model of Nasal Hyperresponsiveness

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Key Words

Allergic rhinitis · Nasal hyperresponsiveness · Nasal mucosa · Histamine H₁ receptor · GTP-binding protein · Sensitized guinea pigs

Abstract

Background: Nasal hyperresponsiveness is a common feature of allergic rhinitis, but the underlying mechanisms have yet to be elucidated. The effects of repeated antigen inhalation on the characteristics of histamine H₁ receptors and expression levels of heterotrimeric guanine 5'-triphosphate-binding proteins in nasal mucosa were investigated to understand the mechanisms of the pathogenesis of nasal hyperresponsiveness in allergic rhinitis. **Methods:** Male Hartley guinea pigs were sensitized by the inhalation of dinitrophenylated ovalbumin antigen (10 mg of protein/ml) and repeatedly challenged by inhaling aerosolized dinitrophenylated ovalbumin antigen for 3 weeks. Twenty-four hours after the last antigen inhalation, in vivo nasal responsiveness to histamine was measured. [³H]Mepyramine binding assays and immunoblotting for α subunits of the G_q protein were also performed using membrane preparations of isolated nasal mucosae. **Results:** The histamine-induced increase in intranasal pressure was significantly aug-

mented after repeated antigen challenge, indicating that nasal hyperresponsiveness was achieved. In saturation binding studies, no significant change was observed in the density and antagonist affinity of H₁ receptors in the hyperresponsive animals. On the other hand, the affinity of histamine for high-affinity agonist binding sites in the hyperresponsive group, measured by histamine competition binding studies, was much greater than that in control animals, and these results were affected by guanosine 5'-O-(3-thiotriphosphate) in both groups. Moreover, G α_q levels in nasal mucosal homogenates were significantly increased after repeated antigen challenge. **Conclusions:** Elevated G protein levels in nasal mucosa might induce an increased binding affinity of histamine to its receptors, resulting in an augmented nasal response to histamine, that is, nasal hyperresponsiveness, in guinea pigs.

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Introduction

Nonspecific airway hyperresponsiveness is one of the important characteristics of allergic respiratory diseases. Nasal hyperresponsiveness to histamine challenge has also been reported in patients with allergic rhinitis [1, 2].

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For example, intranasal application of histamine causes severer sneezing, rhinorrhea and nasal obstruction in those patients than in healthy individuals [2]. There is thus a need to understand the underlying mechanisms of nasal hyperresponsiveness in order to develop therapies for allergic rhinitis.

Histamine is released by the type I allergic reaction and plays an important role in eliciting nasal symptoms of allergic rhinitis, such as sneezing, rhinorrhea and congestion [3]. Histamine receptors are classified as H₁, H₂ and H₃ subtypes [4, 5]. In nasal mucosa, histamine H₁ receptors exist exclusively on the endothelium of nasal vessels [6]. Histamine directly causes increases in blood flow, blood volume and vascular permeability through its action on H₁ receptors of the vascular bed, and produces immediate nasal congestion [7, 8]. Moreover, antihistamine therapy is effective for relieving the symptoms of allergic rhinitis [3, 9, 10]. It is thus suggested that histamine H₁ receptors play an important role in nasal symptoms. So, increases in H₁ receptor density and affinity and/or intracellular signaling in nasal mucosa may be responsible for augmented histamine responsiveness, that is, nasal hyperresponsiveness. However, no quantitative or qualitative change in H₁ receptors was demonstrated in nasal mucosae from patients with allergic rhinitis [11] or from a guinea pig model of toluene diisocyanate-induced nasal hyperresponsiveness [12]. In contrast, a significant increase in H₁ receptor density has been reported in a guinea pig model of dinitrophenylated *Ascaris*-induced nasal hyperresponsiveness [13].

One purpose of the present study was to investigate whether alterations in histamine H₁ receptor properties contribute to the nasal hyperresponsiveness to histamine in a guinea pig model of allergic rhinitis induced by repeated challenge with aerosol of dinitrophenylated ovalbumin antigen. We compared the H₁ receptor density, antagonist affinity and characteristics of agonist binding in membrane preparations of nasal mucosae obtained from nonsensitized normal control and sensitized control guinea pigs and guinea pigs repeatedly challenged with antigen. We also examined the effect of a nonhydrolyzable guanosine 5'-triphosphate (GTP) analogue, guanosine 5'-O-(3-thiotriphosphate) (GTPγS), on agonist binding in order to confirm the possible participation of heterotrimeric G proteins in the pathogenesis of nasal hyperresponsiveness. Moreover, the change in the level of the G protein Gα_q, which is known to be linked with H₁ receptors, in the hyperresponsive nasal mucosa was investigated using immunoblotting.

Materials and Methods

Sensitization and Antigenic Challenge

Male Hartley guinea pigs (230–250 g, Tokyo Laboratory Animals Inc., Japan) were sensitized by inhalation of dinitrophenylated ovalbumin antigen (10 mg of protein/ml) with an ultrasonic nebulizer (TUR-3000, Nihon Kohden, Tokyo, Japan) for 10 min in an anesthetized condition in a plexiglass box (300 × 200 mm, height 150 mm) (day 0). Seven days after the sensitization (day 7), the animals were boosted by the same inhalation procedure as described above. Then, the animals received a total of 21 challenges with aerosolized antigen (each for 3 min) every 24 h for 3 weeks from days 14 to 34. The antigen concentrations used in the challenge period were 0.5 mg of protein/ml at the first antigen challenge (day 14) and 1 mg of protein/ml on the other days (days 15 to 34). Twenty-four hours after the last antigen challenge (day 35), the experiments described below were performed. Control animals received the same immunization, but inhaled saline aerosol instead of dinitrophenylated ovalbumin antigen.

Measurement of Intranasal Pressure

Intranasal pressure was measured as an index of intranasal resistance by the method described in previous papers [14, 15] with minor modifications. Briefly, guinea pigs were anesthetized with urethane (1.6 g/kg, i.p.) and fixed in a supine position. Animals spontaneously respired through a tracheal cannula inserted into the lower trachea. A polyethylene cannula (outer diameter 3 mm, inner diameter 2 mm, length 50 mm) was carefully inserted to a depth of 3 mm into the nasopharynx from the side of the larynx after making a 2-mm incision in the thyroid cartilage. The two duct pores, which are situated in the upper oral cavity wall and lead to the nasal cavities, were obstructed with an adhesive material (Alonalpha, Toua, Tokyo, Japan). Then, a polyethylene cannula (outer diameter 2 mm, inner diameter 1.5 mm, length 20 mm) was carefully inserted into each nostril to a depth of 5 mm. The two nostril cannulae were connected to a Y-shaped cannula with another polyethylene cannula. The nasal cavities were ventilated between both cannulae inserted into the nasopharynx and bilateral nostrils with an artificial respirator (SN-480-7-10, Shinano Apparatus, Tokyo, Japan). A fixed volume of 4 ml of air was insufflated at a frequency of 70 strokes/min from the nasopharynx to the nostrils. The side branch of the nasopharyngeal cannula was connected to a pressure transducer (TM1 BLH, Nihon Kohden) to monitor the intranasal pressure.

To assess the nasal responsiveness, histamine or saline (vehicle for histamine) was inhaled for 15 min into the nasal cavities by aerosolizing solution in a specially devised plastic cylindrical chamber (diameter 27 mm, height 57 mm) which was introduced in an ultrasonic nebulizer (TUR-3200, Nihon Kohden). The ultrasonic nebulizer was placed in the air-insufflating circuit so that the aerosolized mist was inhaled into the nasal cavities each time air was sent out from the respirator. The changes in the intranasal pressure were monitored throughout the experiment and the area under the curve (AUC) over time was calculated between the onset and 15 min after the aerosol provocation.

Membrane Preparations of Nasal Mucosae

Twenty-four hours after the last antigen challenge, animals were sacrificed by exsanguination under anesthesia with urethane (1.6 g/kg, i.p.). The nasal septum was promptly excised and immediately soaked in ice-cold, oxygenated Krebs-Henseleit solution (118.0 mM

NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 1.2 mM KH₂PO₄, 10.0 mM glucose, pH 7.4). The bilateral nasal mucosae were carefully dissected from the septum under stereomicroscopy. These mucosal segments were homogenized with Physcotron (NS-50, Niti-on, Tokyo, Japan; 10 s × 5; level maximum) in 10 volumes of ice-cold 0.32 M sucrose. These mucosal homogenates were centrifuged (1,500 g at 4°C for 10 min), and then the resultant supernatants were recentrifuged (50,000 g at 4°C for 10 min). The resultant pellets were resuspended in 10 volumes of ice-cold 50 mM Na/K phosphate buffer (pH 7.5) and homogenized. After a centrifugation (50,000 g at 4°C for 10 min), the resultant pellets were washed out 3 times by the same procedure. The pellets were resuspended in 1.25 ml of ice-cold 50 mM Na/K phosphate buffer (pH 7.5), and these mucosal membrane preparations were stored at -85°C until use. The protein concentrations of these preparations were determined by the method of Lowry et al. [16] in triplicate with bovine serum albumin as a standard.

Receptor Binding Studies

In saturation binding experiments, the assay tubes (total volume of 500 µl containing 100 µg of protein of the membrane preparation) were incubated in 50 mM Na/K phosphate buffer (pH 7.5) with a wide concentration range of [³H]mepyramine (specific activity 38.8 Ci/mmol, final concentrations 0.03125–2.0 nM) at 25°C for 60 min. At the end of the incubation, the suspension was rapidly filtrated through a polyethylenimine- and mepyramine (10 µM)-coated Whatman GF/B glass fiber filter to separate bound [³H]mepyramine from free [³H]mepyramine. The filter was washed 4 times with 4 ml of 50 mM Na/K phosphate buffer (pH 7.5) and 15 ml of scintillation fluid (Aquasol-2, New England Nuclear, Stevenage, UK) was added. Twenty-four hours after the addition of scintillation fluid, the radioactivity was counted by a liquid scintillation counter (LSA-1600CA liquid scintillation analyzer, Packard, Meriden, Conn., USA). The specific binding was calculated by subtracting nonspecific binding in the presence of cold mepyramine (20 µM) from total binding in the absence of mepyramine.

Competition binding experiments were also performed under the same conditions as described above, except for a fixed [³H]mepyramine concentration (1.0 nM) and the presence of 5 mM MgCl₂ and 10⁹–10² M histamine as a displacer. The histamine competition binding assay was also performed in the presence of a nonhydrolyzable guanine nucleotide, GTPγS. The specific binding was calculated as described above.

All these binding studies were undertaken in duplicate.

Western Blots

Immunoblotting was performed in the membrane preparations of nasal mucosae obtained from the guinea pigs repeatedly challenged with antigen and sensitized control guinea pigs as described above. Briefly, the membrane fractions were dissolved in sodium dodecyl sulfate (SDS) sample buffer and heated at 100°C for 4 min. The samples (10 µg of protein per lane) were subjected to 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then electrophoretically transferred for 18 h onto nitrocellulose membranes (Hybond-ECL, Amersham, UK) in cold transfer buffer [20% methanol containing 25 mM tris(hydroxymethyl)aminomethane (Tris) and 192 mM glycine]. After repeated washing out with Tris buffer (20 mM Tris, 500 mM NaCl, pH 7.5) containing 0.1% Tween 20 (TTBS), the nitrocellulose membranes were incubated with blocking buffer (3% gelatin in TTBS) for 1.5 h at room temperature. The nitro-

cellulose membranes were then incubated with primary antibody (polyclonal rabbit anti-Gα_q, 1/2,500 dilution; Santa Cruz) in antibody buffer (1% gelatin in TTBS) for 2 h at room temperature. The nitrocellulose membranes were then washed 5 times (each for 15 min) with TTBS. They were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1/5,000 dilution; Amersham) for 1.5 h at room temperature, and then washed 5 times with TTBS. The blots were detected using an enhanced chemiluminescent method (ECL System; Amersham) and quantitated by a densitometry system (Atto Densitograph Software version 4.0; Atto Co., Japan). The nitrocellulose membranes were then stripped of the primary and secondary antibodies by incubation with stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris, pH 6.7) at 50°C for 30 min. To normalize the Gα protein content to an internal control protein, β-actin, immunoblotting was also performed on the same gel using monoclonal mouse anti-β-actin N-terminal (1/5,000 dilution; Sigma, St. Louis, Mo., USA) and goat anti-mouse IgG (1/5,000 dilution; Amersham). Under the above conditions, a linear relationship between the band densities of Gα proteins and amounts of loaded proteins was found for protein concentrations ranging between 5 and 25 µg [17]. Similar results were obtained with β-actin. The ratios of corresponding Gα_q/β-actin in each lane were calculated as indexes of Gα_q protein levels.

Statistical Analyses

All the data of the binding experiments were analyzed using the EBDA/LIGAND program (Biosoft, Cambridge, UK) adapted for a Macintosh Computer (Quadra 650) [18]. The individual [³H]mepyramine saturation binding experiments revealed a one-site model, and the dissociation constant (K_d) and maximal density of receptors (B_{max}) were calculated using individual Scatchard analysis. The individual histamine competition curve was analyzed by a computerized iterative procedure using a nonlinear least-squares regression. For those curves best described by a two-site model, pK_H, pK_L, R_H and R_L values were generated; these refer to the logarithms of the high- and low-agonist affinity dissociation constants and the proportions of the receptors in the high- and low-affinity sites, respectively.

All the data were expressed as the mean with SE. Statistical significance of differences was determined by Dunnett's multiple analysis or two-way analysis of variance. A value of p < 0.05 was considered significant.

Results

Change in *in vivo* Nasal Responsiveness to Histamine after Repeated Antigen Challenge

At each antigen challenge, symptoms (e.g. scratching nose, sneezing) were observed in the sensitized guinea pigs. The symptoms observed were remarkable in degree with repetition of the challenge. Inhalation of saline aerosol by sensitized animals (the sensitized control group) caused no remarkable symptoms.

In nonsensitized normal guinea pigs, inhalation of histamine (0.03, 0.1 and 0.3%, for 15 min) aerosol into the nasal cavities under anesthesia induced an increase in intranasal pressure in a concentration-dependent man-

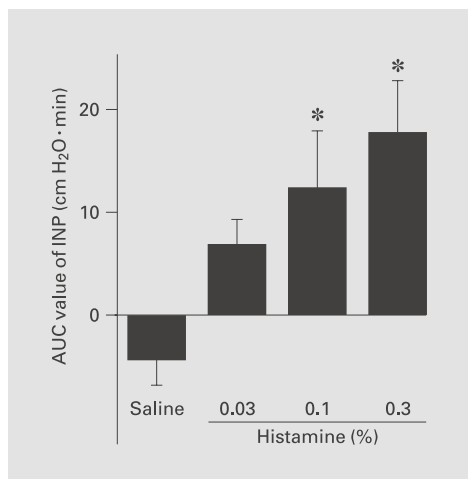


Fig. 1. The AUC values of the time course changes in intranasal pressure (INP) induced by inhaled histamine (0.03–0.3%, each for 15 min) in anesthetized normal guinea pigs. Intranasal pressure was measured as described in Materials and Methods. Each column represents the mean $\pm$ SEM from 4–6 experiments. * $p < 0.05$ compared with the saline inhalation group by Dunnett's multiple analysis.

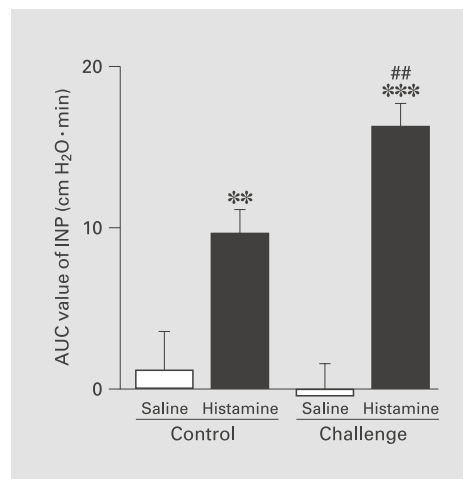


Fig. 2. The AUC values of the time course changes in intranasal pressure (INP) induced by inhaled histamine (0.1%, for 15 min) in anesthetized guinea pigs which were repeatedly challenged with dinitrophenylated ovalbumin antigen (black bars) or saline (white bars) for 3 weeks. Intranasal pressure was measured as described in Materials and Methods. Each column represents the mean $\pm$ SEM from 5–6 experiments. ** $p < 0.01$ and *** $p < 0.001$ compared with the respective saline inhalation groups; ## $p < 0.01$ compared with the control histamine group by Dunnett's multiple analysis.

ner. The AUC values of the time course changes in intranasal pressure induced by histamine are shown in figure 1. The increase in intranasal pressure induced by 0.3% histamine was markedly inhibited by pretreatment with chlorpheniramine (3 mg/kg, i.v.; data not shown), indicating that the response is mainly mediated by histamine H₁ receptors.

Figure 2 shows the AUC values of the time course changes in intranasal pressure induced by inhalation of 0.1% histamine in sensitized control guinea pigs and guinea pigs repeatedly challenged with antigen. The sensitization procedure itself had no significant effect on the nasal response to 0.1% histamine (fig. 1, 2). However, the nasal response to histamine was significantly augmented in guinea pigs repeatedly challenged with antigen as compared to that in the sensitized control animals (fig. 2), indicating that nasal hyperresponsiveness to histamine is obtained after repeated antigen inhalation.

Saturation Binding Study in Membrane Preparations of Nasal Mucosae

The saturation binding curves and Scatchard analysis of [³H]mepyramine binding to membrane preparations obtained from nonsensitized normal control and sensi-

tized control guinea pigs and guinea pigs repeatedly challenged with antigen are shown in figures 3 and 4. In respective groups of animals, [³H]mepyramine was characterized as an interaction with a single population of saturable, high-affinity binding sites. There were no significant differences among the groups with regard to histamine H₁ receptor density ($B_{\max}$) and antagonist affinity (K_d) (table 1).

Histamine Competition Binding Study in Membrane Preparations of Nasal Mucosae

Histamine displacement of [³H]mepyramine binding to H₁ receptors in membrane preparations obtained from sensitized control guinea pigs and guinea pigs repeatedly challenged with antigen was also assayed in the absence and presence of a nonhydrolyzable guanine nucleotide, GTP γ S (fig. 5). The histamine competition binding curves envisaged the existence of two affinity states of H₁ receptors for histamine in both the groups. As shown in table 2, the histamine affinity for high-affinity binding sites of membrane preparations obtained from guinea pigs repeatedly challenged with antigen was significantly greater than that from sensitized control animals in the absence of GTP γ S. No significant difference was ob-

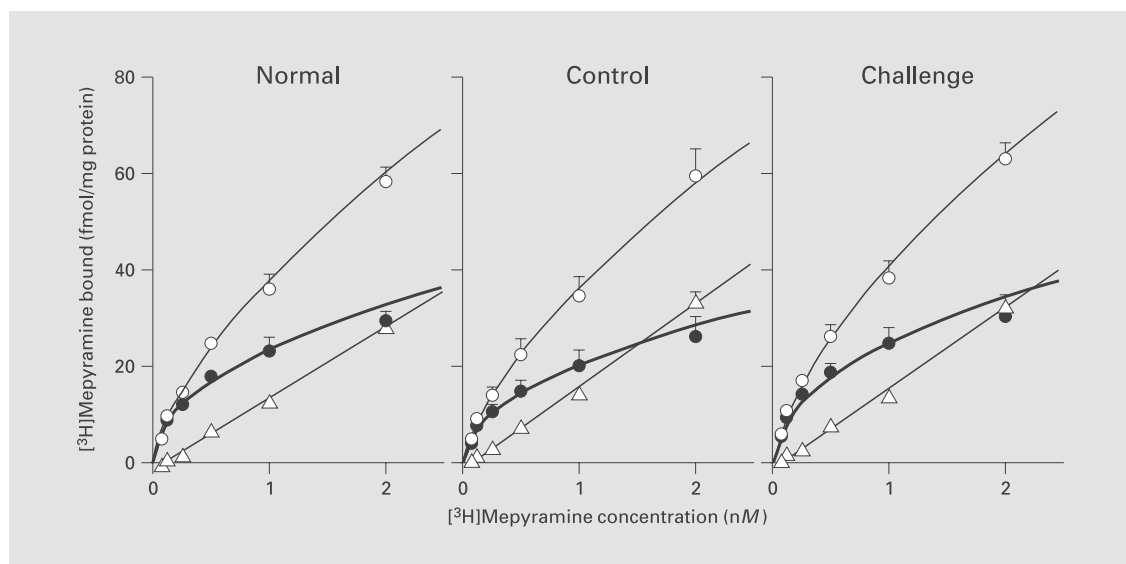


Fig. 3. Saturation curves of [^{3} H]mepyramine binding to membrane preparations of nasal mucosae obtained from nonsensitized normal control (left panel) and sensitized control guinea pigs (middle panel) and guinea pigs repeatedly challenged with antigen (right panel). The membrane preparations were incubated with increasing concentrations of [^{3} H]mepyramine (0.03125–2.0 nM) at 25 °C for 60 min in the presence (nonspecific binding; Δ) or absence (total binding; $\circ$) of 20 μ M cold mepyramine. Specific binding ($\bullet$) is the difference between [^{3} H]mepyramine binding in the absence and presence of cold mepyramine. Each point represents the mean $\pm$ SEM from 5–6 duplicate experiments.

Table 1. Histamine H_1 receptor antagonist affinity (K_d) and receptor density (B_{max}) in membrane preparations of nasal mucosae

Group	n	K_d , nM	B_{max} , fmol/mg of protein
Normal	5	0.40 ± 0.08	31.6 ± 3.6
Control	5	0.44 ± 0.13	28.1 ± 4.8
Challenge	6	0.32 ± 0.04	31.5 ± 4.3

Membrane preparations of nasal mucosae were obtained from nonsensitized normal control (Normal) and sensitized control (Control) guinea pigs and guinea pigs repeatedly challenged with antigen (Challenge). Values are means $\pm$ SEM.

served in the histamine affinity for low-affinity binding sites and in the percentages of high- and low-affinity receptors between the groups.

In the presence of GTP γ S, the histamine competition binding curves of both the groups were shifted to the right and nearly overlapped (fig. 5). No high-affinity binding sites were detected in either group. The slope values (pseudo Hill coefficients) for the displacement curves in

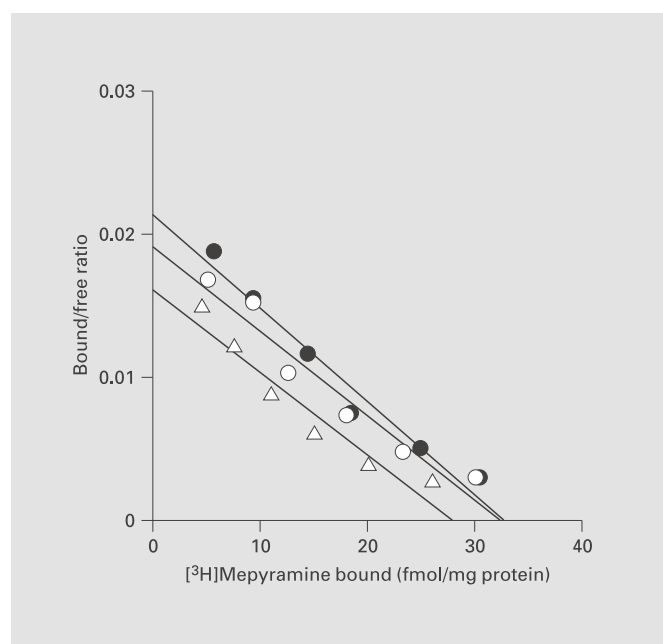


Fig. 4. Scatchard plots of [^{3} H]mepyramine binding to histamine H_1 receptors in membrane preparations of nasal mucosae obtained from nonsensitized normal control ($\circ$) and sensitized control (Δ) guinea pigs and guinea pigs repeatedly challenged with antigen ($\bullet$). Each point represents the mean from 5–6 duplicate experiments.

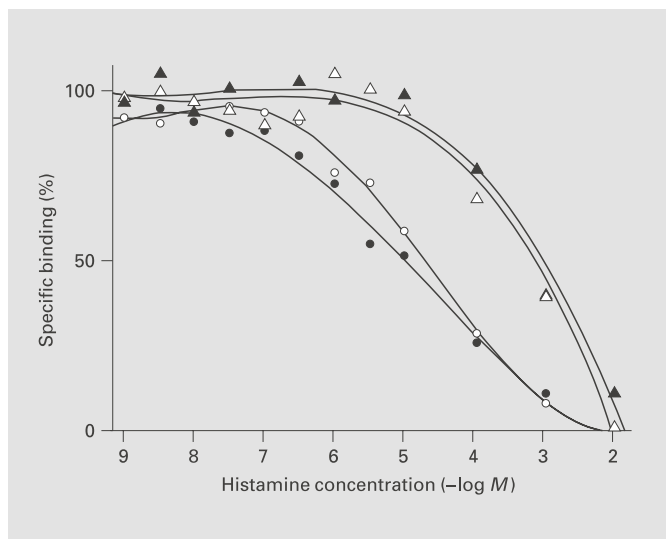


Fig. 5. Histamine displacement curves for [³H]mepyramine binding in membrane preparations of nasal mucosae obtained from sensitized control guinea pigs (○, △) and guinea pigs repeatedly challenged with antigen (●, ▲) in the absence (○, ●) and presence (△, ▲) of 100 μM GTPγS. Each point represents the mean from 3–4 duplicate experiments. In the absence of GTPγS, the high-affinity portion of the displacement curve of the group repeatedly challenged with antigen was shifted significantly ($p < 0.05$) to the left as compared with that of the control animals (table 2), indicating that the level and/or activity of G proteins linked to the high-affinity binding sites of H₁ receptors may be increased in the nasal hyperresponsive group (see Discussion).

the presence of GTPγS were nearer to 1 than those in the absence of GTPγS in both the groups. These findings indicate that the levels of G proteins linked to H₁ receptors might be enhanced by antigen-induced nasal hyperresponsiveness.

Measurement of Levels of G Proteins by Immunoblot Analyses

In the present study, the levels of α-subunits of heterotrimeric G proteins were measured using immunoblot analyses in membrane preparations of nasal mucosae. β-Actin, a constitutive 45-kD protein, was used as an internal standard and was measured in the same gel. Representative immunoblots for Gα_q and β-actin of nasal mucosal membranes obtained from the sensitized control guinea pigs and guinea pigs repeatedly challenged with antigen are shown in figure 6a. Immunoblotting with the antibody against Gα_q showed a single 42-kD band, indicating the existence of Gα_q proteins in the guinea pig nasal mucosae. The ratios of corresponding Gα_q protein/β-actin were calculated and are summarized in figure 6b. Although the sensitization procedure alone had no effect on the level of Gα_q protein (data not shown), the level of Gα_q protein in the guinea pigs repeatedly challenged with antigen was significantly increased as compared with that in the sensitized control animals.

Table 2. Histamine competition binding in membrane preparations of nasal mucosae

Group	GTPγS	n	Slope	pK _H	pK _L	R _H	R _L
Control	–	4	0.56 ± 0.05	6.43 ± 0.14	4.80 ± 0.02	44.5 ± 12.7	55.5 ± 12.7
	+	3	0.71 ± 0.20	–	3.88 ± 0.19	0	100
Challenge	–	4	0.38 ± 0.04	6.87 ± 0.05 ^b	4.89 ± 0.36	43.6 ± 8.4	56.4 ± 8.4
	+	3	1.14 ± 0.29 ^a	–	4.24 ± 0.34	0	100

Membrane preparations of nasal mucosae were obtained from sensitized control guinea pigs (Control) and guinea pigs repeatedly challenged with antigen (Challenge). pK_H = Logarithm of high-affinity agonist dissociation constant; pK_L = logarithm of low-affinity agonist dissociation constant; R_H = percentage of high-affinity binding sites; R_L = percentage of low-affinity binding sites.

^a $p < 0.05$ compared with the respective groups without GTPγS; ^b $p < 0.05$ compared with the control group by Dunnett's multiple analysis. In the absence of GTPγS, the pK_H of the nasal hyperresponsive group was significantly greater than the pK_H of the control animals, indicating that the level and/or activity of G proteins linked to the high-affinity binding sites of H₁ receptors may be increased in the nasal hyperresponsive group.

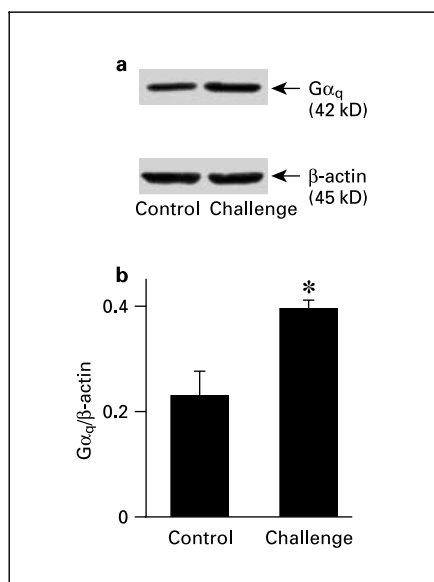


Fig. 6. The effect of repeated antigen challenge on $G\alpha_q$ expression in membrane preparations of guinea pig nasal mucosa. a Typical $G\alpha_q$ immunoblots of membrane preparations of nasal mucosae obtained from sensitized control guinea pigs (Control) and guinea pigs repeatedly challenged with antigen (Challenge). Membrane preparations (10 μ g of protein/lane) were subjected to SDS-PAGE and transferred to nitrocellulose membranes, which were incubated with specific antibody against $G\alpha_q$. After further incubation with horseradish peroxidase-conjugated secondary antibody, a 42-kD band corresponding to $G\alpha_q$ was detected (upper panel). Then, the nitrocellulose membranes were stripped and reprobed by specific antibody against β -actin (a 45-kD band; lower panel). b Relative densities of $G\alpha_q$ to β -actin analyzed by densitometry. The ratios of corresponding $G\alpha_q/\beta$ -actin in respective lanes were calculated as indexes of the $G\alpha_q$ protein level. Each column represents the mean $\pm$ SEM from 5 duplicate experiments. * $p < 0.05$ compared with the control group by Dunnett's multiple analysis.

Discussion

In the present study, we demonstrated that repeated antigen challenge, but not sensitization alone, caused *in vivo* nasal hyperresponsiveness to inhaled histamine in guinea pigs. In this animal model of nasal hyperresponsiveness, the high-affinity histamine binding for H_1 receptors in nasal mucosal preparations was significantly greater than that in the sensitized control group, although no significant change in H_1 receptor density or antagonist affinity was observed between the groups. Furthermore, in the presence of a nonhydrolyzable GTP analogue, GTP γ S, the high-affinity portions of the displacement curves of the groups, especially that of the hyperresponsive group, were shifted to the right; the displacement

curves overlapped each other, and their dissociation constants resulted in the same level. These findings suggest that nasal hyperresponsiveness in guinea pigs cannot be explained by changes in the level of nasal H_1 receptor alone, but might be accounted for by an increased level and/or activity of G proteins linked to H_1 receptors. In fact, the level of $G\alpha_q$ proteins in nasal mucosae determined by immunoblotting was significantly elevated concurrently with the nasal hyperresponsiveness.

Histamine is known to have an important role in eliciting the nasal symptoms of allergic rhinitis (i.e. sneezing, rhinorrhea and nasal congestion). In nasal mucosa, histamine H_1 receptors exist exclusively on the endothelium of nasal vessels [6]. Histamine directly causes increases in blood flow, blood volume and vascular permeability through its action on H_1 receptors of the vascular bed, and produces an immediate nasal congestion which is reflected as an increase in nasal airway resistance [7, 8]. In the current study, histamine aerosol challenge in the non-sensitized normal guinea pigs caused an increase in intranasal pressure in a concentration-dependent manner (fig. 1). Histamine also causes an increase in nasal vascular permeability (Evans blue exudation) and relaxes isolated nasal vascular smooth muscle in guinea pigs, all of which, including the increase in intranasal pressure, are sensitive to H_1 blocking agents [unpubl. observations, in preparation]. It is thus possible that the edematous response and blood vessel dilation via H_1 receptors might contribute to the elevation of intranasal pressure induced by histamine in guinea pigs.

The elevation of intranasal pressure induced by histamine aerosol was significantly augmented in the guinea pigs repeatedly challenged with antigen as compared to that in the sensitized control animals (fig. 2). This finding indicates that nasal hyperresponsiveness, one of the characteristics of patients suffering from allergic rhinitis, is achieved after repetitive exposure to allergen in guinea pigs. The augmented nasal response to histamine reminded us of a speculation that the density and/or affinity of H_1 receptors in nasal mucosa may be increased in this animal model of allergic rhinitis. In the present study, however, no significant difference in either H_1 receptor density or antagonist affinity in the nasal mucosae was observed between the groups. Similar results were reported in nasal mucosa of patients with allergic rhinitis [11]. Therefore, the change in H_1 receptor density appears unlikely to contribute to the augmented nasal responsiveness to histamine. On the other hand, a significant increase in H_1 receptor density has been reported in a guinea pig model of nasal hyperresponsiveness in which

guinea pigs were sensitized and repeatedly challenged with dinitrophenylated *Ascaris* antigen [13]. The differences in the antigen used and/or immunization procedure may influence the results of binding studies in this animal species. Anyhow, the model used in the present study might imitate human allergic rhinitis at least with respect to H₁ receptor expression.

Histamine H₁ receptors are known to couple with a heterotrimeric GTP-binding protein termed G_q. When histamine binds to H₁ receptors, agonist-receptor-G_q protein coupling occurs and the exchange of guanosine 5'-diphosphate for GTP in the α subunit is promoted. The binding of GTP to the α subunit induces a dissociation of the $\alpha\beta\gamma$ holomer to a free, activated, GTP-bound α subunit. Subsequently, the GTP-bound α subunit activates an effector (i.e. phospholipase C) and its downstream pathway of intracellular signal transduction to reveal the response [19]. When the dissociation of the GTP-bound α subunit occurs, the affinity of the agonist for the receptor decreases. In the present study, GTP γ S caused the complete conversion of histamine high-affinity binding sites to low-affinity ones (fig. 5, table 2), indicating that the histamine high-affinity binding states are dependent on receptor-G protein interaction. Furthermore, the effect of GTP γ S was found to be much greater, in particular, in the membrane preparations of nasal mucosae obtained from guinea pigs repeatedly challenged with antigen. These findings suggest that the nasal hyperresponsiveness in guinea pigs might be accounted for by an increased level and/or activity of G proteins linked to H₁ receptors.

In the present study, the G protein levels measured were normalized to the levels of β -actin, a common constitutive protein, to resolve the problem as much as possible. As was expected, the level of G α_q proteins determined by immunoblot analysis was significantly elevated in the

nasal mucosae obtained from the guinea pigs repeatedly challenged with antigen (fig. 6). This is the first study, to our knowledge, that has demonstrated an increase in G α_q protein level concurrently with antigen-induced nasal hyperresponsiveness. As described above, increased high-affinity histamine binding sites for H₁ receptors that were affected by GTP γ S was demonstrated in nasal mucosae of the nasal hyperresponsive animals, although no alterations of H₁ receptor density and antagonist affinity were observed. The observed GTP shift of the competition binding curve means that these receptors couple to G proteins. Thus, the increase in histamine affinity to H₁ receptors in nasal hyperresponsive guinea pigs may reflect the increase in G proteins. In summary, it is suggested that in this animal model of nasal hyperresponsiveness, the enhanced in vivo nasal responses via H₁ receptors may reflect the overexpression of G α_q proteins in nasal mucosa. On the other hand, enhanced G protein levels in airways have also been reported in bronchial hyperresponsiveness in animal models of allergic asthma [17, 20]. The overexpression of G proteins might thus be a universal event in the pathogenesis of upper- and lower-airway hyperresponsiveness in allergic respiratory diseases.

In conclusion, our present study demonstrated the overexpression of G α_q proteins in nasal mucosae of guinea pigs with antigen-induced nasal hyperresponsiveness. The overexpressed G α_q proteins in nasal mucosae may induce an increased binding affinity of histamine to H₁ receptors, resulting in augmented nasal responses, that is, nasal hyperresponsiveness, in guinea pigs.

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Erratum

Figure 2 of the article entitled ‘Hymenoptera-Venom-Induced Upregulation of the Basophil Activation Marker Ecto-Nucleotide Pyrophosphatase/Phosphodiesterase 3 in Sensitized Individuals’ by Platz et al., published in *Int Arch Allergy Immunol* 2001;126:335–342, has been published with a substantial error. Please see below the correct figure:

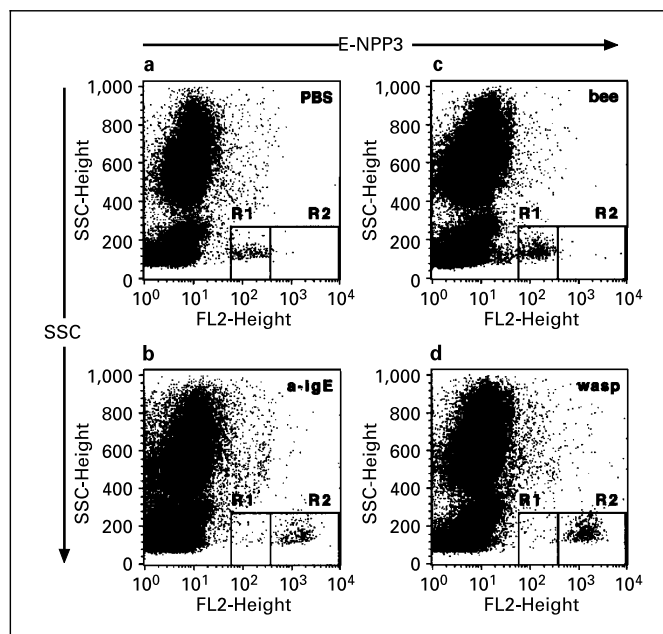


Fig. 2. E-NPP3 expression on basophils upregulated by allergen and anti-IgE antibody. One hundred microliters of whole blood cells from a patient allergic to wasp venom (No. 14) were incubated for 15 min with either PBS (a; negative control), 1 µg/ml of anti-IgE antibody (b; positive control), bee allergen (c), or wasp allergen (d). After staining with 77A6-PE and red blood cell lysis, the cells were analyzed on a FACSCalibur flow cytometer. The SSC versus FL2 (77A6-PE) plots result from 50,000 analyzed cells. Nonstimulated basophils reside in R1 and stimulated basophils in R2.