

Comparison of histamine assay methods in measuring *in vitro*-induced histamine release in patients with allergic rhinitis

R. J. HARVIMA, I. T. HARVIMA, L. TUOMISTO,¹ M. HORSMANHEIMO & J. E. FRÄKI

Departments of Dermatology and ¹Pharmacology, University of Kuopio, Finland

histamine release tests using whole blood were applied in testing *in vitro* Type I birch allergic reaction in two patients with allergic rhinitis. Heparinized blood was incubated with varying dilutions of allergen for 30 min at +37°C. After centrifugation of the blood, plasma was separated and the liberated histamine was analysed by histamine radio enzyme assay (REA), high performance liquid chromatography (HPLC) with post-column derivatization system or radio immuno assay (RIA), and the results obtained by these methods were compared. REA and HPLC gave similar results, while RIA gave somewhat higher values, but was less sensitive.

Key words: histamine; assay methods; histamine release; birch allergy.

Accepted for publication 7 November 1988

Histamine is one of the mediators of the allergic reaction liberated from basophils or mast cells (2, 14). Several assay methods have been employed for histamine. Bioassay (1), fluorimetric assay (15) and gas chromatography (18) are not very sensitive and require extensive sample purification. High performance liquid chromatography (HPLC) with the pre-column derivatization system (6) is hampered by insensitivity. Mass fragmentography (11) is sensitive, but highly specialized laboratory equipment is needed. The most sensitive methods are radio enzyme assay (REA) (3,9) and HPLC with post-column derivatization system (19). Recently, immunological assay methods have been developed for histamine, e.g. enzyme immuno assay (4) and radio immuno assay (RIA) (12) using monoclonal antibody.

We have compared the most sensitive methods (REA and HPLC with post-column derivatization) with the new commercially available RIA method (Pharmacia) in patients with birch allergic rhinitis.

MATERIAL AND METHODS

The *in vitro*-induced histamine release was measured in two patients with allergic rhinitis. Patient I was a 33-year-old woman with mild rhinitis of several years standing but otherwise healthy. Her first severe attack of rhinitis occurred in the birch flowering season 8 months prior to the histamine release tests. Birch allergen (Allergologisk Laboratorium, Copenhagen, Denmark) produced a strong reaction 3 × 4 mm on prick testing. The skin reaction to histamine was 4 × 4 mm. All other 33 allergens tested were negative. The patient had taken no medication during the 4 months prior to the skin and histamine release tests.

Patient II was a 15 year-old boy with birch-allergic rhinitis of 4 years standing but otherwise healthy. The strongest positive prick test reaction was to birch (9 × 6 mm), with a histamine reaction of 4 × 4 mm. He had milder prick test reactions (< 5 mm in diameter) to the following allergens: alder, orchard grass,

meadow fescue, timothy, horse, dog, cat, cow, pea and nut. No medication was taken during the 12 days prior to the skin and histamine release tests.

Chemicals used were: histamine from Fluka (Buchs, Switzerland), 1-methylhistamine from Serva (Heidelberg, FRG), and 3-methylhistamine and N-alpha-methylhistamine from Calbiochem-Behring (La Jolla, CA, USA). All other chemicals of analytical grade were from E. Merck (Darmstadt, FRG) or Sigma (St. Louis, MO, USA). The commercial histamine RIA-kits were from Pharmacia Fine Chemicals (Uppsala, Sweden). Birch antigen was obtained as described by Viander et al. (17).

The whole blood was taken from the patients' antecubital veins and collected into 10 ml Venoject®-tubes containing lithium heparin (or Na₂EDTA for control). One ml of blood was mixed gently in 1.5 ml Eppendorf® polypropylene tube with 50 µl of antigen (allergen) solution dilutions (in 0.9% NaCl). After incubation for 30 min at +37°C the tubes were centrifuged at 900 × *g* for 10 min at room temperature. The clear plasma was collected carefully, avoiding leukocyte contamination, and stored at -80°C until histamine assays (within 2-3 weeks) were performed as described below. To measure whole blood histamine, the blood was mixed with an equal volume of water, frozen and thawed 5 times and centrifuged for 10 min at 10,000 × *g* and the supernatant diluted with 0.9% NaCl.

Histamine assay using REA was performed as described recently in detail by us (9). Histamine assay using HPLC was performed after precipitating the proteins with perchloric acid by the method of Yamatodani et al. (19). Histamine assay using a commercial RIA-method (Pharmacia Fine Chemicals, Uppsala, Sweden) with monoclonal antibody was performed as follows: 200 µl of standard (histamine, 1-methylhistamine, 3-methylhistamine, N-alpha-methylhistamine; at concentration of 10-1000 nM), or samples, were mixed with 200 µl ¹²⁵I-histamine-DSA (dog serum albumin) followed by addition of 200 µl of antibody solution. The mixture was incubated for 16-20 h at +4°C. Two ml of "decanting suspension" was added,

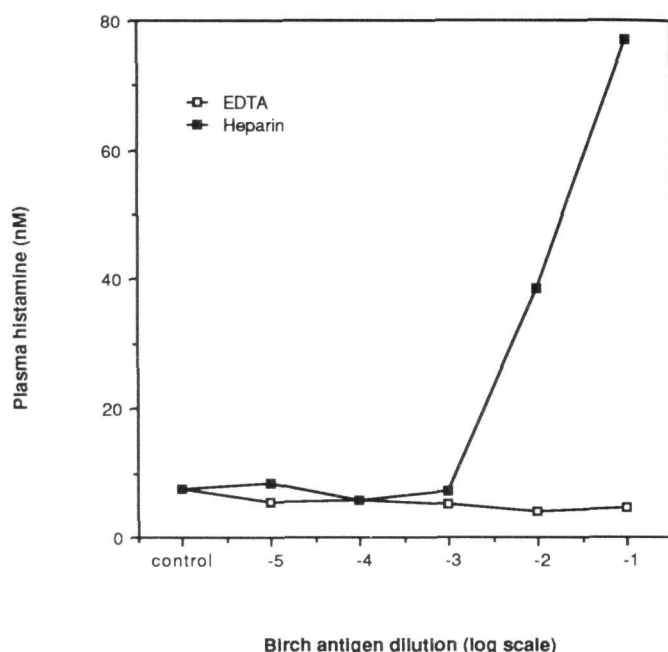


Fig. 1. The release of histamine in one rhinitis patient after birch-induced histamine liberation test (Patient I). 50 µl of birch antigen dilution (in 0.9% NaCl) was incubated with 1 ml blood for 30 min at 37°C, centrifuged for 10 min at 900 × *g*, and plasma histamine was analysed by radio enzyme assay.

followed by 30 min incubation at room temperature. The mixture was centrifuged at 1,500 × *g* for 10 min and the supernatant discarded. The radioactivity of the pellet was measured using Rackgamma-counter (LKB-Wallac, Turku, Finland).

RESULTS

Using EDTA-plasma as control no histamine release from blood leukocytes of Patient I was detected by REA after the birch-induced histamine liberation test (Fig. 1). RIA and HPLC gave similar results. In heparin blood of Patient I, a dose-related histamine liberation in plasma was found by REA (Fig. 1).

Table 1 gives the results of the histamine liberation test in Patient II analysed by three different methods (REA, HPLC, RIA). All assay methods showed a clear increase in plasma histamine. REA and HPLC gave very similar results except for whole blood. The RIA method gave somewhat higher values.

RIA gave higher (27±14 nM, mean±SD) plasma histamine levels in 16 controls than

REA in 35 controls (6.5 ± 4.3 nM, see ref. 9), or HPLC in 31 controls (1.3 ± 1.5 nM). Whole blood histamine in four controls was determined by REA and HPLC and showed a marked difference in the values obtained: results with HPLC were 60–110 % of those with REA.

The relative sensitivity of RIA is shown in Table 2. Our results are compared with those of Pharmacia and of Chevrier et al. (4); the latter used another monoclonal antibody in enzyme immuno assay. As seen in Table 2, the monoclonal antibody used in RIA is not specific for histamine; higher affinity is obtained with 1-methylhistamine and N-alpha-methyl-histamine, whereas very low affinity was obtained with serotonin and histidine. The other monoclo-

nal antibody used in enzyme immuno assay is more specific for histamine, as seen in Table 2.

DISCUSSION

Birch allergy in patients with allergic rhinitis was selected for this study as a model disease. For testing histamine liberation from blood samples, it is necessary to use heparin as an anticoagulant instead of EDTA because histamine release from blood leukocytes is calcium dependent.

HPLC and REA gave very similar results, although whole blood histamine was somewhat lower with HPLC; variations in drug or serotonin levels in blood might possibly influence the REA method. Whole blood histamine levels are probably not very important in testing histamine liberation. The higher results obtained with RIA may be due to the non-specificity of monoclonal antibody used, which cross-reacted also with 1-methylhistamine and histidine. 1-Methylhistamine is a natural metabolite of histamine. Thus the high reactivity to 1-methylhistamine is a serious drawback when using the RIA method. The cross-reactivity to histidine is relevant because the concentration of histidine in plasma is approx. 100 μM, i.e. approx. 10,000-fold higher than that of histamine. However, a monoclonal antibody has been described (4, see also Table 2) which cross-reacts with histidine and 1-methylhistamine to a much smaller degree. N-alpha-methylhistamine, which also

Table 1

Comparison of results of analysis methods used for histamine assay in one rhinitis patient after birch-induced histamine liberation test (Patient II)

Birch antigen dilution	Plasma histamine (nM)		
	REA	HPLC	RIA
Control	12.5	13.0	< 10
1:100	129	137	175
1:10	178	171	208
1:1	> 250	267	410
Whole blood	762	513	1320

REA = radio enzyme assay; HPLC = high performance liquid chromatography with post-column derivatization system; RIA = radio immuno assay.

Table 2
Specificity of histamine RIA

Compound	Relative sensitivity		
	This study	Pharmacia*	Ref. 4
Histamine	1.0	1.0	1.0
1-methylhistamine	17.7	18.0	0.00005
3-methylhistamine	0.9	–	–
N-alpha-methylhistamine	7.3	–	–
Serotonin	–	0.0005	0.00003
Histidine	–	0.00008	0.000005
Spermidine	–	–	< 0.0000005

*Pharmacia Fine Chemical, Uppsala, Sweden. Directions for use: Histamine RIA 50. March 1987.
– = values not determined
The specificity was determined at 50 % uptake.

interferes with the RIA-method, has been detected only in gastric juice (7).

In this study the REA, HPLC and RIA methods all showed an increase in liberated plasma histamine in samples from clinically confirmed birch-allergic patients with rhinitis. Although data from only two such patients have been presented, similar results have since been obtained in other birch-allergic patients.

When choosing assay methods, account should be taken of their various limitations. REA is a simple, sensitive and specific method, but is disturbed by a variety of drugs, for example muscle relaxants (8, 10), serotonin (4, 10) and anti-histamines (10, 16). HPLC with the post-column derivatization system (19) is highly sensitive (approx. 1–2 nM) but requires sophisticated equipment. The HPLC-system can easily be automatized and needs no radioactive compounds. The commercially available histamine-RIA (Pharmacia) method might also be useable in histamine release testing *in vitro*, in which the basic absolute level of histamine is not important. The RIA method is not suitable for measurement of normal plasma or whole blood histamine levels because of its insensitivity and/or non-specificity. In addition, possible interactions or effects of drugs or other compounds can not be excluded.

Histamine release test will not replace the skin prick test, although a close and positive correlation has been found between skin test and histamine release results (13). *In vitro* histamine release test is useful in patients who are expected to react strongly to an allergen (or even a drug), or who in prick tests show a positive reaction to saline as in dermographism.

The present study confirms that the histamine release test can be performed using whole blood (5, 13) instead of isolated leukocytes (5, 13). In addition, we have compared the new commercially available RIA method with REA or HPLC, especially in testing histamine release. RIA gave somewhat higher histamine values, and was less sensitive than REA or HPLC.

ACKNOWLEDGEMENTS

Research grants from Sigrid Juselius Foundation and Helena Vuorenmies Foundation are acknowledged. Phar-

macia is acknowledged for kind gifts of histamine RIA-kits. Ms. Merja Hämäläinen, Ms. Birgitta Markkio and Ms. Anne Hyryläinen are acknowledged for their technical assistance.

REFERENCES

1. Austen, K. F.: Biologic assay of histamine. In Williams, C. A. & Chase, M. W. (eds.): *Methods in immunology and immunohistochemistry*, Vol. 5, pp. 125–129. Acad. Press, New York, 1968.
2. Beaven, M. A.: Histamine: its role in physiological and pathological processes. *Monogr. Allergy* 13, 1–113, 1978.
3. Beaven, M. A., Robinson-White, A., Roderick, N. B. & Kauffman, G. L.: The demonstration of histamine release in clinical conditions: a review of past and present assay procedures. *Klin. Wochenschr.* 60, 873–881, 1982.
4. Chevrier, D., Guesdon, J.-L., Mazie, J.-C. & Avrameas, S.: Enzyme immunoassay for the measurement of histamine. *J. Immunol. Methods* 94, 119–125, 1986.
5. Clinton, P. M., Murdoch, R. D. & Kemeny, D. M.: Development of a microassay for histamine release. *Clin. Allergy* 17, 181–189, 1987.
6. Granerus, G. & Wass, U.: Urinary excretion of histamine, methylhistamine (1-MeHi) and methylimidazole-acetic acid (MeImAA) in mastocytosis: comparison of new HPLC methods with other present methods. *Agents Actions* 14, 341–345, 1984.
7. Haimart, M., Laynay, J. M., Zurcher, G., Caue, N., Dreux, C. & Da Prada, M.: Simultaneous determination of histamine and N-alpha-methylhistamine in biological samples by an improved enzymatic single isotope assay. *Agents Actions* 16, 71–75, 1985.
8. Harle, D. G., Baldo, B. A. & Fisher, M. M.: Inhibition of histamine-N-methyltransferase activity by neuromuscular blocking drugs. *Agents Actions* 17, 27–31, 1985.
9. Harvima, R. J., Harvima, I. T. & Fräki, J. E.: Optimization of histamine radio enzyme assay using purified histamine-N-methyltransferase. *Clin. Chim. Acta* 171, 247–256, 1988.
10. Harvima, R. J., Harvima, I. T., Kajander, E. O., Penttilä, I. M., Horsmanheimo, M. & Fräki, J. E.: Effect of drugs on histamine radio-enzyme assay. *Clin. Chim. Acta* (in press).
11. Keyzer, J. J., Wolthers, B. G., Muskiet, F. A. J., Breukelman, H., Kauffman, H. F. & De Vries, K.: Measurement of plasma histamine by stable isotope dilution gas chromatography-mass spectrometry: methodology and normal values. *Anal. Biochem.* 139, 474–481, 1984.
12. Morel, A. M., Anfosso, F. J. & Delaage, M. A.: Radioimmunoassay for histamine. Application to histamine release. *J. Allergy Clin. Immunol.* 75, (Suppl.), 124, 1985.
13. Nolte, H., Schiotz, O. & Stahl Skov, P.: A new glass microfibre-based histamine analysis for allergy testing in

- children. *Allergy* 42, 366-373, 1987.
14. Schwartz, L. B. & Austen, K. F.: Structure and function of the chemical mediators of mast cells. *Prog. Allergy* 34, 271-321, 1984.
15. Shore, P. A.: Fluorometric assay of histamine. In Williams, C. A. & Chase, M. W. (eds.): *Methods in immunology and immunohistochemistry*, Vol. 5, pp. 129-135. Acad. Press, New York, 1976.
16. Taylor, K. M. & Snyder, S. H.: Histamine methyltransferase: inhibition and potentiation by antihistamines. *Mol. Pharmacol.* 8, 300-310, 1972.
17. Viander, M., Fräki, J. E. & Djupsund, B. M.: Antigens and allergens in birch pollen extract. *Allergy* 34, 289-302, 1979.
18. Wollin, A. & Navert, H.: Quantitation of histamine and some of its basic methylated metabolites in biological materials by gas-liquid chromatography. *Anal. Biochem.* 145, 73-79, 1985.
19. Yamatodani, A., Fukuda, H., Wada, H., Iwaeda, T. & Watanabe, T.: High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. *J. Chromatogr.* 344, 115-123, 1985.

Address:

Rauno J. Harvima, M.Sc., B.Med.

Department of Dermatology

University of Kuopio

Clinical Research Unit

P.O.B. 6

SF-70211 Kuopio

Finland

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.