

Allergens, IgE, mediators, inflammatory mechanisms

Histamine-induced venodilation in human beings involves both H₁ and H₂ receptor subtypes

William D. Dachman, MD, Gabriella Bedarida, MD, Terrence F. Blaschke, MD, and Brian B. Hoffman, MD *Stanford and Palo Alto, Calif.*

Histamine is a potent vasodilatory substance released during anaphylaxis. The purpose of this study was to investigate the mechanism by which histamine produces venodilation in humans in vivo with the use of the dorsal hand-vein compliance technique. In healthy volunteers full dose-response curves were constructed by infusing histamine, before and after administration of an H₁ or H₂ antagonist or both antagonists, into dorsal hand veins precontracted with the α -adrenergic agonist phenylephrine. In the presence of the H₁ antagonist brompheniramine (530 ng/min), the maximal venodilatory response to histamine decreased from 128% $\pm$ 57% to 78% $\pm$ 15% ($p < 0.05$). In the presence of H₂ antagonist cimetidine (49 μ g/min), the maximal venodilatory response to histamine decreased from 120% $\pm$ 33% to 48% $\pm$ 26% ($p < 0.01$). Concurrent infusion of histamine with the combination of cimetidine and diphenhydramine resulted in almost complete abolishment of histamine-induced venodilation. Methylene blue (6.8 μ g/min), an inhibitor of the action of endothelium-derived relaxing factor, decreased the vasodilatory response to histamine from 131% $\pm$ 23% to 73% $\pm$ 24% ($p = 0.01$). The results suggest that the venodilatory response of histamine is mediated through both H₁ and H₂ receptor subtypes and that this response is mediated in part by endothelium-derived relaxing factor. (J ALLERGY CLIN IMMUNOL 1994;93:606-14.)

Key words: Endothelium-derived relaxing factor, dorsal hand-vein compliance technique, cimetidine, brompheniramine

Histamine, an autacoid with potent vascular effects, is released during anaphylactic reactions and induces vasodilation and hypotension. First-line therapy for anaphylactic shock is treatment with epinephrine to reverse the vasodilation and prevent further degranulation of mast cells. Ad-

Abbreviations used

ED ₅₀ :	Dose producing half the maximum effect
EDRF:	Endothelium-derived relaxing factor
E _{MAX} :	Maximum effect of the drug
IC ₅₀ :	Dose rate inhibiting 50% of E _{max}

From the Division of Clinical Pharmacology, Stanford University Medical Center, Stanford; and Geriatric Research, Education and Clinical Center, Veterans Affairs Medical Center, Palo Alto.

Supported by National Institutes of Health grant AG 05627. Dr. Dachman was supported by a Boehringer Ingelheim Centennial Fellowship.

Received for publication Feb. 15, 1993; accepted for publication June 30, 1993.

Reprint requests: Terrence F. Blaschke, MD, Division of Clinical Pharmacology, Stanford University Medical Center, S-169, Stanford, CA 94305.

Copyright © 1994 by Mosby-Year Book, Inc.
0091-6749/94 \$3.00 + 0 1/1/49759

ditional therapy has traditionally utilized H₁ antagonists, but recently it has been suggested that combined therapy with H₁ and H₂ antagonists may be preferable to reverse the vasodilation induced by histamine.¹⁻³

Ash and Schild⁴ postulated the presence of two distinct histamine receptors based on the ability of the classic antihistamines to block only a portion of histamine's effects. Currently, histamine receptors are classified as H₁, H₂, and H₃.^{5, 6} The

H₃ receptor acts as an autoregulator of histamine's release as a neurotransmitter.

The effects of histamine on the vasculature have been extensively studied and found to vary among species and vascular beds. Various animal studies have implicated either the H₁ or the H₂ receptor as the receptor subtype involved in histamine-induced vasodilation. For example, cerebral artery strips from monkeys respond to histamine with full relaxation, which is partially attenuated by the H₁ antagonist chlorpheniramine and the H₂ antagonist cimetidine, when given separately, and fully antagonized by combined treatment with both drugs. In this vessel, removal of the endothelium partially attenuated the vasodilatory response, and the remaining relaxation was abolished by H₂ antagonism but not by H₁ antagonism.⁷ Other investigators have demonstrated histamine-induced prostacyclin release from human pulmonary arteries, which leads to vasodilation.⁸

The aim of this study was to investigate the mechanism by which histamine produces venodilation in human beings. The importance of studying histamine-induced venodilation relates to the major role that histamine plays in the development of anaphylactic shock, a condition in which vascular pooling in the venous system contributes to marked hypotension.⁹ In this study we investigated whether venodilation induced by histamine is mediated through H₁ and/or H₂ receptor subtypes. In addition, we determined the possible roles of endothelium-derived relaxing factor (EDRF) and prostacyclin release in histamine-induced venodilation.

METHODS

Subjects

The subjects, 10 healthy volunteers (mean age $\pm$ SD = 25.8 $\pm$ 3.0 years), were admitted on the morning of the study to the Aging Study Unit at the Palo Alto Veterans Affairs Medical Center. The subjects signed a written informed consent form and underwent a complete physical examination, electrocardiogram, and routine laboratory tests (SMA-20, complete blood count, urinalysis). Exclusion criteria included a history of any significant disease state, use of illicit drugs, alcoholism, or long-term use of any medication including over-the-counter antihistamines. All subjects were nonsmokers, and they were asked to refrain from consuming caffeine for at least 12 hours before the study.

Dorsal hand-vein technique

The dorsal hand-vein technique was used to quantify responsiveness of the dorsal hand-vein to histamine

alone and in combination with its antagonists. This technique, previously modified by Aellig,¹⁰ as used in our laboratory, has been previously described in detail.¹¹ This approach has the advantages of allowing infusion of very small amounts of vasoactive drugs and thus avoiding potentially confounding systemic hemodynamic effects. Furthermore, complete dose-response curves can be generated by administering sequentially increasing concentrations of drugs. From the curves obtained, it is possible to derive parameters that represent the maximum effect of the drug ($E_{\max}$) and the sensitivity to the drug (the dose producing half the maximum effect [ED₅₀]).

Each subject was studied in the supine position with one arm placed on a padded support with an upward angle of 30 degrees from the horizontal to allow for complete emptying of the veins. A suitable vein was chosen on the dorsum of the hand and a 23-gauge needle was inserted. Normal saline solution was infused for at least 30 minutes to allow for equilibration of the vein after the initial vasoconstriction caused by the needle insertion. The flow rate was kept constant throughout the study at 0.27 ml/min by use of a Harvard infusion pump (Harvard Apparatus Inc., South Natick, Ma.). A tripod, holding a linear variable differential transformer (LVDT, Shaevitz Engineering, Pennsauken, N.J.), was mounted on the back of the hand with the central aperture of the LVDT over the top of the vein under investigation at a distance of 10 mm downstream from the tip of the needle. The central aperture of the LVDT contains a freely movable metallic core; the signal output of the LVDT is linearly proportional to the vertical movement of the core and is amplified and recorded on a strip chart recorder. Each reading was taken by measuring the size of the vein under a congestive pressure of 40 mm Hg (i.e., by measuring the difference between the position of the core before and after inflation of the blood pressure cuff positioned on the upper portion of the same arm). Doses of drugs are expressed as rates of infusion. Each dose was infused for a minimum of 5 minutes, which as been shown to be sufficient to reach the maximum effect of each infusion rate,¹⁰ after which a reading was taken. Results are presented as normalized dose-response curves in which venodilation is plotted against the logarithm of the dose. The dilation of the vein during saline infusion was defined as 100% dilation. Phenylephrine, a selective α_1 -agonist, was used to pre-constrict the hand vein. Phenylephrine was infused in the dose range of 12 to 7917 ng/min; the dose of phenylephrine that reduced the size of the vein to 20% of its baseline value was determined. This degree of precontraction was defined as 0% dilation. This dose of phenylephrine was then infused at a constant rate during the subsequent administration of histamine alone or in combination with its antagonists. The vasodilation produced by these drugs was calculated as a percentage of the range between 100% (during saline

infusion) and 0% dilation (during phenylephrine infusion). Blood pressure and heart rate were monitored in the opposite arm with a Dinamap Blood Pressure Monitor (model 845, Critikon, Tampa, Fla.).

Study design

Dose-response curves for the selective H_1 antagonist brompheniramine in the range of 0.07 to 4234 ng/min and for the selective H_2 antagonist cimetidine in the range of 5 ng/min to 49 μ g/min were constructed. The effects of these antagonists were determined in the presence of histamine, infused at a rate of 110 ng/min, which typically produced maximal venodilation.

To evaluate the role of H_1 and H_2 receptors in mediating histamine-induced vasodilation in greater detail, we conducted experiments in which brompheniramine (530 ng/min) or cimetidine (49 μ g/min) was infused at constant rates in excess of their values of the dose rate inhibiting 50% of $E_{\max}$ (IC_{50}), in order to fully block H_1 and H_2 receptors, respectively. After an 80% precontraction of the vein was obtained with phenylephrine, a complete dose-response curve to histamine was constructed (doses ranged from 2 to 136 ng/min). Then the histamine was continued and an antagonist alone was infused for 45 minutes, either the H_2 -antagonist cimetidine (infused at a rate of 49 μ g/min) or the H_1 antagonist brompheniramine (infused at a rate of 530 ng/min). The vein was precontracted again to 80% of the new baseline by adding phenylephrine to the infusion of antagonist, and a second dose-response curve to histamine was constructed. The antagonists did not change baseline venodilation or the vasoconstrictor response to phenylephrine.

We also tested the effect of single or combined antagonists on histamine-mediated dilation. After precontraction of the vein with phenylephrine, histamine was infused at a dose of 74 ng/min to determine the response to histamine in the absence of antagonist(s). The infusion of histamine was discontinued, and inhibitor(s) alone were infused for at least 45 minutes. Histamine was then infused together with the antagonist(s). Three subjects received a combination of the H_1 antagonist, diphenhydramine (530 ng/min) and the H_2 antagonist, cimetidine (49 μ g/min); four subjects received an inhibitor of soluble guanylate cyclase, methylene blue (6.8 μ g/min); and three subjects received an inhibitor of cyclooxygenase, indomethacin (5.4 μ g/min). Preliminary experiments (data not shown) demonstrated that the venodilatory response to histamine was reproducible after a 45-minute saline infusion.

Materials

All drugs were diluted in normal saline solution. The following drugs were used: histamine phosphate (Lilly Pharmaceuticals, Indianapolis, Ind.); phenol-free

cimetidine (Smith Kline French Laboratories, Philadelphia, Pa.); brompheniramine maleate (Central Laboratories, Seymour, Ind.); diphenhydramine (Wyeth Laboratories Inc, Philadelphia, Pa.); phenylephrine hydrochloride (1% injection) (Winthrop Laboratories, New York, N.Y.); indomethacin sodium trihydrate (Merck Sharp & Dohme, West Point, Pa.); methylene blue (1% injection) (Reagent Laboratories, Inc., Shirley, N.Y.).

Data analysis

Results are expressed as means $\pm$ SD. Individual dose-response curves to histamine were fitted to a four-parameter logistic equation with the ALLFIT program.¹² This iterative curve-fitting program provides an estimate of the $E_{\max}$ and of the ED_{50} . A log transformation was performed on individual ED_{50} values, because log values of doses have a more normal distribution,¹³ and the geometric mean was calculated as the anti-log of the mean of the log values. A Student's paired or unpaired two-tailed *t* test was used as appropriate to compare the individual values for ED_{50} (after log transformation) and $E_{\max}$. A value of $p < 0.05$ was considered significant.

RESULTS

Maximal antagonism by brompheniramine decreased the histamine venodilatory response from 185% to 82% ($n = 2$); for cimetidine maximal antagonism decreased the histamine venodilatory response from 168% to 42% ($n = 2$). The infusion rate of brompheniramine that produced 50% of the maximal inhibition of histamine-induced venodilation (IC_{50}) was 4.0 ± 3.2 ng/min; the IC_{50} of cimetidine was 36.4 ± 21.4 ng/min. Individual dose-response curves for brompheniramine- and cimetidine-induced antagonism of histamine-induced venodilation are shown in Fig. 1, A and B.

In the presence of brompheniramine (530 ng/min), the $E_{\max}$ for histamine decreased from $128\% \pm 57\%$ to $78\% \pm 15\%$ ($p < 0.05$, $n = 6$), whereas its log ED_{50} did not significantly change; 1.24 ± 0.41 ng/min (17.4 ng/min) for histamine alone; and 1.31 ± 0.41 ng/min (20.4 ng/min) for histamine infused with brompheniramine. In the studies of antagonism of histamine by cimetidine (49 μ g/min), the $E_{\max}$ decreased from $120\% \pm 33\%$ to $48\% \pm 26\%$ ($p < 0.01$, $n = 6$), whereas the log ED_{50} for histamine alone was 1.18 ± 0.34 ng/min (15.1 ng/min) and 1.29 ± 0.41 ng/min (19.5 ng/min) for histamine infused concurrently with cimetidine ($p = \text{NS}$). Typical dose-response curves to histamine alone and together with fixed concentrations of either brompheniramine or cimetidine are shown in Figs. 2 and 3, respectively.

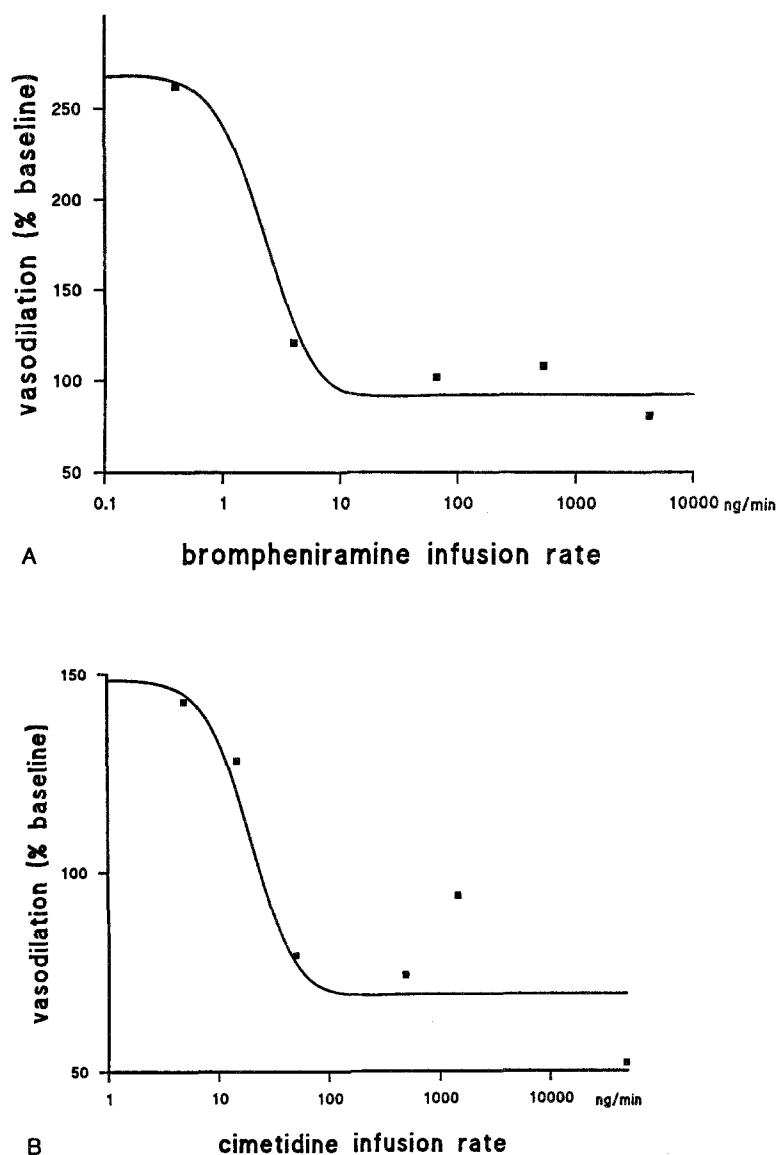


FIG. 1. Effect of increasing doses of brompheniramine and cimetidine on histamine-induced venodilation in a dorsal hand vein precontracted with phenylephrine. **A**, In the presence of histamine, infused at a constant rate of 110 ng/min, a dose-response curve for brompheniramine was generated. **B**, In the presence of histamine, infused at a constant rate of 110 ng/min, a dose-response curve for cimetidine was generated. Dilation of the vein is expressed as a percentage of baseline (before precontraction with phenylephrine) vein diameter. The data are the results in a typical subject. These experiments were repeated two times each with similar results.

Venodilation in response to histamine provided reproducible responses, with the E_{max} to histamine not being statistically different in the two separate sets of studies performed in the same six individuals. However, when the maximal venodilation to histamine obtained in the presence of brompheniramine is compared with that obtained

in the presence of cimetidine in these six subjects, cimetidine inhibited venodilation more than did brompheniramine ($p = 0.008$). These results suggest that the H_2 receptor subtype plays a more prominent role in histamine-induced venodilation than the H_1 receptor subtype.

The results of the next series of studies showed

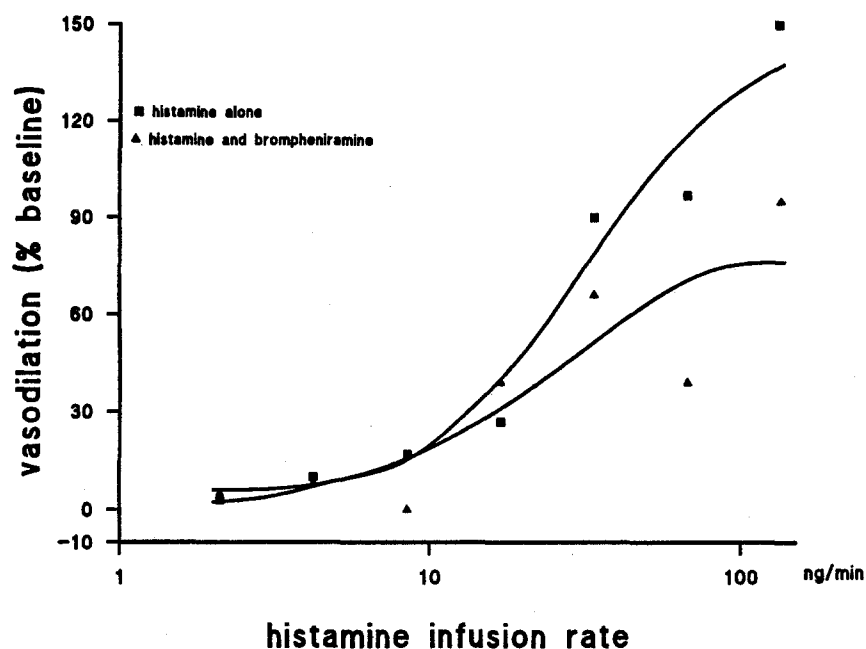


FIG. 2. Effect of brompheniramine on histamine-induced venodilation in a dorsal hand vein precontracted with phenylephrine. Dose-response curves for histamine alone and for histamine in the presence of brompheniramine at 530 ng/min, with a 45-minute infusion of brompheniramine between curves, were constructed. Dilation of the vein is expressed as a percentage of baseline (before precontraction with phenylephrine) vein diameter. Maximal dilation was not fully determined in this particular study and was estimated with the ALLFIT program. This experiment was repeated six times with similar results.

that the combination of diphenhydramine and cimetidine almost completely abolished the venodilatory response to histamine. The venodilatory response for histamine alone in these studies was $185\% \pm 65\%$; and for histamine infused concurrently with cimetidine (49 $\mu\text{g}/\text{min}$) and diphenhydramine (530 ng/min), the venodilatory response was $5\% \pm 10\%$ ($p = 0.03$, $n = 3$), indicating that high concentrations of a combination of H_1 and H_2 antagonists completely block histamine-induced dilation. In experiments in which methylene blue was used, the venodilatory response for histamine alone was $131\% \pm 23\%$, and with methylene blue the response was $73\% \pm 24\%$ ($p = 0.01$, $n = 4$). In the presence of the cyclooxygenase inhibitor indomethacin, the venodilatory response did not change: $106\% \pm 48\%$ for histamine alone and $108\% \pm 59\%$ for histamine simultaneously administered with indomethacin ($n = 3$). The results of these studies suggest that antagonism of both H_1 and H_2 receptor subtypes is necessary for complete abolishment of histamine-induced venodilation.

EDRF, but not prostacyclin, may play a role in mediating the venodilatory response to histamine in human veins.

DISCUSSION

The results demonstrate that histamine acts as a venodilator in humans in vivo via both H_1 and H_2 receptors, with venodilation mediated via H_2 receptors predominating. Essentially complete antagonism of histamine-induced venodilation is produced by administering a combination of H_1 and H_2 antagonists. Methylene blue partially attenuated the venodilatory response to histamine, suggesting that EDRF was involved in a component of the vasodilation.

Histamine is a potent and efficacious venodilator. Indeed, other vasodilators that we have previously studied with the use of the dorsal hand-vein compliance technique provided the following results: isoproterenol produced an E_{max} of 97 ± 3 and ED_{50} of 42 pmol/min in young subjects,¹¹ nitroglycerin caused an E_{max} of 103 ± 65 and an ED_{50} of 16 pmol/min,¹⁴ and prostaglandin E_1

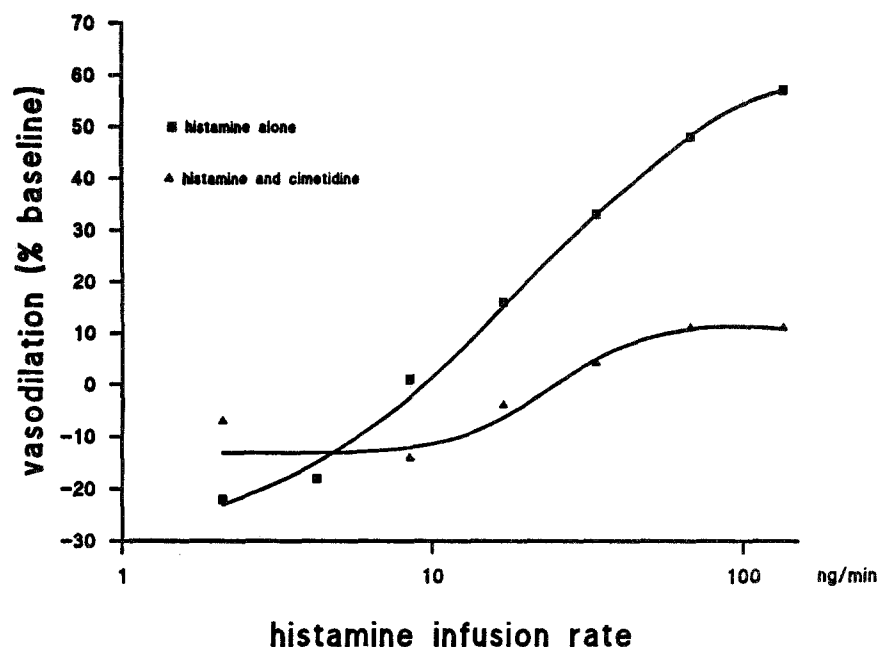


FIG. 3. Effect of cimetidine on histamine-induced venodilation in a dorsal hand vein precontracted with phenylephrine. Dose-response curves for histamine alone and for histamine in the presence of cimetidine at 49 μ g/min, with a 45-minute infusion of cimetidine between curves, were constructed. Dilation of the vein is expressed as a percentage of baseline (before precontraction with phenylephrine) vein diameter. Data are the results in a typical subject. This experiment was repeated six times with similar results.

produced an $E_{\max}$ of 129 ± 29 and an ED_{50} of 0.23 pmol/min.¹⁵ The mean ED_{50} for the 12 histamine dose-response curves performed in the current study was 63 pmol/min, suggesting that histamine is not as potent as other vasodilators previously studied but is highly efficacious, with an $E_{\max}$ greater than 120%.

In a preliminary experiment (data not shown) we demonstrated that brompheniramine was a competitive antagonist of histamine-induced venodilation; when brompheniramine was infused at a constant rate of 6.6 ng/min, the histamine dose-response curve was shifted to the right with no loss in maximal response, which is characteristic of competitive antagonism. In this study we infused either brompheniramine or cimetidine at concentrations greatly in excess of their IC_{50} values to fully block H_1 or H_2 receptors, respectively. Interestingly, in those experiments, histamine's maximal effect was decreased with no loss in sensitivity to histamine. This pattern would be expected for noncompetitive antagonists. However, we think the data are better explained by

pseudoirreversible antagonism, caused by the high concentration of antagonists that we used in these experiments to fully block H_1 and H_2 receptors. In other words, the concentrations of histamine that we were able to infuse into the volunteers were not sufficient to overcome receptor blockade. Both antagonists are very specific: cimetidine's K_d for interaction at the H_1 receptor is 560 times greater than its K_d for interaction at the H_2 receptor.⁶ Similarly, chlorpheniramine, an alkylamine structurally similar to brompheniramine, has a K_d for the H_2 receptor 3000 times greater than that for the H_1 -receptor.⁶

EDRF is an endogenous vasodilator released from the endothelium by certain compounds, such as acetylcholine, bradykinin, adenosine diphosphate, adenosine triphosphate, and substance P.¹⁶ EDRF mediates vasodilation through activation of soluble guanylate cyclase to produce cyclic guanosine monophosphate.¹⁷ We used methylene blue to inhibit soluble guanylate cyclase¹⁸ at a dose rate that was able to inhibit bradykin-stimulated endothelium-dependent dilation with the

dorsal hand-vein technique.¹⁹ We have obtained in our laboratory preliminary data concerning the respective efficacy of methylene blue and N^G-monomethyl-L-arginine (L-NMMA), a nitric oxide synthase inhibitor, as inhibitors of EDRF-mediated dilation. These data confirmed that methylene blue is a specific and efficacious inhibitor of EDRF-mediated dilation. Infusion of methylene blue alone resulted in some antagonism of histamine-induced venodilation. Our results are consistent with the *in vitro* results found in strips of monkey basilar artery.⁷ Histamine infusion resulted in relaxation of these vessel preparations, which was attenuated by cimetidine, chlorpheniramine, and endothelium denudation; but indomethacin had no effect on the response. The relaxation of the vessel after removal of endothelium could be blocked with cimetidine but not with chlorpheniramine, suggesting that the H₁ receptor is endothelium-dependent.

Little is known about the effect of histamine on veins. *In vitro* studies with human internal mammary arteries and saphenous veins obtained during coronary bypass surgery demonstrated that H₁ receptors, in an intact vessel, mediate relaxation; when the endothelium is rubbed off, constriction caused by the H₁ receptors located on the smooth muscle is unmasked.²⁰ Monkey pulmonary veins dilate in response to histamine through both an endothelial H₁ receptor and a smooth muscle H₂ receptor.²¹

Although prostacyclin release has been demonstrated previously from histamine stimulation of human pulmonary arteries,⁸ bovine retinal arteries,²² rat hindlimb vessels,²³ and through stimulation of H₁ receptor in rabbit ear arteries,²⁴ the cyclooxygenase inhibitor indomethacin had no effect on histamine-induced venodilation in our study; the same dose rate was able to block prostaglandin-dependent responses induced by bradykinin when the dorsal hand-vein technique was used.¹⁹ Therefore histamine-mediated vasodilation does not appear to have a significant component mediated through prostacyclin in the human hand vein.

The pharmacologic effects of a systemic infusion of histamine on the peripheral vessels are very important because of the role played by histamine, which, when suddenly released from mast cells, acts as a pathophysiologic mediator of systemic anaphylaxis.³ In studies involving dogs and cats, the intravenous infusion of histamine produces a va-

sodepressor effect, tachycardia and a decrease in total peripheral resistance, which could only be abolished by combination therapy with an H₁ antagonist (mepyramine) and an H₂ antagonist (cimetidine).²⁵ In human beings plasma concentrations of systemically infused histamine of 2.5 ng/ml are necessary to widen the pulse pressure by 30%.²⁶ In other studies investigating the effect of systemic infusions of histamine in human beings, neither H₁ nor H₂ antagonists alone significantly prevented the increase in pulse pressure, which was chiefly manifested as a fall in diastolic blood pressure, but combined pretreatment with both H₁ and H₂ antagonists prevented an increase in pulse pressure.²⁷ Also, elevated concentrations of plasma histamine (20 to 140 ng/ml) have been detected in patients with severe hypotension as a result of systemic anaphylaxis.⁹

The estimated local concentrations of histamine in our studies, given an estimated venous flow rate of 2.5 ml/min in the dorsal hand vein (unpublished observations), ranged from 0.8 ng/ml to 54 ng/ml. Therefore we achieved local concentrations of histamine in the vein comparable to those shown to produce systemic effects²⁶ and anaphylaxis.⁹ Our findings in human hand veins do not exclude the possibility that histamine has a greater or predominant role in other vascular beds such as the upper body or the splanchnic vasculature. Also, because the purpose of our study was to determine accurate pharmacologic responses of histamine receptor subtypes, we did not investigate the effect of histamine on baseline nonprecontracted vessels. However, even if the effects on baseline vessels were less marked, in anaphylaxis, dilation of the whole bed of capacitance vessels may play as large a part in producing hypotension, by causing impaired venous return of blood to the heart and a decrease in preload in addition to dilation of arterial resistance vessels.

A limited number of randomized clinical trials have been conducted to validate the greater efficacy of a combination of H₁ and H₂ antagonists versus H₁ antagonists alone in treating anaphylaxis. Unfortunately, the data are inconclusive, with one study confirming the hypothesis and two failing to find a difference in outcome.¹ Uncontrolled studies have shown that the combination of antagonists was more efficacious than H₁ alone in preventing anaphylactoid reactions to chymopain²⁸ or radiographic contrast media.² In an-

other study, a randomized trial in dogs, experimental, life-threatening anaphylactic reactions that resulted from infusion of the polypeptide polygeline were prevented or greatly diminished by combination therapy with cimetidine and the H_1 antagonist dimethpyrindene.²⁹ Our results provide the experimental evidence for both H_1 - and H_2 -mediated venodilation in human beings and a rationale for the clinical use of a combination of H_1 and H_2 antagonists to treat or prevent anaphylaxis or anaphylactoid reactions.

The standard recommended treatment of systemic anaphylaxis is epinephrine because of its ability to counteract the mediators' effects on target tissues, such as bronchospasm and hypotension, and to inhibit further degranulation of mast cells.³⁰ Recently, some authors have also recommended antihistamine therapy in the form of diphenhydramine 25 to 50 mg given intramuscularly or orally every 6 hours, and cimetidine 300 mg given intravenously or orally every 6 hours as a second-line therapy to antagonize histamine's protracted effects on the myocardium and the vasculature.^{3, 31} This study provides important evidence that combined therapy with H_1 and H_2 antagonists, rather than therapy with an H_1 antagonist alone, may be useful in reversing potentially life-threatening hypotension.

We thank the nurses and staff members of the Palo Alto Veterans Affairs Medical Center Aging Unit for their support.

REFERENCES

- Lieberman P. The use of antihistamines in the prevention and treatment of anaphylaxis and anaphylactoid reactions. *J ALLERGY CLIN IMMUNOL* 1990;86:684-6.
- Ring J, Rothenberg KH, Clauss W. Prevention of anaphylactoid reactions after radiographic contrast media infusion by combined histamine H_1 - and H_2 -receptor antagonists: results of a prospective controlled trial. *Int Arch Allergy Appl Immunol* 1985;78:9-14.
- Bochner BS, Lichtenstein LM. Current concepts: anaphylaxis. *N Engl J Med* 1991;324:1785-9.
- Ash ASF, Schild HO. Receptors mediating some actions of histamine. *Br J Pharmacol Chemother* 1966;27:427-39.
- Haaksma EEJ, Leurs R, Timmerman H. Histamine receptors: subclasses and specific ligands. *Pharmacol Ther* 1990;47:73-104.
- Hill SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol Rev* 1990;42:45-83.
- Toda N. Mechanism underlying responses to histamine of isolated monkey and human cerebral arteries. *Am J Physiol* 1990;258:H311-H317.
- Schellenberg RR, Duff MJ, Foster A, Paddon HB. Histamine releases PGI_2 from human pulmonary artery. *Prostaglandins* 1986;32:201-9.
- Smith PL, Kagey-Sobotka A, Bleecker ER, et al. Physiologic manifestations of human anaphylaxis. *J Clin Invest* 1980;66:1072-80.
- Aellig WH. A new technique for recording compliance of human hand veins. *Br J Clin Pharmacol* 1981;11:237-43.
- Pan HYM, Hoffman BB, Pershe RA, Blaschke TF. Decline in beta adrenergic receptor-mediated relaxation with aging in man. *J Pharmacol Exp Ther* 1986;239:802-7.
- De Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am J Physiol* 1978;E97-102.
- Fleming WW, Westfall DO, De La Lande IS, Jellet LB. Log-normal distribution of effective doses of norepinephrine and acetylcholine in several tissues. *J Pharmacol Exp Ther* 1972;181:339-45.
- Eichler HG, Hiremath A, Katzir D, Blaschke TF, Hoffman BB. Absence of age-related changes in venous responsiveness to nitroglycerin in vivo in humans. *Clin Pharmacol Exp Ther* 1987;42:521-4.
- Vincent J, Blaschke TF, Hoffman BB. Desensitization of β -adrenoceptor- and prostaglandin E1 receptor-mediated human vascular smooth muscle relaxation. *J Cardiovasc Pharmacol* 1992;19:447-52.
- Furchgott RF, Zawadzki JV. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989;3:2007-18.
- Ignarro LJ, Ross G, Tillisch J. Pharmacology of endothelium-derived nitric oxide and nitrovasodilators. *West J Med* 1991;154:51-62.
- Murad F. Cyclic guanosine monophosphate as a mediator of vasodilation. *J Clin Invest* 1986;78:1-5.
- Dachman WD, Ford GA, Blaschke TF, Hoffman BB. Mechanism of bradykinin-induced venodilation in humans. *J Cardiovasc Pharmacol* 1993;21:241-8.
- Schoeffter P, Godfraind T. Histamine receptors in the smooth muscle of human internal mammary artery and saphenous vein. *Pharmacol Toxicol* 1989;64:64-71.
- Matsuki T, Ohhashi T. Endothelium and mechanical responses of isolated monkey pulmonary veins to histamine. *Am J Physiol* 1990;259:H1032-7.
- Benedito S, Prieto D, Nielson PJ, Nyborg MCB. Histamine induces endothelium-dependent relaxation of bovine retinal arteries. *Invest Ophthalmol Vis Sci* 1991;32:32-38.
- Van De Voorde J, Leusen I. Influence of prostaglandin-synthesis inhibitors on carbachol- and histamine-induced vasodilation in perfused rat hindquarters. *Pflügers Arch* 1983;397:290-4.
- Juan H, Sametz W. Histamine-induced release of arachidonic acid and of prostaglandins in the peripheral vascular bed: mode of action. *Naunyn Schmiedeberg's Arch Pharmacol* 1980;314:183-90.
- Owen DAA, Harvey CA, Boyce MC. Effects of histamine on the circulatory system. *Klin Wochenschr* 1982;60:972-7.
- Kaliner M, Shelhamer JH, Ottesen EA. Effects of infused histamine: correlation of plasma histamine levels and symptoms. *J ALLERGY CLIN IMMUNOL* 1982;69:283-9.
- Kaliner MH, 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 pul-

- monary responses. *J ALLERGY CLIN IMMUNOL* 1981;68:365-71.
28. Moss J, Roizen M, Noroby E. Decreased incidence and mortality of anaphylaxis to chymopapain. *Anesth Analg* 1985;64:1197-201.
 29. Schöning B, Lorenz W, Doenicke A. Prophylaxis of anaphylactoid reactions to a polypeptid plasma substitute by H₁-plus H₂-receptor antagonists: Synopsis of three randomized controlled trials. *Klin Wochenschr* 1982;60:1048-55.
 30. Barach EM, Nowack RM, Lee TG, Tennyson G, Tomlanovich MC. Epinephrine for treatment of anaphylactic shock. *JAMA* 1984;251:2118-22.
 31. Stark BJ, Sullivan TJ. Biphasic and protracted anaphylaxis. *J ALLERGY CLIN IMMUNOL* 1986;78:76-83.

Molecular characterization of dog albumin as a cross-reactive allergen

Susanne Spitzauer, MD,^a Christian Schweiger, MD,^a Wolfgang R. Sperr, MD,^b Budhi Pandjaitan,^a Peter Valent, MD,^b Sonja Mühl,^a Christof Ebner, MD,^c Otto Scheiner, PhD,^c Dietrich Kraft, MD,^c Helmut Rumpold, MD,^a and Rudolf Valenta, MD^c Vienna, Austria

*Indoor allergens comprise a group of allergenic proteins that are commonly derived from house dust mite and cat and dog dander. In addition to the two major dog allergens (molecular weights: 19 and 23 kd), dog albumin represents an important allergen for up to 35% of patients who are allergic to dogs. In IgE immunoblot inhibition studies and histamine release tests it has been demonstrated that patients who react to dog albumin exhibit IgE reactivity with purified albumins from cat, mouse, chicken, and rat. The proportion of dog-specific IgE directed against dog albumin was determined for patients allergic to dog albumin, and it ranges from 70% to 90%. By IgE immunoscreening of a lambda gt11 expression library from a dog salivary gland, we identified a number of reactive complementary DNA clones. All patients with IgE reactivity against natural dog albumin displayed IgE reactivity to the β -galactosidase fusion protein encoded by clone 54c, which was therefore assumed to contain major IgE epitopes of dog albumin. The deduced amino acid sequence of clone 54c was compared with the Swiss-Prot library, and significant sequence homologies were found with albumins from different species (human: 82.6%, pig: 81.8%, cattle: 77.3%, sheep: 78.8%, mouse: 75.8%, and rat: 76.2%). Several other IgE-positive clones hybridized with oligonucleotides that were prepared according to this sequence. Partial complementary DNA coding for dog albumin fragments may be considered a useful tool for further characterization of major IgE epitopes of dog albumin. (*J ALLERGY CLIN IMMUNOL* 1994;93:614-27.)*

Key words: Dog allergens, albumin, cDNA cloning, IgE

From ^aInstitute of Medical and Chemical Laboratory Diagnostics; ^bDepartment of Hematology and Hemostaseology; ^cInstitute of General and Experimental Pathology, AKH, University of Vienna, Austria.

Supported by grants S06703 and S06712-Med of the "Fonds zur Förderung der wissenschaftlichen Forschung" and grant 3/8603 of the "Fonds zur Förderung der gewerblichen Wirtschaft," Vienna, Austria.

Received for publication Feb. 19, 1993; revised July 9, 1993; accepted for publication July 20, 1993.

Reprint requests: Rudolf Valenta, MD, Institute of General and Experimental Pathology, AKH, University of Vienna, Währingergürtel 18-20, A-1090 Vienna, Austria.

Copyright © 1994 by Mosby-Year Book, Inc.

0091-6749/94 \$3.00 + 0 1/1/50233

Abbreviations used

cDNA: Complementary DNA
SDS-PAGE: Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Type I allergy to indoor allergens¹ is caused by proteins derived from mites, molds, and animals² such as cats and dogs. Dog allergens have been detected in protein extracts prepared from dander, hair, and saliva by immunochemical techniques,³⁻⁷ and a panel of relevant allergens has