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Note

Simultaneous determination of tryptophan, serotonin and 5-hydroxyindoleacetic acid in rat brain by high-performance liquid chromatography using a weak acidic cation-exchange resin*

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Serotonin (5-HT) synthesis and metabolism in brain tissue are significantly influenced by L-tryptophan. For an understanding of the physiological role of serotonin, the simultaneous determination of tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in brain tissue is important [1—3]. Recently, high-performance liquid chromatography (HPLC) coupled with fluorescence or amperometric detection has simplified the determination of many indoles

*Preliminary reports of this work have appeared previously [S. Hori, S. Ohtani, K. Ohtani and T. Ito, The Fourth Annual Meeting of the Japan Neuroscience Society, Kyoto, Japan, Abstract; Neurosci. Lett., Suppl. 6 (1981) S.51].

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[4—28]. In this paper, we have improved the quantitative determination of tryptophan, 5-HT and 5-HIAA, using a weak acidic cation-exchange resin.

MATERIALS AND METHODS

The weak acidic cation-exchange resin, Hitachi No. 3011C (10—15 μm, spherical), was from Hitachi (Tokyo, Japan). L-Tryptophan, 5-hydroxytryptophan (5-HTP), serotonin creatinine sulphate (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), L-tyrosine, 3,4-dihydroxyphenylalanine (DOPA), 3,4-dihydroxyphenylethylamine (dopamine), norepinephrine and epinephrine were from Nakarai Chemicals (Kyoto, Japan). All other chemicals were of analytical reagent grade and used without further purification.

The liquid chromatograph and detection system consisted of a Hitachi 645A pump, a high-pressure sampling valve (Hitachi) and a Hitachi 3011C weak acidic cation-exchange column (4.6 × 250 mm) with a steel jacket. A Hitachi fluorescence spectrophotometer, Model 650-10S, was used; excitation and emission wavelengths were 280 and 340 nm, respectively.

For the column preparation, the packing material (about 2.5 g of Hitachi 3011C resin) was dispersed in methanol—water (1:1, v/v) and packed into the stainless-steel column under high pressure (less than 150 kg/cm^2) using the slurry packing method.

Rat brain (0.1—0.5 g) was homogenized with 0.1 *M* $HClO_4$ (4:1, v/w) by a Handy sonic homogenizer (Model UR-20P, Tomy Seiko, Tokyo, Japan) [15, 22]. After 5 min centrifugation at 25 000 *g* (4°C), the compounds were determined directly by injecting 20—50 μl of the supernatant into the chromatograph.

RESULTS AND DISCUSSION

Separation of tryptophan, 5-HTP, 5-HT and 5-HIAA

The elution patterns of tryptophan, 5-HTP, 5-HT and 5-HIAA on a column of porous polystyrene—divinylbenzene polymer with —COOH as the active functional group (Hitachi Gel 3011C) are significantly affected by the pH, ionic strength and methanol concentration of the mobile phase [29]. As shown in Fig. 1A, pH 4.0—4.4 was the most convenient range of the mobile phase. Increasing the methanol concentration of the mobile phase resulted in decreased retention times (Fig. 1B); it did not affect the separation sequence or the symmetry of the peaks. Ionic strength has a pronounced effect on the retention times of tryptophan, 5-HTP, 5-HT, and 5-HIAA. With 0.8 *M* citrate buffer, the retention time of 5-HT was markedly reduced (Fig. 1C); with 1.2 *M* citrate buffer, the retention time of tryptophan and 5-HTP was slightly reduced while that of 5-HIAA was slightly increased.

Based on these results, the elution conditions selected were 0.5 *M* citrate—sodium citrate (pH 4) containing 20% methanol; the flow-rate was 0.2 ml/min at 60°C.

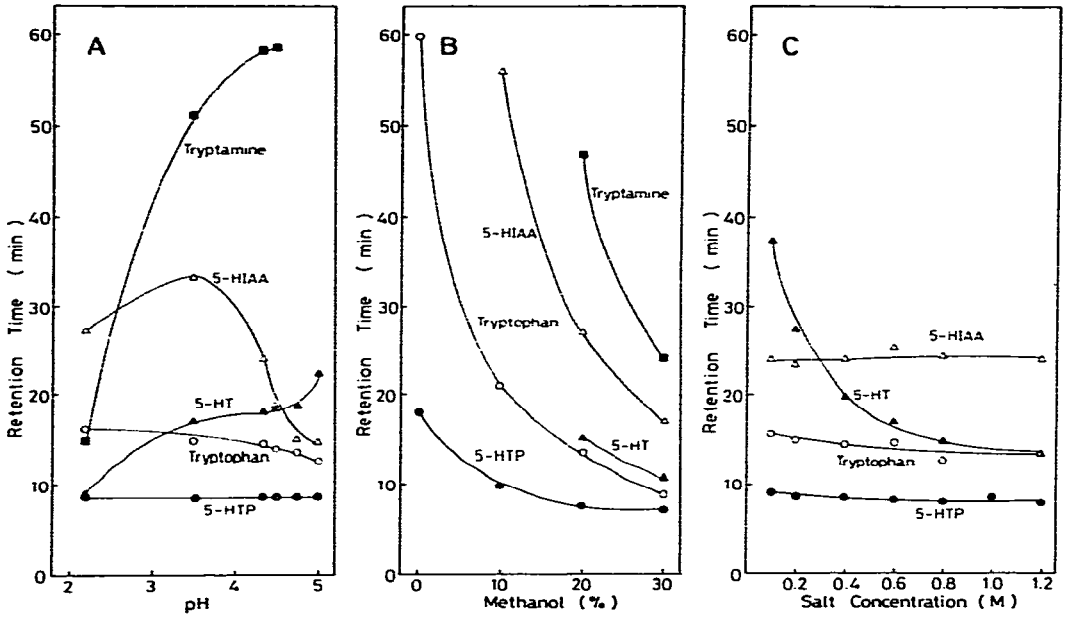

Fig. 1. Retention times of tryptophan, 5-HTP, 5-HT, 5-HIAA and tryptamine. (A) Effect of pH. The mobile phase was 0.5 *M* citrate—sodium citrate containing 20% methanol. (B) Effect of the methanol concentration. The mobile phase was 0.5 *M* citrate—sodium citrate (pH 4). (C) Effect of the salt concentration. The mobile phase was citrate—sodium citrate (pH 4) containing 20% methanol.

Determination of tryptophan, 5-HT and 5-HIAA in rat brain

The standard solution contained 100 pmoles of tryptophan, 5-HTP, 5-HT, 5-HIAA, tyrosine, DOPA, dopamine, norepinephrine, and epinephrine in 1 μl of 0.1 *M* $HClO_4$, and was stored at —80°C in a freezer. Before use, it was diluted to 5, 7.5 and 25 pmoles in 50 μl of 0.1 *M* $HClO_4$. Tyrosine, DOPA, dopamine, norepinephrine, and epinephrine elution preceded other elutions

[redacted]

5-HIAA were eluted at 13.0, 19.8 and 25.6 min, respectively (Fig. 2A). Fig. 2B demonstrates the separation of tryptophan, 5-HT and 5-HIAA from rat brain extract. Compounds in the extract did not affect either the separation or the fluorometric detection* of the indoles. The tryptophan, 5-HT and 5-HIAA content of rat cerebral cortex, brain stem and cerebellum is given in Table I. The values obtained with our HPLC method are close to those reported by others [12, 30—33]. The detection limits (signal-to-noise ratio of 2) of tryptophan, 5-HT and 5-HIAA were 0.25, 0.1 and 0.5 pmole, respectively, which were comparable to or even better than the amperometric detection. In conclusion, the simultaneous quantitation of these indoles was simplified by the combination of the improved acidic weak cation-exchange resin and fluorometric detection.

*But if amperometric detection was used, unknown materials were detected near the peaks of the indoles (data not shown).

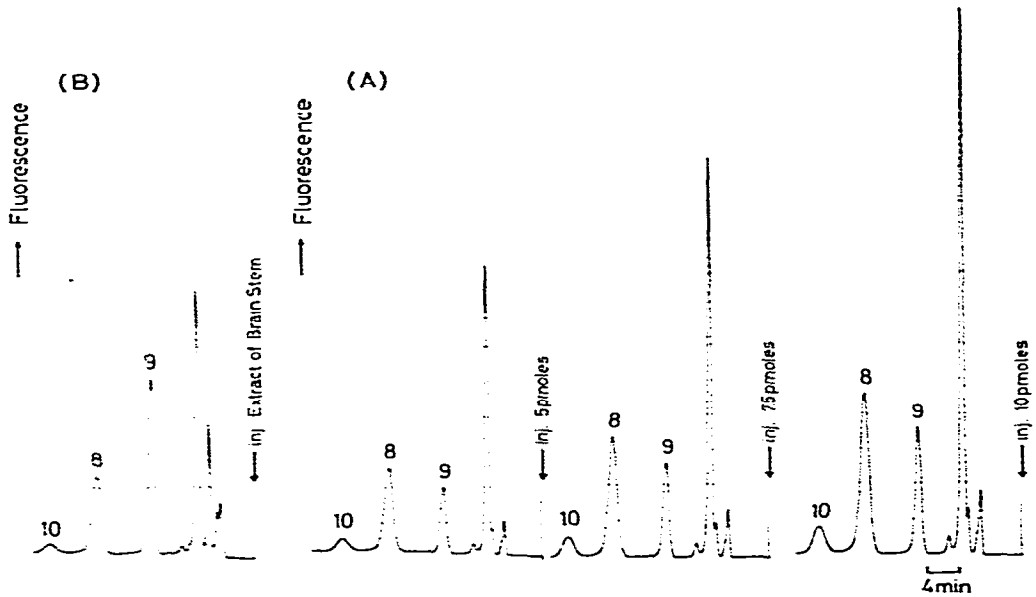

Fig. 2. Separation of tryptophan, 5-HT and 5-HIAA. (A) Separation of the standard solutions. (B) Separation of tryptophan, 5-HT and 5-HIAA in an extract from rat brain stem. Peaks 8, 9 and 10 were identified as tryptophan, 5-HT and 5-HIAA, respectively.

TABLE I

TRYPTOPHAN, 5-HT AND 5-HIAA CONTENT OF RAT BRAIN

Values are nmoles/g wet tissue ± S.D. The number of experiments is given in parentheses.

	Tryptophan	5-HT	5-HIAA
Cerebral cortex	12.8 ± 1.37 (4)	3.51 ± 0.55 (4)	1.95 ± 0.31 (4)
Brain stem	11.5	4.15	2.40
	10.1	3.64	2.15
Cerebellum	13.5	0.45	0.80
	11.6	0.44	0.30

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