

A RAPID, simple and low-cost assay method of histamine-*N*-methyltransferase activity was developed. Methylhistamine, which was separated from the enzymatic reaction system on reversed-phase high-performance liquid chromatography using an ion-paired chromatographic technique, was detected spectrophotometrically at 226 nm. The mobile phase used for the separation of methylhistamine was 0.05 M NH₄H₂PO₄ (pH 3.0) containing 2 mM of sodium octanesulfonate. The new assay technique could detect methylhistamine as an enzyme activity product of histamine-*N*-methyltransferase in the brain and kidney of rats. Chlorpheniramine maleate, an antihistamine, activated the histamine-*N*-methyltransferase. Whether neurotransmitter or neuromodulator, the role of histamine in the brain has not yet been made clear. Therefore, the present method could be applicable for the enzymatic investigation of histamine metabolism in central nervous system or inflammatory reactions.

Key words: Histamine-*N*-methyltransferase, HPLC-UV detection, Histamine, Brain, Methylhistamine

Rapid and simple determination of histamine-*N*-methyl transferase activity by high-performance liquid chromatography with UV detection

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Introduction

Histamine-*N*-methyltransferase (EC 2.1.1.8) is shown to be a histamine-metabolizing enzyme with diamine oxidase (histaminase).^{1,2} Histamine-*N*-methyltransferase can catalyze the methylation of histamine to methylhistamine in the presence of *S*-adenosylmethionine.

The histamine-*N*-methyltransferase activity is measured by the isotope method,³ or by fluorometric detection using an *o*-phthalaldehyde complex with methylhistamine and high-performance liquid chromatography (HPLC) equipped with a fluorometric detector.^{4,5} The isotope method is highly sensitive and specific but the method is expensive to operate. On the contrary, the HPLC fluorometric detection technique is less sensitive because the fluorescence intensity of methylhistamine is lower than that of histamine. Correcting the disadvantage, Granerus and Wass⁶ managed to detect methylhistamine at less than 1 ng/ml (0.1 ng/injection) by improvement of the analysis system. In these procedures,^{4–6} however, it is necessary for methylhistamine to be derivatized using *o*-phthalaldehyde (OPA) and the procedure is very complicated.

So far, the present authors have investigated rapid and specific assays of various enzymatic activities on HPLC.⁷ In the present study, a rapid, simple and low-cost assay method of histamine-*N*-methyltransferase activity has been developed. Therefore, methylhistamine separated from the enzymatic reaction system on reversed-phase HPLC was detected and assayed spectrophotometrically at 226 nm. The new assay method also was applicable for the determination of histamine-*N*-methyltransferase activity in the brain and kidney of rats.

Materials and methods

Reagents and systems

All chemicals were of analytical grade or reagent grade, and were obtained from Sigma Chem. Co. (St. Louis, MO, USA) and from Wako Pure Chem. Co. (Tokyo, Japan). The HPLC column used was filled with Nucleosil-phenyl (7 μm, 4.0 × 200 mm) from SENSYU SCIENTIFIC Co. (Tokyo, Japan). The Extrelute-1 of the extraction tube was obtained from Cica-Merck (Kanto Kagaku Co., Tokyo, Japan) and the HPLC system used a SSC-3110 (SENSYU SCIENTIFIC Co., Tokyo, Japan).

Determination of histamine-*N*-methyltransferase activity by the HPLC-ultraviolet method

*Optimal conditions for histamine-*N*-methyltransferase assay*

Methylhistamine produced after enzymatic reaction was separated from histamine and *S*-adenosylmethionine as the substrate on HPLC using a reversed-phase column, Nucleosil-phenyl. Ion-paired chromatography based 0.05 M NH₄H₂PO₄ (pH 3.0) containing sodium octanesulfonate was used for the separation of methylhistamine. The flow rate of the eluent was 1.0 ml/min at ambient temperature. Methylhistamine was detected with an ultraviolet (UV) detector at 226 nm.

Enzyme source

Histamine-*N*-methyltransferase activities in rat brain and kidney were obtained as follows. The homogenate of excised rat brain and kidney, with five times the volumes of phosphate-buffered saline (pH 7.4) to wet weights, was centrifuged at 18,000 × *g* for 20 min at 4°C. The supernatant was used as the enzyme specimens in the present investigation.

Enzymatic reaction

Incubation mixture consisted of histamine-*N*-methyltransferase specimens (0.6 ml of supernatant in the case of the brain or 0.1 ml of supernatant in the case of the kidney, and 0.1 M sodium phosphate buffer (pH 7.4) 0.5 ml), 0.1 ml of 1 mM *S*-adenosylmethionine and 0.1 ml of 0.25 mM histamine. The reaction mixture was incubated for 60 min at 37°C and then stopped with 0.2 ml of 2.5 M sodium hydroxide.

Extraction of methylhistamine

Methylhistamine was extracted from the incubation mixture with the following procedure. The reaction mixture that was made up to 1 ml in total with 2.5 M sodium hydroxide was adsorbed on Extrelute-1 for 5 min at ambient temperature. Methylhistamine, as the product of enzyme reaction charged on Extrelute-1, was eluted with various organic solvents to extract selectively. The solvent of the elute containing methylhistamine was removed to dryness, and the residue was resuspended in the mobile phase and HPLC separation and determination were performed.

Correlation between the isotope method and the HPLC-UV method

The isotopic assay was performed following the method reported by Beaven and Horakova.³

Results and discussion

Separation of methylhistamine from the substrates on HPLC

Methylhistamine, histamine and *S*-adenosylmethionine were separated on reversed-phase HPLC column 0.05 M NH₄H₂PO₄ (pH 3.0) containing sodium octanesulfonate of various concentrations. Methylhistamine and histamine were not sufficiently retained on Nucleosil-phenyl column when the mobile phase was composed only of 0.05 M NH₄H₂PO₄ (pH 3.0). Therefore, sodium octanesulfonate was added to the mobile phase as the counter ion. It was found that 0.05 M NH₄H₂PO₄ (pH 3.0) containing 2 mM of sodium octanesulfonate was the best solvent for the present purpose.

Detection of methylhistamine after the separation on HPLC

The spectral absorption maximum of methylhistamine and histamine is at OD 226 nm and can be detected by a UV detector. Therefore, for the present purpose of quantitative detection of methylhistamine after separation on HPLC, UV 226 nm was found to be satisfactory.

Calibration of methylhistamine levels by HPLC

Using the HPLC conditions already described, a linear calibration for methylhistamine was obtained from 0.6 to 10.0 ng/injection using 0.001 AUFS of detection range. The detection limit was 0.6 ng/injection (4.8 pmol/injection) as methylhistamine and was comparable with the HPLC fluorometry method reported by Tsuruta *et al.*⁴ Fukuda *et al.*⁵ also reported that the detection limit of methylhistamine/OPA derivatives was 50 pmol (6.25 ng) per injection. Furthermore, Granerus and Wass⁶ developed a more sensitive procedure for methylhistamine detection, but the detection limit was approximately 0.1 ng/injection nevertheless. Generally, histamine/OPA derivatives have stronger fluorescence intensity (> 1000-fold) than methylhistamine/OPA ones. It is thought this is due to the structures.

Optimum conditions for the histamine-*N*-methyltransferase activity assay

The incubation conditions for the histamine-*N*-methyltransferase were performed according to the method described by Beaven and Horakova³ and Tsuruta *et al.*⁴ The enzymatic reaction was linear up to 120 min of incubation. Therefore, the conditions for histamine-*N*-methyltransferase activity determination of the HPLC-UV method were the following: 0.1–0.6 ml of specimens, 0.1 ml of 1 mM

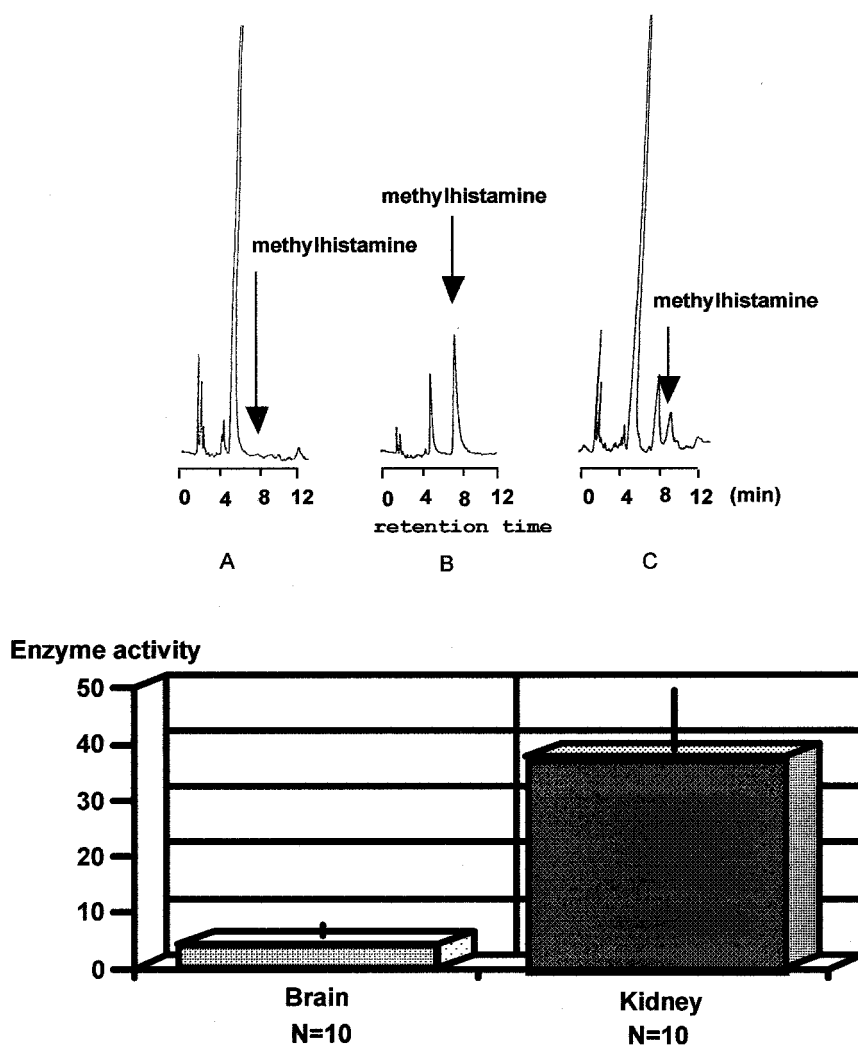


FIG. 1. Typical chromatogram of methylhistamine (upper part) and histamine-*N*-methyltransferase activity in rat brain and kidney (lower part). Upper part: (A) substrate blank, (B) standard of methylhistamine, and (C) rat kidney. Lower part: enzyme activity on the vertical axis shows histamine-*N*-methyltransferase activity as nmol/h/mg protein. Each column represents the mean \pm SD.

S-adenosylmethionine and 0.1 ml of 0.25 mM histamine were mixed and adjusted to 0.8 ml with 0.1 M sodium phosphate buffer (pH 7.4). The mixture was incubated for 120 min at 37°C and then terminated with 0.2 ml of 2.5 M sodium hydroxide. The methylhistamine, which was the reaction product, was purified from the substrate.

Correlation between the present technique and enzyme isotopic assay

The methylhistamine peak on the HPLC chromatogram increased with incubation time of these enzyme mixture at 37°C. The peak, which was suggested to be methylhistamine, was identified by the retention time as well as mass spectrometry spectrum analysis of the elute fraction. The correlation between the HPLC-UV method and the isotope method was satisfactory ($r = 0.912$; $p < 0.01$).

Extraction of methylhistamine with Extrelute-1

The recovery of methylhistamine by Extrelute-1 was determined using various organic solvents; 59.1% of the best recovery was obtained using chloroform. The chloroform layer containing methylhistamine was evaporated to dryness or re-extracted with a small portions of 0.1 N HCl. The former method needs the concentration of the chloroform layer but no interfering peaks did appear, and the time required for one run was less than 10 min. The latter method did not alter the recovery of methylhistamine, but gave many interfering peaks on the HPLC chromatogram, and the elution of all peaks required more than 20 min. It was found that the combination of Extrelute-1 and chloroform extraction was the best procedure for methylhistamine detection in the incubation mixture.

Beaven and Horakova³ washed the chloroform layer with alkaline solution to decrease the blank levels, or

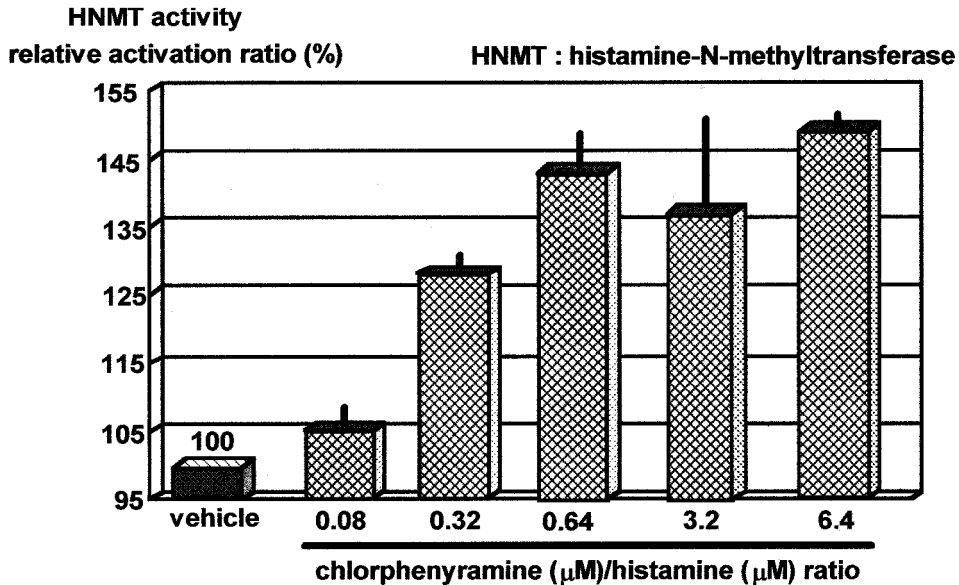


FIG. 2. Activation of histamine-*N*-methyltransferase activity in rat brain by chlorpheniramine maleate as an antihistamine. The vertical axis represents the relative activation ratio compared with value obtained without antihistamines; the horizontal axis represents the chlorpheniramine/histamine ratio.

extracted methylhistamine with toluene/iso-amylalcohol = 1/1. Tayler and Snyder⁸ separated methylhistamine from the incubation mixture by paper chromatography. The present method, however, separated methylhistamine in the chloroform extracts on HPLC and then detected it at OD 226 nm. The blank levels did not increase as in the case of the isotope method. Conditions for evaporating the chloroform layer containing methylhistamine were examined by three methods. Recovery of methylhistamine was 59.1% when chloroform was removed under reduced pressure, it was 42.6% by overnight standing at ambient temperature, and was 33.3% by removal of chloroform on a heating block at 60°C. The coefficients of variation were ± 10.2 , ± 10.2 and $\pm 12.3\%$, respectively. Using chloroform, Extrelute-1 and chloroform drying under reduced pressure was found to be the best procedure for methylhistamine extraction.

Application of the method for detection of histamine-*N*-methyltransferase activity in biological specimens

*Content of histamine-*N*-methyltransferase in biological specimens*

The present method was used for the measurement of histamine-*N*-methyltransferase activity in the kidney and brain of rats. The typical chromatograms are shown in Fig. 1. The levels of histamine-*N*-methyltransferase activity could be specifically determined, as shown in Fig. 2. The data obtained were the same as the levels reported by Tayler and Snyder⁸ or Brown *et al.*⁹

*Influence of antihistamines on histamine-*N*-methyltransferase activity*

The histamine-*N*-methyltransferase activity was activated by various concentrations of chlorpheniramine maleate, an antihistamine. The stimulation of histamine-*N*-methyltransferase activity was dependent on the molecular ratio between histamine and antihistamine. Tayler and Snyder and other authors¹⁰⁻¹² have also reported similar results. The histamine-*N*-methyltransferase activity in rat kidney and brain, and its activation by an antihistamine, is shown in Fig. 2. It is known that administration of antihistamines induces central nervous system (CNS) depression in an early stage but the CNS depression is recovered with routinely administration. The tolerance of CNS depression with continuous administration of antihistamines may be caused by activation and/or induction of histamine-*N*-methyltransferase in the brain. New antihistamines that suppress CNS depression may influence not only BBB function, but may also enzymatic activity of histamine-*N*-methyltransferase. Whether neurotransmitter or neuromodulator, the role of histamine in the brain has not yet been made clear. Therefore, the present method may be useful for the enzymatic investigation of histamine metabolism.

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