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SIMULTANEOUS DETERMINATION OF HISTAMINE AND N^{τ}-METHYLHISTAMINE IN HUMAN PLASMA AND URINE BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

A new and sensitive method is described for the determination of histamine and N^{τ}-methylhistamine in human plasma and urine by gas chromatography—mass spectrometry. $^{15}N_2$-Labeled histamine and N^{τ}-[methyl-d_3]methylhistamine were used as internal standards. Histamine and N^{τ}-methylhistamine were converted to the derivatives N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine and N^{α}-heptafluorobutyryl-N^{τ}-methylhistamine, respectively. After these derivatives had been purified on a small column packed with CPG-10, the molecular ions were monitored during selected ion monitoring. Linear standard curves were obtained in the range of 0.5—10 ng/ml for both compounds. The reliability of the histamine analysis was demonstrated by using two different ion pairs, while a comparison with results from two different derivatizations on the same urine sample also established the specificity of the N^{τ}-methylhistamine analysis. An increase of 1 ng of histamine in the plasma could be precisely determined by the present method. The histamine content of plasma from five normal subjects was determined as 0.83 ± 0.37 (S.D.) ng/ml and the N^{τ}-methylhistamine content in most subjects was below the limits of this measurement. High excretion of histamine was noted in the urine collected in the early morning from a patient with nephritis.

INTRODUCTION

Histamine is released as a chemical mediator from mast cells and basophils during inflammatory and allergic reactions. The released histamine is metabolized via two enzymatic pathways. Histamine is deaminated by diamine oxidase (histaminase) to form imidazoleacetic acid. Alternatively, histamine is methylated to form N^{τ}-methylhistamine by the enzyme, histamine-N-methyltransferase. N^{τ}-Methylhistamine is subsequently deaminated by monoamine oxidase to form N^{τ}-methylimidazoleacetic acid. Previous studies [1—3] have

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shown that ring N^{τ}-methylation represents the principal pathway of histamine metabolism in a variety of mammalian species and it is known that N^{τ}-methylhistamine, N^{τ}-methylimidazoleacetic acid and imidazoleacetic acid are the main metabolites of histamine in man [4, 5]. Since it has been suggested that histamine plays a role in the function of the central nervous system, the metabolism of histamine is of psychiatric interest. Determination of histamine and N^{τ}-methylhistamine by colorimetric methods has been reported by White [6] and Yamatodani et al. [7]. However, these methods raise some questions as regards specificity and, moreover, they lack the necessary sensitivity for low-level assays.

The present authors have reported a method for the quantitative determination of biological samples containing a relatively large amount of histamine by selected ion monitoring [8]. This paper describes a method for the simultaneous determination of histamine and N^{τ}-methylhistamine in human plasma and urine by gas chromatography—mass spectrometry.

EXPERIMENTAL

Materials

Histamine dihydrochloride was purchased from E. Merck (Darmstadt, G.F.R.) and N^{τ}-methylhistamine dihydrochloride was synthesized in our laboratory. Heptafluorobutyric anhydride (HFBAn) and pentafluoropropionic anhydride (PFPAn) were purchased from Wako (Osaka, Japan). Ethyl chloroformate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan) and distilled before use. Ethyl acetate was stored over a molecular sieve type 3A. CPG-10 (120—200 mesh) was obtained from Electro-Nucleonics (Fairfield, NJ, U.S.A.). $^{15}N_2$-Labeled histamine dihydrochloride was synthesized and used as an internal standard [8]. Deuterated N^{τ}-methylhistamine dihydrochloride was synthesized using dimethyl-d_6 sulfate (99 atom% D; Merck Sharp & Dohme, Montreal, Canada) by the method of Rothschild and Shayer [9] as modified in our laboratory, and it was dissolved in 0.01 *N* hydrochloric acid solution to give a concentration of 800 ng/ml. [2,5-^{3}H]Histamine dihydrochloride was purchased from the Radiochemical Centre (Amersham, Great Britain). Synthesis of N^{τ}-[methyl-^{14}C]methylhistamine was examined using histamine, S-adenosyl-L-[methyl-^{14}C]methionine and the enzyme histamine-N-methyltransferase [10].

Analytical procedures

About 7 ng of N^{τ}-[methyl-Cd_3]methylhistamine and 10 ng of $^{15}N_2$-histamine were added as internal standards to 1 ml of plasma or 100 μl of urine. Protein was removed by centrifugation after the addition of 10 ml of ethanol. This deproteinization step can be omitted in the analysis of urine. After reducing the volume of the supernatant to 1 ml under reduced pressure, the concentrated supernatant was added to a mixture of 2 g of sodium chloride, 0.5 g of sodium carbonate and 2 ml of 0.25 *M* borate buffer (pH 10). The mixture was then extracted with 5 ml of butanol. The organic layer was transferred to a tube containing 7 ml of heptane and 1.5 ml of 1 *N* hydrochloric acid solution. After the histamine and N^{τ}-methylhistamine had been returned to the hydrochloric acid phase, the aqueous phase was removed and evaporated to dryness

under reduced pressure. The residue mixed with 50 μl of a mixture of HFBAn and ethyl acetate (1:1, v/v) was heated at 90°C for 30 min. After allowing the mixture to come to room temperature, the excess reagent was removed under a nitrogen stream. The residue was treated with 50 μl each of ethyl chloroformate and ethyl acetate 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 solution and 2 ml of dichloromethane were added. The mixture was shaken, centrifuged, and the aqueous phase was discarded. Following removal of the organic phase to dryness under a nitrogen stream, the residue re-dissolved in a small volume of dichloromethane was loaded onto a small column of CPG-10 (1.5 × 0.5 cm I.D., equilibrated with hexane) and 2 ml of hexane and benzene were successively passed through the column. HA-HFB-ETO was eluted with 2 ml of ethyl acetate—ether (1:1, v/v). The column was next washed with 2 ml of isopropanol, and N^{α}-heptafluorobutyryl-N^{τ}-methylhistamine (MEHA-HFB) was eluted from the column with 1.5 ml of methanol. After the ethyl acetate—ether fraction and the methanol fraction had been evaporated to dryness under a nitrogen stream, the residues re-dissolved in ethyl acetate were injected into a gas chromatograph—mass spectrometer. When plasma was analyzed, the re-dissolved residues could be mixed before measurement. In the case of urine samples, the measurement should be carried out without mixing of the residues.

Samples

Blood samples were collected from five healthy individuals who were members of the laboratory staff. Venous blood was drawn into a plastic syringe containing a small amount of heparin and centrifuged at room temperature. Urine was collected in the early morning. All samples were stored at −20°C until assay.

Gas chromatography—mass spectrometry

A Shimadzu LKB 2091 gas chromatograph—mass spectrometer and data processing system (Shimadzu PAC500FDG) connected to a minicomputer (Okitac-4300b; Oki Electric Industry, Tokyo, Japan) were 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 flow-rate of carrier gas (helium) was about 12 ml/min, the trap current was 50 μA and the ionization energy was 20 eV. The temperatures employed were as follows: injection, 240°C; oven, 200°C; separator, 250°C; and ion source, 220°C.

RESULTS AND DISCUSSION

For quantification of histamine, HA-HFB-ETO was prepared and the molecular ions at *m/z* 379 and 381 were used for monitoring histamine and $^{15}N_2$-labeled histamine, respectively, while N^{τ}-methylhistamine was converted to MEHA-HFB. Fig. 1 shows a mass spectrum of the deuterated MEHA-HFB; the base peak at *m/z* 111 is formed by the elimination of $NHCOC_3F_7$ with hydrogen. The peak height ratio of *m/z* 321/324 was less than 0.0013 in deuterated MEHA-HFB. This product is a satisfactory internal standard, since

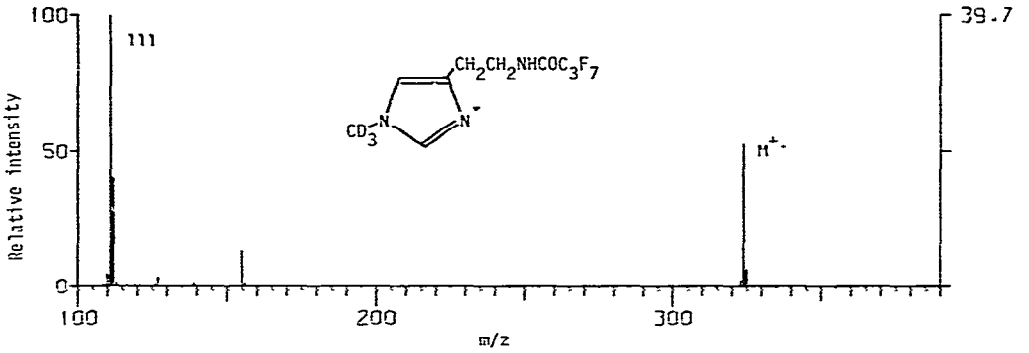

Fig. 1. Mass spectrum of the derivative of deuterated N^{τ}-methylhistamine.

a low background contribution from the internal standard is of value for analyses requiring high sensitivity. The quantification of N^{τ}-methylhistamine was performed by measuring the molecular ions at *m/z* 321 and 324 for the internal standard.

The histamine and N^{τ}-methylhistamine concentrations were determined from standard curves, constructed by plotting the peak height ratio of *m/z* 379/381 and *m/z* 321/324, respectively, against concentration. Linear standard curves were obtained in the concentration range 0.5—10 ng/ml for both compounds by adding known amounts to 1 ml of distilled water and treating the solutions as described above.

The detection limit was 20 pg of N^{τ}-methylhistamine, with a signal-to-noise ratio of 6:1. Higher sensitivity was found for N^{τ}-methylhistamine than for histamine.

The derivatives of histamine and N^{τ}-methylhistamine were purified by chromatography on CPG-10. Recently, Hashimoto and Miyazaki [11] reported a clean-up method for catecholamines with a CPG-10 column and stated that such CPG-10 column chromatography afforded some advantages over silica gel for purifying the biogenic amines. The CPG-10 column was also useful for the clean-up procedure of the derivatives of histamine and N^{τ}-methylhistamine. When the derivative of [^{3}H]histamine was chromatographed on CPG-10, 95.4 ± 8.2% (S.D.; n=5) of the radioactivity was eluted with ethyl acetate—ether. On the other hand, the recovery was 64.0 ± 13.0% (S.D.; n=5) by elution with methanol for ^{14}C-labeled MEHA-HFB. When the organic eluates from the CPG-10 column were combined and concentrated, a reduced sensitivity, which was considered to be due to decomposition of the derivative, was observed for the determination of histamine. Based on these results, after drying the solvents, each fraction re-dissolved in ethyl acetate was simultaneously drawn into a syringe and injected into the gas chromatograph—mass spectrometer. With the clean-up procedure described here, selected ion profiles free from any interfering peak were obtained for the plasma extracts. However, certain urine components which eluted with the ethyl acetate—ether from CPG-10 interfered with the determination of N^{τ}-methylhistamine. Thus, in the case of urine analysis, each residue must not be mixed before measurement. A selected-ion profile recorded during the analysis of a plasma extract is shown in Fig. 2.

The specificity of the proposed method was tested as follows. If compounds

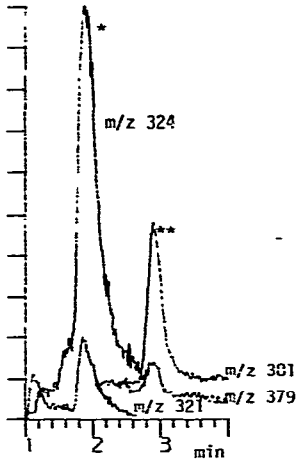

Fig. 2. Selected-ion profile obtained by analysis of a human plasma extract. * = N^{α}-Heptafluorobutyryl-N^{τ}-methylhistamine; ** = N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine.

other than histamine were present in the peak, the relative ion intensity would be different from the authentic histamine and the sample extracts. As shown in Table I, the intensity ratio at the molecular ion and the fragment ion resulting from elimination of the ethoxycarbonyl group in the authentic histamine was identical to that of histamine extracted from plasma and urine. This finding indicates that fragments from compounds other than histamine did not interfere with these ions, and so confirms the specificity of the determination of histamine. An attempt was made to assess the specificity for N^{τ}-methylhistamine determination in the same manner. However, since there are no proper fragment ions for this purpose in the higher mass region as shown in Fig. 1, the following experiment was performed. Since a very small amount of N^{τ}-methylhistamine was detected in plasma, 100 μl of urine were used instead of plasma as the sample for the experiment. An aliquot of the sample was analyzed by the procedure described under Experimental. A second aliquot of the same sample was extracted, derivatized with PFPAn in place of HFBAn, and the molecular ions at *m/z* 271 and 274 were monitored for unlabeled and labeled MEHA-PFP, respectively. If compounds other than N^{τ}-methylhistamine were not present in this peak, the values obtained by the two different methods would be consistent with each other. The measured values by derivatization with HFBAn were, in fact, consistent with those from the PFP-derivative (Table II), so the specificity of the determination of N^{τ}-methylhistamine was confirmed.

To estimate assay precision, histamine in amounts of 0.71 and 1.42 ng was

TABLE I

SPECIFICITY OF HISTAMINE DETERMINATION BY SELECTED ION MONITORING

Data are expressed as means ± S.D. (n=5).

	m/z 381/*m/z* 308	*m/z* 379/*m/z* 306
Authentic	1.17 ± 0.04	1.15 ± 0.03
Plasma	1.20 ± 0.11	1.22 ± 0.06
Urine	1.18 ± 0.15	1.16 ± 0.06

TABLE II

COMPARISON OF RESULTS WITH PENTAFLUOROPROPIONYL AND HEPTAFLUOROBUTYRYL DERIVATIVES OF N^{τ}-METHYLHISTAMINE FOR THE SAME URINE SAMPLE

The results, in ng/100 μl, were obtained by derivatization with heptafluorobutyric anhydride (for HFB) and pentafluoropropionic anhydride (for PFP).

Urine	HFB	PFP	HFB/PFP
A	10.6	11.4	0.93
B	23.1	24.0	0.96
C	22.3	22.1	1.01
			0.97 ± 0.04 (S.D.)

TABLE III

ACCURACY OF DETERMINATION OF HISTAMINE IN HUMAN PLASMA

Added (ng)	Found (ng/ml)		Recovery (%)
—	0.71 0.63 0.45	0.60 ± 0.13 (21.7)*	
0.71	1.34 1.16 1.34	1.28 ± 0.10 (7.8)*	95.8
1.42	2.05 2.32 2.40	2.26 ± 0.18 (8.0)*	116.9

*Coefficient of variation (%).

added to 1-ml aliquots of pooled plasma. The amount of histamine in the samples was measured by the present method. The results are shown in Table III. The histamine content of the pooled plasma measured in triplicate was 0.60 ± 0.13 ng/ml. The amounts of histamine added agreed well with the amounts of histamine measured. The recoveries of the 0.71 and 1.42 ng of histamine added to the plasma pool were found to be 95.8 and 116.9%, respectively. The precision of this method was within acceptable limits. To elucidate the pathogenesis of an asthmatic patient, it is important to trace the variance in the plasma histamine level. It was proved experimentally that an increase of 1 ng of histamine could be detected precisely by the present method.

Although values for the concentration of N^{τ}-methylhistamine in human urine have been reported by Fram and Green [12], no comparable report for the N^{τ}-methylhistamine levels in human plasma has yet appeared. Preliminary results for five normal plasma samples gave values of 0.83 ± 0.37 ng/ml for histamine. On the other hand, the N^{τ}-methylhistamine content was below the limits of this measurement in three of five subjects. The plasma of subjects A and E contained 1.41 and 0.59 ng/ml of N^{τ}-methylhistamine, respectively (Table IV).

Next, urine collected in the early morning was analyzed. It was found that the concentrations of histamine and N^{τ}-methylhistamine in the urine varied. A high

TABLE IV

CONTENT OF HISTAMINE AND N^{τ}-METHYLHISTAMINE IN HUMAN PLASMA AND URINE

Urine was collected in the early morning and the results are expressed in ng/ml.

Subject*	Sex	N^{τ}-Methylhistamine	Histamine
Plasma			
A	F	1.14	0.77
B	M	n.d.**	0.89
C	F	n.d.	1.43
D	F	n.d.	0.63
E	M	0.59	0.45
Urine			
E***	M	105.8	24.1
E***	M	222.9	42.0
F	M	230.8	48.4
G	F	68.9	22.5
H	M	99.0	733.0

*A—G, normal healthy subjects; H, patient with nephritis.
**n.d. = Not detectable.
***Collected on two consecutive days.

excretion of histamine, 733 ng/ml, was found in a patient with nephritis, but there was no concomitant increase in N^{τ}-methylhistamine.

The method described here has a high sensitivity, permits the determination of both histamine and N^{τ}-methylhistamine in the same sample, and is thus expected to facilitate the measurement of both amines in tissues.

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