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Note

Determination of histamine and methylhistamines by dansylation and its application to biological specimens

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Histamine is of interest as a putative neurotransmitter in the mammalian central nervous system[1]. In the brain, endogenous histamine is catabolized almost entirely by ring-N methylation[2,3] to form N^{τ}-methylhistamine**. A sensitive assay method for N^{τ}-methylhistamine is therefore indispensable for studying histamine metabolism in the brain. In spite of many attempts to separate and determine N-methylated histamines[5–7], lack of specificity and sensitivity makes it difficult to apply these methods to biological specimens, and so far no assay method has sufficient sensitivity to determine them in the brain.

We have already reported that a carboxylic acid type resin, Amberlite IRC-50 is useful for the chromatographic separation of 5-dimethylaminonaphthalene-1-sulphonyl (Dns; dansyl) derivatives of amino acids and amines[8,9]. In this report, we describe a chromatographic system for the complete separation of histamine, N^{τ}-methylhistamine and N^{π}-methylhistamine as their Dns derivatives. By this method, as little as 10 pmoles of the amines could be measured, and determination of the amines in whole mouse-brain was successful.

EXPERIMENTAL

Chemicals and reagents

Histamine diphosphate was obtained from Wako (Osaka, Japan) and N^{τ}-methylhistamine dihydrochloride from Calbiochem (La Jolla, Calif., U.S.A.); N^{π}-methylhistamine was a kind gift from Dr. T. Nakajima (Department of Neuropharmacology, Osaka University, School of Medicine). Dns chloride was purchased from Seikagaku Kogyo (Tokyo, Japan), and organic solvents and other chemicals were of the special grades for liquid chromatography or for use with amino acid analyzers, and were used without further purification. Redistilled water was used throughout.

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** The nomenclature for histamine derivatives is that proposed by Black and Ganellin[4].

Dansylation of histamine and methylhistamines

Solutions of 10 to 500 pmoles of histamine and the methylhistamines dissolved in 10 μl of 0.01 *M* hydrochloric acid were mixed with 100 μl of 0.5 *M* sodium carbonate and 50 μl of an acetone solution of Dns chloride (50 mg/ml), and several drops of acetone were added to the mixture for clarification. After incubation at 4° for 16 to 20 h in the dark, were acetone was removed from the mixture by a stream of nitrogen, and the Dns derivatives of amines were extracted into 5 ml of benzene. The benzene was separated and evaporated to dryness under a nitrogen stream; this procedure converted the derivatives of histamine and the methylhistamines into the sole N^{α}-mono-Dns derivatives[10] as described by Tamura *et al.*[11].

Preparation of biological specimens

A portion (50 to 500 mg) of whole mouse-brain was homogenized with 4.0 ml of ice-cold 3% perchloric acid in a glass homogenizer, and the mixture was centrifuged at 10,000 *g* for 30 min. To the deproteinized supernatant liquid was added 1.0 ml of 0.5 *M* sodium phosphate buffer of pH 6.5, and the pH of the solution was adjusted to 6.5 with 5 *M* potassium hydroxide. After being kept in an ice bath for *ca.* 2 h, the precipitate was removed by brief centrifugation.

Histamine and the methylhistamines in the supernatant solution were purified by two-step column chromatography. The whole supernatant was applied to a column (9.5 × 0.4 cm) of Amberlite CG-50 type II resin (Na^+) that had been equilibrated with 0.2 *M* sodium phosphate buffer of pH 6.5. After the column had been washed with 10 ml of water and 10 ml of 0.1 *M* hydrochloric acid, the amines were eluted with 3.0 ml of 0.5 *M* hydrochloric acid ("amine fraction"). This "amine fraction" contained histamine, the methylhistamines and such other amines as 5-hydroxytryptamine, tyramine, dopamine and various polyamines. To separate histamine and the methylhistamines from other amines, the "amine fraction" was applied to a column (5.0 × 0.4 cm) of Dowex 50W-X8 (Na^+) that had been equilibrated with 0.2 *M* sodium phosphate buffer of pH 6.5. After the column had been washed with 10 ml of 2 *M* hydrochloric acid, histamine and the methylhistamines were eluted with 5.0 ml of 3 *M* hydrochloric acid ("HA fraction"). This "HA fraction" was evaporated to dryness under reduced pressure and dansylated as described above.

Chromatography of Dns-histamine and Dns-methylhistamines

Column chromatography of the Dns derivatives was performed according to the method of Seki and Wada[8], with slight modifications. Amberlite IRC-50 (Na^+) (particle size 40 to 50 μm) was packed into a chromatographic tube (10 × 0.8 cm) and two tubes were connected in series to reduce the flow resistance. The eluent was 0.1 *M* sodium citrate buffer of pH 5.6–tetrahydrofuran–ethyl methyl ketone–acetone–methanol–2,2′-thiodiethanol (12:1:2:3:2:0.05, by vol.). The eluent was pumped into the column at a flow-rate of 14 ml/h using a JEOL constant-flow pump (Model P-2705S-A). The column temperature was maintained at 42° by means of a Tamson circulator (model TX3). The eluate from the column was monitored with a JEOL fluorimeter (model JLC-FL) (excitation wavelength 230–400 nm, emission wavelength 410–800 nm) equipped with a recorder.

RESULTS AND DISCUSSION

As shown in Fig. 1, the Dns derivatives of N$^{\tau}$-methylhistamine, N$^{\pi}$-methylhistamine and histamine were completely separated in this system, their elution times being 110, 135 and 170 min, respectively; a calibration curve for each amine is shown in Fig. 2. There was a linear relationship between the amount of each amine and the fluorescence intensity, and as little as 10 pmoles of each amine could be assayed precisely and reproducibly. This improvement in sensitivity as compared with our previous method[10] was achieved by using a highly sensitive and stable fluorimeter.

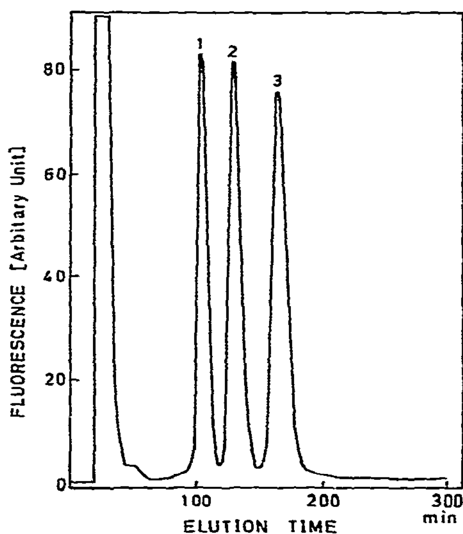

Fig. 1. Elution pattern of Dns derivatives of histamine and methylhistamines. 1 = Dns-N$^{\tau}$-methylhistamine (400 pmoles); 2 = Dns-N$^{\pi}$-methylhistamine (400 pmoles); 3 = Dns-histamine (400 pmoles). For chromatographic conditions, see text.

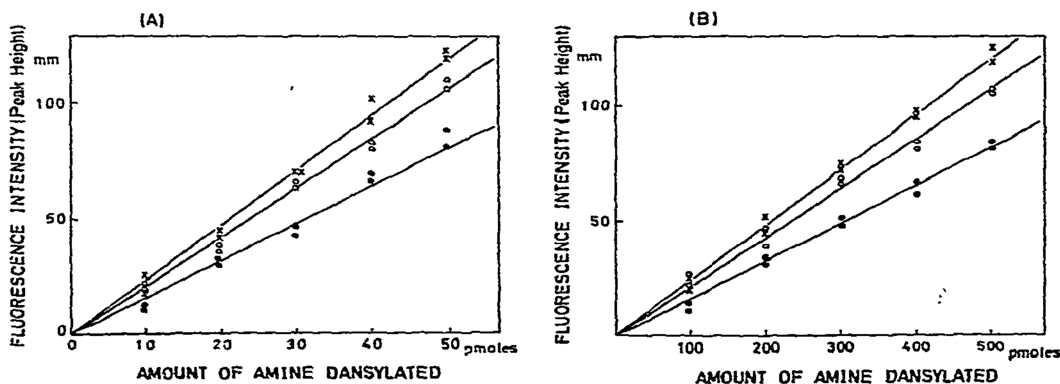

Fig. 2. Calibration graphs for histamine and methylhistamines: (A) standards (0–50 pmoles); (B) standards (0–500 pmoles). Fluorescence intensity is expressed in terms of peak height in mm. ● = histamine; ○ = N$^{\pi}$-methylhistamine; × = N$^{\tau}$-methylhistamine.

Fig. 3 shows the typical elution pattern of the dansylated "HA fraction" of a whole mouse-brain. Peaks for histamine (3) and N^{τ}-methylhistamine (1) were observed, but there was no peak at the elution time (135 min) corresponding to N^{π}-methylhistamine. These peaks were confirmed by the addition of authentic samples to the dansylated "HA fraction". A relatively high peak (shown by an asterisk) was present in all the samples tested; it had an elution time of 150 min and has not yet been identified.

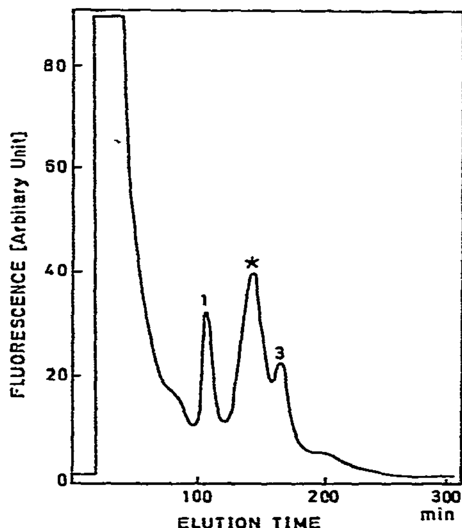

Fig. 3. Typical elution pattern of dansylated "HA fraction" of 142 mg of the whole brain of a mouse (No. 2 in Table I). 1 = N^{τ}-Methylhistamine (112 pmoles); * = unidentified peak; 3 = histamine (51 pmoles). For sample preparation and chromatographic conditions, see text.

When 23,300 dpm of ^{14}C-histamine were added to brain homogenate before the analysis, 15,830 dpm of radioactivity were recovered from the column in the Dns-histamine peak; so, the recovery through the whole procedure was calculated as being 67.9%.

Table I summarizes the amounts of histamine and N^{τ}-methylhistamine in whole mouse-brain (five male mice were used). On average, whole mouse-brain contained (per g of wet tissue) 0.31 nmole of histamine and 0.72 nmole of N^{τ}-methyl-

TABLE I

HISTAMINE AND N^{τ}-METHYLHISTAMINE CONTENTS IN WHOLE MOUSE-BRAIN

Mouse No.	*Histamine (nmole/g)*	*N^{τ}-Methylhistamine (nmole/g)*
1	0.30	0.69
2	0.36	0.79
3	0.36	0.70
4	0.23	0.64
5	0.28	0.79
Mean ± S.D.	0.31 ± 0.05	0.72 ± 0.06

histamine. The histamine content of whole mouse-brain determined by this method is in good agreement with that obtained by the enzymic method of Taylor and Snyder[12] (0.43 ± 0.02 nmole per g of wet tissue).

We are currently using the method described here for studying the metabolism of endogenous histamine.

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