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Note

Simultaneous determination of histidine-containing dipeptides, histamine, methylhistamine and histidine by high-performance liquid chromatography

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Carnosine (β-alanylhistidine) appears to play an important role as a reservoir pool for histidine in the synthesis of histamine [1,2]. The determination of carnosine has previously been carried out either by cation-exchange chromatography, which was time-consuming (130 min [3]), or by colorimetry, which suffers from interfering substances [4]. Seiler et al. [5] have recently published a rapid high-performance liquid chromatographic (HPLC) procedure for carnosine, but presented no data for its application to various tissues. Several methods are available for histamine determination in tissues and body fluids. The fluorimetric assay for histamine in tissues developed by Shore et al. [6], or its modification, has been extensively used in the past. This method is sensitive but not sufficiently selective and reproducible. The enzymatic–radioisotope method [7], on the other hand, detects lower levels of histamine than the fluorimetric method, however it is time-consuming, relatively expensive and only one compound, histamine, can be measured.

The introduction of HPLC has improved the sensitivity of histamine assays but significant limitations remain. Current HPLC methods usually determine

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only histamine and one metabolite (3-methylhistamine), often involve complex sample preparation and several have a tendency to limit column usage and lifespan by operating at the extremes of pH [8–11].

There is an important need for the rapid and sensitive quantification of carnosine, histidine, as well as histamine and its methylated degradation products in the same tissue sample. We now describe such an HPLC method. The procedure is simple, rapid, economical, sensitive and selective. Successful analyses have been carried out on muscle, brain, stomach and heart tissue of growing rats.

EXPERIMENTAL

Reagents

HPLC-grade methanol, acetonitrile and reagent-grade triethylamine were obtained from Fisher Scientific (Springfield, NJ, U.S.A.), histidine, histamine, carnosine and 2-mercaptoethanol from Sigma (St. Louis, MO, U.S.A.), 3-methylhistamine and 1-methylhistamine from Boehring Diagnostics (San Diego, CA, U.S.A.) and *o*-phthalaldehyde (OPA), 0.8 mg/ml in 1 *M* potassium borate buffer, pH 10.4, containing Brij 35 and mercaptoethanol from Pierce (Rockford, IL, U.S.A.).

Instrumentation

A Waters Assoc. (Milford, MA, U.S.A.) HPLC system, equipped with the following components was used: a Waters 840 data and chromatography control station, a Wisp Model 712 sample processor, a Model 510 solvent delivery system and a Kratos Spectroflow 980 fluorescence detector. Recording was done with a Digital LA 50 printer.

The analytical column used was a 5-μm Ultrasphere ODS high-resolution end-capped column (250 mm$\times$4.6 mm) from Beckman Instruments (Somerset, NJ, U.S.A.).

Chromatography

Solvent A, a 0.06 *M* solution of potassium phosphate monobasic, was prepared using HPLC-grade water. In turn, this was used to prepare a solution of 0.4% triethylamine. Solvent B was methanol–acetonitrile (60:40, v/v). The mobile phase composition, 50% B in A, was degassed and the pH adjusted to 6.4. The flow-rate was 0.7 ml/min. The column temperature was ambient. Detection wavelengths were set at 310 nm for excitation and 375 nm for emission.

Derivatization procedure

To 10 μl prepared, filtered sample mixture, 45 μl reagent buffer and 45 μl OPA reagent were added, allowed to stand at room temperature for 1 min and then a 10-μl sample was injected.

Reaction buffer

Reaction buffer was prepared by mixing 5 ml of borate buffer (0.5 *M* sodium tetraborate), 5 ml of methanol and 0.1 ml of 0.5% 2-mercaptoethanol in methanol.

Preparation of tissue extracts

Male Sprague–Dawley rats (body mass 100–110 g) were killed by decapitation. Hindleg muscle, whole brain, stomach and heart were excised (70–100 mg each), washed, minced and homogenized in 1.5 ml of ice-cold 0.3 *M* perchloric acid. However, samples weighing as little as 10 mg can be prepared and analyzed by this procedure. Polyethylene 13-ml test tubes, covered with marbles, were placed in a boiling water bath for 5 min followed by centrifugation (Sorvall) at 11 360 *g* at 0°C for 10 min. The supernatant was then filtered through a 0.2-μm filter and the pH adjusted to 6.4 with 33% sodium hydroxide.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms for standards and tissue extracts for histidine, histamine, 1- and 3-methylhistamine, as well as the dipeptides carnosine, anser-

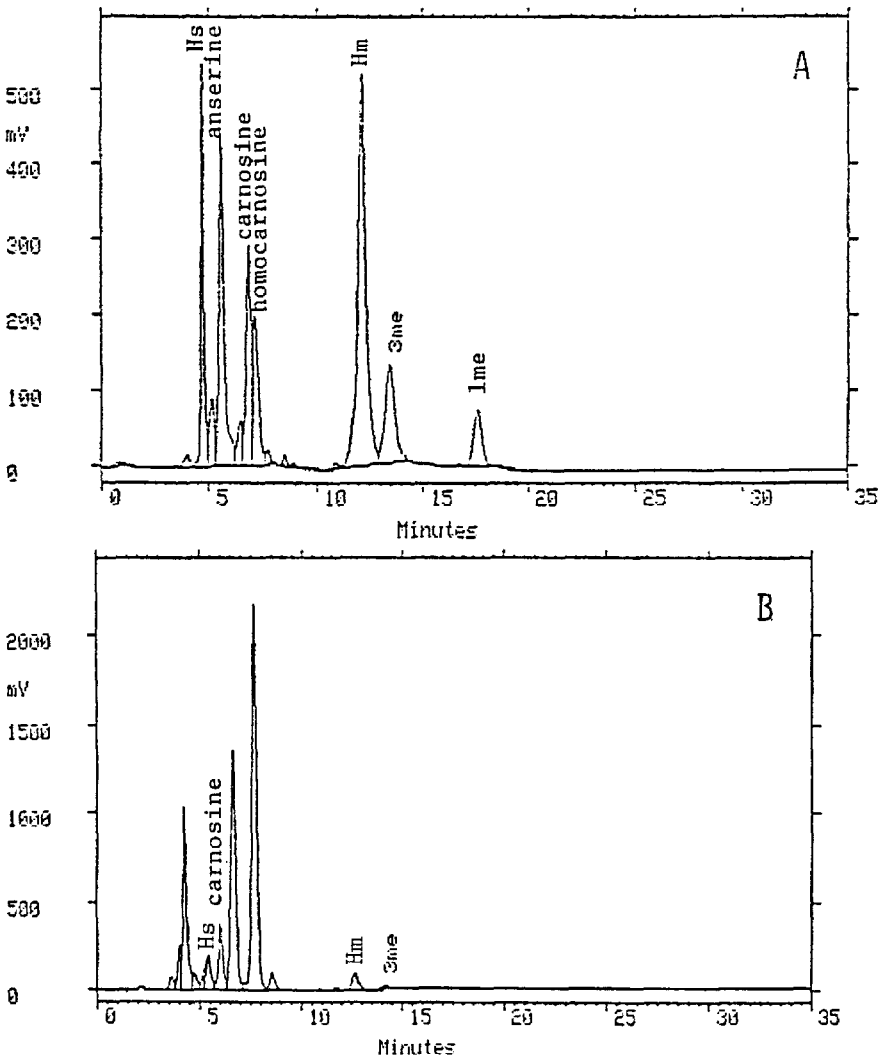

Fig. 1.

(Continued on p. 318)

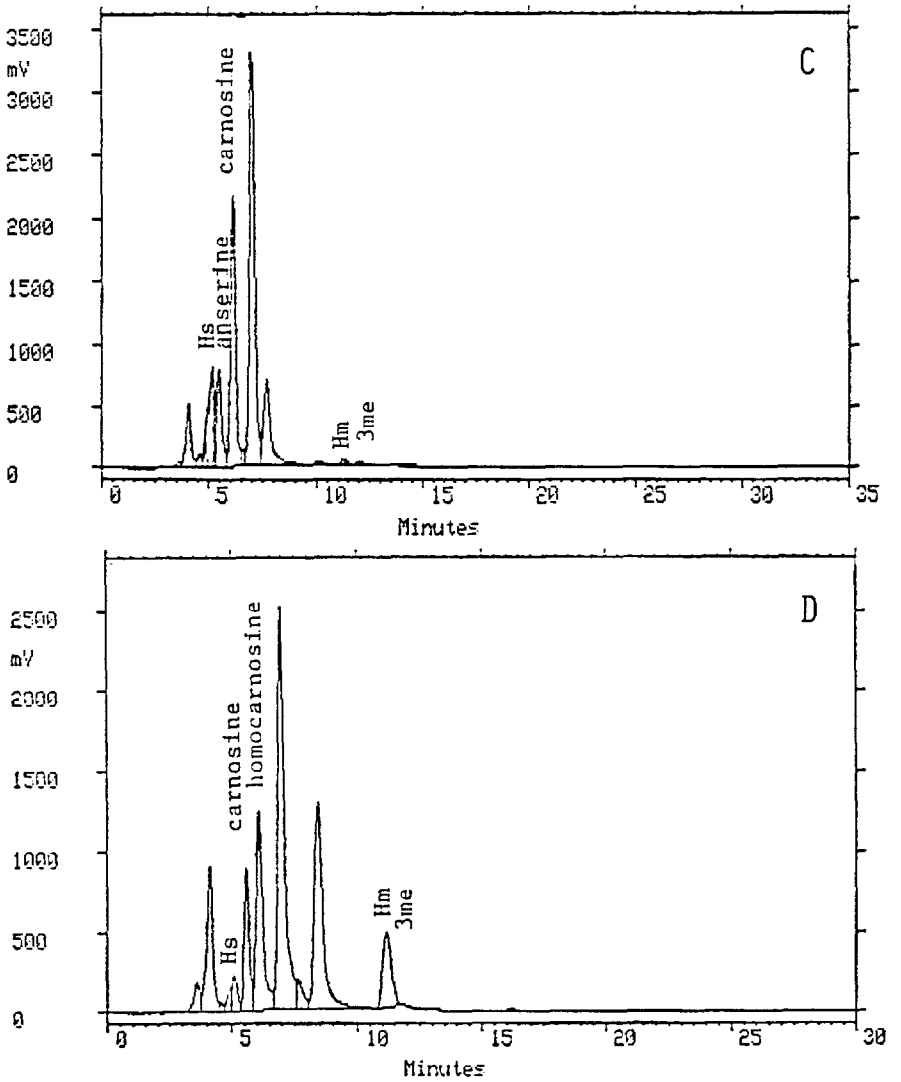

Fig. 1. Chromatograms of (A) standard solutions of histidine, histamine, 1- and 3-methylhistamine, carnosine, anserine and homocarnosine, (B) kidney, (C) leg muscle and (D) brain. Retention times: histidine (Hs), 4.69 min; anserine, 5.39 min; carnosine, 6.38 min; homocarnosine, 6.71 min; histamine (Hm), 11.04 min; 3-methylhistamine (3me), 11.92 min; 1-methylhistamine (1me), 16.18 min.

ine and homocarnosine. The clarity of resolution is noteworth for the tissue extracts which were not purified prior to HPLC injection. For the complete separation of all compounds, 15 min were required while carnosine appeared after 6.4 min and the histamine peak was eluted in 11 min. The latter finding is similar to other reported HPLC procedures for histamine [8,9]. Very low levels of 1-methylhistamine were observed in the tissue samples studied.

The relative fluorescence intensity for the different imidazole compounds of major interest in this study was linear over a range of 0.4 ng to 15 μg, which covered the needs for the tissues studied.

Table I lists histamine, 3-methylhistamine, histidine, carnosine, anserine and

TABLE I

HISTAMINE, METHYLHISTAMINE, HISTIDINE AND CARNOSINE CONTENT OF RAT TISSUES

Each value, expressed as μg/g of wet tissue, represents the mean ± standard deviation for tissue analyses from four male rats weighing between 100 and 110 g.

Tissue	Histamine	3-Methylhistamine	Histidine	Carnosine	Anserine	Homocarnosine
Brain	5.0 ± 0.5	2.1 ± 0.2	6.6 ± 1.0	21.8 ± 4.8	–	24.6 ± 1.2
Heart	4.5 ± 0.2	14.5 ± 1.1	36.6 ± 8.2	10.7 ± 1.0	43.9 ± 11.0	–
Kidney	5.2 ± 0.3	5.9 ± 0.8	35.0 ± 0.7	30.2 ± 2.6	–	–
Leg muscle	4.9 ± 1.3	7.6 ± 1.9	30.1 ± 1.0	40.7 ± 8.4	93.5 ± 10.2	–
Stomach	6.3 ± 1.3	1.7 ± 0.5	10.9 ± 1.5	11.2 ± 1.8	8.8 ± 3.1	–

homocarnosine concentrations for various rat tissues. The histamine values are similar to other published results for this compound in which HPLC methods were used [8,9]. They are also in the same range as values previously reported from our laboratory and by others using radioisotope techniques [12]. Ronnberg et al. [8] and Arakawa and Tachibana [9] suggested that there may be interference from other fluorescing compounds in tissue extracts unless a clean-up procedure is first carried out. However, excellent resolution of peaks was obtained with all tissue extracts in our studies. Earlier workers observed no need for purification with blood, gastric tissue and mast cells, but did find it neccesary to purify a brain extract [8]. The carnosine values for leg muscle and brain (Table I) are in the same range as previously determined in our laboratory by ion-exchange chromatography [13]. Anserine was found in heart, leg muscle and stomach, but not in kidney or brain. Homocarnosine was detected only in brain.

Table II shows recovery values for histamine added to muscle tissue prior to homogenization. The recovery values ranged between 93 and 117%. Devalia et al. [14] recently reported histamine and 1-methylhistamine recoveries of 86% by a different HPLC procedure. We believe that it is important to spike the sample with different concentrations of standards prior to homogenization so that all compounds are exposed to the same conditions. This has generally not been done by previous workers. Since 1-methylhistamine was absent from tissue exctracts, it was used as an internal standard in the present procedure.

General comments

The precision of the present method is attested to by the constancy of the retention times for the different compounds from sample to sample as well as by the excellent recovery values obtained for histamine (Table II). Carnosine is eluted in 6.4 min instead of more than 2 h by the better of the earlier methods [3], but in a similar time frame (13 min) as reported by Seiler et al. [5], who did not separate histamine in their procedure. The present procedure for analyzing histamine also offers advantages over earlier HPLC procedures. Perhaps of greatest significance is the maintenance of operating conditions at an essentially neutral pH (6.4) instead of at the extremes of pH 2.25 [8] and pH 12.7 [8] where the column degenerates after only a few analyses. The separation and simulta-

TABLE II

HISTAMINE RECOVERY FROM MUSCLE TISSUE

Each value represents the average ± standard deviation of three tissue samples.

Histamine added* (μg)	Total histamine measured (μg/g)	Recovery (%)
0	2.7±0.22	
5	7.4±0.40	93
10	13.0±0.88	97
15	20.2±4.3	117

*To muscle before homogenization.

neous determination of carnosine, histamine, 1- and 3-methylhistamine and histidine makes the present procedure clearly more useful since it permits the study of histamine metabolism in relation to histidine and carnosine. This is of particular importance since carnosine has been shown to act as a readily available reservoir for histidine during periods of stress when histamine synthesis is increased [15].

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