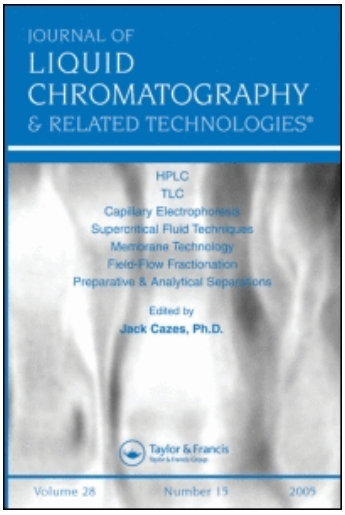

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Application of a Novel Technique: The Determination of Histamine Content of Urine, Brain, and Cerebral Spinal Fluid

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APPLICATION OF A NOVEL TECHNIQUE: THE DETERMINATION OF HISTAMINE CONTENT OF URINE, BRAIN, AND CEREBRAL SPINAL FLUID

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ABSTRACT

The application of a novel high performance liquid chromatographic (HPLC) technique for the determination of histamine in urine, cerebral spinal fluid (CSF), and brain tissue from rat is been reported. The determination of histamine in these biological samples was accomplished by injecting the samples into a Dionex Bio-HPLC system. Using this method 780 pmols/5 μl, 25.8 pmols/5 μl, 819 pmoles/mg of tissue/5 μl of histamine in urine, CSF, and brain tissue, respectively, were detected. The retention time for histamine is less than 5 minutes. This reported method proves to be suitable for the quantitation of histamine in urine, cerebral spinal fluid, and brain tissue.

INTRODUCTION

For the last decade, interest has been focused on the development of a HPLC method suitable for determining histamine in biological samples [1-3]. Of the various methods reported for the determination of histamine, many of these methods were applied only for the determination of histamine in plasma samples [4-13].

We recently reported a modified HPLC-EC method for a qualitative and quantitative determination of histamine in small plasma volume which prove to be more sensitive than other reported methods [14-18]. Because of the sensitivity and reproducibility of our reported method in quantitating histamine in small plasma volume, we investigated the use of this method in determining histamine in urine, CSF, and brain tissue from rats. The results suggest that the reported method is also applicable for determining histamine in these three biological samples.

Methods and Materials

Reagents

Histamine dihydrochloride and methylhistamine were purchased from Sigma Chemical Co., St. Louis,

MO. All other chemicals were "HPLC grade" or reagent grade and were obtained from commercial sources.

Sample Preparation

A. Urine

Urine samples were collected from Sprague Dawley rats and histamine was quantitated by adding 200 pmols of methylhistamine, the internal standard, to 500 μl of urine in a 1.5ml heparinized microcentrifuge tube. The samples were centrifuged (20,000 RPM) for 15 min at 4°C. The supernatant from the samples were removed and diluted with phosphate buffer to make a 1:99 (v/v) dilution. This mixture was then vortexed for approximately 30 seconds. After vortexing, the samples were heated in boiling water for 3-4 minutes to denature protein in the urine. The boiled mixtures were centrifuged for 10 minutes to separate the protein residue from the samples. The supernatant from the preparation was removed and stored on ice until injected into the HPLC for histamine determination.

B. CSF

CSF samples were obtained according to the

method of Rezvani et al. [19,20]. According to this technique, 20 μl of dialysate (CSF) was collected from the cerebral cortex by microdialysis from Sprague Dawley rats in a CM/100 refrigerated fraction collector (Carnegie Medicin). To these 20 μl samples, 200 pmols of methylhistamine was added and 5 μl was injected into the HPLC for histamine determination.

C. Brain tissue

Whole brain was removed from Sprague Dawley rats and weighed. The brain tissue was homogenized in 0.4N perchloric acid 10X the volume of weight which contained methylhistamine, the internal standard. The homogenate was centrifuged at 20,000 rpm for 20 minutes at 4 °C. The supernatant (1 ml) was removed and, to the supernatant, 100 μl of concentrated KOH was added to neutralize the PCA. The samples were then vortexed and centrifuged again at 20,000 rpm for 2 min. The supernatant was removed and heated in boiling water for 4 mins to precipitate proteins. The supernatant was then diluted with phosphate buffer as needed for injection into the HPLC for the determination of histamine.

Instrumentation

The determination of histamine was accomplished by using a Dionex BioLC Instrument with a quaternary gradient pump and a pulsed amperometric detector which selectively determines histamine at the picomole levels. The potential voltage was set at +1.05 volts with an output range of 30nA throughout the experiments. The basic chromatographic module contained a metal-free, high pressure injector. The column used for detection of histamine was a stainless steel C-18 Zorbax ODS 4.5mm ID x 25cm (5 microns).

The analytical mobile phase was a phosphate buffer which consisted of 0.12M NaH_2PO_4, 0.1M NaOH, 19μM of Lauryl Sulfate and 50% methanol:water (79:21, v/v). The pH was adjusted to 5.6. This solution was filtered under vacuum through a 0.45 micron millipore filter prior to use.

Standard Curve

According to the procedure previously reported [14], the standard curve was derived with a range of concentrations from 1.2 ng/μl-0.075 ng/μl. To construct the standard curve, 10 μl

portions of each histamine standard were injected into the HPLC.

RESULTS AND DISCUSSION

We have previously described a rapid and sensitive method for the determination of histamine in small plasma volume [14, 18]. The reproducibility of this method was demonstrated by consistent retention times for the detection of histamine when samples were injected into the HPLC. The results presented in this paper show that the method can also be used for the determination of histamine in rat urine, CSF, and brain tissue.

A plot of peak heights for a series of standard solutions of histamine detected at 30 nA was constructed. Good linearity was observed between 0.075 ng/μl-1.2 ng/μl and the linear regression analysis of data points showed a linear relationship with an excellent correlation coefficient ($r=0.955$).

Figure 1 shows chromatograms of the separation of (A) standard histamine and methyl-histamine, and (B) histamine and methlyhistamine

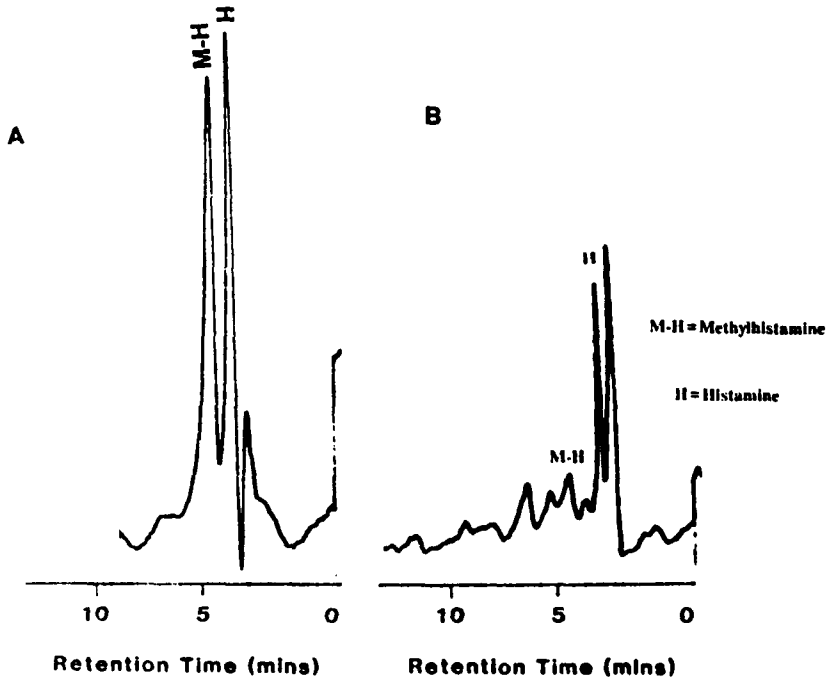

Figure 1. HPLC chromatograms (A) of standard histamine and methylhistamine and (B) histamine detected in urine samples obtained from rat. The chromatographic conditions: 79:21 (0.1 M phosphate buffer and 50% methanol and H_2O) as mobile phase with a flow rate of 0.6 ml/min. The detector was set at 30nA.

in rat urine. Five microliters of supernatant from rat urine was injected directly into the HPLC and 780 pmols of histamine was quantitated. Histamine in the urine samples was confirmed by adding increasing amounts of standard histamine to each sample and injecting 10 μl of each into the HPLC,

there by increasing the suspected histamine peak height. Figure 2 is a representative chromatogram of CSF injected into the HPLC for histamine determination. Determining the histamine content in CSF presented less of a problem than in urine and brain samples because with the use of a microdialysis probe to collect samples, many of the interfering proteins were removed. In a 5 μl injection of CSF, as much as 25.8 pmols of histamine were quantitated. Figure 3 is a representative chromatogram of supernatant from rat brain tissue injected into the HPLC for the determination of histamine. The clean-up of these samples was necessary in order to obtain detection of histamine and methylhistamine from other detectable substances in the samples. When 5 μl of supernatant from brain tissue was injected into the HPLC, as much as 819 pmols/mg tissue of histamine were quantitated.

The retention time for histamine during the analysis of urine, CSF and brain tissue was less than 5 minutes which was comfirmed on the day of an experiment by injecting standards of histamine into the HPLC. The separation of histamine from

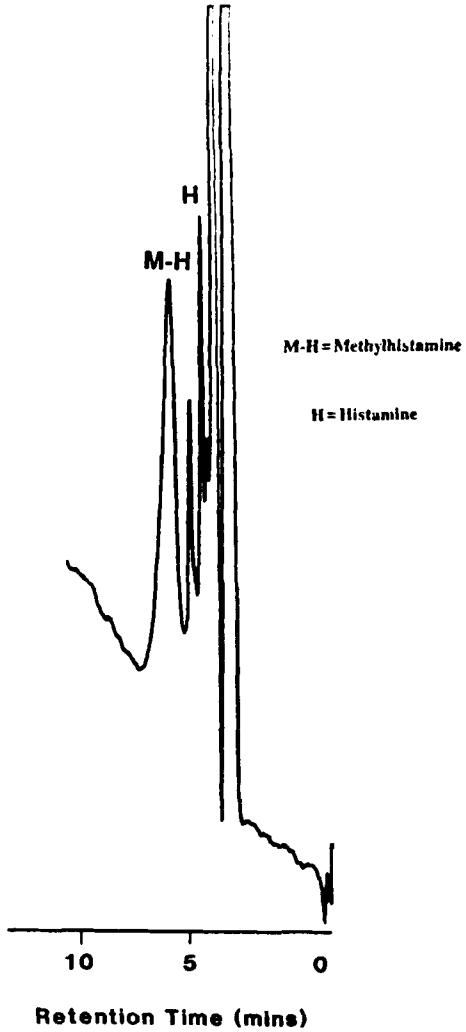

Figure 2. HPLC chromatogram of histamine detected in CSF obtained from rat. The chromatographic conditions: 79:21 (0.1 M phosphate buffer and 50% methanol and H_2O) as mobile phase with a flow rate of 0.6 ml/min. The detector was set at 30nA.

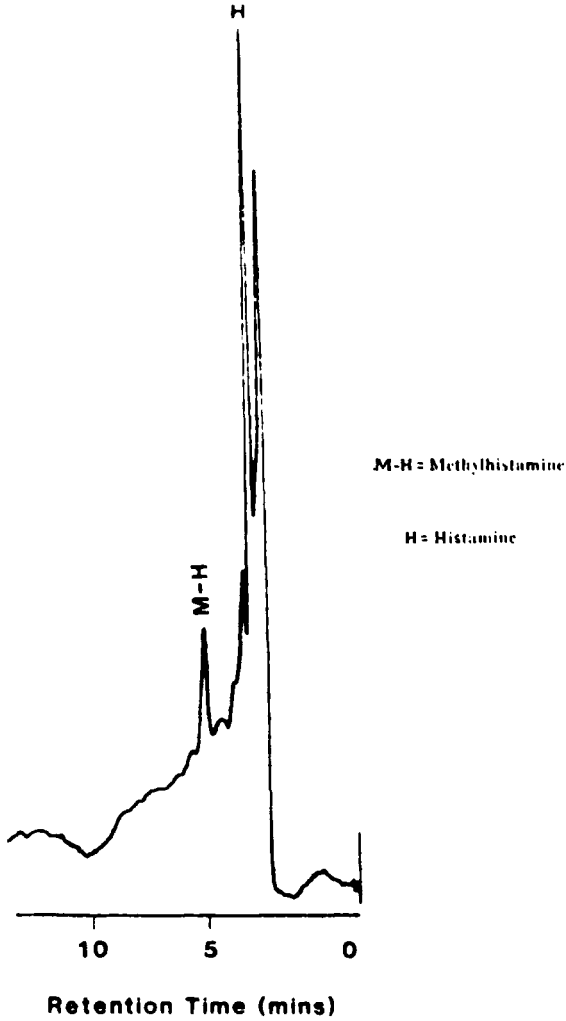

Figure 3. HPLC chromatogram of histamine detected in rat brain. The chromatographic conditions: 79:21 (0.1 M phosphate buffer and 50% methanol and H_2O) as mobile phase with a flow rate of 0.6 ml/min. The detector was set at 30nA.

other substances in these samples was adequate with the use of this procedure and histamine could be quantified in all three biological samples. These results show clearly that this method is applicable for the determination of histamine in urine, CSF, and brain tissue. The method is simple, selective and sensitive for the routine analysis of histamine.

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REFERENCES

1. Tsuruta, Y., Kohashi K., and Ohkura, Y., Determination of histamine in plasma by high-speed liquid chromatography, J. Chromatography, 146,490-493, 1978.

2. Leroy, P., Nicolas, A., and Moreau, A., Electrochemical detection of sympathomimetic drugs, following pre-column o-phthalaldehyde derivatization and reversed-phase high performance liquid chromatography, J. Chromatogr., 282,561-569, 1983.

3. Harsing, L.G., Jr., Nagashima, H., Vizi, E.S., Duncalf, D., Vizi, E. and Goldiner, P., Determination of histamine concentrations in plasma by liquid chromatography/electrochemistry, Clinical Chemistry., 32,1823-1827, 1986.

4. Adam, H.M., Hardwick, H.T., and Spencer, K.E.V., A method of estimating histamine in plasma, Br. J. Pharmacol. 12,397-405, 1975.

5. Noah, J.W., and Brand, A., A fluorimetric method to determine the levels of histamine in human plasma, J. Allergy, 32,236-242, 1961.

6. Graham, H., Scarpellini, J.A.D., Hubka, B.P., and Lowry, O.H., Measurement of the normal range of free histamine in human blood plasma, Biochem. Pharmacol., 17,2271-2280, 1968.

7. Lorenz W., Reimann, H.J., and Barth, H., A sensitive and specific method for the determination of histamine in human whole blood and plasma, Hoppe- Seyler's Z Physiol. Chem., 353,911-920, 1972.

8. Lorenz, W., Schoning, B., Schwarz, B., and Neugebauer, E., Increase in sensitivity of the fluorometric plasma histamine determination: routine assay in the femtomol range, Naunyn-Schmiedeberg's Arch. Pharmacol. Suppl., 302,363, 1978.

9. Miller, R.L., McCord, C., Sanda, M., Bourne, H.R., and Melmon, K.L., Application of the enzymatic double isotope dilution assay for the study of histamine in plasma, J. Pharmacol. Exp. Ther., 175,228-234 1970.

10. Bruce, C., Taylor, W.H., and Westwood, A., An improved radioenzymatic assay for histamine in human plasma, whole blood, urine and gastric juice, Ann Clin. Biochem., 16,259-264, 1979.

11. Brown, M.J., Ind, P.W.., Barnes, P.J., Jenner, D.A., and Dollery, C.T., A sensitive and specific radiometric method for the measurement of plasma histamine in normal individuals, Anal. Biochem., 109,142-146, 1980.

12. Morel, A.M., and Delaage, M.A., Immunoanalysis of histamine through a novel chemical derivatization, J. Allergy Clin. Immunol., 82, 646-654, 1988.

13. Keyzer, J.J., Wolthers, B.G., Muskiet, F.A.J., Breukelman, H., Kauffman, H.F., and 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.

14. Washington, B., Smith, M.O., Robinson, T.J., Olubadewo, J.O. and Ochillo, R.F., A rapid quantitative and qualitative method for histamine in small plasma volume. J. Liq. Chromatogr., 14(11), 2189-2200, 1991.

15. Washington, B., Smith, M.O., Robinson, T.J., Olubadewo, J.O. and Ochillo, R.F., A novel rapid qualitative microassay of underivatized histamine in small plasma volume, Europ. J. Pharmacol., Abstract, 183: 707, 1990.

16. Smith, M.O., Washington, B., Robinson, T.J., and Ochillo, R.F., Does stress alter plasma catecholamine and histamine levels in Sprague Dawley, Rats, Southeastern Pharmacology Society, (SEPS) Abstract #12, 1989.

17. Ochillo, R.F., Smith, M.O., Tsai, C.S., Washington, B., Robinson, T.J and Olubadewo, J.O., Pharmacological basis of stress-induced hypotension, Europ. J. Pharmacol., Abstract, 183: 862, 1990.

18. Washington, B., Smith, M.O., Robinson, T.J., and Ochillo, R.F., Selection of a suitable solvent system for the separation and quantitation of histamine, J. Liq. Chromatogr., 14(7), 1417-1424, 1991.

19. Rezvani, A.H., Beleslin, D.B. and Meyers, R. D., Neuroanatomical mapping of hypothalamic regions mediating verapamil hyper- and hypothermia in the cat. Brain Res. Bull. 17, 249-254, 1986.

20. Rezvani, A.H., MavManus, K.T., and Myers, R.D., Rate of In-vivo Verapamil Exchange within the hypothalamus of the Cat as examined by push-pull perfusion, Neurochem. Res 11 1643-1651, 1986.

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