

The effects of gender on allergen-induced histamine release in ongoing allergic cutaneous reactions

Paul C. Atkins, MD, Carolyn von Allmen, BA, Mary Valenzano, and
Burton Zweiman, MD Philadelphia, Pa.

Background: Marked variability in the amount of histamine released in the first hour of ongoing cutaneous reactions has been noted. This variability occurs even among subjects with similar degrees of skin test reactivity to antigen.

Methods: To determine gender effects on mediator release, we retrospectively compared: (1) skin chamber histamine release after a 0 to 1-hour and 1 to 5-hour exposure to antigen; (2) neutrophil accumulation after 5 hours of antigen exposure and skin test reactivity to antigen, histamine, and codeine in 91 male and 60 female subjects.

Results: There was no difference in skin test reactivity to antigen, histamine, or codeine between male and female subjects. However, in the group as a whole, male subjects released higher amounts of histamine in the first hour (74 ± 4 ng/ml) than female subjects (55 ± 4 ng/ml), ($p < 0.01$). When subjects were matched for equivalent skin reactivity to antigen, male subjects who were sensitive to 10 PNU/ml and 1 PNU/ml released more histamine (67 ± 9 ng/ml and 82 ± 9 ng/ml) than female subjects (51 ± 7 ng/ml and 55 ± 7 ng/ml) ($p < 0.05$ and < 0.01). In the most sensitive subjects, those with skin sensitivity to 0.01 PNU/ml of antigen, there was not a significant difference between the histamine release in the first hour in male (79 ± 12 ng/ml) or female (69 ± 9 ng/ml) subjects. No difference was observed between male and female subjects in either neutrophil or histamine accumulation in the 1- to 5-hour period.

Conclusions: Since the first hour release of histamine is secondary to mast cell activation and the 1- to 5-hour histamine release is secondary to basophil activation, we conclude that the gender of the subject influences the degree of in vivo antigen-induced histamine release from mast cells. (*J ALLERGY CLIN IMMUNOL* 1993;91:1031-4.)

Key words: Gender, histamine, antigen, neutrophils, skin chamber

We have previously noted the marked variability in the amount of histamine released in the first hour during ongoing cutaneous allergic reactions.¹ This variability occurred even among subjects with similar degrees of skin test reactivity to antigen, as defined by extinction dilution skin testing (the lowest dose of antigen that results in a 10 mm whealing response after intradermal injection).¹

In this study we wanted to determine whether the gender of the subject had an effect on the amount of mediator release after antigen exposure. We therefore retrospectively analyzed all of the data accumulated in our laboratory since 1984 and compared: (1) gender; (2) age; (3) skin test responses to histamine, codeine, and antigen; and (4) skin chamber release of histamine and accumulation of neutrophils at sites of antigen and buffer administration.

METHODS

Subjects

Ninety-one male and 60 female subjects who had undergone skin chamber challenges with antigen (100 PNU/ml of grass or ragweed pollen) and buffer (phosphate-buffered saline) form the basis of this study. All subjects were sensitive to intradermal injection of < 10 PNU/ml of ragweed or grass pollen antigen. All subjects manifested clinical symptoms in the relevant pollen season. The mean age of male subjects

From the Division of Allergy and Immunology, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia.

Supported by a grant from the National Institutes of Health AI 14332.

Accepted for publication Nov. 20, 1992.

Reprint requests: Paul C. Atkins, MD, Department of Medicine, Division of Allergy & Immunology, Hospital of the University of Pennsylvania, Silverstein Pavilion, Suite B, 3400 Spruce St., Philadelphia, PA 19104-4283.

Copyright © 1993 by Mosby-Year Book, Inc.

0091-6749/93 \$1.00 ± 0.10 1/1/44594

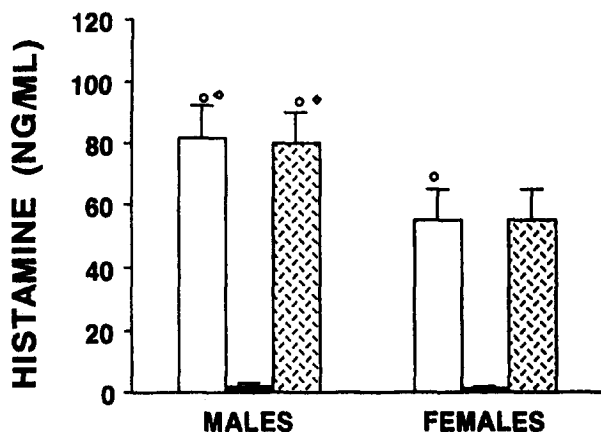


FIG. 1. The skin chamber fluid histamine levels at sites exposed for 1 hour to antigen (*open bar*) or buffer (*solid bar*) are shown on the ordinate in the 91 male and 64 female subjects. The solid bars indicate the net histamine in the chamber fluids (antigen-buffer site). The open circle denotes values significantly ($p < 0.001$) different from those at buffer sites. The diamond denotes values in male subjects that are significantly different ($p < 0.01$) from those in female subjects.

(28.6 ± 0.4 years; range 19 to 32 years) was not significantly different from that of the female subjects (27 ± 1 year; range, 18 to 46 years).

Skin test reactivity

The wheal and flare skin test reactivity was measured 20 minutes after intradermal injection of 0.02 ml of (1) buffer (phosphate-buffered saline with human serum albumin, which is used as the diluent for the antigen, histamine, and codeine skin test solutions); (2) histamine (0.02 mg/ml); (3) codeine (1 mg/ml); and (4) antigen (serial 10-fold dilution of 10 PNU/ml to 1×10^{-6} PNU/ml of ragweed or grass pollen antigen). The extinction dilution was defined as the lowest concentration of antigen that results in a wheal >10 mm.

Skin chamber challenges

Skin chamber challenges were performed as previously described.^{1,2} In brief, the epidermis was denuded from skin blisters that were formed by gentle heat and suction, removed, and discarded. After a 5-minute exposure to buffer, the fluid was removed and discarded. The dermis was exposed to antigen (100 PNU/ml of ragweed or grass pollen extract) diluted in phosphate-buffered saline with 10 units of heparin (buffer) or buffer alone for 1 hour. The fluid was removed, frozen at -70°C to be analyzed for histamine, and replaced with antigen or buffer solutions for 1 to 5 hours. At the end of the fifth hour, the fluid was removed, and an aliquot was analyzed for his-

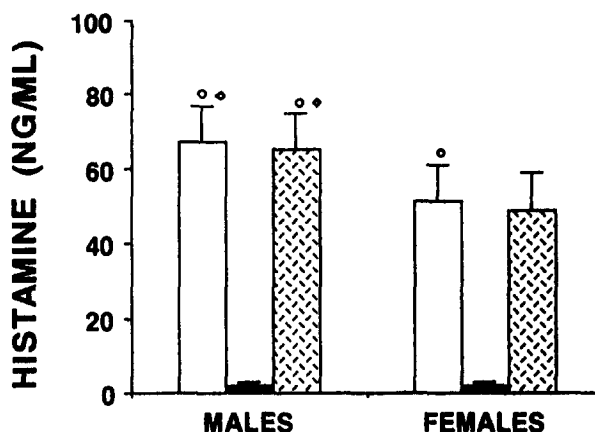


FIG. 2. The skin chamber fluid histamine levels (mean $\pm$ SEM) in 28 male and 25 female subjects with extinction dilution on skin test reactivity. Values of 1 PNU/ml are shown on the ordinate. Sites exposed for 1 hour to antigen are represented by open bars, buffer sites by solid bars, and net histamine levels (antigen-buffer sites) by hatched bars. Open circles denote values significantly ($p < 0.001$) different from those at buffer sites, and the diamond denotes values in male subjects that are significantly different from those in female subjects.

tamine, the remainder was used to measure cellular content. Cells were counted with an automated cell counter, and smears were stained with Diff Quick (W. H. Thomas, Philadelphia, Pa.) and analyzed for differential cell counts. More than 95% of the cells were neutrophils, as previously reported. Therefore cell counts are reported as neutrophil counts and calculated by multiplying the recovered number of cells by the differential percentage of neutrophils per milliliter of chamber fluids.^{3,4}

Histamine

Histamine was measured by radioenzymatic assay as previously reported.⁵

Analysis of data

The data were normally distributed as determined by the Kolmogorov-Smirnov test. All of the data were analyzed initially with two-way analysis of variance. The statistically significant groups were distinguished by Student's t test.

RESULTS

Skin test responses

There was no significant difference between male and female subjects in the extinction dilution skin test reactivity to antigen, expressed as the log of the lowest concentration (in protein nitrogen units per milliliter) that results in a 10 mm whealing reaction

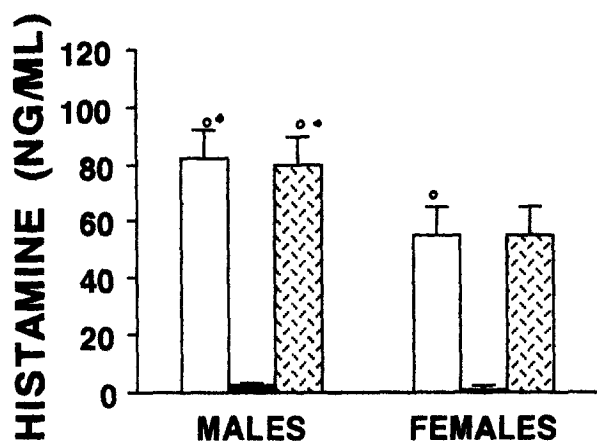


FIG. 3. The skin chamber fluid histamine levels (mean $\pm$ SEM) in 31 men and 18 women with extinction dilution skin test reactivity = 0.1 PNU/ml are shown on the ordinate. Sites exposed for 1 hour to antigen are represented by open bars, buffer by solid bars, and net histamine levels (antigen-buffer) by hatched bars. Open circles denote values significantly ($p < 0.001$) different from those at buffer sites; the diamond denotes values in male subjects that are significantly ($p < 0.01$) different from those in female subjects.

(men = 0.19 ± 0.1 ; women = 0.26 ± 0.1). The wheal and flare responses in mean diameter (in millimeters) to histamine (men = 11.43 ± 0.22 wheal, 32.54 ± 1.11 flare; women = 11.36 ± 0.24 wheal, 32.61 ± 1 flare) and to codeine (men = 13.56 ± 0.42 wheal; 36.71 ± 1 flare; women = 14.04 ± 0.4 wheal, 36.6 ± 1.1 flare) were virtually identical.

Skin chamber histamine release in the first hour

The skin chamber fluid histamine (in nanograms per millimeter) release in response to the first hour of antigen exposure was higher in the male (74 ± 4) than in the female (55 ± 4) subjects ($p < 0.01$), as shown in Fig. 1.

Since this greater histamine release in men could have been due to the presence of more sensitive subjects in the male population, we matched our male and female subjects for antigen skin test reactivity and analyzed the antigen-induced histamine release. We had 28 male and 25 female subjects who exhibited skin sensitivity to antigen levels as low as 1 PNU/ml (Fig. 2). In this group male subjects exhibited significantly greater histamine release in the first hour (67 ± 9 ng/ml) than female subjects (51 ± 7 ng/ml) ($p < 0.05$). An additional 31 men and 18 women exhibited skin sensitivity to antigen as low as 0.1 PNU/ml (Fig. 3). In this group also male subjects

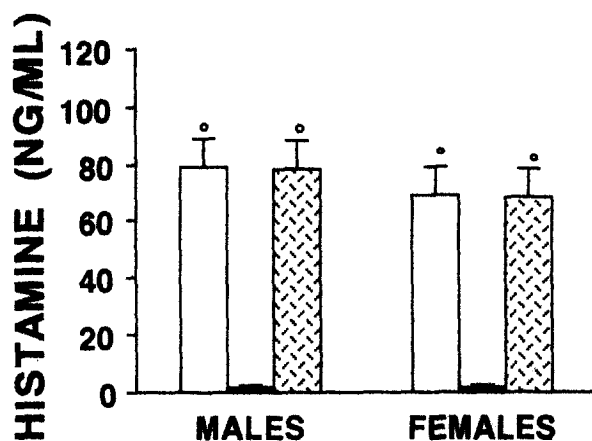


FIG. 4. The skin chamber fluid histamine levels (mean $\pm$ SEM) in 17 men and 13 women with extinction dilution skin test reactivity = 0.01 PNU/ml are shown on the ordinate. Sites exposed for 1 hour to antigen are represented by open bars, buffer by solid bars, and net release (antigen-buffer) by hatched bars. Open circles denote values significantly ($p < 0.001$) different from those at buffer sites.

released significantly higher quantities of histamine in the first hour (82 ± 6 ng/ml) than female subjects (55 ± 7 ng/ml) ($p < 0.01$). In a third group of subjects (17 men and 13 women) with skin sensitive to as little as 0.01 PNU/ml of antigen, the difference in the first-hour histamine concentrations between the men (79 ± 12 ng/ml) and women (69 ± 9 ng/ml) was not statistically significant (Fig. 4).

Histamine and neutrophil concentrations during the second to fifth hours of antigen exposure

The cumulative concentrations of histamine released during the second to fifth hours was not significantly different in the male (44 ± 3 ng/ml) and female (39 ± 3 ng/ml) subjects. Nor was there any difference in the concentrations of neutrophils (10^6) per milliliter recovered at the end of the fifth hour of antigen exposure in male (1.6 ± 0.1) or female (1.8 ± 0.3) subjects. Similarly, buffer site histamine levels throughout the study period were virtually identical in the male and female subjects.

DISCUSSION

In this study we have demonstrated for the first time that men release higher amounts of histamine than women during the first hour of antigen exposure even in subjects matched for equivalent skin test reactivity to antigen. It is not surprising that in subjects who were exquisitely sensitive to antigen (those sensitive to 0.01 PNU/ml) there was no significant difference

between male and female subjects in histamine release in response to skin chamber challenge with 100 PNU/ml of antigen. The reasons for this could be: (1) the group was too small to detect a difference (2) skin chamber antigen concentration (100 PNU, 5 logs higher than the concentration that induces skin test reactivity) may have been great enough to mask any significant differences.

Since there was no difference in skin test reactivity to histamine or codeine, it is unlikely that the difference between men and women reflects differences in distribution of mast cells or end organ responsiveness. A question can be raised as to why men did not exhibit greater skin test reactivity to antigen than women, if indeed they had greater amounts of mediator release. This question cannot be answered directly by this study. It may be that the differences in histamine release noted may not be discernible with the doses of antigen used in intradermal skin testing. Another possibility is that the diffusion or recovery of histamine is different in men than in women. Although this cannot be approached directly in this study, the volumes of fluids recovered did not vary between male and female subjects. Furthermore, the response to intradermal injection of histamine and codeine in men and women implies no discernible difference in diffusion of these substances after intradermal injection. We think it is more likely that the differences detected in histamine release reflect true differences in mast cell reactivity. This impression is reinforced by the fact that there was no difference between men and women in the amount of local histamine release or accumulation of cells during the continued exposure of antigen after the first hour. We have previously demonstrated that the persistent histamine release after continuous antigen skin chamber challenge is from basophils, since it does not occur during codeine administration, is not associated with tryptase release, and is reduced by oral corticosteroid administration.⁶

In reviewing the literature we could not find studies that directly compare in vivo allergen-induced histamine release in male and female subjects. Several studies have compared the incidence of allergen skin test reactivity in men and women. In 365 French subjects there was no difference in the incidence of skin test reactivity between male and female subjects.⁷ In a survey of 659 patients in Peru, the authors concluded that there was no difference between men and women in the incidence of skin test reactivity to allergen or in the size of the histamine-induced wheal and flare response.⁸ In one study of endomyocardial biopsies (100 normal hearts surveyed at autopsy) a greater number of mast cells per high-power field was seen in women than in men,⁹ whereas no difference between mast cell numbers in men or women was documented in skin biopsy specimens of sun-exposed and

nonsun-exposed skin in 55 subjects analyzed at autopsy.¹⁰ In one study from Spain, in 226 individuals who were sensitive to dust mite, there was no influence of gender on the degree of in vitro dust-mite-induced basophil histamine release.¹¹

Therefore the findings of our study that there was no influence of gender on the degree of skin whealing to histamine or to the amount of histamine released during in vivo continuous antigen challenge after the first hour (which we have shown is secondary to basophil activation) are supported by other studies in the literature. Our new finding, that in vivo antigen-induced mast cell histamine release during the first hour of challenge is lower in women than in men warrants further prospective studies to determine whether it is associated with different degrees of clinical reactivity in male and female subjects.

REFERENCES

1. Atkins PC, von Allmen C, Valenzano M, Olson R, Shalit M, Zweiman B. Determinants of in vivo histamine release in cutaneous allergic reactions in humans. *J ALLERGY CLIN IMMUNOL* 1990;86:371-9.
2. Talbot SF, Atkins PC, Valenzano M, Zweiman B. Correlations of in vivo mediator release with late cutaneous allergic responses in humans. I. Kinetics of histamine release. *J ALLERGY CLIN IMMUNOL* 1984;74:819-26.
3. Fleekop PD, Atkins PC, von Allmen C, Valenzano M, Shalit M, Zweiman B. Cellular inflammatory responses in human allergic skin reactions. *J ALLERGY CLIN IMMUNOL* 1987;80:140-6.
4. Shalit M, Valone FH, Atkins PC, Goetzl EJ, Ratnoff WD, Zweiman B. Late appearance phospholipid platelet activating factor and leukotriene B1 in human skin after repeated antigen challenge. *J ALLERGY CLIN IMMUNOL* 1989;83:691-6.
5. Atkins PC, Valenzano M, Zweiman B. Plasma concentrations of histamine by radioenzymatic assay: effects of histaminase incubations. *J ALLERGY CLIN IMMUNOL* 1982;69:39-45.
6. Atkins PC, Schwartz LB, Adkinson NF, von Allmen C, Valenzano M, Zweiman B. In vivo antigen-induced cutaneous mediator release: simultaneous comparisons of histamine, tryptase, and prostaglandin D2 release and the effect of oral corticosteroid administration. *J ALLERGY CLIN IMMUNOL* 1990;86:360-70.
7. Skassa-Brocietz W, Manderscheid JC, Michel FB, Bousquet J. Skin test reactivity from infancy to old age. *J ALLERGY CLIN IMMUNOL* 1987;80:711-6.
8. Lopez LR, Noriega Y, Losno R. Immediate skin test reactivity to common aeroallergens in patients with respiratory allergens: a comparative analysis of allergen induced skin reactions and their histamine controls. *J ALLERGY CLIN IMMUNOL* 1988;81:1143-8.
9. Turlington BS, Edwards WD. Quantitation of mast cells in 100 normal and 92 diseased human hearts. Complications for interpreting endomyocardial biopsies. *Am J Cardiovasc Pathol* 1988;2:151-7.
10. Rosen LB, Frank B. Mast cells in sun-exposed and non sun-exposed skin. An autopsy study. *Am J Dermatopathol* 1987;9:208-11.
11. Gambo PM, Castello JG, Wong E, de la Cuesta G, Oehling A. Variation in histamine release in an atopic population. II. Clinical situation according to age and duration of immunotherapy. *Allergol Immunopathol (Madr)* 1989;77:279-83.