

Histamine H₂ receptor-mediated airway goblet cell secretion and its modulation by histamine-degrading enzymes

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Background: Airway goblet cell hypersecretion may contribute to the pathophysiology of asthma. However, it is unknown whether histamine affects goblet cell secretion and, if so, which subtype of histamine receptor is involved and whether endogenous histamine-degrading enzymes modulate these actions.

Methods: We morphometrically assessed goblet cell secretion in the guinea pig trachea stained with alcian blue and periodic acid Schiff stains by measuring the mucus score, which was inversely related to the degree of mucus glycoprotein discharge.

Results: Inhalation of histamine caused a dose-dependent decrease in mucus score, an effect that was inhibited by pretreatment with the H₂-receptor antagonist cimetidine but not with the H₁-receptor antagonist mepyramine or the H₃-receptor antagonist thioperamide. Inhaled Dimaprit, a selective H₂-receptor agonist, likewise decreased mucus score; whereas stimulation of H₁- and H₃-receptors with 2-methylhistamine and (R)- α -methylhistamine, respectively, had no effect. Pretreatment with the histamine N-methyltransferase inhibitor SKF 91488, but not the diamine oxidase inhibitor aminoguanidine, potentiated the dose-dependent effect of histamine on goblet cell secretion, causing a decrease in the concentration of inhaled histamine required to produce a half-maximal effect from 0.80 ± 0.12 to 0.48 ± 0.09 mg/ml ($p < 0.01$). The histamine methyltransferase activity in the tracheal mucosa was 29 times higher than diamine oxidase activity.

Conclusion: These findings suggest that histamine stimulates airway goblet cell secretion through H₂-receptors and that this effect may be modulated principally by endogenous histamine methyltransferase through a degradation of histamine. (*J Allergy Clin Immunol* 1997;99:233-8.)

Key words: Mucus secretion, histamine receptor, histamine N-methyltransferase, diamine oxidase, asthma

It has been generally accepted that submucosal glandular cells are the major source of mucus glycoprotein secretion in normal airways, and morphologic studies suggest that contribution of goblet cells to the production of mucin is probably less than that of submucosal glands. However, marked hyperplasia of airway goblet cells has been found in patients who died of severe asthma¹ and chronic bronchitis,² suggesting that these cells may play a significant part in the pathophysiology of airway hypersecretion under certain conditions.

Histamine is one of the most important chemical mediators in the initiation and development of antigen-induced immediate asthmatic responses, including airway smooth muscle contraction and microvascular leakage.³ However, the effect of histamine on mucus glycoprotein secretion from airway goblet cells remains uncertain. In addition, histamine can be metabolized by histamine N-

Abbreviations used

AU:	Arbitrary units
DAO:	Diamine oxidase
HMT:	Histamine N-methyltransferase

methyltransferase (HMT, EC 2.1.1.8) into N-methylhistamine and by diamine oxidase (DAO, EC 1.4.3.6), also called histaminase, to imidazole acetic acid.^{4,5} Recent evidence suggests that these enzymes, particularly HMT, protect against bronchoconstriction^{6,7} and microvascular leakage⁸ induced by histamine and antigen challenge by degrading histamine. Therefore the first objective of this study was to determine whether histamine affects airway goblet cell secretion and, if so, to determine the subtype of histamine receptor involved. The second objective was to elucidate the modulatory role of histamine-degrading enzymes in histamine-mediated goblet cell secretion. To accomplish this, we studied guinea pig tracheal mucosa by means of a semiquantitative morphometric analysis of mucus discharge from goblet cells.

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METHODS

General procedure

The experiments were approved by the Ethical Committee of Tokyo Women's Medical College. Pathogen-free male Dunkin-Hartley guinea pigs, weighing 250 to 280 gm, were anesthetized with an intraperitoneal injection of urethane (500 mg/kg) and α -chloralose (50 mg/kg). The larynx and upper trachea were exposed, the trachea just below the larynx was incised, and a polyethylene tube was inserted 5 mm into the trachea. The guinea pigs were artificially ventilated (frequency, 90 breaths/min; tidal volume, 10 ml/kg) with a constant-volume ventilator (model SN-480-7; Shinano Co., Tokyo, Japan).

To study the effect of histamine on goblet cell secretion, histamine was given by inhalation. Histamine diphosphate (Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile saline solution (3 mg/ml), and aerosols of the solution were generated by an ultrasonic nebulizer (model 5000D; Shimadzu Medical Co., Tokyo) and delivered by the respirator (10 breaths). In a control experiment the same volume of sterile saline solution was likewise given. The nebulizer output was 200 ml/min, with particles that had a mass median aerodynamic diameter of 7.6 μ m (the manufacturer's specification).

To determine which subtype of histamine receptor was involved in histamine-induced goblet cell secretion, the H_1 -receptor antagonist mepyramine (1 to 10 mg/kg, administered intraperitoneally [Rhone-Poulenc Ltd., Dagenham, U.K.]), the H_2 -receptor antagonist cimetidine (1 to 10 mg/kg, administered intraperitoneally [Sigma]), or the H_3 -receptor antagonist thio-peramide (1 to 5 mg/kg, administered intraperitoneally [Sigma])⁹ was given; and 30 minutes later, histamine (3 mg/ml, 10 breaths) was given. The maximum dose and the route of histamine receptor antagonists were chosen according to the previous studies,^{9,10} and our preliminary experiment showed that these antagonists per se had no effect on goblet cell secretion in the guinea pig trachea. Moreover, the effects of various doses (1 to 10 mg/ml, 10 breaths) of histamine, the H_1 -receptor agonist 2-methylhistamine (Wako Chemicals, Tokyo),³ the H_2 -receptor agonist Dimaprit hydrochloride (ICN Pharmaceuticals Inc., Tokyo),¹¹ and the H_3 -receptor agonist (*R*)- α -methylhistamine (Nakalai Tesque, Kyoto, Japan)¹² on goblet cell secretion were examined.

To assess the modulatory role of histamine-degrading enzymes, the effects of SKF 91488 (S-[4-(*N,N*-dimethylamino)-butyl] isothioureia; Smith Kline Co., Philadelphia, Pa.), an inhibitor of HMT,¹³ and aminoguanidine (Sigma), an inhibitor of DAO,¹⁴ on histamine-induced goblet cell secretion were examined. To do so, SKF 91488 or aminoguanidine (10^{-2} mol/L) was delivered by the respirator (90 breaths), and 15 minutes later, various doses of histamine (0.1 to 10 mg/ml, 10 breaths) were given. The dose of each inhibitor was chosen according to the previous study,⁸ and our separate studies showed that inhalation of these inhibitors by themselves did not significantly affect goblet cell secretion.

Preparation of tissues

One minute after the administration of histamine, saline solution, or histamine receptor agonists, the left ventricle was incised, a blunt-ended needle was inserted into the aorta, and the ventricles were cross-clamped. Blood was drained from the incised right atrium with 10% formaldehyde at a pressure of 120 mm Hg, and when the perfusate became clear, the trachea was removed and fixed for 24 hours in 10% formaldehyde. The tracheal sections were then cut longitudinally in the coronal plane (5 μ m thick) and stained with alcian blue and periodic

acid Schiff stain in sequence to demonstrate the acidic and neutral intracellular glycoprotein of the secretory cells. Slides were coded to avoid observer bias during quantification and were observed at a magnification of $\times 400$ (Optiphot-NFXII; Nikon, Tokyo).

Analysis of mucus discharge

The intracellular mucus glycoproteins of the epithelial secretory cells were recognized as purple-colored, oval-shaped disks of varying size. To analyze secretory responses of goblet cells, a mucus score¹⁵ was determined from the histologic sections by grading the amount of mucus in each secretory cell as follows. For grade 1, the vertical distance of the stained area was within one third of the epithelial layer, measured from basement membrane to cell apices, indicating that goblet cells had discharged their mucus. For grade 2, the vertical distance of the stained area exceeded one third of the epithelial layer, indicating that goblet cells had not discharged their mucus. The stained areas were graded in 20 consecutive high-power fields along the two walls of the lower trachea revealed in coronal section, starting at the carina and moving cranially (a total of 40 fields). In each guinea pig, mucus score (in arbitrary units, AU) was calculated as $n_1 + 2n_2$, where n_1 and n_2 were the total number of cells for grade 1 and grade 2, respectively. Because there were 25 to 30 epithelial cells in each high-power field, we examined 1000 to 1200 epithelial cells for each animal. For example, if there were 300 goblet cells and all the cells contained large amounts of mucus, then presumably there were 300 grade 2 cells. Thus $n_1 = 0$ and $n_2 = 300$, and the mucus score was 600. If 100% of these cells discharged their mucus completely, then none of the cells were stained. Thus $n_1 = 0$ and $n_2 = 0$, and the mucus score was 0. If 50% of these cells completely discharged their mucus and the remaining 50% discharged a moderate amount of mucus and became grade 1 cells, then $n_1 = 150$ and $n_2 = 0$, and the mucus score was 150. The mucus score was thus inversely related to the degree of goblet cell discharge (i.e., the lower the score, the greater the degree of discharge and vice versa).

To assess a possible contribution of airway smooth muscle contraction to mucus discharge, mucus score was determined in selected animals in epithelium overlying cartilage (cartilaginous epithelium) and in epithelium overlying the muscular interstitium between cartilage (muscular epithelium) in 20 fields along each side of trachea.

Measurement of HMT and DAO activities

The measurements of HMT and DAO activities have been described in detail previously.^{16,17} Briefly, guinea pigs were anesthetized with an intraperitoneal injection of pentobarbital sodium (35 mg/kg), and after the drainage of circulating blood with saline solution, the trachea was removed. For the measurement of HMT activity, the tracheal mucosa was excised and homogenated with a glass homogenizer in ice-cold potassium phosphate buffer (100 nmol/L, pH 6.8) containing 0.2 mmol/L dithiothreitol, 0.01 mmol/L pyridoxal 5'-phosphate, 1% polyethylene glycol, 0.01 mmol/L aminoguanidine, and 20 μ g/ml phenylmethanesulfonyl fluoride. The homogenate was centrifuged at 4°C (12,000 g, 15 minutes), and the supernatant was dialyzed three times for 8 hours against 100 volumes of the buffer. The reaction of HMT was carried out by incubating the supernatant at 37°C for 120 minutes with 10 mmol/L histamine and 100 mmol/L S-adenosyl-L-methionine. For the measurement of DAO activity, the mucosal tissues were homogenated with potassium phosphate buffer and centrifuged, after which

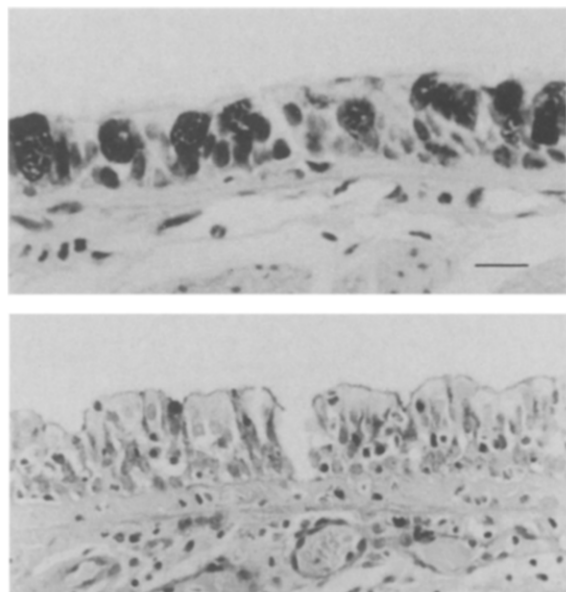


FIG. 1. Photomicrographs of airway epithelium of lower trachea in guinea pigs. Sterile saline solution (**upper panel**) or histamine (3 mg/ml, 10 breaths) (**lower panel**) was given by inhalation, and the trachea was removed and stained with alcian blue and periodic acid Schiff stain. Scale bar = 10 mm.

the supernatant was dialyzed against the buffer. The DAO reaction was performed by incubating the supernatant for 120 minutes with 10 mmol/L histamine at 37° C in the presence of 0.01 mmol/L SKF 94188. After incubation, N-methylhistamine and imidazole acetaldehyde were separated from histamine by high-performance liquid chromatography on a weak cation exchanger and with an automated *o*-phthalaldehyde fluorometric system (Toyo-Soda Co., Tokyo). The HMT and DAO activities were expressed as picomoles of N-methylhistamine and imidazole acetaldehyde, respectively, formed per minute per milligram of protein, as determined by the method of Lowry et al.¹⁸

Statistical analysis

All values were expressed as means $\pm$ SEM. One-way analysis of variance and Fisher's multiple range tests were used to determine statistically significant differences among/between groups, and a *p* value of less than 0.05 was considered significant.

RESULTS

Effect of histamine on goblet cell secretion

As demonstrated in Fig. 1, the distribution of goblet cell secretory granules in the guinea pig trachea was consistent with previous reports^{19,20} and formed the basis of our grading of 2 for undischarged mucus and of 1 for discharged mucus, respectively. There was a preponderance of grade 2 cells under unstimulated conditions, which produced mean mucus score values of approximately 600 AU. Inhalation of histamine (3 mg/ml, 10 breaths) reduced the amount of stained intracellular mucus in the goblet cells: the values of mucus score

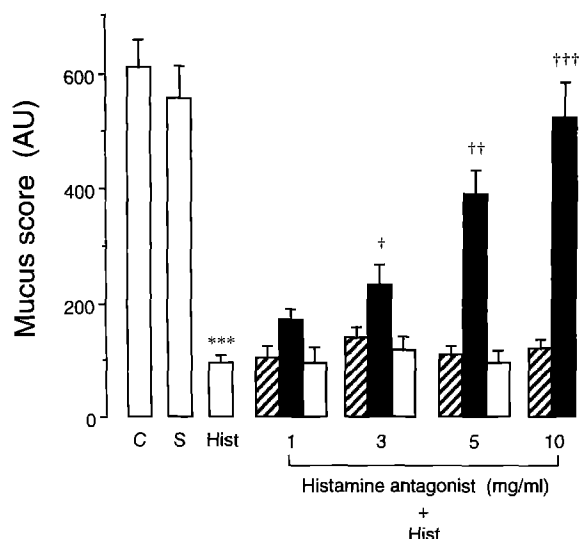


FIG. 2. Responses of mucus discharge from guinea pig tracheal goblet cells to sterile saline solution (*S*) and histamine (*Hist*) (3 mg/ml, 10 breaths) and dose-dependent effects of pretreatment with intraperitoneal mepyramine (*hatched bars*), cimetidine (*filled bars*), and thioperamide (*open bars*) on histamine-induced mucus discharge. Responses are expressed as mucus score in arbitrary units (AU). Data are expressed as means $\pm$ SEM; *n* = 8 for each column. ****p* < 0.001 (significantly different from control (*C*) values). †*p* < 0.05, ††*p* < 0.01, †††*p* < 0.001 (significantly different from the response to histamine alone).

were decreased from 610 ± 47 to 96 ± 12 AU (*p* < 0.001, *n* = 8), indicating an increase in goblet cell secretion, but the vehicle sterile saline solution alone had no effect (Fig. 2). Pretreatment of guinea pigs with cimetidine inhibited the effect of histamine on goblet cell secretion. The histamine-induced (3 mg/ml, 10 breaths) decrease in mucus score was restored by cimetidine (10 mg/kg, given intraperitoneally) (*p* < 0.001, *n* = 8), whereas mepyramine and thioperamide had no effect. Inhalation of histamine and Dimaprit (3 mg/ml, 10 breaths), but not 2-methylhistamine or (*R*)- α -methylhistamine, stimulated mucus discharge in a dose-dependent manner (Fig. 3).

Inhaled histamine decreased the value for mucus score of both cartilaginous epithelium and muscular epithelium, where the ratio of mucus score of muscular epithelium to that of cartilaginous epithelium in saline controls (0.95 ± 0.06 , *n* = 7) was not significantly different from that in histamine-treated animals (0.91 ± 0.05 , *n* = 7) (Fig. 4).

As shown in Fig. 5, pretreatment with inhaled SKF 91488 potentiated the stimulatory effect of histamine on goblet cell secretion: the histamine-induced maximal decrease in mucus score was further decreased, and the concentration of inhaled histamine required to produce a half-maximal effect was decreased from 0.80 ± 0.12 mg/ml to 0.48 ± 0.09 mg/ml (*p* < 0.01, *n* = 7). On the other hand, aminoguanidine did not significantly alter the response of mucus score to histamine.

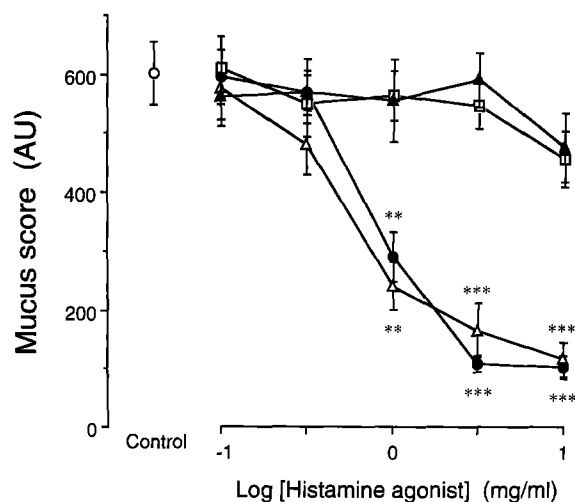


FIG. 3. Dose-dependent effect of histamine (filled circles), 2-methylhistamine (open squares), Dimaprit (open triangles), and α -methylhistamine (closed triangles) on goblet cell secretion in guinea pig trachea. Various concentrations of the drug were given by inhalation (10 breaths). Responses are expressed as mucus score in arbitrary units (AU). Data are expressed as means $\pm$ SEM; $n = 8$ for each point. ** $p < 0.01$ and *** $p < 0.001$, significantly different from control values (open circle).

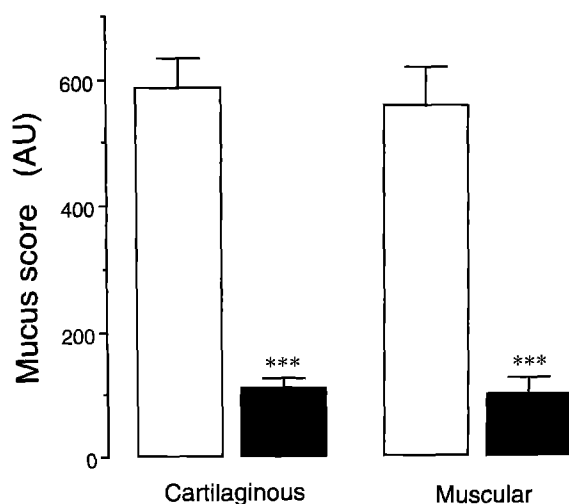


FIG. 4. Effects of inhaled histamine (3 mg/min, 10 breaths) (filled columns) on goblet cell secretion in cartilaginous epithelium and muscular epithelium of guinea pig trachea. Responses are expressed as mucus score. Data are expressed as means $\pm$ SEM; $n = 7$ for each column. *** $p < 0.001$, significantly different from saline control values (open columns).

Activities of histamine-degrading enzymes

The activity of HMT in the guinea pig tracheal mucosa determined by high-performance liquid chromatography was 29 times higher than that of DAO (Fig. 6).

DISCUSSION

A marked hyperplasia of airway goblet cells can frequently be found in patients who died of severe acute

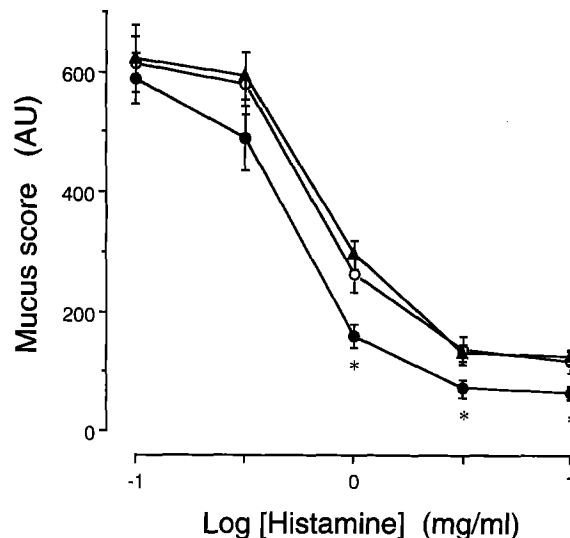


FIG. 5. Effects of pretreatment with SKF 91488 (filled circles) and aminoguanidine (filled triangles) on dose-dependent stimulation of goblet cell secretion in guinea pig trachea induced by inhaled histamine. Guinea pigs received 90 breaths of each inhibitor, and 15 minutes later, 10 breaths of various concentrations of histamine. Responses are expressed as mucus score. Data are expressed as means $\pm$ SEM; $n = 7$ for each point. * $p < 0.05$, significantly different from corresponding responses to histamine alone (open circles).

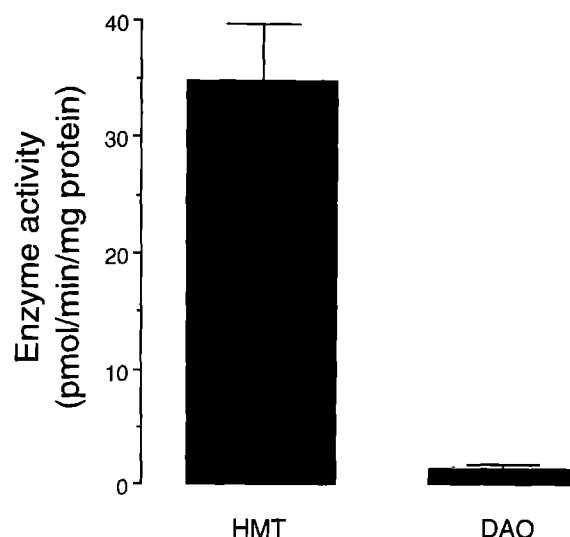


FIG. 6. Activities of HMT and DAO in guinea pig tracheal mucosa. Enzyme activities were measured by high-performance liquid chromatography on the basis of postcolumn derivatization with o-phthalaldehyde. Data are expressed as means $\pm$ SEM; $n = 8$ for each column.

asthma,¹ suggesting that hypersecretion of mucus glycoprotein from these cells may be involved in the formation of mucus plugs and the resultant aggravation of asthmatic symptoms. Histamine is released from lung mast cells and blood basophils on activation with antigen bridging of membrane-bound IgE and produces a variety

of airway responses associated with acute asthma, such as airway smooth muscle contraction and vascular leakage. In addition, Shelhamer et al.²¹ showed that exogenous histamine added to cultured human bronchi increased mucus glycoprotein release and that this effect was mediated by H₂-receptors. However, there are at least three sources of mucus production in the airway: mucous and serous cells in the submucosal glands, goblet cells in the surface epithelial cells, and the external surface of apical membrane of ciliated cells. This semiquantitative morphometric study enabled us to discriminate the secretory responses of goblet cells, and we found that inhalation of histamine caused a rapid decrease in the amount of stainable intracellular mucus of guinea pig tracheal goblet cells in a dose-dependent fashion, indicating an increased mucus discharge by these cells.¹⁵

Moreover, the histamine-induced goblet cell secretion was abolished by pretreatment with cimetidine, a selective H₂-receptor antagonist, but not with mepyramine, an H₁-receptor antagonist, or thioperamide, an H₃-receptor antagonist.⁹ We also found that inhaled Dimaprit, a selective agonist for H₂-receptor,¹¹ decreased the mucus score to the same extent as did histamine; whereas stimulation of H₁- and H₃-receptors with 2-methylhistamine and (*R*)- α -methylhistamine, respectively,¹² had no effect. These results suggest that histamine causes airway goblet cell secretion through stimulation of H₂-receptors.

Because intracellular mucus may also be released by a change in cell shape or mechanical stretch on plasma membrane,²² it is possible that the observed mucus discharge could be accomplished by squeezing the granules toward the airway lumen through the histamine-induced contraction of underlying airway smooth muscle. However, this possibility seems unlikely, because stimulation of H₂-receptors with Dimaprit, which is supposed to have little effect on airway smooth muscle contraction, greatly decreased mucus score and because mepyramine, which inhibits histamine-induced bronchoconstriction, did not alter the response of goblet cells. Furthermore, the mucus score was comparable in cartilaginous (i.e., noncontracted) and muscular (i.e., contracted) epithelium in response to histamine. We thus speculate that histamine may have exerted its effect by directly stimulating goblet cell H₂-receptors, although the localization of this receptor subtype to airway goblet cells remains to be elucidated.

Histamine can be metabolized by two major pathways in the body^{4,5}: 50% to 70% of histamine is metabolized by HMT located in the small intestine, liver, kidneys, and leukocytes into N-methylhistamine, which is further metabolized to N-methylimidazole acetic acid; and the remaining 30% to 45% is metabolized by DAO located in the intestinal mucosa, placenta, liver, skin, kidneys, neutrophils, and eosinophils to imidazole acetic acid. In this study pretreatment of guinea pigs with SKF 91488, a potent noncompetitive inhibitor of HMT with a micromolar affinity constant,¹³ potentiated the response of

goblet cells to histamine. SKF 91488 is a structural analog of histamine H₂-receptor,¹³ but this agent has been shown to possess no histamine agonist activity,²³ and we found that inhalation of SKF 91488 per se did not cause goblet cell secretion. Thus the potentiation was not due to further stimulation of H₂-receptor by SKF 91488, but rather to the inhibition of HMT activity and the resultant blockade of inactivation of histamine. However, the response of mucus discharge reached plateau by the inhalation of 3 mg/ml histamine, indicating that histamine receptors may be saturated. It is therefore difficult to consider that blocking of histamine degradation could potentiate the responses to excessive amount of histamine. The reason for the observed potentiation is unknown, but one possibility would be that SKF 91488 could also have acted on goblet cells and changed the sensitivity to histamine.

In contrast to the effect of HMT inhibitor, the DAO inhibitor aminoguanidine¹⁴ failed to alter the histamine-induced decrease in mucus score. Lindström et al.²⁴ showed that DAO may contribute to the inhibitory effect of the epithelium on the contractile responses of guinea pig trachea to histamine, but more recent studies on bronchoconstriction,^{6,7} electrolyte transport in airway epithelial cells,²⁵ and microvascular permeability⁸ have indicated that HMT plays a major role compared with DAO in the modulation of histamine actions. The relative concentrations of HMT and DAO in the airway vary among species. Studies on HMT activity and on cellular sites of HMT biosynthesis by *in situ* hybridization in the guinea pig trachea showed that HMT is present in the epithelial and endothelial cells,⁶ but the level of DAO activity in the tracheal mucosa has not been reported. In our study DAO activity was 29 times lower than HMT activity. This result may be compatible with previous findings that HMT activity was higher than DAO activity by 13 times in guinea pig lungs²⁶ and by 50 times in human nasal mucosa.¹⁷ Therefore HMT is a principal enzyme that inactivates histamine in guinea pig tracheal mucosa, thereby regulating histamine-induced airway responses including stimulation of goblet cell secretion.

In conclusion, histamine produces a rapid stimulation of mucus discharge from airway goblet cells in guinea pigs, presumably through H₂-receptors, and the histamine-degrading enzyme HMT in the airway mucosa may play a role in limiting goblet cell hypersecretion through a metabolism of histamine.

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REFERENCES

1. Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T. Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 1992;101:916-21.
2. Thurlbeck WM, Malaka D, Murphy M. Goblet cells in the peripheral airways in chronic bronchitis. *Am Rev Respir Dis* 1975;112:65-9.

3. White MV, Slater JE, Kaliner MA. Histamine and asthma. *Am Rev Respir Dis* 1987;135:1165-76.
4. Beaven MA. Histamine: its role in physiologic and pathologic process. *Monogr Allergy* 1978;13:1-20.
5. Zeinger RS, Yurdin DL, Colten HR. Histamine metabolism. II. Cellular and subcellular localization of the catabolic enzymes, histaminase and histamine methyltransferase in human leukocytes. *J Allergy Clin Immunol* 1976;58:172-9.
6. Ohnishi T, Yamauchi K, Sekizawa K, Ohkawara Y, Maeyama K, Sasaki M, et al. Histamine N-methyltransferase controls the contractile responses of guinea pig trachea to histamine. *J Pharmacol Exp Ther* 1992;261:1268-72.
7. Sekizawa K, Nakazawa H, Ohnishi T, Yamauchi K, Ohkawara Y, Maeyama K, et al. Histamine N-methyltransferase modulates histamine- and antigen-induced bronchoconstriction in guinea pigs in vivo. *Am Rev Respir Dis* 1993;147:92-6.
8. Sekizawa K, Nakazawa H, Morikawa M, Yamauchi K, Maeyama K, Watanabe T, et al. Histamine N-methyltransferase inhibitor potentiates histamine- and antigen-induced airway microvascular leakage in guinea pigs. *J Allergy Clin Immunol* 1995;96:910-6.
9. Arrang J-M, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, et al. Highly potent and selective ligands for histamine H₃-receptors. *Nature* 1987;327:117-23.
10. Ichinose M, Stretton CD, Schwartz JC, Barnes PJ. Histamine H₃-receptors inhibit cholinergic bronchoconstriction in guinea pig airways. *Br J Pharmacol* 1989;97:13-5.
11. Black JW, Duncan WAM, Durant CJ, Ganellin R, Parsons EM. Definitions and antagonism of histamine H₂-receptors. *Nature* 1972;236:385-90.
12. Burgaud JL, Oudart N. Effect of an histaminergic H₃ agonist on the non-adrenergic non-cholinergic contraction in guinea-pig perfused bronchioles. *J Pharm Pharmacol* 1993;45:955-8.
13. Beaven MA, Shaff RE. Inhibition of histamine methylation in vivo by the Dimaprit analog, SKF compound 91488. *Agents Actions* 1979;9:455-60.
14. Schuler W. Zur Hemmung der Deaminooxydase (Histaminase). *Experientia* 1952;8:230-2.
15. Tokuyama K, Kuo HP, Rohde JAL, Barnes PJ, Rogers DF. Neural control of goblet cell secretion in guinea pig airways. *Am J Physiol* 1990;259:L108-15.
16. Fukuda H, Yamatodani A, Imamura I. High-performance liquid chromatographic determination of histamine N-methyltransferase activity. *J Chromatogr* 1991;567:459-64.
17. Abe Y, Ogino S, Irifune M, Imamura I, Fukui H, Wada H, et al. Histamine content, synthesis and degradation in human nasal mucosa. *Clin Exp Allergy* 1993;23:132-6.
18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
19. McDermott RP, Donaldson RM Jr, Trier JS. Glycoprotein synthesis and secretion by mucosal biopsies of rabbit colon and human rectum. *J Clin Invest* 1974;54:545-54.
20. McDonald DM. Neurogenic inflammation in the rat trachea. I. Changes in venules, leukocytes and epithelial cells. *J Neurocytol* 1988;17:583-603.
21. Shelhamer JH, Marom Z, Kaliner M. Immunologic and neuropharmacologic stimulation of mucus glycoprotein release from human airways in vitro. *J Clin Invest* 1980;66:1400-8.
22. Verdugo P. Goblet cells secretion and mucogenesis. *Annu Rev Physiol* 1990;52:157-76.
23. Bertaccini G, Molina E, Vitali T, Zappia L. Action of histamine receptor agonists and antagonists on the rat uterus. *Br J Pharmacol* 1979;66:13-7.
24. Lindström EG, Andersson RGG, Granérus G, Grundström N. Is the airway epithelium responsible for histamine metabolism in the trachea of guinea pigs? *Agents Actions* 1991;33:170-2.
25. Yamada K, Shimura S, Satoh M, Sasaki T, Yamauchi K, Takishima T. HMT regulates histamine-induced Cl⁻ secretion across the canine tracheal epithelium. *Respir Physiol* 1994;97:105-9.
26. Abe Y, Ogino S, Irifune M, Imamura I, Liu YQ, Fukui H, et al. Histamine content, synthesis and degradation in nasal mucosa and lung of guinea pigs treated with toluene diisocyanate (TDI). *Clin Exp Allergy* 1993;23:512-7.