

Localization of histamine N-methyltransferase messenger RNA in human nasal mucosa

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Background: Histamine is metabolized mainly by histamine N-methyltransferase (HMT) to N^ε-methylhistamine in human nasal mucosa. Human HMT cDNA has been cloned and expressed in COS cells. The purpose of this study was to determine the localization of HMT mRNA in human nasal mucosa.

Methods: The fragment (nucleotide residues 430-1055) of human HMT cDNA was subcloned in a Bluescript vector (Stratagene, La Jolla, Calif.), and HMT sense and anti-sense RNA probes were made with T₇ and T₃ RNA polymerases. In situ hybridization with digoxigenin-labeled RNA probes was performed on surgical specimens of human nasal turbinates.

Results: HMT mRNA was localized in cells in the epithelium and submucosa, and densely in endothelial cells of vessels. No HMT mRNA was identified in the submucosal glands. The presence of HMT mRNA was confirmed by Northern blot analysis, and HMT activities were also detected in nasal mucosa.

Conclusion: Our study indicates that endothelium expresses HMT mRNA, whereas cells in the epithelium and submucosa, which remain unidentified, are an additional source of HMT mRNA. (*J ALLERGY CLIN IMMUNOL* 1995;95:96-102.)

Key words: Histamine N-methyltransferase, in situ hybridization, nasal mucosa

Histamine plays an important role in the nasal symptoms of allergic rhinitis (i.e., pruritus, sneezing, rhinorrhea, and congestion) through its direct action on the vascular bed. A previous autoradiographic study revealed that histamine H₁ receptors exist exclusively on the endothelium of vessels in human nasal mucosa,¹ supporting the findings that histamine directly increases vascular permeability through H₁ receptor.²⁻⁵

Primary enzymes degrading histamine are histamine N^ε-methyltransferase (HMT) (EC 2.1.1.8) and diamine oxidase (DAO) (EC 1.4.3.6).⁶⁻⁸ The

Abbreviations used

DAO:	Diamine oxidase
HMT:	Histamine N ^ε -methyltransferase
PBS:	Phosphate-buffered saline
SSC:	Standard saline citrate

principal route involved in the catabolism of histamine varies depending on species and organs.^{9,10} In human nasal mucosa, it was reported that HMT activity was 50 times higher than that of DAO,¹¹ suggesting that HMT is the major histamine-metabolizing enzyme in human nasal mucosa. However, the localization of HMT or of the biosynthesis of HMT in human nasal mucosa is unknown.

Recently, we cloned human HMT cDNA, which is confirmed to encode human HMT by transient expression of HMT activity in COS cells.¹² In this study the localization of HMT mRNA in human nasal mucosa was examined by in situ hybridization. Northern blot analysis was also done to confirm its presence. In addition, histamine con-

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tent and HMT activity in nasal mucosa were measured.

METHODS

Human tissue preparation

Human inferior turbinates were obtained at the time of surgery from eight patients with nasal obstruction and nasal allergy. No patient had had a recent infection or had been taking antihistamines during the 4 weeks before surgery. The turbinates were injected with 2 to 4 ml of 1% lidocaine with 1:100,000 epinephrine. An incision was made from the lateral wall of the inferior turbinate at the level of the infundibulum through the inferior conchal bone and then inferiorly along the medial aspect of that bone. The remaining medial flap of turbinate was wrapped superiorly and laterally to close the wound. Within 20 minutes of surgical excision, the nasal mucosa was dissected from the inferior conchal bone and quickly washed with cold saline solution to remove blood contamination. Mucosal specimens for measurements of histamine and HMT activity and for Northern blot analysis were frozen in liquid nitrogen on dry ice for 20 seconds and then stored at -80°C until use.

Tissue fixation

For in situ hybridization, the specimens were fixed in 4% paraformaldehyde solution with phosphate-buffered saline (PBS) at 4°C for 4 to 8 hours. They were washed in 30% sucrose in PBS at 4°C for 4 hours, embedded in OCT compound (Miles; Elkhart, Ind.), rapidly frozen in liquid nitrogen on dry ice, and stored at -80°C until use. Frozen sections 10 μm thick were mounted on poly-L-lysine-coated glass slides, air-dried for 1 hour, and further dried at 45°C for 3 hours. All of the buffers and glassware used for the detection of mRNA were RNase-free.

Preparation of RNA probes

An Nde I/Nde I fragment of human HMT cDNA (625 bp) was inserted into the multiple cloning site of a plasmid, Bluescript SK⁺ (Stratagene, La Jolla, Calif.). Sense (having an identical sequence to mRNA) and anti-sense (complementary to mRNA) RNA probes were transcribed from linearized plasmid containing HMT cDNA fragment by T₇ or T₃ RNA polymerase. These probes were labeled with α -phosphorous 32-labeled cytosine triphosphate (New England Nuclear Co., Boston, Mass.) for Northern blotting or with digoxigenin-11-uridine triphosphate for in situ hybridization according to the manufacturer's manual (DIG RNA Labeling kit SP6/T7; Boehringer, Mannheim, Germany). The size of the probes was reduced to an average of 150 bp by alkaline treatment.¹³

In situ hybridization

In situ hybridization procedures were similar to those previously described¹⁴⁻¹⁶ with some modifications. After

drying, the specimens were rehydrated in PBS to remove OCT compound. They were immersed in 0.2N HCl for 20 minutes and then incubated in proteinase K (1 $\mu\text{g}/\text{ml}$; Sigma Chemical Co., St Louis, Mo.) for 10 minutes at 37°C . After washing in PBS, the specimens were refixed in freshly prepared 4% paraformaldehyde in PBS for 5 minutes and then immersed in glycine (2 mg/ml) in PBS twice for 15 minutes. They were then immersed in 50% deionized formamide and $2\times$ standard saline citrate (SSC) for 1 hour at room temperature. The specimens were incubated with a hybridization mixture for 16 hours at 45°C . The composition of the hybridization mixture was as follows: 1 mg/ml yeast tRNA, 20 mmol/L Tris-HCl buffer (pH 8.0), 2 mmol/L ethylenediaminetetraacetic acid, $1\times$ Denhardt's solution, 300 mmol/L NaCl, 50% deionized formamide, and 10% dextran sulfate. The final concentration of the probe was 100 ng/ml. After hybridization, the specimens were washed as follows: (1) 50% formamide and $2\times$ SSC for 1 hour at 45°C ; (2) 0.5 mol/L NaCl, 10 mmol/L Tris-HCl buffer (pH 8.0), twice for 5 minutes; (3) RNase A (Sigma Type 1a, 20 $\mu\text{g}/\text{ml}$) treatment for 30 minutes at 37°C to remove unhybridized single-stranded RNAs; (4) 50% formamide and $2\times$ SSC for 1 hour at 45°C ; (5) 50% formamide and $1\times$ SSC for 1 hour at 45°C ; and (6) 50% formamide and $1\times$ SSC for 30 minutes at room temperature.

Immunologic detection of hybridized RNA probes was done with a Nucleic Acid Detection Kit (Boehringer, Mannheim, Germany). Alkaline phosphatase-conjugated F(ab) fragments of anti-digoxigenin antibody were applied at a dilution of 1:500 for 1 hour. Alkaline phosphatase was detected by incubating with 5-bromo-4-chloro-3-indolyl phosphate, enzyme substrate, and nitroblue tetrazolium chromogen. After the development of the precipitation of the color reagent, the specimens were mounted with 50% glycerol and 50% Tris ethylenediaminetetraacetic acid.

Northern blot analysis

Total RNA samples were prepared from homogenized frozen tissues or cultured KU-812F cells¹⁷ with the method of Chomczynski and Sacchi.¹⁸ We evaluated high HMT activity in human kidneys, although no activity was detected in KU-812F cells in a preliminary study. Therefore we used the RNA from kidneys as a positive control and RNA from KU-812F cells as a negative control in Northern blot analysis. Purity was assessed by A_{260}/A_{280} spectrophotometric measurements, and 20 μg samples were applied to 1.5% denaturing agarose gels, electrophoresed, and blotted onto Hybond-N (Amersham, Buckinghamshire, England). After prehybridization for 4 hours at 55°C in buffer containing 50% formamide, 0.1% sodium pyrophosphate, 50 mmol/L Tris-HCl (pH 7.5), $5\times$ SSC, $5\times$ Denhardt's solution, 1% sodium dodecyl-sulfate, 5 mmol/L ethylenediaminetetraacetic acid, 50 $\mu\text{g}/\text{ml}$ yeast tRNA, and 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA, blots were incubated with ^{32}P -labeled human HMT cRNA probe at a concentration of 1 to 2×10^6 cpm/ml

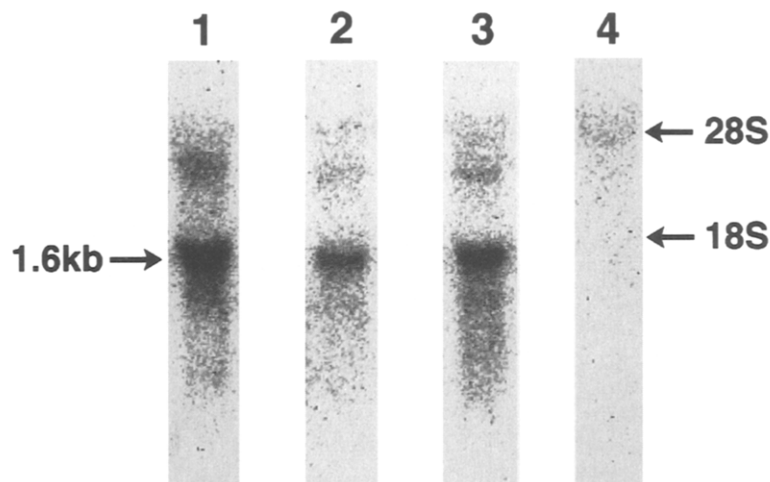


FIG. 1. Expression of HMT mRNA in human tissues. Each lane contains 20 μ g of total RNA extracted from the kidneys and nasal mucosa. Northern blot analysis of HMT mRNA was performed with anti-sense RNA probe as described in the Methods section. The source of RNA in each lane is: 1, kidney; 2 and 3, nasal mucosa from two patients; and 4, KU-812F cells as a negative control. HMT mRNA was detected in the RNA from human kidneys and nasal mucosa but not from KU-812F cells. HMT mRNA (1.6 kb) and 28S and 18S ribosomes are indicated by arrows.

at 50° C for 16 hours. After hybridization, filters were washed twice at room temperature in 2 $\times$ SSC, 0.1% sodium dodecylsulfate, and once at 50° C in 0.1 $\times$ SSC, 0.1% sodium dodecylsulfate. In addition, they were treated with 1 μ g/ml RNase A in 1 $\times$ SSC at 37° C for 5 minutes and then washed at 60° C in 0.1 $\times$ SSC, 0.1% sodium dodecylsulfate. The filters were exposed to Kodak film (Eastman Kodak Co., Rochester, N.Y.) at -80° C with an intensifying screen for 2 days.

Measurement of HMT activity

The frozen samples were excised and homogenized in 4 volumes of cold phosphate buffer (0.1 mol/L at pH 7.0 and 4° C) with a Polytron homogenizer (Kinematica, Lucerne, Switzerland). Some of the homogenate was used for measurement of histamine as mentioned hereafter. The homogenate was centrifuged at 10,000 *g* for 15 minutes at 4° C, and the supernatant was used for measuring HMT activity. The supernatant was dialyzed two times for 8 hours, against 100 volumes of the buffer, with a cellophane membrane. To measure the HMT activity, incubation was carried out at 37° C with 0.5 ml of a mixture of 0.3 ml of 0.1 mol/L potassium phosphate buffer (pH 7.4), 0.05 ml of 1 mmol/L histamine, 0.05 ml of 2.5 mmol/L S-adenosyl-L-methionine, and 0.1 ml of the supernatant for 120 minutes in the presence of 0.1 mmol/L aminoguanidine. The product, *N*⁷-methylhistamine, was separated from histamine in a high-performance liquid chromatography system on a weak cation exchanger, TSK gel CM-2SW (Toyo Soda Manufacturing Co., Tokyo, Japan), with 37.5 mmol/L citric acid, 1.25% imidazole, and 20% acetonitrile at pH 6.8 as the mobile phase at a flow rate of 1.0 ml/min, and then measured fluorometrically with a postcolumn derivatiza-

tion system with *o*-phthalaldehyde and 2-mercaptoethanol.^{19,20} HMT activity was expressed as picomoles of *N*⁷-methylhistamine formed per minute per milligram of protein. Protein concentrations were measured with the method of Lowry et al.²¹ with bovine serum albumin as a standard.

Measurement of histamine

The homogenate was centrifuged at 10,000 *g* for 15 minutes at 4° C, and the deproteinized supernatant was assayed for histamine, as described previously.²² In brief, histamine was partially purified by Bio-Rad AG-50 (X8; Bio-Rad, Richmond, Calif.) column chromatography, concentrated, and subjected to high-performance liquid chromatography (TSK, IEX-510SP; Toyo Soda Ltd.). Then histamine was measured by the *o*-phthalaldehyde method of Shore et al.²³ in an autoanalyzer system.

Statistical analysis

All values are expressed as means $\pm$ SD.

RESULTS

Northern blot analysis

Northern blot analysis with human HMT cRNA probe indicated that 1.6 kb HMT mRNA transcripts were expressed in the human nasal mucosa (Fig. 1). A similar size of mRNA was also expressed in human kidney tissues. In addition, a minor band of 4 kb HMT mRNA was detected in these tissues. No HMT mRNA was detected in the RNA from KU-812F cells (negative control).

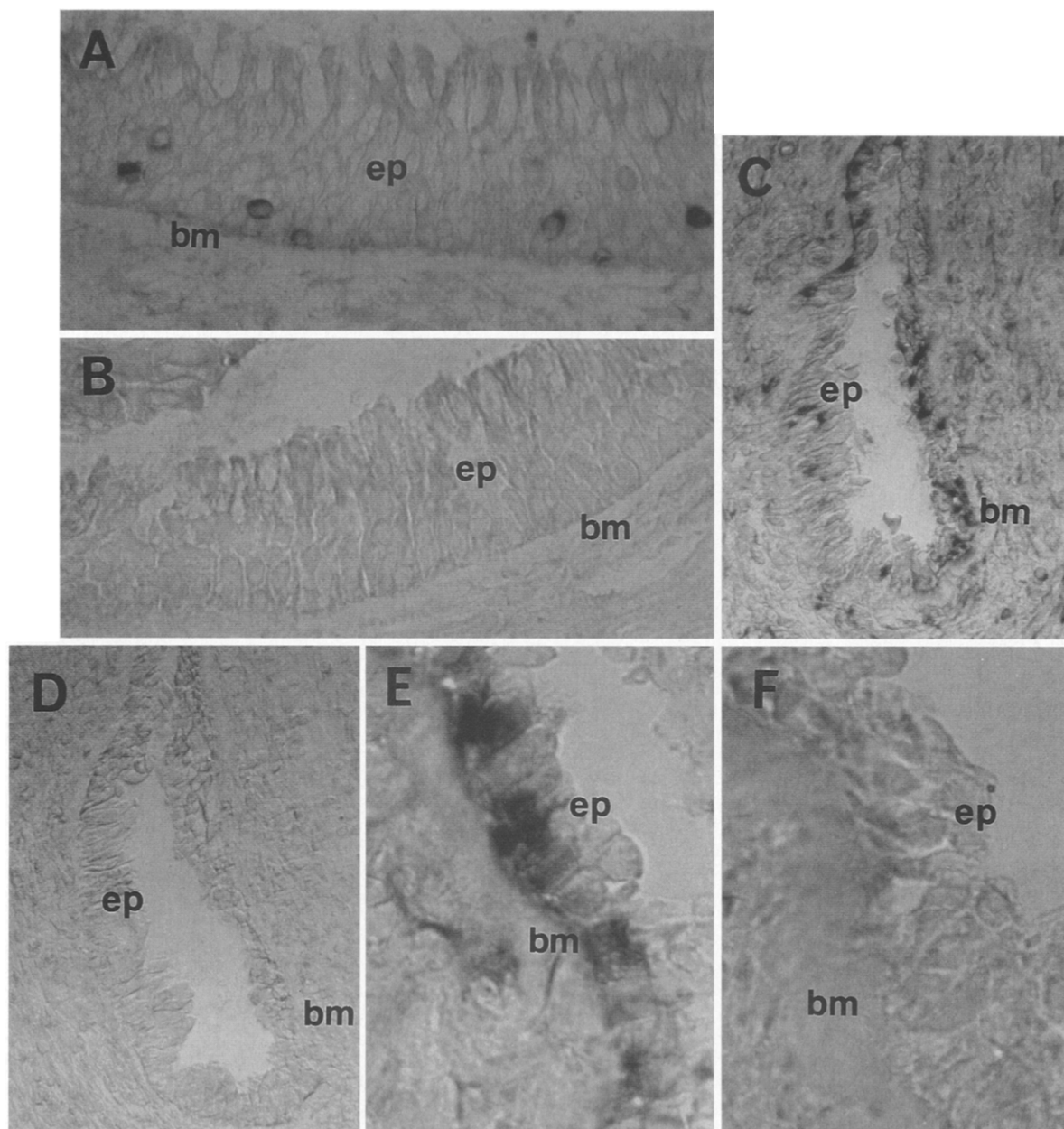


FIG. 2. Localization of human HMT mRNA in the epithelium of human nasal mucosa. **A**, In situ hybridization with anti-sense HMT probe. Some cells in the epithelium (*ep*) were positive. **B**, Negative control with sense HMT probe in the adjacent section. No signals were detected. (Original magnification $\times 200$.) **C**, In situ hybridization with anti-sense HMT probe in the epithelium of another specimen of human nasal mucosa. Some cells in the epithelium (*ep*) were strongly stained. Some cells were shed. **D**, Negative control with sense HMT probe in the adjacent section. No signals were detected. (Original magnification $\times 100$.) **E**, High-powered magnification of the epithelium shown in **C**. **F**, High-powered magnification of the epithelium shown in **D**. (Original magnification $\times 400$.) *bm*, Basement membrane; *ep*, epithelium.

In situ hybridization

In situ hybridization with the anti-sense probe revealed that some cells in the epithelium (Fig. 2, *A*, *C*, and *E*), endothelial cells of vessels including capillaries, small arteries (Fig. 3, *B* and *D*), arte-

rioles, and veins (Fig. 3, *B* and *D*) and spindle-shaped cells and infiltrating cells in submucosa (Fig. 3, *B* and *D*) were positive. In particular, endothelial cells of veins were strongly stained (Fig. 3, *B* and *D*). The specificity of the hybridiza-

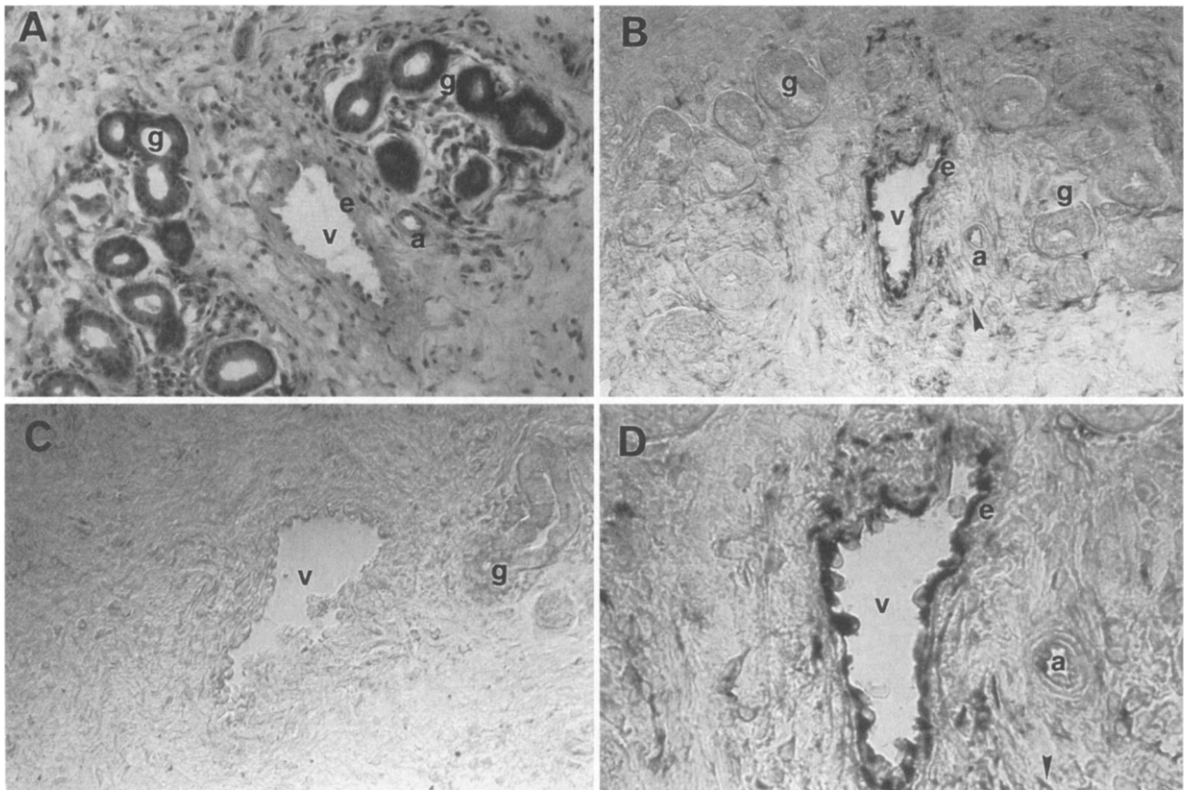


FIG. 3. Localization of human HMT mRNA in vessels of human nasal mucosa. **A**, Hematoxylin and eosin stain of nasal mucosa. **B**, In situ hybridization with anti-sense HMT probe. Endothelial cells (*e*) of a vein (*v*) and a small artery (*a*) were positive. Arrow indicates a positive cell in the submucosa. No positive signals were detected on submucosal glands (*g*). **C**, In situ hybridization with sense HMT probe with adjacent section. No signals were detected. (Original magnification $\times 100$.) **D**, High-powered magnification of the vein shown in **B**. Endothelial cells (*e*) of the vein (*v*) were strongly stained. Arrow indicates a positive cell in the submucosa. (Original magnification $\times 400$.) *v*, Vein; *e*, endothelial cells; *a*, small artery; *g*, submucosal glands.

tion was confirmed by the absence of signals when the preparations were hybridized with a sense probe (Fig. 2, *B*, *D*, and *F* and Fig. 3, *C*). No specific signal was identified in the submucosal glands (Fig. 3, *B* and *C*).

HMT activity

HMT activity of human nasal mucosa was 49.7 ± 9.8 pmol/min/mg protein ($n = 4$).

Histamine content

Histamine content of human nasal mucosa was 72.8 ± 16.4 nmol/gm tissue ($n = 4$).

DISCUSSION

This study is the first to demonstrate the localization of human HMT mRNA in human nasal mucosa. The cells in the epithelium, some cells in the submucosa, and vascular endothelial cells ex-

pressed human HMT mRNA. No HMT mRNA was identified in the submucosal glands.

In human beings, there are two major enzymatic pathways of histamine metabolism: *N*-methylation of histamine to *N*⁷-methylhistamine catalyzed by HMT and the oxidative deamination of histamine to imidazole acetic acid, catalyzed by the enzyme DAO, which is primarily, histaminase.⁶⁻⁸ The more important one is catalyzed by HMT, localized in human small intestine,¹⁰ liver,¹⁰ kidneys,²⁴ lungs,¹⁰ skin,²⁵ nasal mucosa,¹¹ and monocytes.⁸ In the other pathway histamine undergoes oxidative deamination catalyzed mainly by the nonspecific enzyme DAO, located in human intestinal mucosa,¹⁰ and eosinophils and neutrophils.⁸

HMT activity was reported to be 50 times higher than that of DAO,¹¹ suggesting that HMT is more important than DAO in the degradation of histamine in human nasal mucosa. In this study HMT

activity was detected in human nasal mucosa with values compatible with previous data.¹¹ In addition, the histamine content in human nasal mucosa with nasal allergy was also compatible with previous data.¹¹

We have cloned human cDNA, which is confirmed to encode human HMT by transient expression of HMT activity in COS cells.¹²

Most of the previous studies performed with the *in situ* hybridization technique used radioactive probes for the detection of mRNA in tissues,²⁶ whereas this *in situ* hybridization method with a nonradioactive RNA probe is useful because of its high resolution power for cell identification.

In situ hybridization clearly indicated that HMT mRNA was present in the endothelial cells of vessels, especially capillaries and veins, and cells in the epithelium and submucosa, but not in the submucosal glands in human nasal turbinate mucosa. In nasal mucosa histamine released from mast cells by antigen or other stimuli diffuses into the extracellular milieu. Histamine directly causes vascular permeability through histamine H₁ receptors on the endothelium of vessels.²⁻⁵ Histamine simultaneously causes nasal glandular secretion by stimulating a nasopal reflex²⁷ but not through its direct action on submucosal glands, as supported by the finding that there was no localization of H₁ receptors on submucosal glands by autoradiographic study.¹ Present studies of HMT activity and of cellular sites of biosynthesis of HMT by *in situ* hybridization in human nasal mucosa indicate that HMT is likely present predominantly in the endothelium of vessels. In addition, it is likely present in cells in the epithelium and submucosa but not in submucosal glands. Thus the endothelial cells may contribute to the degradation of histamine in nasal mucosa. The previous finding that human vascular endothelial cells²⁸ have HMT activity supports our finding. Further, the finding that HMT mRNA was expressed in the epithelium and endothelium of guinea pig trachea²⁸ also supports our finding. It is also reported that degradation of histamine by a target cell serves to terminate its biologic activity at or near its site of direct action on receptors such as histamine H₁ receptors.²⁹ In this context, it is interesting that histamine acts directly on the endothelial cells through H₁ receptors located there and that its metabolism seems to be carried out at the level of these target cells (i.e., endothelial cells) but not in submucosal glands in human nasal mucosa. Thus the dense localization of HMT mRNA on the endothelium of vessels suggests that HMT may modulate vascular perme-

ability by its enzymatic activity in human nasal mucosa. Further immunohistochemical study is required to determine the localization of HMT.

HMT mRNA was also detectable in cells in the epithelium. Stimulating mast cells with antigen causes the release of histamine into the extracellular milieu. Mast cells were widely distributed in the nasal mucosa, such as in the epithelium and in the lamina propria, with some variation in distribution according to heterogeneity, mucosal type, or connective tissue type mast cells.³⁰ HMT in the epithelium may play a pathophysiologic role in inactivating histamine released from intraepithelial mast cells. It remains unclear whether some cells containing HMT mRNA in the epithelium (Fig. 2, A) are the constituent cells of epithelium or resident inflammatory cells in the epithelium. Further study with immunostaining and *in situ* hybridization is needed to determine the identity of these cells.

In conclusion, this study, which clearly demonstrates the dense localization of HMT mRNA on the endothelium of vessels and the HMT activity in human nasal mucosa, suggests that HMT may modulate vascular permeability by inactivating histamine in human nasal mucosa. In addition, this study indicates that cells in the epithelium and submucosa, which are still unidentified, are an additional source of HMT mRNA.

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