

Expression and localization of histamine H₂ receptor messenger RNA in human nasal mucosa

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Background: Histamine is an important chemical mediator in allergic rhinitis. Histamine H₂ receptor (H₂R) stimulation controls histamine release and mediates mucus secretion, suggesting that H₂R might play some role in allergic rhinitis.

Objective: The objective of this study was to examine whether H₂R mRNA expression is increased in patients with allergic rhinitis compared with normal subjects and to determine the localization of H₂R mRNA in the human nasal mucosa.

Methods: We extracted RNA from scrapings of the inferior turbinate mucosa of 11 patients with allergic rhinitis. H₂R and β -actin mRNA were amplified by RT-PCR. In situ hybridization with a digoxigenin-labeled H₂R cRNA probe was performed on surgical specimens of nasal turbinate of patients with allergic rhinitis.

Results: The mean $\pm$ SD of H₂R/ β -actin ratios were 0.12 ± 0.16 for the scrapings of normal subjects and 0.35 ± 0.20 for the scrapings of patients with allergic rhinitis; the difference was statistically significant ($P < .01$). H₂R mRNA was localized in the epithelium, serous cells, and mucous cells.

Conclusion: These data suggest that H₂R mRNA is localized in the epithelium and the submucosal glands and that increased H₂R may be playing some role in patients with allergic rhinitis. (*J Allergy Clin Immunol* 1999;103:944-9.)

Key words: Histamine H₂ receptor, mRNA, allergic rhinitis, reverse transcription-polymerase chain reaction, nasal scrapings, nasal mucosa, in situ hybridization

Histamine plays an important role in eliciting the nasal symptoms of allergic rhinitis (ie, pruritus, sneezing, rhinorrhea, and congestion).¹ Histamine receptors are classified as H₁, H₂,² and H₃.³ Histamine H₁ receptor (H₁R) stimulation contracts tracheal and vascular smooth muscles, increases vascular permeability, and stimulates sensory nerve endings.⁴ Histamine H₂ receptor (H₂R) mainly mediates gastric acid secretion and relaxes a number of tissues, including airway and vascular smooth

Abbreviations used

H₁R: Histamine H₁ receptor
H₂R: Histamine H₂ receptor
H₃R: Histamine H₃ receptor
HDM: House dust mite

muscles.⁴ Histamine H₃ receptor (H₃R) stimulation inhibits synthesis and release of histamine itself.⁴ Human H₁R and H₂R genes have been isolated and cloned by screening a human genomic library with a bovine H₁R and H₂R probe, respectively.^{5,6} The H₃R gene has not been cloned yet. The nucleotide sequence of human H₂R shows little similarity to that of human H₁R (27.9%).⁵

It has been postulated that released histamine acts as a feedback inhibitor of histamine release and that this control mechanism is mediated by H₂R.⁷⁻⁹ It was reported that H₂R stimulation can mediate mucus secretion¹⁰ and airway goblet cell secretion.^{11,12} Nakazawa and Umegae¹³ reported that histamine suppressed specific IgE production by means of H₂R, and Fujimoto and Kimata¹⁴ reported that histamine has a direct inhibitory effect on B cells by means of H₂R. Clinical studies in subjects with seasonal rhinitis suggested that treatment with an H₂R antagonist produced some symptomatic benefit,^{15,16} but pretreatment with an oral H₂R antagonist did not prevent the measured rise in nasal airway resistance induced by allergen challenge.¹⁷ Satoh and Shimada¹⁸ reported that the number of H₂Rs was increased in the nasal allergic group compared with the nonallergic group. These previous reports suggest that H₂R plays some role and is increased in allergic rhinitis. However, to our knowledge, there has not been a report concerning the localization and the expression of H₂R mRNA in the human nasal mucosa. We hypothesized that the H₂R mRNA is localized in the human nasal mucosa, and the expression of H₂R mRNA is increased in the nasal mucosa of patients with allergic rhinitis compared with normal subjects.

This study was designed to examine the localization of H₂R mRNA in human nasal mucosa by in situ hybridization and to compare the expression of H₂R mRNA in nasal mucosa from normal subjects and patients with allergic rhinitis. Because it is impossible to obtain large specimens from normal human subjects, we amplified mRNA by RT-PCR.

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METHODS

Subjects

To examine the expression of H₂R mRNA, scrapings were obtained by using curettes from nasal mucosa in the inferior turbinates of 11 healthy volunteers (18 to 45 years of age; mean age $\pm$ SD, 22.1 $\pm$ 7.8 years; 5 men and 6 women) and 11 patients with allergic rhinitis (16 to 67 years of age; mean age $\pm$ SD, 28.1 $\pm$ 14.2 years; 7 men and 4 women). To examine the localization of H₂R mRNA, inferior turbinate mucosal samples were obtained at the time of surgery from the patients with allergic rhinitis who had severe nasal congestion.

All the patients were allergic to house dust mite (HDM). The diagnosis of allergic rhinitis was based on a compatible history, anterior rhinoscopic examination, and the following 3 criteria: (1) eosinophilia in nasal smears; (2) RAST class 2 or more for HDM; and (3) a positive nasal provocation test response for HDM. Tests were performed several weeks before obtaining samples. The patients with allergic rhinitis were moderately symptomatic and received neither antihistamine therapy nor topical steroids in the 6 months before the study. The normal subjects did not have nasal symptoms, physical examination findings peculiar to allergic rhinitis, or eosinophils in nasal scrapings.

Informed consent was obtained from each patient and healthy volunteer, and the protocol was approved by the Ethics Committee of Mie University School of Medicine.

Preparation of oligonucleotide primers

Oligonucleotide primers were constructed from the published cDNA sequences of H₂R⁶ and β -actin cDNA.¹⁹ The β -actin primers were selected to span an intron-exon boundary to distinguish between amplification of mRNA and any contaminating genomic DNA, and these 2 pairs of primers were selected as close as possible to the poly-A tail to increase the efficiency of the RT-PCR by using oligo d(T) as a primer for reverse transcription. The sequence of the H₂R primers were 5'-GCGGTCCTCATCCTCATCACC-3' (sense), corresponding to bases 76-96 of the cloned full-length sequence, and 5'-TTCCTGTTGCCTGGGTTCTC-3' (antisense), which anneals to bases 989-1008. The β -actin primers were 5'-ATGGATGATGATATCGCCGCG-3' (sense) and 5'-ATGTCGTC-CCAGTTGGTGACGAT-3' (antisense). The predicted size of the amplified H₂R and β -actin DNA products were 933 and 254 bp, respectively.

Isolation of RNA and RT-PCR

After samples were scraped or resected, isolation of total cellular RNA was performed as previously described.^{20,21} Reverse transcription of RNA and amplification of H₂R and β -actin cDNA were performed as previously described,²¹ with minor modifications.

The amplification profile consisted of denaturation at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds, and extension at 72°C for 1.5 minutes. The reaction was terminated by chilling at 4°C. The negative controls contained all the necessary components for PCR except template DNA. As positive controls, human genomic DNA was used as a template for H₂R, and human β -actin plasmid DNA was used for β -actin. DNase-treated genomic DNA and β -actin plasmid DNA were amplified to test the adequacy of DNase treatment.

Previous studies have shown that the amount of amplified fragment is proportional to the quantity of input mRNA during the exponential phase of the amplification process.^{22,23} We have also examined every 5 cycles of amplification to determine the point at which amplification is within an exponential phase. We confirmed that the amplification of H₂R and β -actin mRNA remained in an exponential phase at 33 and 27 cycles, respectively. The reaction mixtures were amplified for each cycle.

The PCR products were electrophoresed and blotted onto nylon membranes.

Hybridization with end-labeled oligonucleotide probes

In the experiments to validate the PCR products, a sense 30-mer oligonucleotide probe internal to the amplified fragment of H₂R sequence 5'-AGAGGATCAATCACATTAGCTCCTGGAAGG-3', corresponding to nucleotide 641-670 of the cDNA sequence, was synthesized. The oligonucleotide probe used to validate the β -actin PCR fragment was 5'-GTCCACTCTCAATCACTCTCAGTTCTTTGA-3'. One hundred nanograms of each probe was end-labeled, and the nylon membranes were prehybridized and hybridized as previously described²¹ at 65°C for H₂R or at 61°C for β -actin. Intensity of the bands of H₂R and β -actin in autoradiographs was quantitated with a densitometer.

Statistical analysis

The Mann-Whitney *U* test was used to test for differences in H₂R/ β -actin ratio between the 2 groups. Significance was accepted at the 5% level of confidence.

Preparation of cRNA probes

The cDNA fragment of human H₂R was amplified by PCR with a pair of previously described primers. This cDNA fragment of human H₂R (933 bp) was inserted into a pCR II vector (Original TA Cloning Kit, Invitrogen). Digoxigenin-labeled sense and antisense RNA probes were transcribed *in vivo* from the linearized plasmid by SP6 or T7 RNA polymerase according to the manufacturer's manual (DIG RNA Labeling Kit SP6/T7, Boehringer Mannheim). The size of the probes was reduced to an average of 200 nucleotides by the alkaline treatment.²⁴

In situ hybridization

For *in situ* hybridization, nasal mucosal tissue was dissected from inferior turbinate of the patients with allergic rhinitis by using scissors. They were trimmed and fixed in 4% paraformaldehyde solution with 0.1 mol/L PBS, pH 7.4, at 4°C for 16 hours. After being rinsed in the same buffer, they were dehydrated by a graded ethanol series and xylene, embedded in paraffin, cut at 4 μ m thickness, and placed on glass slides coated with silan-coated slide glass (Dako Japan, Kyoto). All of the buffers and glasses used for the detection of mRNA were RNase-free.

In situ hybridization procedures were similar to those previously described,²⁴⁻²⁶ with minor modifications. Sections were deparaffinized with xylene and digested with 0.3 U/mL proteinase K (Boehringer Mannheim) for 20 minutes at 37°C, post-fixed with 4% paraformaldehyde, washed in PBS 3 times, and then treated in 0.2 mol/L HCl for 10 minutes. After being washed again, they were treated in 0.1 mol/L triethanolamine (pH 8.0), treated with 0.1 mol/L triethanolamine/0.25% acetic anhydride for 10 minutes, dehydrated, and air dried. The specimens were prehybridized with hybridization buffer for 2 hours at 50°C and incubated with 40 μ L of hybridization buffer containing 100 ng of alkaline-hydrolyzed digoxigenin-labeled antisense or sense probes for 16 hours at 50°C. After hybridization, the specimens were rinsed and incubated. To remove unhybridized RNA, the sections were incubated with 20 μ g/mL RNase A (Sigma) for 30 minutes at 37°C. Immunologic detection of hybridized RNA probes was done with a Nucleic Acid Detection Kit (Boehringer Mannheim). When satisfactory signals were obtained, the sections were washed and counterstained with 0.1% nuclear fast red in 5% aluminum sulfate solution, air dried, and mounted.

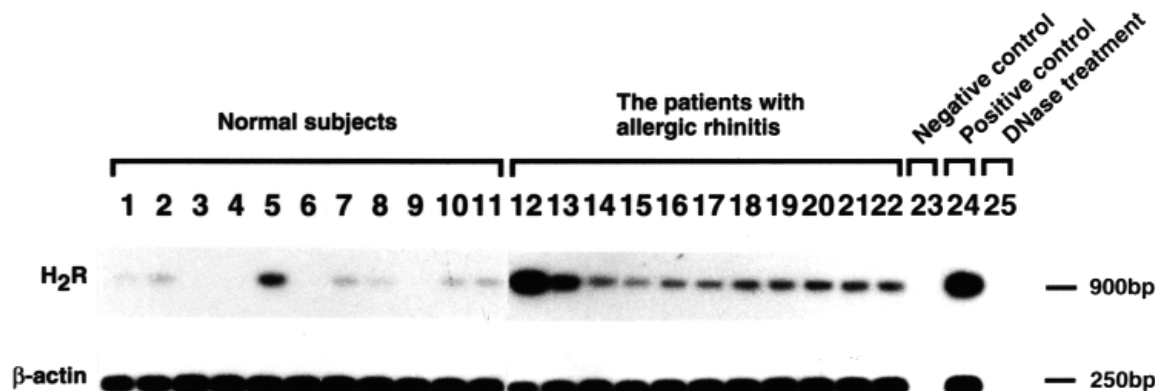


FIG 1. Southern blotting bands of amplification products of H₂R mRNA (upper row) and β-actin mRNA (lower row) are shown in the vertical lanes 1 to 25. Lanes 1 to 11, Scrapings of the normal subjects; lanes 12 to 22, scrapings of the patients with allergic rhinitis; lane 23, negative control containing no DNA; lane 24, genomic DNA for H₂R and β-actin plasmid DNA for β-actin were amplified as positive control; lane 25, DNase-treated genomic DNA and β-actin plasmid DNA as a template.

RESULTS

Verification of PCR products as predicted segments of H₂R and spliced β-actin mRNA

The bands of β-actin were observed in all the samples. The 7 bands of H₂R in normal subjects and all the bands of H₂R in patients with allergic rhinitis were observed. The bands were detected in the positive control lane but not in the DNase-treated genomic DNA lane, indicating that DNase treatment was adequate (Fig 1). Although some lanes appear to have no obvious bands for H₂R mRNA, faint bands could be observed on an original film. The H₂R and β-actin probes did not hybridize to unrelated DNA molecules under the conditions used.

Kinetics and yields of H₂R and β-actin mRNA

As illustrated in Fig 2, the amplification of H₂R and β-actin mRNA remained in an exponential phase at 33 and 27 cycles, respectively.

Comparison of the expression of H₂R mRNA in normal subjects and patients with allergic rhinitis

The H₂R and β-actin mRNA were amplified separately for 33 and 27 cycles, respectively. Samples from the normal subjects and the patients were always run in parallel. The H₂R/β-actin ratios were quantitated in the 11 healthy volunteers and the 11 patients with allergic rhinitis. As shown in Fig 3, the means ± SD of H₂R/β-actin ratios were 0.12 ± 0.16 for the scrapings of normal subjects and 0.35 ± 0.20 for the scrapings of patients with allergic rhinitis. The group of patients with allergic rhinitis had significantly higher H₂R/β-actin ratios compared with the normal subjects ($P < .01$).

In situ hybridization

In situ hybridization with the antisense probe revealed that signals were observed in the surface epithelia,

including goblet cells (Fig 4, B, arrows) and the serous (Fig 4, C) and mucous cells (Fig 4, D) in the submucosal glands. Some types of inflammatory cells in the stroma are also positive. Considering their morphology and localization, the cells are presumed to be plasma cells (arrowhead in Fig 4, D) and mast cells (arrowhead in Fig 4, D). The specificity of the hybridization was confirmed by the absence of signals when the preparations were hybridized with a sense probe (Fig 4, E and F). Strong hybridization signals are clearly visible in the goblet cells, serous cells, and mucous cells.

DISCUSSION

There have been many reports about the roles of H₂R,^{7-10,13,14,27,28} and an investigator has reported the quantitation of H₂R,¹⁸ but to our knowledge, this study is the first report that specifically demonstrated the localization of human H₂R mRNA in the human nasal mucosa and the expression of H₂R mRNA in the nasal scrapings and mucosae of inferior turbinates of both patients with allergic rhinitis and normal subjects.

Most of the previous studies performed with the in situ hybridization technique used radioactive probes for the detection of mRNA in tissues because nonradiolabeled hybridization is inferior to radiolabeled hybridization in the sensitivity and the incorporation of the label into the probe.²⁹ It is reported that the in situ hybridization method with a nonradioactive RNA probe is useful because of its high resolution power for cell identification.^{25,26} Our in situ hybridization results clearly indicated that H₂R mRNA was present in the goblet cells, serous cells, mucous cells, and infiltrating cells in the submucosa but not in the endothelial cells of vessels in the human nasal turbinate mucosa.

Knight et al²⁷ reported that H₂Rs are resident on the epithelium. Takeyama et al¹¹ and Tamaoki et al¹² reported that histamine increases airway goblet secretion by stimulating H₂R on goblet cells. These previous findings

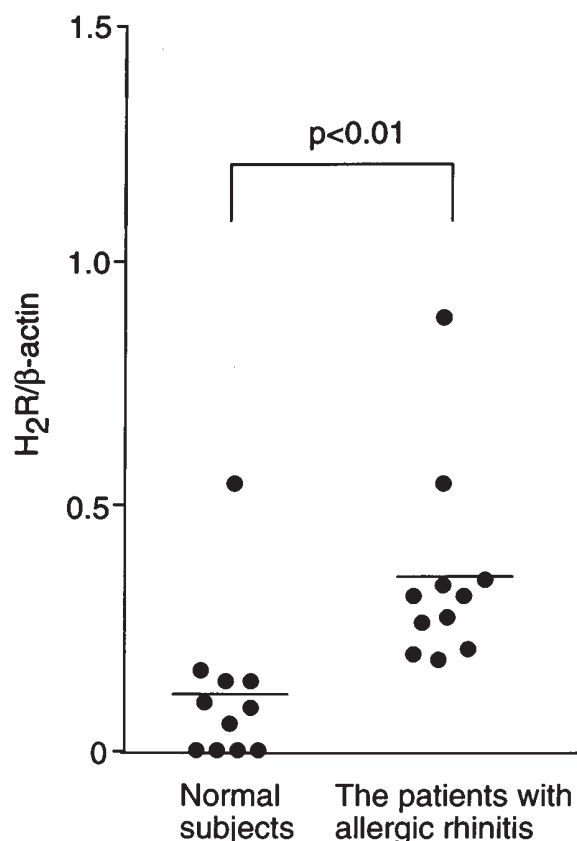


FIG 3. Differences in H₂R gene expression determined by analysis of scrapings from normal individuals and scrapings from patients with allergic rhinitis. The quantitative results are expressed as ratios of the intensity of the H₂R band at 33 cycles to the intensity of the β -actin band at 27 cycles.

apical membrane.¹² We suggest that each cell type has H₂R, and histamine increases mucus secretion by stimulating H₂R.

H₂R mRNA was also detectable in infiltrating cells in the submucosa. Considering their morphology and localization, the cells are presumed to be plasma cells and mast cells. The previous findings that histamine suppressed IgE production by means of H₂R⁷ and that H₂-mediated effects include inhibition of mast cells by histamine release^{9-10,13} support our finding that H₂R mRNA was present in the plasma cells and mast cells, but we cannot draw a firm conclusion about which infiltrating cells expressed H₂R mRNA. Therefore further study with immunohistochemistry/in situ hybridization is needed to determine the identity of these cells.

The role of H₂R remains questionable, whereas the role of H₁R in allergic rhinitis is clearly established. We tried to identify H₁R mRNA in the nose by in situ hybridization but have not succeeded in doing so thus far. The failure to identify H₁R mRNA in the nose may be due to technical reasons, such as the number of H₁R mRNA in the nose being too small to be identified by in situ hybridization.

In this study we developed a semiquantitative assay

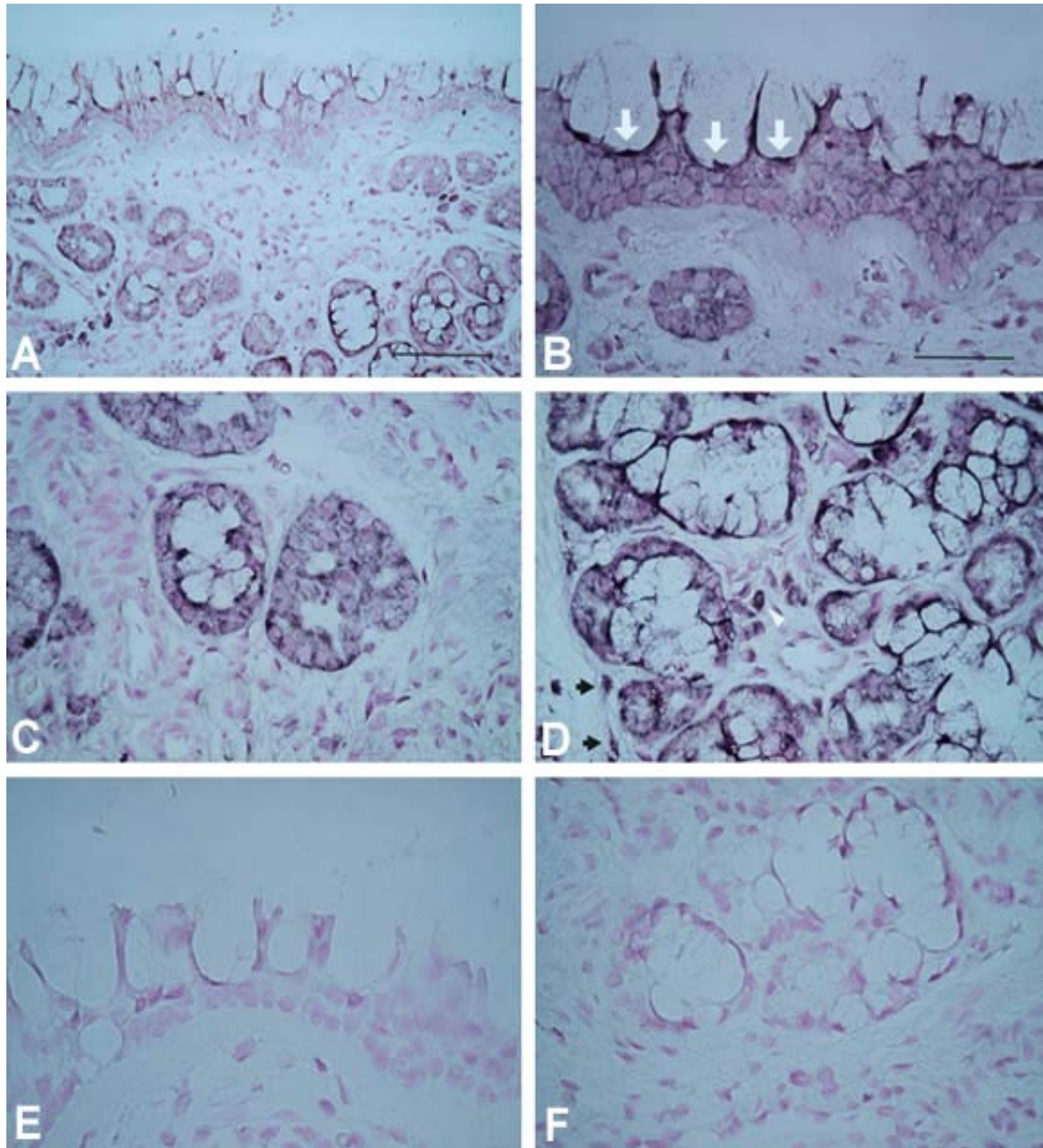


FIG 4. Localization of human H₂R mRNA in human nasal mucosa. **A**, In situ hybridization with antisense H₂R probe. The surface epithelia containing goblet cells, serous cells, mucous cells, and infiltrating cells were positive. **B**, High-powered magnification of the surface epithelia containing the goblet cells. **C**, High-powered magnification of the serous cells. **D**, High-powered magnification of the mucous cells and infiltrating cells. Considering their morphology and localization, the cells are presumed to be plasma cells and mast cells. **E** and **F**, In situ hybridization with sense H₂R probe. No signals were detected. Arrows indicate goblet cells (open arrows), plasma cells (open arrowheads), and mast cells (filled arrows). Bar in **A** represents 100 μ m, and bar in **B** through **F** represents 50 μ m.

for H₂R mRNA to examine the expression of H₂R in the nasal mucosa immediately after sampling from the inferior turbinates. The expression of receptor mRNA was quantitated by RT-PCR. Such an approach has been shown to be a useful method for detecting small amounts of transcripts when applied to the quantification of RNA from small numbers of cells or to characterize low abun-

dant transcripts.³⁰⁻³² Because human H₂R genes lack introns,⁶ we treated RNA with DNase to remove genomic DNA, and we confirmed that the amplification of H₂R and β -actin mRNA remained in an exponential phase at 33 and 27 cycles, respectively.

We demonstrated that the H₂R mRNA was expressed in larger amounts in the nasal mucosa from the patients

with allergic rhinitis than in the nasal mucosa from the normal subjects. Likewise, Satoh and Shimada¹⁸ reported that the number of H₂Rs was increased in the nasal allergic group compared with the nonallergic group by a binding assay. It may be difficult to compare mRNA with receptor protein and to make a firm conclusion. There are many steps in the pathway leading from DNA to protein. A cell can regulate the proteins it makes at the transcriptional level, the RNA processing level, the RNA transport level, the translation level, the mRNA degradation level, or the protein activity level.^{33,34} Because transcriptional controls are paramount for most genes,^{33,34} we suggest that the number of H₂Rs is increased in patients with allergic rhinitis. We believe that increased H₂R in patients with allergic rhinitis plays some role in the development of nasal symptoms. Cytology of nasal scrapings from normal individuals and patients with allergic rhinitis shows that 95.1% and 84.8% of the total cells were epithelial cells, and the remaining 4.9% and 15.2% were infiltrating cells, respectively (unpublished data). In this study most of the H₂R mRNA signal-containing cells were resident cells, such as goblet cells and submucosal gland cells. Thus the increase of H₂R mRNA in the allergic subjects could be due to the induction of H₂R mRNA expression in these resident cells.

In conclusion, we demonstrated in this study that H₂R mRNA was localized in the epithelium, the serous cells, and the mucous cells and that the expression of H₂R mRNA was increased in nasal mucosa from the patients with allergic rhinitis in comparison with those from the normal subjects. We suggest that localized and increased H₂R mRNA in the nasal mucosa may play some role in the nasal symptoms found in patients with allergic rhinitis.

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