

Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: Characterization of factors influencing histamine releasability

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Background: The purposes of the study were to characterize allergen-induced histamine release in intact human skin in vivo by using a novel microdialysis technique and to study covariates influencing histamine releasability.

Methods: Hollow microdialysis fibers were inserted into the upper dermis in 15 timothy-sensitive subjects. Up to 12 fibers were inserted in each subject. Each fiber was perfused with Krebs-Ringer's solution at a rate of 3.0 μ l/min. Three to four serial dilutions of allergen were applied to the skin by intracutaneous injections or skin prick test above individual fibers. Samples were collected in two 2-minute fractions before skin challenge and in 10 consecutive samples for 20 minutes after skin challenge. Histamine was assayed spectrofluorometrically.

Results: A significant dose-response relationship for histamine release was demonstrated with intracutaneous tests and skin prick tests. The time to reach peak histamine release after an intracutaneous test was 4 to 8 minutes, compared with 12 to 14 minutes for a skin prick test. Histamine release correlated significantly with wheal size. Intrasubject coefficient of variation on histamine release was about 20%. A substantial intersubject variation in histamine releasability was observed. Seventy to seventy-five percent of the variation could be accounted for by a combination of gender, total and allergen-specific IgE, and an in vitro basophil histamine release test.

Conclusion: Using a skin microdialysis technique, we have described in detail histamine release in intact human skin by allergen. The microdialysis method proved to be a reproducible technique for monitoring histamine release in allergic skin reactions and for studying histamine releasability of skin mast cells in vivo. (*J ALLERGY CLIN IMMUNOL* 1996;97:672-9.)

Key words: Hypersensitivity, histamine liberation, microdialysis, mast cells, skin test methods

Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine remains the best

Abbreviations used

ICT: Intracutaneous test
SPT: Skin prick test

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characterized and most potent vasoactive mediator implicated in the acute phase of type I allergic reactions. The importance of histamine in the skin is supported by findings showing that H₁-receptor antagonists (antihistamines) most effectively block the signs and symptoms of type I allergic skin reactions: the wheal, the flare, and the itch.^{1,2}

To better understand the pathophysiology of allergic skin reactions in vivo, methods have been introduced to characterize the events of mediator release. Previous studies have shown release of

histamine and other mediators in suction blister fluid, skin chambers, subcutaneous adipose tissue, and the systemic circulation draining the challenged skin site.³⁻¹⁰ No methods have allowed selective and continuous measurement of mediator release into the extracellular water space in intact human skin.

Microdialysis is a technique originally developed for the monitoring of neurotransmitters and drugs in the extracellular compartment of animal brains.^{11, 12} During the last 10 years, the technique has been extensively used in peripheral tissues, including subcutaneous adipose, liver, eye, blood, and muscle tissue.^{13, 14} A skin microdialysis technique, which allows analysis of low molecular weight substances in the extracellular water space in intact human skin, has recently been developed.^{15, 16} The technique provides a detailed kinetic measurement of mediator release directly into the extracellular water compartment of intact human skin and enables a correlation to visible skin reactions. With this technique, histamine release by substance P¹⁷ and codeine¹⁸ has been demonstrated. The purpose of this study was to characterize allergen-induced histamine release by means of intradermal injection and skin prick tests (SPTs).

METHODS

Subjects

The study included 15 subjects (3 women and 12 men) allergic to timothy grass (*Phleum pratense*). The diagnosis of allergy was based on symptoms of allergic rhinoconjunctivitis during the grass pollen season, a positive skin test response to timothy grass, and IgE antibodies against timothy grass (RAST class >2; CAP system; Pharmacia, Uppsala, Sweden). A positive (class 3 on a scale of 0 to 3) diagnostic basophil histamine release test response to timothy grass was found in 12 of 15 subjects; three were nonresponders to anti-IgE. Mean age was 23.6 years (standard deviation, 5.8 years; range, 19 to 44 years). No subject had dermatographism or took any medication known to interfere with mast cell degranulation and/or interpretation of skin test responses. The investigation was performed 3 months after the end of the grass pollen season. Informed consent was obtained, and the study was approved by the local ethics committee.

Microdialysis instrumentation

Dialysis probes were made from single dialysis fibers from a hemodialysator (model GFE 18; Gambro Dialysaten AG, Hechingen, Germany). The fibers were glued to gas-tight nylon tubing and sterilized. The final probes had an outer diameter of 216 μ m, a molecular weight cutoff of 2 kd, a length of 20 mm available for diffusion, and an outlet of 30 mm.^{15, 17} As many as 12 23-gauge

cannulas (Terumo, Europe N.V., Leuven, Belgium) were introduced into the upper dermis in the radial/ventral surface of the forearms of each subject. Each dialysis fiber was introduced into the skin through a guide cannula, which was then removed. The distance between each cannula was at least 3 cm. Local anesthesia (EMLA cream; Astra AB, Södertälje, Sweden, under occlusion with Tegaderm; 3M Healthcare Ltd., Loughborough, Leicestershire, U.K.) was applied for 45 minutes and removed before insertion of fibers. After insertion, a recovery phase of at least 2 hours was allowed before skin challenge. This interval has been shown to be sufficient for local anaesthesia to wear off (so that axon reflex vasodilation returns to normal) and for the implantation-induced hyperemia to resolve.¹⁷ The inlet of each dialysis probe was connected to individual syringes mounted in micro-injection pumps (CMA/100; CMA/Microdialysis, Stockholm, Sweden). The fiber was perfused in parallel at a rate of 3.0 μ l/min with an isotonic, Krebs-Ringer's bicarbonate solution. Dialysates were collected directly from the efferent tube into microtiter wells coated with a glass fiber matrix used for analysis of histamine. Dialysate was collected at 2-minute intervals (6 μ l fractions). Before skin challenge, two baseline values of 6 μ l were collected over 4 minutes. After skin challenge, dialysate was collected at 2-minute intervals (6 μ l) for 20 minutes.

Skin challenge

A stock solution of timothy grass pollen allergen (Aqua-gen; ALK, Copenhagen, Denmark), 1,000,000 SQ-U/ml, was made in ALK diluent (0.3 mg human serum albumin and 5 mg phenol per 1 ml) and used for intracutaneous tests (ICTs) and SPTs after dilution. For ICTs, 25 μ l replicates of three to four 10-fold serial dilutions of allergen were injected intradermally. A 1 mm tip lancet was used (ALK) for SPTs. We attempted to obtain a wheal of 15 to 20 mm in diameter for the highest antigen concentration used for each subject. To avoid puncture of the fiber, the skin challenge was placed 1 mm from the fiber at the midpoint of the 20 mm dialysis fiber. The challenge sequence was randomized. Wheal sizes were recorded 20 minutes after skin challenge. The contours of the wheals were outlined by a fine pen and transferred with adhesive tape to the record form; the area was calculated with the cutting-weighing method. The presence and size of flare reactions were not recorded.

Histamine analysis

A spectrofluorometric assay of histamine in microtiter wells coated with a glass fiber matrix was performed.^{19, 20} The specificity of this assay for measuring histamine in skin dialysate has been confirmed.¹⁷ The limit of detection of this assay is approximately 5 ng/ml.

Statistics

Descriptive statistics included calculation of mean $\pm$ SEM, if not otherwise stated. Coefficient of variation was

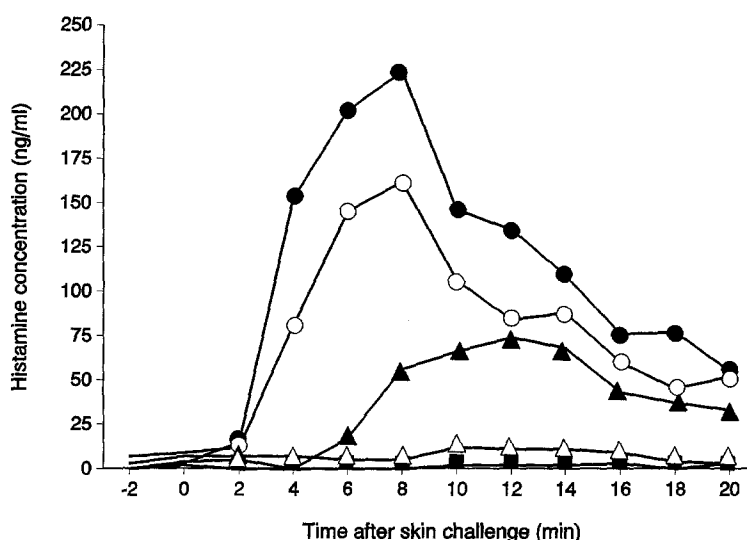


FIG. 1. An example of histamine release after intradermal injection of serial dilutions of allergen in a single subject. Two 6 μ l baseline samples (labeled -2 and 0 min) were collected over a 4-minute period before skin challenge. Thereafter, samples were collected from each fiber every 2 minutes, and the histamine concentrations were assayed spectrofluorometrically. Filled circles, 10 SQ-U/ml; open circles, 1 SQ-U/ml; filled triangles, 0.1 SQ-U/ml; open triangles, 0.01 SQ-U/ml; and filled squares, vehicle.

calculated as: $(SD \div \text{mean}) \times 100$. Analytical statistical methods included an exponential dose-response model, which allows estimation of dose-response relationships, as well as calculation of individual sensitivities of response variables in relation to allergen load. The analysis has been described elsewhere.^{21,22} This analysis was preferred to conventional analysis of variance because subjects were challenged with different numbers and concentrations of allergen. Furthermore, the analysis permits calculation of relative sensitivities. To estimate significant covariates for individual variations in sensitivities, the calculated sensitivities and covariates were entered into multiple regression analyses with backward stepwise elimination of least significant covariates. Probability (p) values of less than 0.05 were considered statistically significant.

RESULTS

Fig. 1 shows histamine release after intradermal injection of allergen in a single subject. For each experiment, peak histamine concentration and cumulative histamine release over the 20-minute sampling period were calculated and corrected for baseline release and vehicle responses. The peak and cumulative histamine release variables represent two measures from the same experiments. Because these two measures correlated significantly for both routes of administration of allergen ($p < 0.001$), we have chosen to use peak histamine values in the statistical calculations. The equations for the correlation between peak and cumulative

histamine release were $y = 5.21x - 13.41$ ($r = 0.999$), and $y = 5.94x - 8.81$ ($r = 0.977$) for ICT and SPT, respectively (Tables I and II).

Dose-response relationship

A statistically significant dose-response relationship for increasing histamine release with increasing concentrations of injected allergen was seen for ICT ($F(1,27) = 454.0$, $p < 0.001$). With SPT, only data from seven subjects were available for analysis, but a similar dose-response relationship was observed ($F(1,12) = 304.1$, $p < 0.001$).

Kinetics of histamine release

The time to reach peak histamine release in consecutive samples after skin challenge is shown in Fig. 2. To ensure a valid representation of histamine kinetics, only experiments showing a net histamine release of 10 ng/ml or more were included in the analysis. Fifty of 72 ICT experiments and 14 of 36 SPT experiments fulfilled this criterion. After intradermal injection of allergen, a rapid increase in histamine level was observed, and most peak values were observed 4 to 8 minutes after the application. The decay of histamine after reaching peak values showed a half-life of approximately 7 minutes. The kinetics of histamine release with SPT were quite different from those of the ICT experiments because most peak heights

TABLE I. Individual histamine release data obtained with intradermal injection of the allergen

Subject No.	Concentration of allergen (SQ-U/ml)				
	0.01	0.1	1	10	100
1	5.0 (34)	24.5 (145)	59.5 (349)	154.5 (830)	ND
2	7.5 (21)	6.0 (36)	35.5 (141)	56.0 (269)	ND
3	1.0 (0)	2.5 (12)	43.0 (108)	66.0 (288)	ND
4	0.0 (0)	5.0 (4)	1.5 (0)	27.5 (116)	ND
5	7.0 (18)	72.0 (379)	154.0 (755)	222.5 (1177)	ND
6	5.0 (35)	20.0 (52)	82.5 (436)	171.0 (704)	ND
7	8.0 (48)	9.0 (43)	67.5 (255)	63.0 (294)	ND
8	ND	7.0 (34)	30.8 (177)	180.5 (956)	260.8 (1454)
9	1 (0)	4 (15)	26 (129)	45 (232)	ND
10	12 (51)	15 (84)	96 (528)	126 (557)	ND
11	ND	0.0 (0)	6.3 (36)	31.3 (175)	77.3 (407)
12	ND	ND	14.5 (94)	46.0 (192)	88.0 (444)
13	ND	0.5 (0)	22.0 (80)	41.0 (174)	ND
14	ND	ND	22.0 (104)	74.0 (302)	96.0 (573)
15	ND	10.5 (38)	39.5 (240)	92.5 (461)	ND

Peak histamine release (ng/ml) and cumulative release (ng/ml over 20 min) in parentheses. All values are corrected for baseline and vehicle responses.

Values from subjects 8 to 11 are mean values of duplicate skin challenges.

ND, Not done.

TABLE II. Individual histamine release data obtained with administration of allergen by SPTs

Subject No.	Concentration of allergen (SQ-U/ml)			
	100	1,000	10,000	100,000
1	4.0 (0)	8.0 (28)	2.0 (6)	36.0 (250)
2	3.0 (8)	1.5 (0)	3.5 (0)	6.5 (37)
3	0.5 (0)	0.0 (0)	3.0 (0)	17.5 (83)
4	0.0 (20)	0.5 (0)	4.5 (0)	3.0 (4)
5	1.5 (11)	11.5 (26)	21.0 (136)	66.5 (352)
6	1.5 (1)	6.0 (28)	13.0 (87)	20.0 (96)
7	0.5 (3)	5.5 (19)	3.0 (0)	17.0 (93)
8	ND	ND	ND	17.0 (73)
9	ND	ND	ND	15.5 (77)
10	ND	ND	ND	36.0 (233)
11	ND	ND	ND	10.3 (43)

Peak histamine release (ng/ml) and cumulative release (ng/ml over 20 min) in parentheses.

Values for subjects 8 to 11 are mean values of duplicate skin challenges.

ND, Not done.

were observed 12 to 14 minutes after skin challenge. The time to reach peak histamine values was not related to the magnitude of histamine release.

To compare the kinetics of histamine release by allergen and nonimmunologic stimulation, codeine (1 mg/ml) was injected intradermally in three subjects (subjects 5, 6, and 7). In all cases peak histamine release was observed in the second sample (2 to 4 minutes after ICT), showing peak release of 382.7 ± 4.9 ng/ml histamine.

Reproducibility

In four subjects (subjects 8, 9, 10, and 11) skin challenges were performed in duplicate for studies of intrasubject reproducibility. ICTs were performed with four concentrations of allergen, whereas SPTs were done with one concentration of allergen only (100,000 SQ-U/ml). The coefficient of variation on peak and cumulative histamine release was 14.5% and 17.9% for ICT ($n = 16$ comparative data sets), and 10.6% and 27.0% for SPT ($n = 4$), respectively.

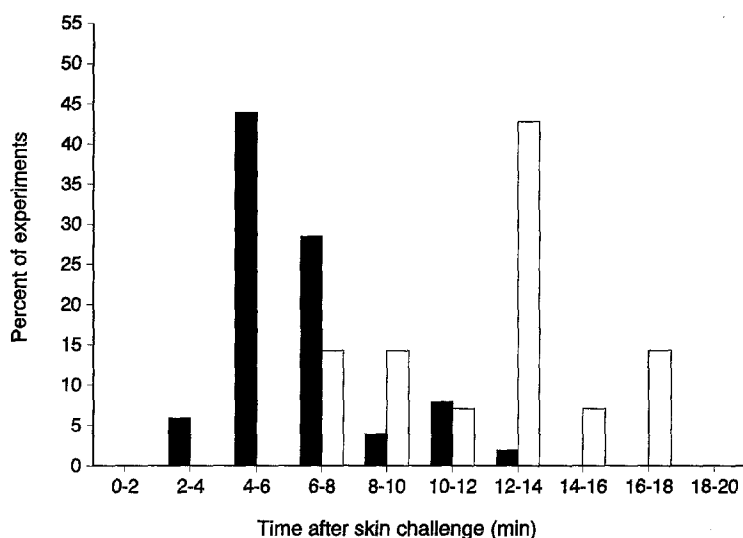


FIG. 2. Distribution of the time to reach peak histamine concentration after ICT (filled bars) and SPT (open bars) in percent of eligible experiments.

Skin reactions

There was a statistically significant correlation between allergen concentrations and wheal sizes by both routes of administration of allergen (data not shown). The relations between logarithmically transformed peak histamine release and wheal sizes obtained with ICTs and SPTs are presented in Fig. 3. For both routes of administration of allergen, there was a statistically significant correlation (ICT: $r = 0.81$, $p < 0.001$; SPT: $r = 0.70$, $p < 0.001$).

Histamine releasability and skin sensitivity

To quantify the substantial difference in histamine releasability and skin sensitivity among the subjects, we used the exponential dose-response analysis to calculate individual sensitivities. We performed calculation of sensitivities for ICT experiments only. After statistical confirmation that all dose-response curves could be joined into a common curve by parallel adjustment along the x axis, it was possible to express the relative sensitivity in histamine release and wheal formation by parallel adjustment of individual curves along the x axis. The individual sensitivity in histamine release showed an interindividual variation of 208%, and a more than 2000-fold variation between the least and most sensitive subjects. The individual sensitivities with respect to wheal formation showed a 91% variation and a 48-fold variation between the least and most sensitive subjects. A high sensitivity

means that a low concentration of allergen is needed to elicit the mean response level.

To determine whether any covariates had a significant impact on histamine releasability and skin sensitivity, the logarithmically transformed sensitivities were entered into a multiple regression analysis with demographic and biochemical covariates. This analysis showed that a combination of gender, total IgE, allergen-specific IgE, and the outcome of a basophil histamine release test could account for 70.1% of the variation in individual sensitivity in skin histamine release. Age had no statistically significant effect on histamine releasability. Women were less sensitive than men ($p = 0.005$). Increasing specific IgE caused increasing sensitivity ($p = 0.04$), whereas increasing total IgE showed decreasing sensitivity ($p = 0.02$). The subjects showing a class 3 basophil histamine release test result were significantly more sensitive than nonresponders to anti-IgE ($p = 0.02$). Because allergen-specific and total IgE showed opposite effects on sensitivity, we calculated the ratio between specific IgE and total IgE (IgE ratio) and entered this figure in the regression analysis. In this analysis 74.4% of the total variation in histamine release could be accounted for by a combination of gender, basophil histamine release, and IgE ratio. None of the covariates (age, gender, specific IgE, total IgE, IgE ratio, basophil histamine release test result, or histamine releasability) turned out to be significant covariates for skin sensitivity.

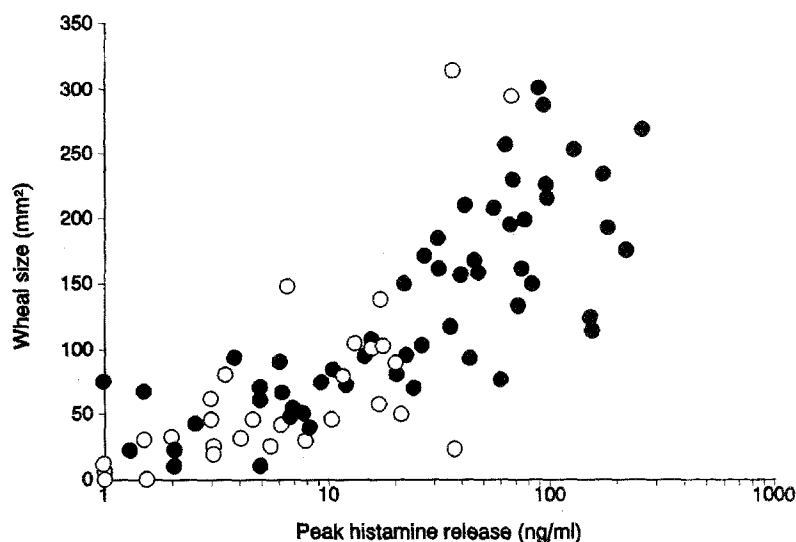


FIG. 3. Correlation of wheal size with histamine peak concentrations. For ICT (filled circles): $r = 0.81$, $p < 0.001$, and for SPT (open circles): $r = 0.70$, $p < 0.001$.

DISCUSSION

By using a skin microdialysis technique, we have described the events of allergen-induced histamine release in intact human skin. We characterized the dose-response relationship and the kinetics of histamine release and demonstrated the correlation between histamine release and skin response. Furthermore, the magnitude of the variation in histamine releasability was described and partly explained.

The histamine concentration in dialysate draining unchallenged skin was 3.5 ± 0.4 ng/ml, which was below the sensitivity of approximately 5 ng/ml histamine of our assay. Using an enzyme immunoassay, we have found resting histamine values of about 1 to 2 ng/ml (Petersen et al. Unpublished observations). Despite the finding that baseline values and most vehicle responses showed histamine values below the sensitivity of the assay, the data were corrected for these figures. The data showed a clear dose-response relationship in almost all subjects who had ICTs. When SPTs were used, only the highest allergen concentration showed notable histamine release. The statistical analysis showed significant dose-response relations for both routes of administration of allergen.

The histamine concentrations found in the dialysis fluid before and after skin challenge were most probably an underestimate of the histamine levels actually present in the skin. Recovery of histamine in vitro is approximately 80%,¹⁷ but for reasons discussed elsewhere^{15, 16} this figure is probably much lower in vivo than in vitro. Still, the dialysate

histamine concentrations directly reflect relative changes in skin histamine concentrations.

The short sampling intervals and the finding that changes in histamine concentrations are instantly reflected in the dialysate¹⁷ allowed a detailed description of the kinetics of histamine release. Maximum histamine concentrations were observed 4 to 8 minutes after intradermal injection of allergen, followed by a decline with a half-life of approximately 7 minutes. The kinetics of histamine release as determined by microdialysis are in accordance with sequential histologic examinations of mast cell degranulation after injection of ragweed.⁶ In comparison with nonimmunologic stimulation with substance P¹⁷ and codeine,¹⁸ allergen-induced histamine release was somewhat slower. Intradermal injection of nonimmunologic mast cell secretagogues induced maximal histamine release 2 to 4 minutes after skin challenge, as confirmed in three subjects in this study. The difference in the rate of degranulation of skin mast cells in response to immunologic and nonimmunologic stimuli is in accordance with results of previous in vivo studies^{4, 23-26} and studies on dispersed human skin mast cells.²⁷

Histamine release elicited by allergen SPT showed a different temporal pattern compared with that determined by ICT. A delayed appearance of histamine after allergen SPT has previously been described,²⁸ indicating that the route of administration of allergen has an effect on the kinetics of histamine release. The protracted histamine release elicited by SPT might be due to slow

absorption of allergen from the SPT pouch to the surrounding skin or diffusion of histamine from the point of stimulus to the dialysis probe. The latter explanation seems less likely because histamine release is localized very distinctly to the site of stimulus administration, and virtually no histamine diffusion occurs within the skin.²⁹

The skin microdialysis technique makes it possible to describe the relationship between biochemical events within the skin and clinical measures. The results of this study showed that wheal size correlated significantly with histamine release. The findings support the supposition that histamine is the main mediator in the wheal.^{1,2} However the squared r values of 50% to 65% indicate that 35% to 50% of the wheal size cannot be explained by histamine alone. End-organ sensitivity to histamine or other mediators may be important.

A pronounced interindividual variation was observed in histamine release, and to a lesser extent, in wheal sizes. The intraindividual variation was modest. Substantial variation in histamine release from the skin has also been demonstrated by other groups^{3,4,23-26} and by us with a microdialysis technique.^{17,18} Releasability,³⁰ that is the ability of inflammatory cells such as basophils^{30,31} and mast cells³²⁻³⁵ to release their inflammatory mediators, can be influenced by a number of complex factors. A combination of gender, total IgE, allergen-specific IgE (or the ratio between allergen-specific and total IgE), and the results of a basophil histamine release test could account for 70% to 75% of the variation in histamine release. Therefore we confirm results of previous studies suggesting that gender and basophil histamine release have an impact on histamine releasability from skin mast cells in vivo.^{25,26}

It was demonstrated that total and allergen-specific IgE were significantly correlated to histamine releasability. So far, data on the role of IgE in skin mast cell releasability are sparse. It is difficult to apply the results from numerous in vitro studies on basophils, and to a certain degree on harvested and isolated mast cells, to the mediator secretagogue profile of mast cells in the skin in situ. Primarily, skin mast cells are morphologically, biochemically, and pharmacologically different from basophils and distinct from mast cells in other organs.³⁶ Secondly, the local microenvironment presents to the resident mast cells an unknown scenario of cytokines, growth factors, chemokines, integrins, and neurogenic stimuli of which the impact and magnitude on histamine releasability in

situ is unknown. The influence of the local environment on histamine releasability can only be laboriously investigated, but the concept of resident skin mast cell releasability and the control of these events may be clinically important.^{9,37}

Previous studies have demonstrated that skin sensitivity is correlated to allergen-specific serum IgE, as well as to basophil histamine release, bronchoprovocation, and symptom-medication score in ragweed-sensitive subjects.^{38,39} Whether such correlations exist for other allergens and diseases remains to be elucidated, because other studies, including this one, have not been able to confirm the results.^{40,41} Organ sensitivity may be determined by in vitro parameters, but the results from challenge procedures in various organs can not be compared directly.⁴²

In conclusion, we have applied a skin microdialysis technique for the study of histamine release by allergen in intact human skin. The technique is minimally invasive, has no cosmetic consequences, allows insertion of a great number of fibers in each individual, and permits comparison of biochemical and clinical measures in normal and diseased skin. Thus the method seems promising for use in immunopharmacologic investigations, as well as for the study of the pathogenesis of various skin diseases.

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