

Histamine alters gene expression in cultured human nasal epithelial cells

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Background: Airway epithelial cells produce cytokines and participate in the regulation of mucosal immunity. Although nasal epithelial cells express histamine receptors, it is not exactly known how nasal epithelial cells respond to histamine. **Objective:** The objective of this study was to examine whether histamine can alter the expression of the 4 genes encoding H₁ receptor, IL-8, TNF- α , and ZO-1 tight-junction protein in cultured nasal epithelial cells.

Methods: We added histamine or vehicle to cultured human nasal epithelial cells and extracted RNA from them 4 hours later. After DNase treatment, mRNAs of β -actin, H₁ receptor, IL-8, TNF- α , and ZO-1 tight-junction protein were amplified by using RT-PCR.

Results: Histamine significantly upregulated IL-8 mRNA expression and significantly downregulated ZO-1 mRNA expression. The latter effect was blocked by pretreatment with mepyramine, an H₁ receptor antagonist.

Conclusion: The reduction of ZO-1 mRNA by histamine may cause increased permeability of the mucosa during allergic reactions in the nose. (*J Allergy Clin Immunol* 2001;107:310-4.)

Key words: Histamine receptors, mRNA, allergic rhinitis, reverse transcription-polymerase chain reaction, nasal mucosa, ZO-1, IL-8

Histamine plays an important role in eliciting nasal symptoms of allergic rhinitis: 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 muscles.⁴ Histamine H₃ receptor 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 bovine H₁R and H₂R probes, respectively.^{5,6} H₃R gene has not been cloned yet. The nucleotide sequence of human H₂R shows little similarity to that of human H₁R (27.9%).⁵

Abbreviations used

H₁R: Histamine H₁ receptor

H₂R: Histamine H₂ receptor

Recently, it became apparent that airway epithelial cells produce many cytokines and participate in the regulation of mucosal immunity.⁷ The traditional view of the epithelium as a relatively passive physical barrier to the external environment has been superseded by the concept that the epithelial cell plays a key role in regulating airway inflammation. It is reported that IL-8, IL-10, IL-13, and RANTES mRNA levels in the nasal mucosa are increased after nasal allergen provocation⁸ and that airway epithelial cells produce cytokines by their interaction with inhaled allergen extracts.⁹ Moreover, eosinophils induce release of IL-8 and GM-CSF.¹⁰

Previously, we reported that human nasal epithelial cells express both H₁R¹¹ and H₂R.¹² It is probable that nasal epithelial cells change the expression of various genes in response to histamine by means of H₁R and H₂R. However, to our knowledge, there have been only a few reports¹³ concerning the effect of histamine on the expression of genes in the epithelial cells. We hypothesized that nasal epithelial cells alter expression of various genes in response to activation of their histamine receptor.

The effects of histamine have been extensively studied in microvascular endothelial cells and blood cells. In cultured retinal microvascular endothelial cells, histamine reduces ZO-1 tight-junction protein expression.¹⁴ In human umbilical vein endothelial cells, histamine induces IL-8 secretion.¹⁵ Histamine suppresses gene expression and synthesis of TNF- α by means of H₂R in peripheral blood mononuclear cells¹⁶ and inhibits TNF- α release by mast cells through H₂ and H₃ receptors.¹⁷ We thought that histamine might also have similar effects on epithelial cells. Moreover, we hypothesized that histamine might increase the expression of H₁R in the epithelial cells because the expression of H₁R is increased in nasal scrapings of patients with nasal allergies.¹¹ In this study we examined the expression of ZO-1, IL-8, TNF- α , and H₁R mRNAs in the cultured nasal epithelial cells to determine whether their expressions can be altered by stimulation of histamine.

METHODS

Cell isolation

Human nasal polyps were obtained from patients with chronic sinusitis at clinically indicated surgery. The specimens were rinsed

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3 times with Dulbecco's modified Eagle's medium/Ham's F12 (Sigma, St Louis, Mo) containing penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL), and the nasal epithelial cells were isolated from the tissue sample by using the protease treatment.¹⁸ Briefly, the excised tissue was exposed to a 0.1% protease solution in F12 medium for 16 hours at 4°C. After incubation, FBS was added to a concentration of 10%, and epithelial cells were detached from the stroma with gentle agitation. The cell suspensions were centrifuged at 1200 rpm for 10 minutes at 4°C, and the pellet was resuspended in growth medium and was centrifuged again. The second cell pellets were suspended again in the growth medium supplemented with FBS and were cultured. The cell viability (trypan blue dye exclusion) was greater than 90%.

Cell culture

Cells were grown on Transwell-Col tissue culture inserts (Coster Co, Cambridge, Mass), which are supplied by the manufacturer coated with a combination of monomeric type I and III bovine placental collagens. All experiments were performed by using 24.5-mm diameter membranes with a 0.45-µm pore size. Dulbecco's modified Eagle's medium/Ham's F12 medium supplemented with growth factors, hormones, and bovine pituitary extract was used. The basic medium (No. D-9785; Sigma, St Louis, Mo) was supplemented with L-leucine (0.45 mmol/L), L-lysine (0.5 mmol/L), L-methionine (0.12 mmol/L), MgCl₂ (0.3 mmol/L), MgSO₄ (0.4 mmol/L), CaCl₂ (1.05 mmol/L), phenol red (8.6 mg/L), NaHCO₃ (1.2 g/L), and retinoic acid (5 × 10⁻⁸ mol/L). The growth factors and hormones included epidermal growth factor (25 ng/mL), endothelial cell growth supplement (7.5 µg/mL), insulin (10 µg/mL), hydrocortisone (0.1 µg/mL), and transferrin (5 µg/mL). The other additives were L-glutamine (6.5 mmol/L), penicillin-streptomycin (50 U/mL to 50 µg/mL), cholera toxin (0.1 µg/mL), and HEPES (final, 30 mmol/L). All the reagents were tissue culture grade and were purchased from Sigma, except for penicillin-streptomycin, which was from GIBCO (Grand Island, NY), and bovine pituitary extract, which was from Collaborative Biomedical Products (Bedford, Mass).

Cells were plated onto the apical surface of membranes in 1 mL of medium with serum (1 × 10⁵ cells/cm²). After 24 hours, medium in both compartments was removed and replaced with serum-free medium. The medium was changed every other day, with 2 and 0.5 mL in the bottom and top compartments, respectively. An air-liquid interface at the level of cell layer was created at day 7. Cultures were incubated in a humid environment in 5% CO₂ at 37°C.

Histamine activation of cultured nasal cells

Histamine (10⁻⁴ mol/L) was added for 4 hours to confluent cells. Cells were harvested and subjected to an RNA-extracting process. In separate studies cells were pretreated with histamine-receptor antagonists to determine whether histamine receptors mediate the changes in their mRNA expression. Mepyramine (H₁R blocker, dissociation constant = 0.8 nmol/L) or ranitidine (H₂R blocker, dissociation constant = 200 nmol/L) at 10⁻³ mol/L, respectively, was applied 30 minutes before adding 10⁻⁴ mol/L histamine to allow for receptor binding.

Isolation of total cellular RNA

Samples (with membrane) were put into ice-cold denaturing solution (4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate [pH 7.0], 0.5% sarcosyl, and 0.1 mol/L 2-mercaptoethanol). After vortexing the mixtures, we followed a single-step method of RNA isolation by Chomczynski and Sacchi.¹⁹ One microgram of RNA pellets was resuspended in 20 µL of 10× buffer, added to 1 U of DNase I, and incubated at 37°C for 30 minutes to distinguish RNA from contaminating genomic DNA. The RNA was extracted with phenol/chloroform/isoamyl alcohol (25/24/1 vol/vol/vol),

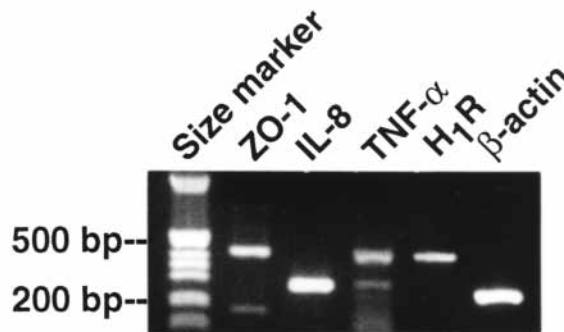


FIG 1. Amplification products obtained with 5 gene-specific primers and RNA derived from cultured cells. RNA extracted from unstimulated cultured cells were reverse transcribed to cDNA and were amplified for 30 cycles. The PCR products were electrophoresed through a 2% agarose gel.

TABLE I. PCR primers used for RT-PCR

mRNA species	Fragment size (bp)	Primer sequences
ZO-1	434	U: 5'-CATAGAATAGACTCCCCTGG-3' L: 5'-CTGCTGGCTTGTCTCTAC-3'
TNF-α	444	U: 5'-GAGTGACAAGCCTGTAGCCCAT-GTTGTAGCA-3' L: 5'-GCAATGATCCCAAAGTAGACCTGCCAGACT-3'
IL-8	289	U: 5'-ATGACTTCCAAGCTGGCCGTGGCT-3' L: 5'-TCTCAGCCCTCTTCAAAACTTCTC-3'
H ₁ R	401	U: 5'-AGCCAATCCTTCTCTCGA-3' L: 5'-TAAGGAGCGAATATGCAG-3'
β-Actin	246	U: 5'-ATGGATGATGATATCGCCGCG-3' L: 5'-ATGTCGTCCAGTTGGTGACGAT-3'

ethanol precipitated, centrifuged at 15,000 rpm for 10 minutes, rinsed with 70% ethanol, and dried.

Preparation of oligonucleotide primers

Oligonucleotide primers were designed from the published cDNA sequences of ZO-1,²⁰ IL-8,²¹ TNF-α,²² H₁R,⁵ and β-actin.²³ The sequences of all primers are shown in Table I.

Reverse transcription of RNA

The RNA pellets prepared as above were reverse transcribed into cDNAs by using the GeneAmp RNA PCR Kit (Takara Biomedicals, Tokyo, Japan). Oligo d(T) primer was used for generation of cDNAs. After incubation at 42°C for 30 minutes, the samples were heated for 5 minutes at 99°C to terminate the reactions and were stored at -20°C until used.

Amplification of ZO-1, IL-8, TNF-α, H₁R, and β-actin cDNAs

Fresh tubes containing the upstream and downstream primers (25 µmol/L of each primer) spanning the given sequence for amplification, 200 µmol/L of each deoxyribonucleoside triphosphate, 50 mmol/L KCl, 10 mmol/L TRIS-HCl (pH 8.3), 1.5 mmol/L MgCl₂, and 1 U of *Thermus aquaticus* (Taq) DNA polymerase, were heated to 94°C, and 1 µL of cDNA were added to a final volume of 50 µL. One drop of mineral oil was added to the reaction mixture and amplified in a Perkin-Elmer/Cetus (Norwalk, Conn) thermal cycler. The amplifica-

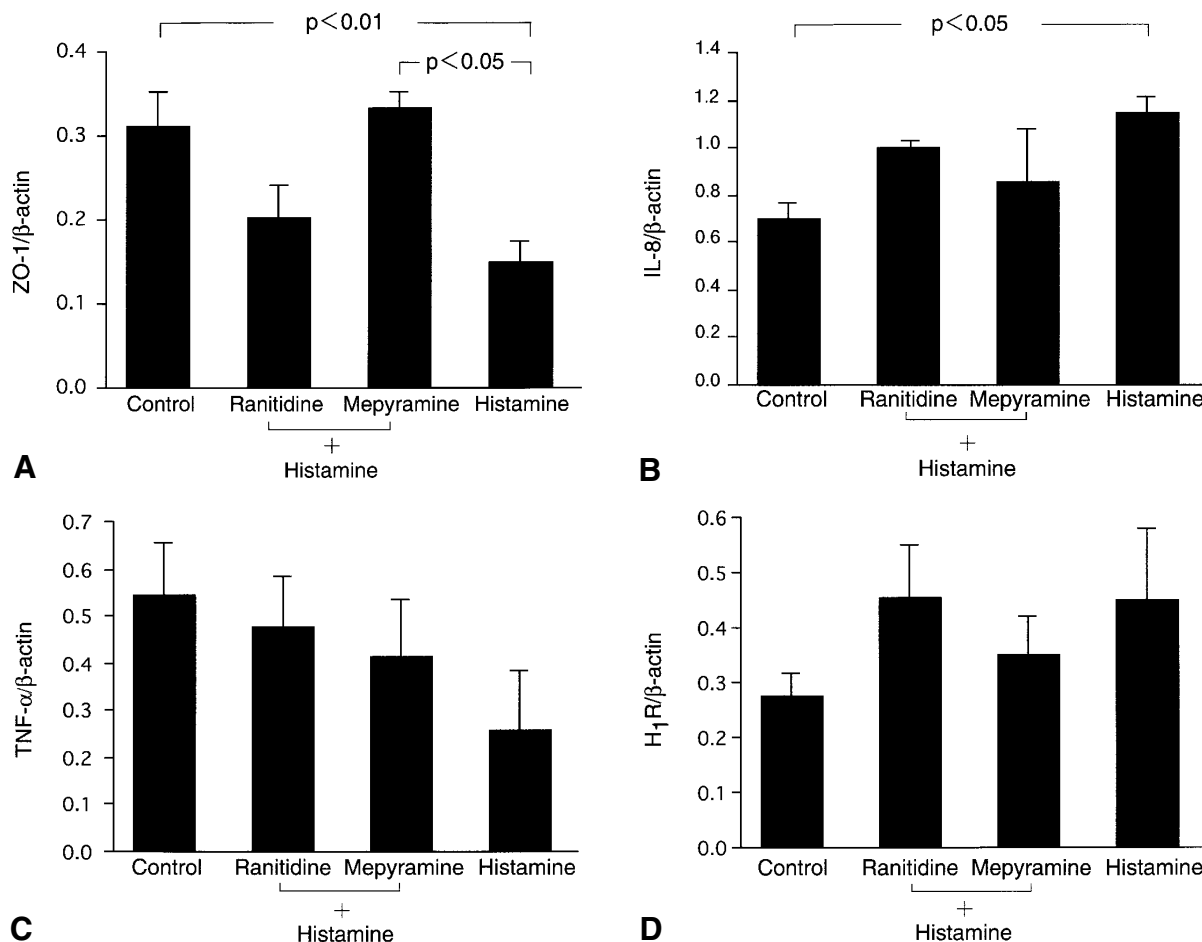


FIG 2. Effects of histamine on ZO-1 mRNA (**A**), IL-8 mRNA (**B**), TNF- α mRNA (**C**), and H₁R mRNA (**D**) corrected for β -actin mRNA expression in cultured epithelium cells and of pretreatment with mepyramine (H₁R antagonist) and ranitidine (H₂R antagonist). Data are expressed as mean $\pm$ SEM of 7 experiments for each column.

tion 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 minute. The reaction was terminated by chilling at 4°C. The negative controls contained all the necessary components for PCR except template DNA. It has been reported that the amount of amplified fragments is proportional to the quantity of input mRNA during the exponential phase of the amplification process.^{24,25} We have also examined every 5 cycles of amplification and confirmed that the amplification of each mRNA remained in the exponential phase at 30 cycles.

The PCR products were electrophoresed through a 2% (wt/vol) agarose gel containing 0.5 μ g/mL of ethidium bromide in 40 mmol/L TRIS-acetate buffer, pH 8.0, containing 1 mmol/L EDTA. Intensity of the bands in the gels was quantitated by using a densitometer. The band intensities (in arbitrary units) of the 4 cDNAs were adjusted on the basis of the intensity of the actin bands to represent equivalent mRNA loaded in each lane. The PCR products were confirmed to be the correct products by using Southern blotting with internal probes.

Statistical analysis

Data are expressed as means $\pm$ SEM. Because the data were normally distributed, the statistical significance of the differences was assessed with the Student *t* test. Differences with a *P* value of less than .05 were considered significant.

RESULTS

As can be seen in Fig 1, the size of the amplification products of 5 mRNAs were identical to the predicted sizes (Table I).

Treatment of cultured nasal cells by histamine significantly decreased the expression of ZO-1 mRNA ($P < .05$; Fig 2, A). This change was significantly inhibited by pretreatment with the H₁R antagonist mepyramine ($P < .01$) but not by pretreatment with the H₂R antagonist ranitidine (Fig 2, A).

The expression of IL-8 mRNA was significantly upregulated by histamine (Fig 2, B). This upregulation was not significantly inhibited either by mepyramine or ranitidine.

Histamine tended to decrease the expression of TNF- α mRNA expression, but the decrease was not statistically significant (Fig 2, C).

The expression of histamine H₁R mRNA tended to increase by histamine administration, but the increment was not statistically significant (Fig 2, D).

DISCUSSION

The present study clearly indicates that administration of histamine to cultured nasal cells significantly reduces the ZO-1 mRNA expression and significantly increases IL-8 mRNA expression. In this study histamine was used at only one concentration (10^{-4} mol/L) because the maximum effect has been obtained around this concentration in the previous studies,¹⁴⁻¹⁷ in which a gradient of concentrations of histamine was used. However, this concentration (10^{-4} mol/L) may be too high to be of physiologic significance.

This study was performed with epithelium from nasal polyps because nasal polyps are more available than nasal mucosa of patients with allergic rhinitis, and nasal polyps lack seromucous glands. Although the stroma of nasal polyps is markedly edematous, the surface epithelium is intact and composed of a respiratory epithelium.²⁶ Thus the epithelium from nasal polyps is similar to that from the nasal mucosa. Moreover, cells from nasal polyps cultured under the same conditions revealed differentiation into ciliated cells and secretory cells,²⁷ which are the 2 major components in the epithelium of nasal mucosa in patients with allergic rhinitis. Yankaskas et al²⁸ examined bioelectric properties of turbinate epithelial cells from normal subjects and of nasal polyp epithelial cells from atopic subjects, both of which were cultured on collagen matrix supports. Both epithelial cells showed similar transepithelial electric potential difference and response to amiloride, which means that physiologic features are the same in both epithelia. Therefore our findings may be applicable to the nasal epithelium.

ZO-1 is one of the tight-junction proteins, which are cytoplasmically located. Tight junctions are macromolecular assemblies of proteins that form contiguous rings at the apices of epithelial cells.²⁹ At least 9 protein constituents of the tight junction have been identified.³⁰⁻³² Of these, ZO-1, a 220-kd phosphoprotein, has been the best characterized.³³ The functional importance of ZO-1 is suggested by its increased expression in parallel with transepithelial electrical resistance in cultured rat brain capillary endothelial cells.³⁴ ZO-1 therefore participates in the regulation of paracellular permeability through tight junctions.

The result of the present study suggests that histamine released in the early-phase reaction of nasal allergy may increase the paracellular permeability of the mucosa by reducing ZO-1 mRNA. Previously, Kawaguchi et al³⁵ examined the nasal epithelial permeability by comparing horseradish peroxidase penetration into the intercellular spaces in allergic and nonallergic guinea pigs. They found that the horseradish peroxidase reaction products in the intercellular spaces in the allergic groups were significantly increased, but they could not find any epithelial injury, even in the allergic guinea pigs, with transmission electron micrographs. It is probable that increased penetration of horseradish peroxidase in their allergic animals may have been due to the decreased ZO-1 expression caused by locally released histamine. As in this case, locally released histamine may cause increased mucosal

permeability. The increase of the permeability may facilitate penetration of antigens through a paracellular pathway, which eventually aggravate allergic reaction.

There are probably 2 different mechanisms of increased epithelial permeability by histamine with H_1R . One occurs rapidly after antigen challenge, with histamine appearing to increase permeability, acting through the actin cytoskeleton to induce physical gaps between the cells. In human umbilical vein endothelial cells, activation of H_1 receptors is associated with an increase in endothelial permeability, reorganization of the F-actin cytoskeleton, and an increase in Ca^{2+} and phosphoinositide turnover.³⁶ Similar mechanisms are expected to operate in epithelial cells. The other mechanism may take place for hours, as observed in this experiment. Reduced ZO-1 mRNA expression caused by H_1R activation may cause reduced ZO-1 protein expression, which increases paracellular epithelial permeability.

Another observation made during the present study is that nasal epithelial cells increase IL-8 mRNA expression by histamine. IL-8 is a major cytokine of human nasal epithelium, constitutively expressed and readily secreted on virus infection or stimulation with IL-1 and TNF.³⁷ Jeannin et al¹⁵ reported that histamine induces IL-8 secretion by endothelial cells, the effect of which is inhibited by H_1R and H_2R antagonists. In the present study the upregulation of IL-8 mRNA by histamine could not be inhibited significantly either by H_1R or H_2R antagonists. A possibility remains that this effect could have been significantly inhibited if both H_1R and H_2R antagonists had been added simultaneously.

The production of IL-8 can have several consequences on the tissue environment. IL-8 is a major cytokine in the recruitment of neutrophils to areas of inflammation. It also activates T lymphocytes and cytokine-primed basophils and eosinophils and therefore may be implicated as an effector in allergic inflammation. Douglass et al³⁸ used IL-8 for challenge in the nasal mucosa and observed that a significant increase in cumulative eosinophil recruitment occurred over the challenge period.³⁸ Therefore locally released histamine itself probably plays a role to accumulate neutrophils and eosinophils by making nasal epithelial cells to increase the production of IL-8.

In the present study histamine failed to alter TNF- α mRNA expression. In mast cells histamine reduced TNF- α mRNA, suggesting that histamine released from mast cells may paradoxically limit the extent of inflammatory and immune reaction.¹⁷ This immunoregulatory effect could not be reproduced in the nasal epithelial cells. This may be due to difference in cell types.

Histamine did not affect H_1R mRNA expression either. Thus previous observation of increased H_1R mRNA expression in allergic nasal epithelia¹¹ could not be explained by stimulation by histamine itself. Other locally released mediators, such as platelet-activating factor,³⁹ may probably upregulate H_1R mRNA expression in nasal epithelia in allergic individuals.

In summary, locally released histamine causes nasal epithelial cells to decrease ZO-1 mRNA expression and

IL-8 mRNA expression. The former effect may increase the mucosal permeability and penetration of additional antigen through paracellular spaces. The latter effect may eventually result in the local accumulation of eosinophils, which enhance local allergic inflammation.

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