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QUANTITATIVE ANALYSIS OF HISTAMINE IN BIOLOGICAL SAMPLES BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

A mass fragmentographic method for the quantitative analysis of histamine in the supernatant from antigen-challenged leukocytes, whole blood, and urine is described. Histamine labeled with two ^{15}N atoms was synthesized and added to the sample as an internal standard. N^{α}-Heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine was prepared for mass fragmentographic analysis and the molecular ions at *m/z* 379 and 381 were used for monitoring histamine and $^{15}N_2$-labeled histamine, respectively. The quantitation limit of histamine was 2 ng by this method. The experimental error of the method was less than 7% at the level of 5 ng in the supernatant from antigen-challenged leukocytes. The value obtained by this method correlated well with that from radioisotopic enzymatic assay (r=0.990).

INTRODUCTION

It is known that histamine is released from mast cells in tissue, basophils in blood, which is mediated by IgE antibody. A number of studies on histamine have been reported and it is generally accepted that histamine is a mediator of immediate type hypersensitivity [1, 2].

Histamine has been measured by a number of techniques including biological [3], colorimetric [4], and fluorimetric assay [5], and by enzymatic assay using radioisotopes [6]. Biological and colorimetric procedures are laborious and may give rise to the question of specificity. The fluorimetric assay of Shore et al. [5] has been adopted for assaying histamine. However, the butanol extract contained not only histamine but spermidine, which interfered with the measurement of histamine, especially in tissues with a low content of histamine [7]. Recently, the application of high-speed liquid chromatography was introduced to overcome this disadvantage [8]. The radioisotopic enzymatic assay originally described by Snyder et al. [6] is a sensitive and specific method but

it involves the hazards associated with radioactivity and radioactive waste.

It is generally recognized that determination by mass fragmentography is a most specific and sensitive method, and its application for many biologically important compounds has been reported. Nevertheless, the quantitation of histamine by mass fragmentography has not yet been reported. The purpose of this study is to develop a method which allows the quantitation of histamine.

EXPERIMENTAL

Materials

Histamine dihydrochloride was purchased from Merck (Darmstadt, G.F.R.). Heptafluorobutyric anhydride (HFBA) was obtained from Wako Pure Chemical (Osaka, Japan) and ethyl chloroformate (ECF) was from Tokyo Kasei Kogyo (Tokyo, Japan). All organic solvents used were of analytical grade. Ethyl acetate was stored over molecular sieve type 3A (Wako).

$^{15}N_2$-Labeled histamine used as an internal standard was synthesized using potassium [^{15}N]phthalimide (99.6 atom per cent; Prochem, London, Great Britain) as the starting material according to the method of Fraser and Raphael [9]. The labeled histamine was dissolved in 0.01 *N* hydrochloric acid solution to make a concentration of 600 ng/ml and kept at 4°.

The radioisotope-labeled compounds, [2,5-^{3}H]histamine dihydrochloride (specific activity, 7.7 Ci/mmol) and S-adenosyl-L-[methyl-^{14}C]methionine (specific activity, 58 mCi/mmol) used for the enzymatic assay were purchased from the Radiochemical Centre, Amersham, Great Britain.

Preparation of the crude extract from the Japanese cedar pollens

Japanese cedar pollens were collected and defatted with diethyl ether. The defatted pollens were extracted with 0.125 *M* ammonium hydrogen carbonate solution and the extract was dialyzed against 5 m*M* ammonium hydrogen carbonate solution. The dialyzate was lyophilized and kept at 4° until use.

Samples

Supernatant released from antigen-challenged leukocytes. In vitro histamine release reaction from washed leukocytes was carried out as follows. Venous blood of a patient hypersensitive to cedar pollen was drawn into a plastic syringe containing heparin. The leukocyte suspension, obtained by the procedure of Siraganian and Brodsky [10], was incubated with the crude extract of Japanese cedar pollens at 37° for 1 h in a tube. The tubes were centrifuged and the supernatant was assayed for histamine.

Whole blood. The venous blood obtained from a patient was repeatedly frozen and thawed, and used for the determination.

All the samples were stored at −20° until assay.

Analytical procedure

An internal standard solution (30 ng), 0.5 ml of 4 *N* sodium hydroxide solution, and 0.5 g of sodium chloride were added to 0.25 ml of sample. The mixture was shaken with 2 ml of ethyl acetate. The organic layer was discarded and the aqueous phase was extracted with 2 ml of butanol. The extraction step

with ethyl acetate can be omitted. The organic layer was transferred to a tube containing 2 ml of heptane and 1.5 ml of 1 *N* hydrochloric acid solution. The mixture was shaken and centrifuged. The aqueous phase was removed and evaporated to dryness under reduced pressure. The residue was mixed with HFBA—ethyl acetate (1:1, v/v) and heated at 80° for 30 min. After removing excess reagent under a nitrogen stream, the residue was treated with 50 μl of a mixture of ECF—ethyl acetate (1:1, v/v) at room temperature for 30 min to form N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine (HA-HFB-ETO), to which 1 ml of 10% sodium carbonate and 2 ml of dichloromethane were added. The mixture was shaken, centrifuged and the aqueous phase was discarded. The organic phase was removed to dryness under a nitrogen stream; the residue was dissolved in ethyl acetate, and the solution was analyzed by gas chromatography—mass spectrometry.

Gas chromatography—mass spectrometry

An LKB 2091 mass spectrometer coupled with a Shimadzu 7A Model gas chromatograph and data processing system (Shimadzu PAC500FDG) connected to a minicomputer (Okitac-4300b,Oki Electric Industry Co., Tokyo, Japan) was used. The glass column (2 m × 2.4 mm I.D.) was packed with 5% SE-30 on Supelcoport (80—100 mesh; Supelco, Bellefonte, Pa., U.S.A.). The trap current was 50 μA, electron energy 20 eV, flow-rate of carrier gas (helium) ca. 12 ml/min, column oven temperature 200°, injection temperature 240°, separator temperature 250° and ion source temperature 220°.

Radioisotopic enzymatic assay of histamine

Enzymatic assay was performed by a slight modification [11] of the method of Beaven et al. [12]. Briefly, histamine was converted to [^{14}C]methylhistamine by incubation with S-adenosyl-L-[methyl-^{14}C]methionine and the enzyme histamine N-methyltransferase prepared from pig brain. A tracer amount of [^{3}H]histamine was added to correct the efficiency of the reaction. The [^{14}C,^{3}H]methylhistamine formed was extracted from the mixture with chloroform and its radioactivity assayed by liquid scintillation spectrometry. Determinations were done in duplicate.

RESULTS AND DISCUSSION

Mass spectra of the N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonyl derivative of histamine and [$^{15}N_2$]histamine as an internal standard are shown in Fig. 1. The intensity of the molecular ion was about 40% of the most abundant peak at *m/z* 166 which resulted from the elimination of $NHCOC_3F_7$ with hydrogen. The molecular ions at *m/z* 379 and 381 were used for monitoring histamine and [$^{15}N_2$]histamine, respectively.

The response at *m/z* 379 of $^{15}N_2$-labeled HA-HFB-ETO was about 2% of *m/z* 381 which was identical with the peak height ratio at *m/z* 379 and 377 for HA-HFB-ETO (Fig. 2A and B). The intensity ratio of *m/z* 379 and 381 was almost the same as the mixing ratio of histamine and [$^{15}N_2$]histamine. Because the peak of $^{15}N_2$-labeled HA-HFB-ETO at *m/z* 379 was about 0.4% when it was measured with hardware multiple ion detector, the contribution to *m/z* 379 was

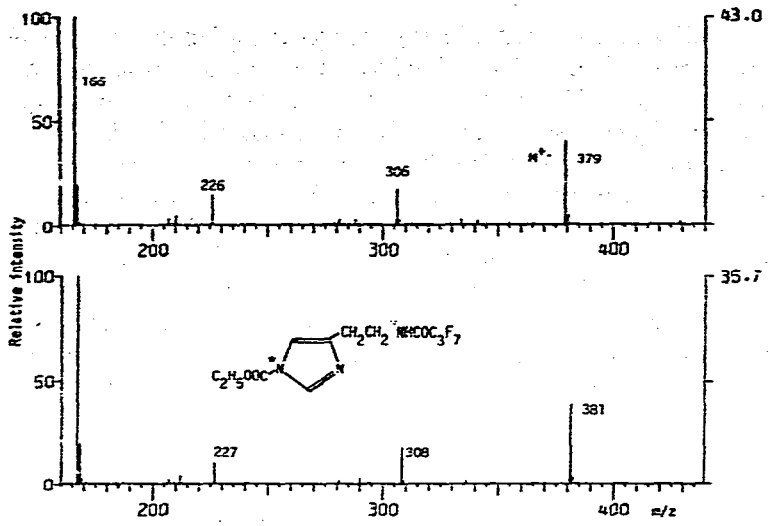

Fig. 1. Mass spectra of N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonyl derivative prepared from histamine (upper) and $^{15}N_2$-labeled histamine (lower). Asterisks (*) indicate ^{15}N.

due to the computer error which can not be resolved at the present time. As the contribution to *m/z* 379 was observed in $^{15}N_2$-labeled HA-HFB-ETO, the amount of internal standard added has to be reduced if greater sensitivity is desired, but when 1.9 ng of histamine were mixed with 30 ng of the internal standard an increase in the peak height ratio was easily detected (Fig. 2C).

For gas chromatographic analysis, HA-HFB-ETO is better than the trimethylsilyl and heptafluorobutyryl derivative of histamine, which fact has been reported [13]. In a two-step derivatization procedure, the yield becomes a seri-

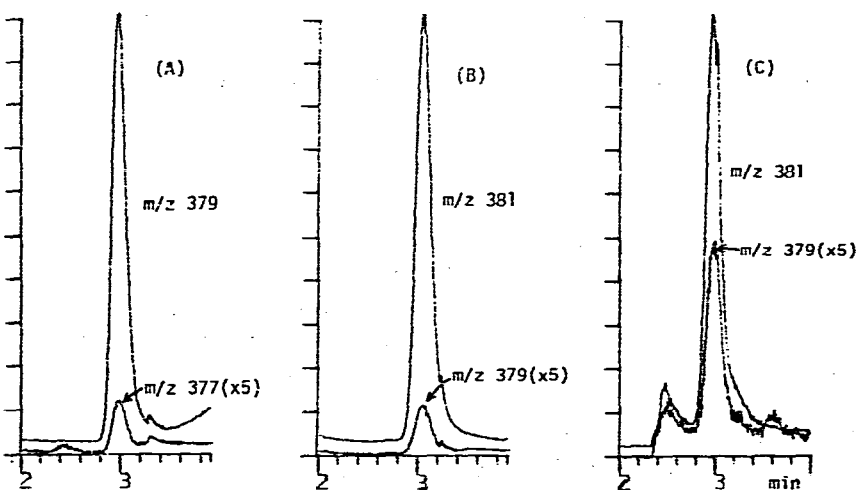

Fig. 2. Mass fragmentograms of the derivative of (A) authentic histamine, (B) $^{15}N_2$-labeled histamine used as an internal standard, and (C) mixture containing histamine (1.9 ng) and $^{15}N_2$-labeled histamine (30 ng).

ous problem. To estimate the recovery of histamine in the present procedure, [^{3}H]histamine was added to the leukocyte suspension. After the sample containing [^{3}H]histamine was extracted and converted to HA-HFB-ETO, the recovery was calculated from the tritium radioactivity. The total recovery, including extraction, clean-up, and two-step derivatization procedures, was about 70%. Hence, this procedure would be applicable for the determination of histamine.

The detection limit was 50 pg of histamine, with a signal-to-noise ratio of 5:1, which permits the quantitative determination of histamine present in whole blood and urine, and in the in vitro histamine release experiment.

In the quantitative determination of histamine, a good linear relationship was obtained between the ratio of the peak heights and amount of histamine in the range of 2—20 ng. A standard solution containing 5.55 ng of histamine was measured by the above method and the value was 5.71 ± 0.17 ng (n=5). This is nearly the theoretical value.

To test the specificity of the determination, the mass spectrometer was focused on the m/z 379, 381 and 306, 308 for a sample of supernatant from antigen-challenged leukocytes. The ratio between the peak heights at both pairs was the same. As shown in Table I, the values obtained were 5.93 ± 0.37 and 5.71 ± 0.29 ng (mean ± S.D.) at m/z 379/381, and 5.59 ± 0.23 at m/z 306/308. This indicates that fragments from compounds other than histamine do not interfere with the quantitation of histamine. Next, this method was compared with the radioisotopic enzymatic assay of histamine. The mass fragmentographic analysis resulted in apparently the same level of histamine measured by radioisotopic enzymatic assay (Table I). The experimental error was less than 7% for mass fragmentography, while it was about 10% for radioisotopic enzymatic assay when five samples were analyzed. The quantitated values obtained by mass fragmentography and radioisotopic enzymatic assay were well correlated (r=0.990), as seen in Fig. 3. It is clear from Fig. 3 and Table I that mass fragmentographic measurement is a specific method for the determination of histamine and more accurate than the radioisotopic enzymatic assay.

TABLE I

COMPARISON OF MASS FRAGMENTOGRAPHY AND RADIOISOTOPIC ENZYMATIC ASSAY

Results are expressed in ng. Samples A, B, C, D, and E were taken from the same supernatant from antigen-challenged leukocytes.

Sample	Mass fragmentography			Radioisotopic enzymatic assay	
	m/z 379 / 381 ($M^{+\cdot}$)		m/z 306/308		
A	5.67	5.78	5.67	4.00	5.13
B	5.58	5.63	5.63	4.16	5.82
C	6.03	5.39	5.26	5.01	5.43
D	5.85	5.59	5.89	5.69	6.13
E	6.51	6.17	5.52	5.10	5.97
Mean	5.93	5.71	5.59	4.79	5.70
S.D.	0.37	0.29	0.23	0.70	0.41
C.V. (%)	6.24	5.08	4.11	14.61	7.19

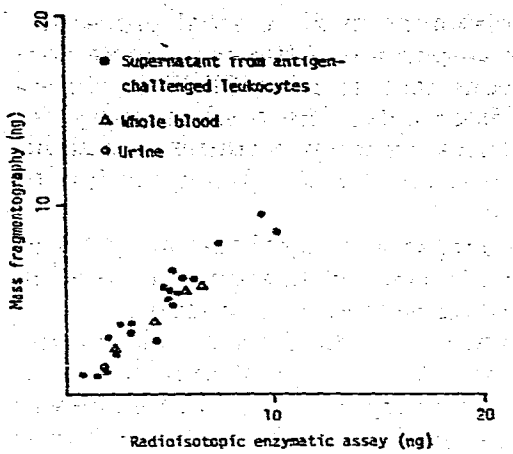

Fig. 3. Correlation between mass fragmentographic measurement and radioisotopic enzymatic assay (r=0.990).

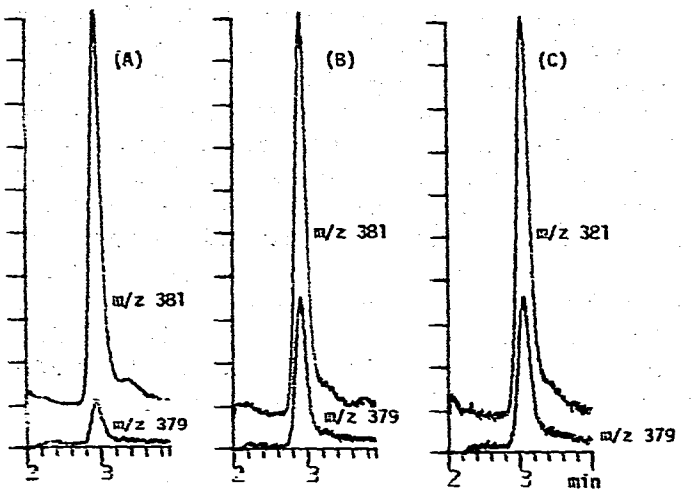

Fig. 4. Mass fragmentograms of histamine extracted from the supernatant from antigen-challenged leukocytes: (A) spontaneous release, (B) 0.01 μg, (C) 0.1 μg antigen challenged. Challenged antigen: crude extract of the Japanese cedar pollens.

Histamine release from leukocytes by antigen challenge has been extensively used for in vitro studies of allergy. After leukocytes were incubated with the crude extract of cedar pollens at 37° for 1 h in a tube, the tube was centrifuged, and the histamine released into the supernatant was determined by mass fragmentography (Fig. 4). When the allergen was challenged at concentrations of 0.01 and 0.1 μg, histamine was released from the leukocytes (basophils), indicating that the patient was hypersensitive to cedar pollens.

The availability of this new method for the analysis of histamine in biological samples will aid clinical research into the action of histamine in allergic patients.

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REFERENCES

1 M.A. Beaven, N. Engl. J. Med., 294 (1976) 30.
2 M.A. Beaven, N. Engl. J. Med., 294 (1976) 320.
3 G.S. Barsoum and J.H. Gaddum, J. Physiol., 85 (1935) 1.
4 T. White, Brit. J. Pharmacol., 26 (1966) 494.
5 P.A. Shore, A. Burkhalter and V.H. Cohn, Jr., J. Pharmacol. Exp. Ther., 127 (1959) 182.
6 S.H. Snyder, R.J. Baldessarini and J. Axelrod, J. Pharmacol. Exp. Ther., 153 (1966) 544.
7 I.A. Michaelson, Eur. J. Pharmacol., 1 (1967) 378.
8 Y. Tsuruta, K. Kohashi and Y. Ohkura, J. Chromatogr., 146 (1978) 490.
9 M.M. Fraser and R.A. Raphael, J. Chem. Soc. London, (1952) 227.
10 R.P. Siraganian and M.J. Brodsky, J. Allergy Clin. Immunol., 57 (1976) 525.
11 H. Mita, H. Yasueda, H. Nishimura and T. Shida, Arerugi (Jap. J. Allergy), 27 (1978) 671.
12 M.A. Beaven, S. Jacobson and Z. Horakova, Clin. Chim. Acta, 37 (1972) 91.
13 H. Mita, H. Yasueda and T. Shida, J. Chromatogr., 175 (1979) 339.