

Characterization and autoradiographic localization of histamine H₁ receptors in human nasal turbinates

Michiko Okayama, MD, James N. Baraniuk, MD, FRCP(C),*
Jefferey N. Hausfeld, MD, FACS,** Marco Merida, MD,*** and
Michael A. Kaliner, MD**** Bethesda, Md., and Washington, D.C.

To examine the localization of histamine H₁ receptors (H₁R) in human nasal mucosa, the autoradiographic distribution of H₁R was studied in human nasal inferior turbinates. Cryostat sections were incubated with various concentration of [³H]pyrilamine in saturation-binding studies and with 1 nmol/L of [³H]pyrilamine for autoradiography. Nonspecific binding was determined by adding 2 μmol/L of pyrilamine. Scatchard analysis demonstrated high-affinity binding sites with a maximum binding capacity of H₁R of 193 ± 46 fmol/mg of protein, and dissociation constant was 0.6 ± 0.1 nmol/L. Autoradiograms indicated H₁R exist exclusively on the endothelium of vessels. No specific labeling could be observed in the submucosal glands or epithelium. These results extend and support our previous finding that histamine directly causes vascular permeability through H₁R and stimulates nasal glandular secretion indirectly through reflexes. (J ALLERGY CLIN IMMUNOL 1992;89:1144-50.)

Key words: Human nasal mucosa, H₁ receptors, [³H]pyrilamine, autoradiography, histamine, vascular permeability, nasal secretion

Histamine plays an important role in eliciting the nasal symptoms of allergic rhinitis: pruritus, sneezing, rhinorrhea, and congestion. Antihistamine therapy is effective in relieving many of the symptoms of allergic rhinitis. Histamine nasal provocation produces an immediate increase in nasal congestion via its direct action on the vascular bed, which reflects simultaneous increases in blood flow, blood volume, and/or vascular permeability, as assessed by an increase in nasal airway resistance.^{1,2} Histamine-induced vascular permeability leads to the production of secretions rich in albumin and other vascular proteins³⁻⁵ and an increase in the secretion of intravenously injected, radiolabeled molecules.⁶ In addition, histamine simultaneously

Abbreviations used

H₁R: H₁ receptor
B_{max}: Maximum binding capacity
K_d: Dissociation constant

causes secretion of glandular products^{1,5} by stimulating a nasonasal reflex.^{1,7} Pretreatment with an H₁ antihistamine, such as chlorpheniramine, inhibits both clinical symptoms and nasal protein secretion, suggesting that these responses are produced through stimulation of H₁R.^{5,8} In vitro, histamine has virtually no effect on human nasal mucus secretion.⁹

These data suggest that in human nasal mucosa, the major target of histamine is the vascular bed, but, as yet, no careful analysis of histamine-receptor localization in the nasal mucosa has been reported. In this study, histamine receptors in the human inferior turbinate have been characterized and localized with [³H]pyrilamine.

MATERIAL AND METHODS

Human tissue preparation

Human inferior turbinates were obtained at the time of surgery from six patients with nasal obstructive syndromes.

From the Allergic Disease Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Md.; *Lung Biology Laboratories, Georgetown University, Washington, D.C.; **Department of Otolaryngology, Washington Hospital Center, Washington, D.C.; and ***Departments of Otolaryngology and Pediatrics, Georgetown University Hospital, Washington, D.C.

Received for publication Oct. 7, 1991.

Revised Jan. 31, 1992.

Accepted for publication Feb. 6, 1992.

Reprint requests: Michael A. Kaliner, MD, Allergic Diseases Section, Laboratory of Clinical Investigation, NIAID, Bethesda, MD 20891.

1/1/36990

No patient had had a recent infection or had been taking antihistamines. At the time of surgery, 2% tetracaine HCl and 0.25% phenylephrine HCl were applied topically on nasal packs. 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 medial flap of turbinate that remained 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. Mucosal specimens for radioligand-binding assay and autoradiography were frozen in 2-methyl-butane on dry ice for 20 seconds and then stored at -70°C until use.

Pharmacologic agents

[³H]Pyrilamine (specific activity, 23.9 Ci/mmol) was obtained from E. I. Du Pont. (Pittsburg, Pa.). Pyrilamine was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Radioligand-binding assay on cryostat sections

Cryostat sections (6 μm) were cut, thawed, and mounted on gelatin-coated slides. Slides were stored at -70°C and used within 12 hours of preparation. Binding of [³H]pyrilamine to cryostat sections was performed with the conditions according to those described by Chang et al.¹⁰ and Hill et al.¹¹ for membrane preparations. For Scatchard analysis, slides bearing four to six sections were incubated in 10 ml of 5 mmol/L of sodium potassium phosphate buffer (pH 7.5) at 25°C . [³H]Pyrilamine from 0.5 to 10 nmol/L was added and incubated for 30 minutes at 25°C . Specific binding of [³H]pyrilamine was determined by the difference between radioligand bound in the absence and presence of 2 $\mu\text{mol/L}$ pyrilamine. All measurements were made in duplicate, and each experiment was repeated three or more times. After incubation, slides were washed twice in sodium potassium phosphate buffer at 4°C for 10 minutes to remove unbound radioligand. Sections were then scraped from the slide into scintillation vials. After solubilization (Protosol, New England Nuclear Co., Boston, Mass.) and addition of 10 ml of scintillant (Ready-Solv HP, Beckman Instruments, Inc., Fullerton, Calif.), counting was performed in a liquid scintillation analyzer (model 2200 CA, Tri-Carb, Packard Instrument Co., Sterling, Va.).

Several parallel sections were collected for protein determination according to the technique of Lowry et al.¹² with bovine serum albumin as a standard.

Autoradiography

Slides bearing four sections were warmed to room temperature and preincubated in sodium potassium phosphate buffer, pH 7.5, containing 1 mmol/L of phenylmethylsulfonylfluoride, and 0.01 mmol/L of leupeptin for 5 minutes at 25°C . Slides were incubated for 30 minutes at 25°C in a solution of 1 nmol/L of [³H]pyrilamine. Nonspecific binding was determined by parallel incubation of slides in the

presence of 2 $\mu\text{mol/L}$ of pyrilamine. After incubation, the slides were washed twice in pH 7.5 sodium potassium phosphate buffer for 10 minutes each at 4°C and quickly washed in distilled water to remove the salts. The slides were dried rapidly in a stream of cold dry air. Nuclear track emulsion (NTB-2, Eastman Kodak, Rochester, N.Y.) was melted in the dark for 4 hours at 45°C and mixed with 1% glycerol in water. In a darkroom, slides were dipped in emulsion, the backs of the slides wiped clean, and the slides placed vertically for 2 hours to allow the emulsion to run off in an even fashion and dry.¹³ Coated slides were placed in plastic boxes. Sections were exposed for 2 to 4 weeks at 4°C and warmed to room temperature in the dark. Slides were then developed in D-19 developer (Eastman Kodak) for 1 minute at 22°C , washed in water for 30 seconds, fixed in Kodak fixes for 4 minutes, and finally washed in water for 15 minutes. Slides were counterstained with toluidine blue.

Statistical analysis

All values are expressed as mean $\pm$ SEM. Standard analysis of receptor binding was used.¹⁴

RESULTS

Radioligand-binding assay

Specific [³H]pyrilamine binding to cryostat sections of [³H]human nasal mucosa was saturable, and Scatchard analysis demonstrated distinct high- and low-affinity binding sites. A B_{max} and a K_d for the high-affinity component was 193 ± 46 fmol/mg of protein and 0.6 ± 0.1 nmol/L, respectively ($n = 3$). The B_{max} and K_d for the low-affinity component was 772 ± 206 fmol/mg of protein and 12.5 ± 2.4 nmol/L, respectively ($n = 3$). A representative experiment with B_{max} of 147 fmol/mg of protein and K_d of 0.5 nmol/L for the high-affinity component, and 772 fmol/mg of protein and 12.5 nmol/L for the low-affinity component is illustrated in Fig. 1.

Autoradiography

Autoradiograms of the human nasal mucosa demonstrated that [³H]pyrilamine binding sites were distributed with dense labeling on vessels, including arterioles, veins, and sinusoids. [³H]Pyrilamine binding sites were localized exclusively on the endothelium and not on the smooth muscle of veins (Fig. 2, A and B). The addition of excess cold pyrilamine completely displaced binding to veins (Fig. 2, C). By contrast, there was no specific labeling either on the epithelium (Fig. 3) or submucosal glands (Fig. 4).

DISCUSSION

This study demonstrates two new findings regarding histamine H₁R in human nasal mucosa. First, distinct higher and lower affinity binding sites are demonstrable by Scatchard analysis of saturation curves.

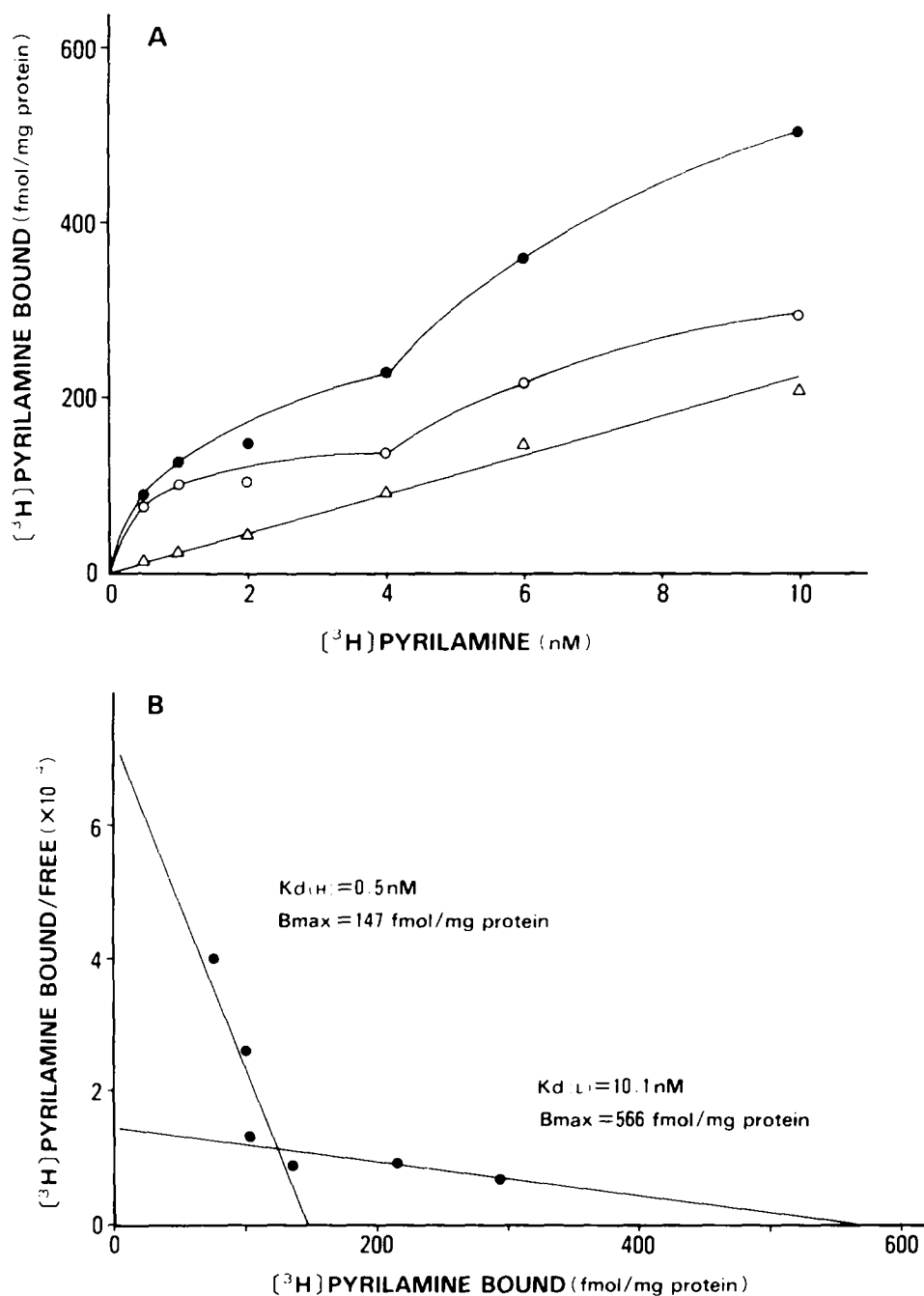


FIG. 1. A, Equilibrium binding of [³H]pyrilamine to cryostat sections of human nasal turbinate mucosa. Sections were incubated with increasing concentrations of [³H]pyrilamine; total (●), specific (○), and nonspecific (△) binding, respectively. Nonspecific binding was determined with 2 μmol/L of pyrilamine. *Each point* is mean of duplicate determinations in a single representative experiment. **B,** Scatchard plot of equilibrium binding; K_d (H), high-affinity component; K_d (L), low-affinity component.

Second, histamine H₁R exist exclusively on the vessels, especially in the endothelium but not in the sub-mucosal glands or epithelium. The receptor-binding study demonstrated that specific binding of

[³H]pyrilamine to cryostat sections of human nasal turbinate mucosa was saturable and had distinct higher and lower affinity binding sites with K_d values 0.6 ± 0.1 and 12.5 ± 2.4 nmol/L, respectively.

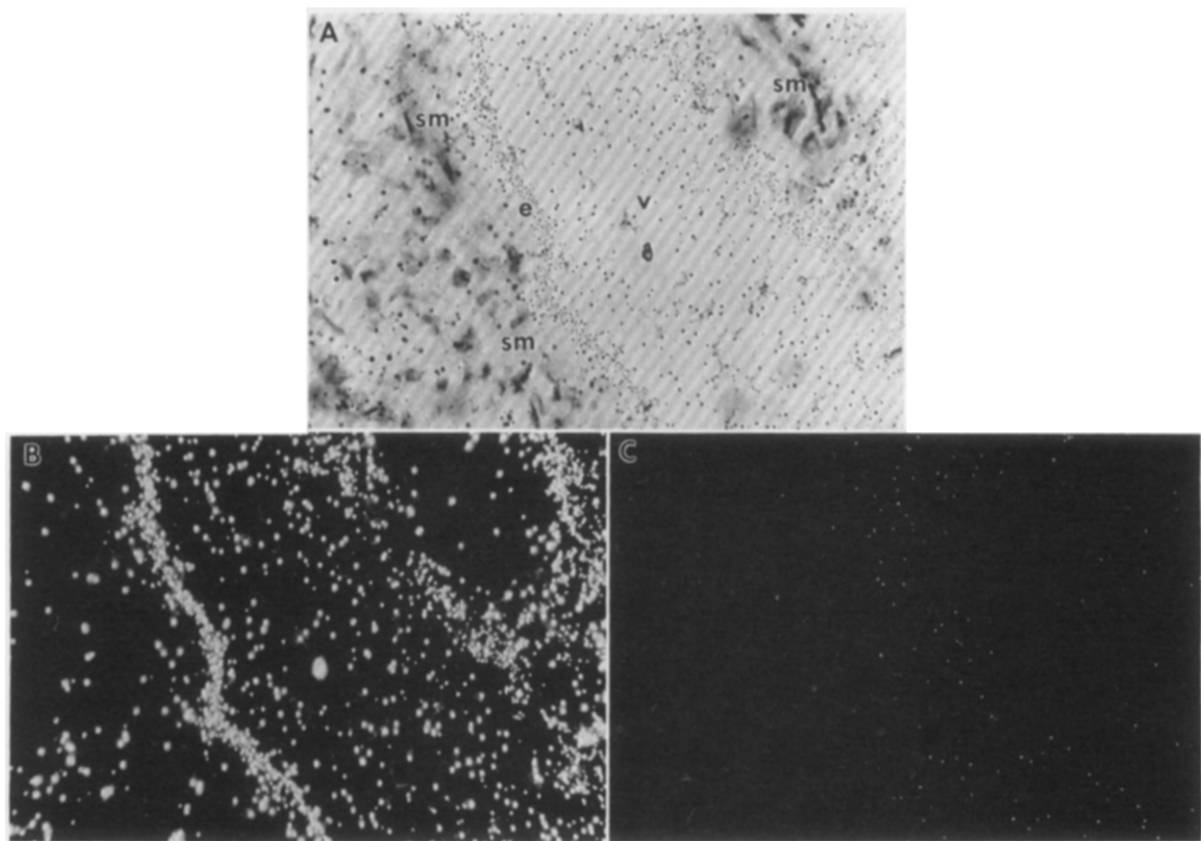


FIG. 2. [³H]Pyrilamine binding sites to venous structure in human nasal turbinate mucosa. **A.** Brightfield image of total binding to vein (*V*). **B.** Darkfield image of total binding of 1 nmol/L of [³H]pyrilamine of same field as **A**. Binding sites are distributed on endothelium (*e*) of the vein but not on the smooth muscle (*sm*). Nonspecific binding (determined by 2 μmol/L pyrilamine). Excess pyrilamine completely displaced binding, suggesting that these binding sites were specific. (Original magnification × 400.)

There has only been one report of H₁R characterization in nasal mucosa. Tosaka and Ishikawa¹⁵ reported a single class of [³H]mepyramine binding sites with a K_d value of 5 nmol/L in human nasal mucosa with an isolated membrane preparation. However, their Scatchard analysis excluded binding produced by the lower [³H]mepyramine concentrations, and therefore, these investigators eliminated the high-affinity H₁R from their calculation. The K_d values for the high-affinity component in the human nasal mucosa agrees with [³H]mepyramine binding studies in brain,¹⁶ intestinal smooth muscle,¹¹ and aorta.^{17, 18}

Multiple H₁R are indicated by binding studies in the tissues. Chang et al.¹⁰ reported high- and low-affinity binding sites with K_d values for the high-affinity component of 1.1 ± 0.3 nmol/L and for the low-affinity component of 9.3 ± 1.8 nmol/L, respectively.¹⁰ Casale et al.¹⁹ described three distinct [³H]pyrilamine binding sites in human peripheral lung

homogenates with K_d values of 81 pmol/L and 7 and 320 μmol/L. Human peripheral blood mononuclear cells also have two binding sites with K_d of 4 nmol/L and 55 μmol/L.²⁰ Despite differences in technique (tissue slices on slides in the current study versus membrane preparations), the results are comparable, and it appears plausible that there are multiple binding sites for [³H]pyrilamine in human nasal turbinate mucosa.

Autoradiographic analysis clearly indicated that H₁R exist on the vessels but not in the submucosal glands or epithelium in human nasal turbinate mucosa. Pyrilamine bound to endothelial more than to smooth muscle cells. Tosaka and Ishikawa¹⁵ reported localization of H₁R in nasal glands and glandular ducts but not in vessels. They could not explain the difference between their autoradiographic localization and the physiologic and pharmacologic roles of H₁R. The autoradiographic procedure used by Tosaka and Ish-

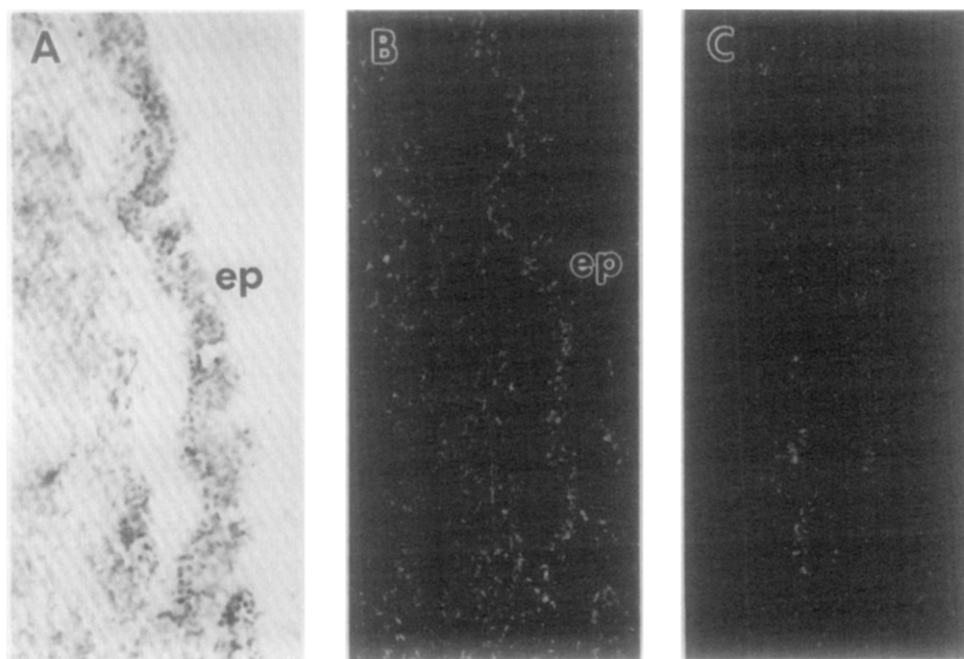


FIG. 3. [^3H]pyrilamine binding sites in epithelium. **A**, Brightfield image of total binding in epithelium (*ep*). **B**, Darkfield image of total binding of [^3H]pyrilamine (1 nmol/L) of same field as **A**. **C**, Nonspecific binding. Note that there is little specific binding to epithelium. (Original magnification $\times 125$.)

ikawa involved formaldehyde fixation for 48 hours and embedding in paraffin before autoradiography. These procedures might affect [^3H]mepyramine binding. By contrast, in the present study, the tissue was fresh frozen and prepared on cryostat sections without fixation. Thus, these procedural differences might influence the results of these studies. Potential histamine binding sites on nerves^{5, 15} could not be resolved by the present methods.

The pharmacologic actions of histamine on blood vessels have been demonstrated to be mediated primarily by H_1R .^{21, 22} In the nasal vasculature of experimental animals, H_1 and H_2R have been identified pharmacologically and physiologically and cause vasodilatation in the cat,²³ dog,²⁴ and possibly in the human.^{8, 25} H_1R have been detected in the membrane fraction of feline vessels with [^3H]mepyramine.^{17, 18} Endothelial cell-dependent vasodilatation by histamine occurs in rat thoracic aorta²⁶ and guinea pig pulmonary artery preparations,²⁷ suggesting that H_1R are present not only in the vascular smooth muscle but also in the vascular endothelium. Toda²⁸ concluded that relaxation in human coronary arteries might be mediated by H_1R in endothelium and H_2R in smooth muscle. Hide et al.²⁸ found that the predominant location of H_1R was endothelial cells and not smooth

muscle cells in guinea pig aorta with a [^3H]mepyramine binding assay.¹⁸ The localization of both H_1 and H_2R on microvascular endothelial cells has been directly detected with electron-opaque conjugates of histamine.²⁹ In a unique immunohistochemical study of histamine-induced increases in cyclic guanosine monophosphate, the most responsive cells to histamine stimulation in guinea pig lung were alveolar and parenchymal macrophages, pleural lining cells, and endothelial and epithelial cells, with little or no increases noted in bronchial or vascular smooth muscle cells.³⁰ Therefore, the presently described autoradiographic results that indicate that H_1R exist mainly on the endothelium of vessels and not in the vascular smooth muscle are in good agreement with previous receptor-binding assays and histochemical findings.

In submucosal glands, there was no localization of [^3H]pyrilamine binding, an observation compatible with *in vitro* functional studies. *In vitro*, histamine has virtually no effect on human nasal glandular secretion, but it has a profound effect on mucous secretion from human bronchi.³¹ The absence of specific binding of pyrilamine to the nasal epithelium also suggests that epithelial cells are not directly responsive to histamine stimulation. Thus, the present study sup-

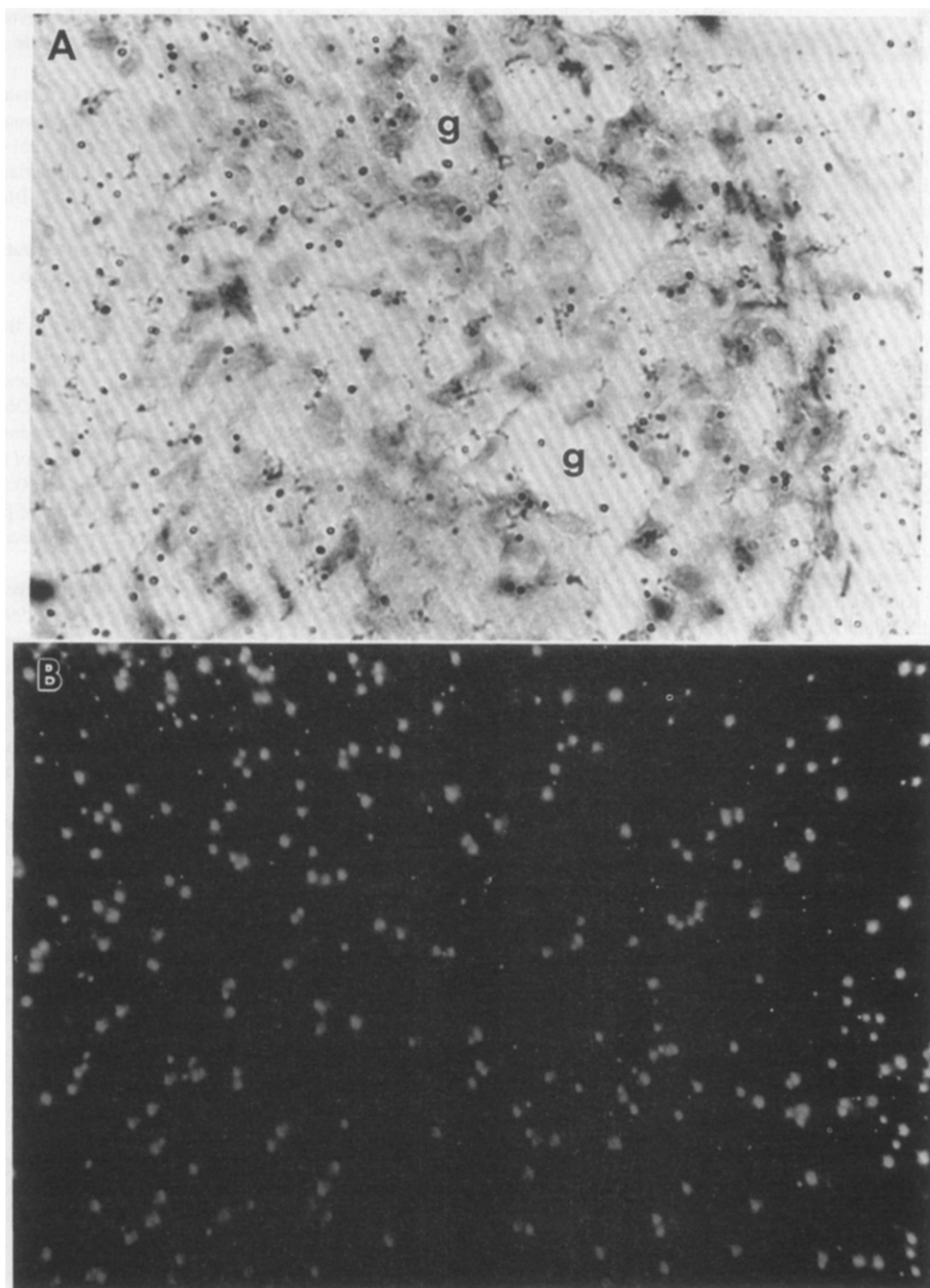


FIG. 4. [^3H]Pyrilamine binding sites in submucosal glands. **A**, Brightfield image of total binding in submucosal glands (*g*). **B**, Darkfield image of total binding of [^3H]pyrilamine (1 nmol/L) of same field as **A**. Note that there are no binding sites in submucosal glands. Many nonspecific grains were observed over field. (Original magnification $\times 400$.)

ports our previous findings that histamine acts directly on vascular receptors to produce vascular permeability and indirectly through cholinergic reflexes to produce glandular secretion.

Dr. M. Okayama was supported by grants from Muro Pharmaceuticals and The Procter and Gamble Co. and Dr. J. N. Baraniuk was supported by grants from The Procter and Gamble Co., Schering Research, and the Wiggins Foundation.

REFERENCES

- Borum P, Gronberg H, Brofeldt S, Mygind N. Nasal reactivity in rhinitis. *Eur J Respir Dis* 1983;64(suppl 128):65-71.
- Corrado OJ, Gould CAL, Kassab JY, Davies RJ. Nasal response of rhinitic and nonrhinitic subjects to histamine and methacholine: a comparative study. *Thorax* 1986;41:863-8.
- Brofeldt S, Mygind N, Sorensen CH, Readman AS, Marriott C. Biochemical analysis of nasal secretions induced by methacholine, histamine, and allergen provocations. *Am Rev Respir Dis* 1986;133:1138-42.
- Togias A, Proud D, Kagey-Sobotka A, Norman P, Lichtenstein L, Naclerio R. The effect of a topical antihistamine on the response of the nasal mucosa to challenge with cold, dry air and histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:599-604.
- Raphael GD, Meredith SD, Baraniuk JN, Druce HM, Banks SM, Kaliner MA. The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis* 1989;139:791-800.
- Bisgaard H, Krogsgaard OW, Mygind N. Measurement of secretion in nasal lavage. *Clin Sci* 1987;73:217-22.
- Konno A, Togawa K. Role of the vidian nerve in nasal allergy. *Ann Otol Rhinol Laryngol* 1979;88:258-66.
- Secher C, Kirkegaard J, Borum P, Maansson A, Osterhammel P, Mygind N. Significance of H_1 and H_2 receptors in the human nose: rationale for optical use of combined antihistamine preparations. *J ALLERGY CLIN IMMUNOL* 1982;70:211-8.
- Mullol J, Raphael GD, Lundgren JD, et al. Comparison of human nasal mucosal secretion in vivo and in vitro. *J ALLERGY CLIN IMMUNOL* 1992;89:584-92.
- Chang RSL, Tran VT, Snyder SH. Characteristics of histamine H_1 receptors in peripheral tissues labeled with [^3H]-mepyramine. *J Pharmacol Exp Ther* 1979;209:437-42.
- Hill SJ, Young JM, Marrian DH. Specific binding of [^3H]-mepyramine to histamine H_1 receptors in intestinal smooth muscle. *Nature* 1977;270:361-3.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagent. *J Biol Chem* 1951;193:265-71.
- Rogers AW. Practical autoradiography: review 20. Arlington Heights, Ill.: Amersham Corp., 1984.
- Wescott S., Kaliner M. The histamine H_1 binding site on human polymorphonuclear leukocytes. *Inflammation* 1983;7:291-300.
- Tosaka K, Ishikawa T. Direct evidence of histamine H_1 -receptors in human nasal mucosa by receptor binding assay and autoradiography. *Kumamoto Med J* 1985;38:37-45.
- Tran VT, Chang RSL, Snyder SH. Histamine H_1 receptors identified in mammalian brain membranes with [^3H]-mepyramine. *Proc Natl Acad Sci USA* 1978;75:6290-4.
- Carman-Krzan M. Specific binding of [^3H]-mepyramine to histamine H_1 -receptors in vascular smooth muscle membranes. *Agents Actions* 1983;13:162-6.
- Hide M, Fukui H, Watanabe T, Wada H, Yamamoto S. Histamine H_1 -receptor in endothelial and smooth muscle cells of guinea pig aorta. *Eur J Pharmacol* 1988;148:161-9.
- Casale TB, Rodbard D, Kaliner M. Characterization of histamine H_1 receptors on human peripheral lung. *Biochem Pharmacol* 1985;34:3285-92.
- Casale TB, Wescott S, Kaliner M. Characterization of histamine H_1 receptors on human mononuclear cells. *Int J Immunopharmacol* 1985;7:639-45.
- Kaliner M, Sigler R, Summers R, Shelhamer JH. Effects of infused histamine: analysis of the effects of H_1 - and H_2 -histamine receptor antagonists on cardiovascular and pulmonary responses. *J ALLERGY CLIN IMMUNOL* 1981;68:365-71.
- Garrison JC, Rall TW. In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. *The pharmacological basis of therapeutics*. 8th ed. New York: Macmillan Publishing, 1990:574-99.
- Hiley R, Wilson H, Yates MS. Identification of β -adrenoceptors and histamine receptors in the cat nasal vasculature. *Acta Otolaryngol* 1978;85:444-8.
- Ichimura K, Jackson Rt. H_1 and H_2 histamine receptors in the in vitro nasal mucosa. *Acta Otolaryngol* 1985;99:610-9.
- Havas TE, Cole P, Parker L, Oprysk D, Ayiomamitis. The effects of combined H_1 and H_2 histamine antagonists on alterations in nasal airflow resistance induced by topical histamine provocation. *J ALLERGY CLIN IMMUNOL* 1986;78:856-60.
- van de Voorde J, Leusen I. Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur J Pharmacol* 1983;87:113-20.
- Satoh H, Inui J. Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea pig pulmonary artery. *Eur J Pharmacol* 1984;97:321-4.
- Toda N. Mechanism of histamine actions in human coronary arteries. *Circ Res* 1987;61:280-6.
- Heltianu C, Simionescu M, Simionescu N. Histamine receptors of the microvascular endothelium revealed in situ with a histamine-ferritin conjugate: characteristic high-affinity binding sites in venules. *J Cell Biol* 1982;93:357-64.
- Serfl K, Casale TB, Wescott LS, Kaliner MA. Immunohistochemical localization of histamine-stimulated increases in cyclic GMP in guinea pig lung. *Am Rev Respir Dis* 1987;135:456-62.
- Shelhamer JH, Marom Z, Kaliner M. Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. *J Clin Invest* 1980;66:1400-8.