Journal of Chromatography, 567 (1991) 254–260
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5855

Short Communication

High-performance liquid chromatographic determination, with ultraviolet detection, of S-adenosyl-L-methionine and S-adenosyl-L-homocysteine in rat tissues and simultaneously of normetanephrine and metanephrine for phenylethanolamine-N-methyltransferase or catechol-O-methyltransferase activities

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(First received November 5th, 1990; revised manuscript received January 21st, 1991)

ABSTRACT

A high-performance liquid chromatographic method, with ultraviolet detection, for the determination of S-adenosyl-L-methionine and S-adenosyl-L-homocysteine, and simultaneously of normetanephrine and metanephrine, is presented. The separation was carried out by reversed-phase ion-pair isocratic chromatography. This procedure was applied to the study of the content of S-adenosyl-L-methionine and S-adenosyl-L-homocysteine in rat brain and adrenal glands and to the measurement of phenylethanolamine-N-methyltransferase or catechol-O-methyltransferase activities in rat adrenal gland.

INTRODUCTION

Methyltransferases are enzymes that use S-adenosyl-L-methionine (SAM) as a methyl donor and produce S-adenosyl-L-homocysteine (SAHc), the demethylated product of SAM [1,2]. Transmethylations are involved in several metabolic processes catalysed by enzymes, such as catechol-O-methyltransferase (COMT) [3] and phenylethanolamine-N-methyltransferase (PNMT) [4], and in membrane fluidity [5]. All these are subjects of interest in our research [6,7]. SAHc is a potent inhibitor of the methyltransferases *in vitro* [8] and *in vivo* [9,10]. The SAHc reversible transformation from adenosine and homocysteine (HCy) is catalysed by SAHc hydrolase [11,12]. The administration of HCy to mice was shown to induce an increase of the brain SAHc content.

Several high-performance liquid chromatographic methods with UV detection (HPLC–UV) have been described for the quantitation of SAM and SAHc in

0378-4347/91/$03.50

biological samples [13–17]. These methods require sample preparation with Bond Elut cartridges [14,15] or gradient elution [16,17]. This paper describes an isocratic HPLC–UV method, using an octadecyl-bonded reversed-phase column, which enables the separation of SAM and SAHc in perchloric acid extracts of rat brain and adrenal glands.

EXPERIMENTAL

HPLC system

An M590 solvent-delivery system with a U6K injector, a Nova-Pak C_{18} steel column (Waters Assoc., Milford, MA U.S.A.), a UV detector (Linear UVIS 200) and a recorder set at 10 mV (Linear) were used. Column sizes (150 mm × 3.9 mm I.D. or 300 mm × 3.9 mm I.D.) and UV wavelengths and ranges (a.u.f.s.) are indicated in the figure legends. The mobile phase consisted of 40 m*M* ammonium dihydrogenphosphate ($NH_4H_2PO_4$), 6 m*M* heptanesulphonic acid sodium salt monohydrate and 6% (v/v) methanol; the pH was adjusted to 4.2 by addition of hydrochloric acid, and then the mobile phase was filtered and degassed under vacuum through a 0.45-μm Millipore membrane filter. The flow-rate was set at 1.4 ml/min. The standard compounds tested were adenosine, S-adenosyl-L-homocysteine, S-adenosyl-L-methionine, normetanephrine and metanephrine, all from Sigma (St. Louis, MO, U.S.A.); they were dissolved in water at a concentration of 1 m*M* and then diluted in 0.4 *M* perchloric acid to the final concentration used during HPLC analysis. The injection value was 25 μl. Adenosine, SAM and SAHc were identified in biological samples during HPLC analysis on the basis of their retention times and co-chromatography with reference compounds. The purity of peaks was verified by variation of the mobile phase pH and the concentrations of heptanesulphonic acid and methanol.

Animals

Male rats (250–300 g) were obtained from Iffa-Credo (Saint-Germain sur l'Arbresle, France). Before use, they were kept for one week under standard laboratory conditions. L-Homocysteine thiolactone (Sigma) was administered intraperitoneally (i.p.) (0.2 ml per 100 g body weight) after dissolution in saline, 30 or 60 min before sacrifice. Rats were killed by decapitation, and adrenals (free of fat) and brains were immediately dissected, frozen and stored at −60°C.

Sample preparation

Tissues were weighed, homogenized with an Ultra-Turrax in 0.4 *M* perchloric acid containing 0.1% (w/v) EDTA and 0.1% (w/v) sodium metabisulphite, adrenals in 1.75 ml and brains in 10.0 ml. Samples were then centrifuged at 10 000 *g* for 20 min at 4°C, the supernatants were filtered through a 0.45-μm membrane into vials, which were capped and immersed in water at 40°C for 90 min and then frozen (−60°C) until HPLC analysis.

Enzyme assay

The adrenals of one rat were dissected after sacrifice and immediately homogenized in a Potter tube containing 0.5 ml of ice-cold 5 m*M* Tris buffer (pH 8.0)

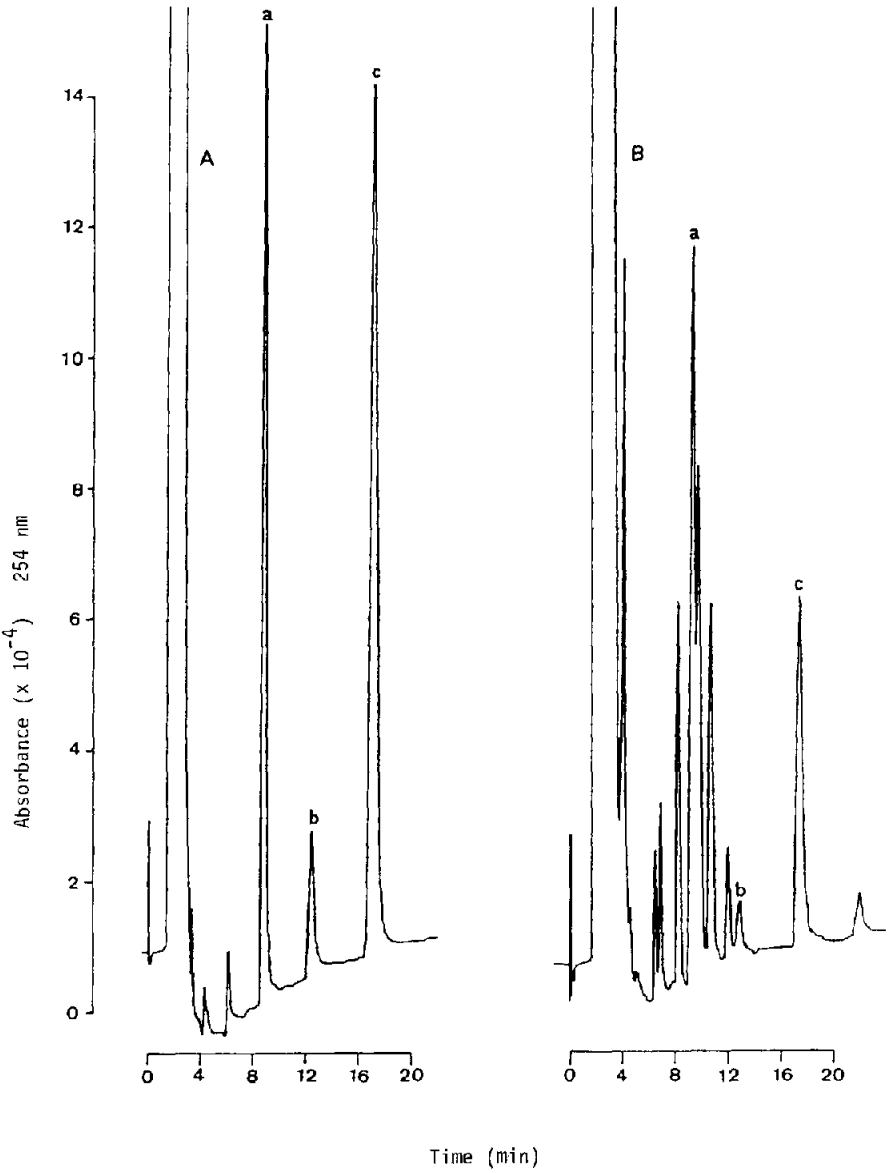

Fig. 1. (A) Chromatogram of the standard solution containing 2 μM adenosine and 5 μM SAM in a 25-μl injection. Peaks: a = adenosine; b = SAHc; c = SAM. Absorbance was monitored at 254 nm and 0.002 a.u.f.s.; the column was a 300 mm × 3.9 mm I.D. Nova-pak C_{18}. (B) Chromatogram of a control rat adrenal sample in a 25-μl injection.

containing 0.5% of Triton X-100. Enzyme activity was estimated in an incubation mixture of the following composition: 20 μl of 1 m*M* normetanephrine, 15 μl of 1 m*M* SAM, 10 μl of 10 m*M* pargyline (as monoamine oxidase inhibitor), 50 μl of 0.5 *M* Tris buffer (pH 8.0), 50 μl of adrenal homogenate and water to make a final volume of 250 μl. Incubation was carried out at 37°C, and the reaction was stopped by the addition of 600 μl of 0.4 *M* perchloric acid. The mixture was then filtered through a 0.45-μm membrane and frozen until HPLC analysis.

RESULTS

HPLC elution profiles

Fig. 1A shows a chromatogram of a solution of adenosine (2 μM) and SAM (5 μM) (the SAHc is contained in the SAM powder). The retention times are: adenosine (peak a), 8.6 min; SAHc (peak b), 12.4 min; SAM (peak c), 16.8 min. For these compounds, the response curves were linear from 0.1 to 50.0 μM for a 25-μl injection volume. Fig. 1B shows a chromatogram of a control rat adrenal sample; SAHc (peak b) and SAM (peak c) are well resolved from unknown compounds. Values obtained for adrenals are given in Table I, which shows that L-homocys-

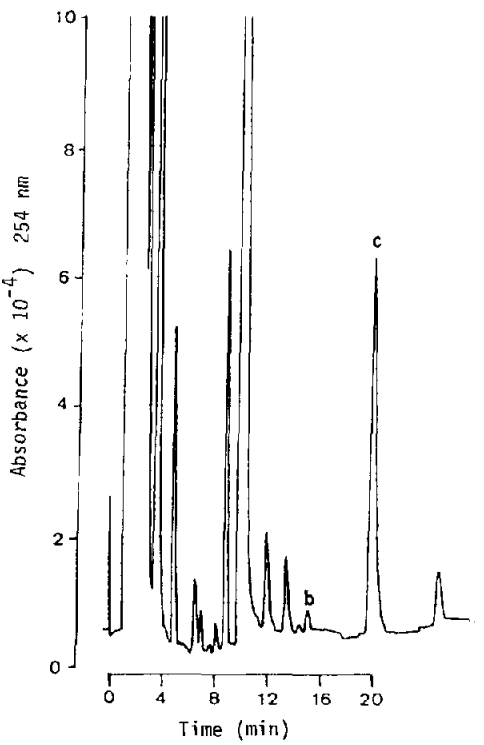

Fig. 2. Chromatogram of a control rat brain sample in a 25-μl injection. Absorbance monitoring and column as in Fig. 1.

TABLE I

SAM AND SAHc CONCENTRATIONS IN RAT ADRENAL AT VARIOUS TIMES AFTER TREATMENT WITH L-HOMOCYSTEINE

Values represent the mean ± S.D. and n the number of animals. Rats were treated with L-homocysteine (200 mg/kg) and were killed 30 or 60 min later. The control animals received an equal volume of saline.

Treatment	n	Concentration (nmol/g wet adrenal tissue)	
		SAM	SAHc
Saline	12	66.8 ± 1.8	7.6 ± 0.8
Homocysteine			
30 min	6	70.0 ± 2.8	226.0 ± 15.8[a]
60 min	4	72.7 ± 2.6	159.8 ± 7.3[a]

[a] Significantly different from saline-injected animals (Student's t-test, $p < 0.001$).

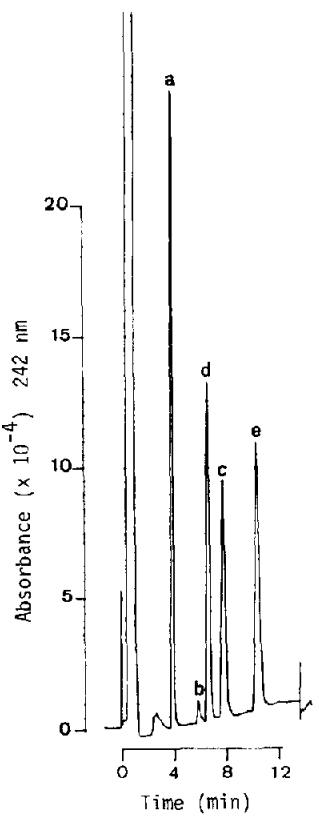

Fig. 3. Chromatogram of the standard solution containing 10 μM adenosine, normetanephrine, SAM and metanephrine in a 25-μl injection. Peaks: a = adenosine; b = SAHc; c = SAM; d = normetanephrine; e = metanephrine. Absorbance was monitored at 242 nm and 0.005 a.u.f.s.; the column was a 150 mm × 3.9 mm I.D. Nova-Pak C_{18}.

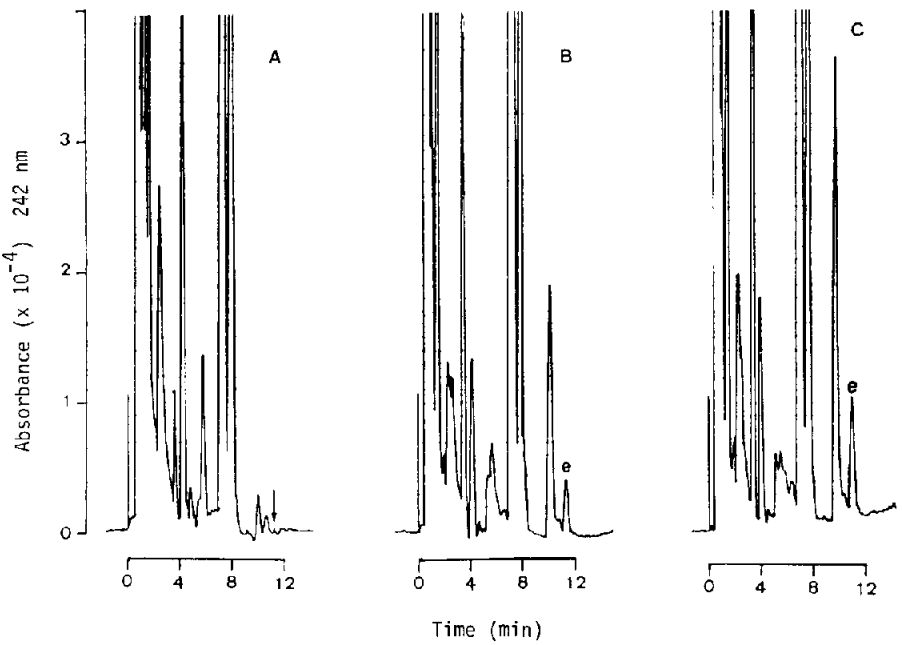

Fig. 4. Chromatograms of the enzymic incubation mixture of crude extract of rat adrenal. (A) Perchloric acid was added before adrenal crude extract; the arrow denotes the absence of metanephrine. (B) Perchloric was added 1 h after the reaction was started by addition of the adrenal crude extract to the incubation mixture. (C) Perchloric acid was added 2 h after the reaction was started. Absorbance was monitored at 242 nm and 0.001 a.u.f.s.; the column was a 150 mm × 3.9 mm I.D. Nova-Pak C_{18}.

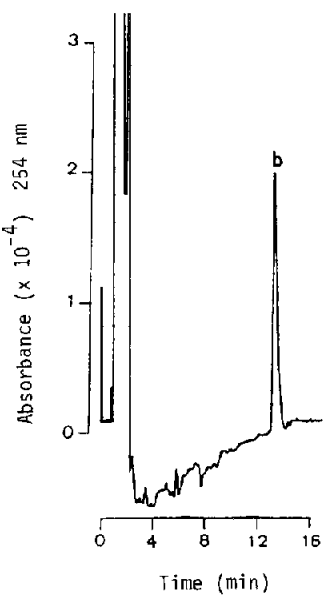

Fig. 5. Chromatogram of the standard 1 μM SAHc in a 25-μl injection. Absorbance was monitored at 254 nm and 0.001 a.u.f.s.; the column was a 300 mm × 3.9 mm I.D. Nova-Pak C_{18}.

teine, after i.p. administration, causes a significant increase of adrenal SAHc and a slight increase of SAM content that is not statistically significant. Fig. 2 shows a chromatogram of a rat brain sample; control values are 23.5 ± 0.5 nmol/g for SAM and 1.25 ± 0.05 nmol/g for SAHc. Fig. 3 shows a chromatogram of a mixed solution of standard (10 μM each) adenosine, SAM, normetanephrine and metanephrine. As the column used was shorter the retention times are decreased: adenosine, 3.8 min; SAHc, 5.8 min; normetanephrine, 6.6 min; SAM, 7.8 min; and metanephrine, 10.2 min. Fig. 4 shows the enzymic production of metanephrine in an incubation mixture containing adrenal crude extract. Fig. 5. shows a chromatogram of standard SAHc injected by itself in order to obtain a quantitative determination, as the SAM powder contains an amount of SAHc as shown in Fig. 1A.

DISCUSSION

The method reported here provides a simple and rapid procedure to study the variations of SAM and SAHc levels in rat adrenals after *in vivo* administration of a pharmacological agent. This method can be applied without modification to quantitation of SAM and SAHc in other rat tissues, *e.g.* brain, and, with adjustment of the homogenization volume, to brain areas. Because the enzyme PNMT produces SAHc and metanephrine from SAM and normetanephrine, and COMT produces metanephrine and SAHc from epinephrine, and also because adrenal crude extract contains epinephrine, additional work is necessary to determine the role of these two enzymes in the production of metanephrine that is observed in Fig. 4.

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