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Note

Analysis of salsolinol in human brain using high-performance liquid chromatography with electrochemical detection

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Catecholamines and aldehydes are known to undergo a Pictet–Spengler type condensation to form tetrahydroisoquinolines [1]. One of the most extensively studied condensation products is salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), which is formed from the condensation of dopamine with acetaldehyde [2]. Salsolinol and other tetrahydroisoquinolines were detected in the urine of Parkinsonian patients who have taken ethanol during L-DOPA therapy [3]. This compound was studied mainly in relation to alcoholism because acetaldehyde that came from ingested alcohol was supposed to react with endogenous dopamine. In chronic ethanol-treated rats the level of salsolinol was increased markedly in various brain regions [4], and it was also higher in the brain of alcoholism patients [5]. It is considered that salsolinol acts as a false neurotransmitter. Salsolinol is known to be taken up and stored in rat brain synaptosomes. Furthermore, it blocks the uptake of catecholamines, causes release of stored catecholamines [6], and inhibits tyrosine hydroxylase [7] and monoamine oxidase activities [8]. In addition, it may have antinociceptive effects and a potency for displacing naloxone from synaptic plasma membranes [9].

Several methods for the analysis of salsolinol have been reported, including radioenzymatic [10,11], high-performance liquid chromatographic [12,13], gas

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chromatographic [14], and gas chromatographic–mass spectrometric (GC–MS) methods [15,16]. However, for studying the physiological role of salsolinol, a simple and sensitive assay method is needed.

This paper describes an improved method for determination of salsolinol using high-performance liquid chromatography with electrochemical detection (HPLC–ED).

EXPERIMENTAL

Materials

Salsolinol hydrochloride, dopamine hydrochloride and isoproterenol hydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.), norepinephrine hydrochloride from Aldrich (Milwaukee, WI, U.S.A.), epinephrine from Wako (Osaka, Japan), serotonin creatinine sulphate from Merck (Darmstadt, F.R.G.), and sodium 1-octanesulphonate from Nakarai (Kyoto, Japan). Amberlite CG-50 was purchased from Rohm and Haas (Philadelphia, PA, U.S.A.). A Shim-pack CLC-ODS packed column (particle size 5 μm, 150 mm $\times$ 6 mm I.D.) was obtained from Shimadzu (Kyoto, Japan). All other chemicals used were of analytical grade.

Sample preparation

Human brain was dissected at autopsy from patients without history of any neurological disorders and stored at $-80\,^{\circ}$C. We dissected the caudate nucleus and putamen from the stored brain prior to assay.

To the brain tissue (ca. 100 mg wet weight) were added four volumes (ca. 400 μl) of 0.32 *M* sucrose containing 50 pmol of isoproterenol as an internal standard. The mixture was homogenized in a sonicator (Branson, Danbury, CT, U.S.A.) for a few seconds, and after addition of 1 ml of 0.4 *M* perchloric acid containing EDTA (2.5 mg/ml) and sodium metabisulphite (5.0 mg/ml), it was placed for 10 min in an ice-water bath. The homogenate was then centrifuged at 1600 *g* for 10 min at 4°C, and the supernatant was transferred to a conical tube, followed by 100 μl of 0.5 *M* sodium phosphate buffer (pH 6.5) and 200 μl of 1 *M* potassium carbonate. The sample was degassed with a vacuum pump for 1 min to remove carbon dioxide that appeared after addition of potassium carbonate, and centrifuged at 1600 *g* for 10 min at 4°C. The clear supernatant was passed through a column containing 130–200 μl of Amberlite CG-50 type II (6 mm $\times$ 5 mm I.D.), and the effluent was discarded. The column was washed with 1 ml of 10 m*M* sodium phosphate buffer (pH 6.5) and 1 ml of water, and eluted with 0.5 ml of 0.5 *M* hydrochloric acid. A 50–100 μl aliquot of the eluate was injected into the HPLC system.

Chromatographic conditions

The HPLC system consisted of a Yanaco L-4000W liquid chromatograph, a VMD 501 electrochemical detector (Yanagimoto, Kyoto, Japan), a graphite electrode EC-100 (Eicom, Kyoto, Japan), a Rheodyne 7125 injector with a 100-μl sample loop (Berkeley, CA, U.S.A.) and a Shim-pack CLC-ODS analytical column. The mobile phase was 0.1 *M* potassium phosphate buffer (pH 2.2) contain-

ing 100 μM EDTA and sodium 1-octanesulphonate (60 mg/l) mixed with acetonitrile (94:6, v/v). The flow-rate was 1.0 ml/min, and the detector potential was set at 0.7 V against an Ag/AgCl reference electrode.

RESULTS AND DISCUSSION

Fig. 1A shows the separation of standard samples of norepinephrine, epinephrine, dopamine, salsolinol, isoproterenol and serotonin, and chromatograms for the extract from human caudate nucleus and putamen are shown in Fig. 1B and C, respectively. The detection limit for salsolinol was ca. 40 fmol per injected sample at a signal-to-noise ratio of at least 3. The absolute recovery of salsolinol by this method was 64.1 ± 2.9% (mean ± S.D.) and its relative yield to isoproterenol as an internal standard was 129.2 ± 19.0% (mean ± S.D.). The reproducibility of the assay with replicates of the same samples was 100 ± 4.5% (coefficient of variation for five determinations). By this method salsolinol in human brain could be detected quantitatively and was found to be 218 ± 95 and 207 ± 86 pmol/g of wet tissue (mean ± S.D.) in caudate nucleus and putamen, respectively. These values agree well with those of a previous report [5].

The present method has several advantages. A GC–MS procedure is useful for monitoring salsolinol in brain, but it needs a relatively large amount of tissue and

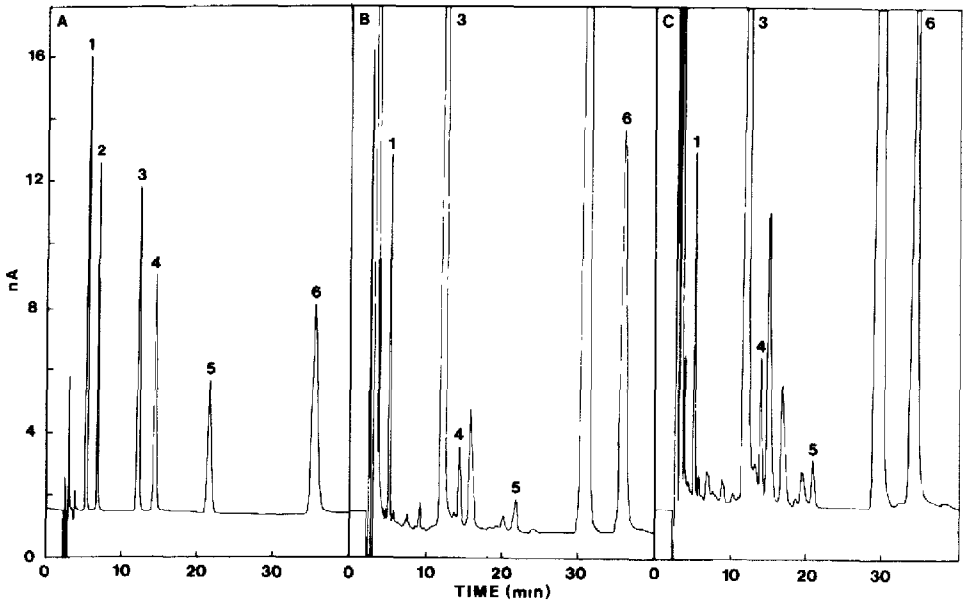

Fig. 1. HPLC profiles of authentic compounds and extracts from human brain. The extraction procedure and analytical conditions are described in Experimental. (A) Authentic compounds dissolved in 0.1 M hydrochloric acid (200 pmol/ml); these were injected in the same amounts (5 pmol per injection). (B) Caudate nucleus (161 mg): norepinephrine, 1.55 nmol/g; dopamine, 17.8 nmol/g; salsolinol, 137 pmol/g; serotonin, 1.03 nmol/g (C) Putamen (105 mg): norepinephrine, 1.7 nmol/g; dopamine, 25.9 nmol/g; salsolinol, 255 pmol/g; serotonin, 2.05 nmol/g. Peaks: 1 = norepinephrine; 2 = epinephrine; 3 = dopamine; 4 = salsolinol; 5 = isoproterenol; 6 = serotonin.

elaborate sample preparation. It was difficult to detect salsolinol quantitatively from a small amount of tissue. The one-step sample clean-up procedure using cation-exchange chromatography in the present method allows the quantitative determination of salsolinol in ca. 100 mg of human brain tissue. Therefore detailed studies of its distribution in the brain can be performed.

To confirm identification of the peak that has the same retention time as authentic salsolinol, we also analysed the same sample by fluorimetric detection and the result was in complete agreement with that by electrochemical detection (data not shown). Similar assay procedures with HPLC–ED were reported by Riggin and Kissinger [12] and Odink et al. [13]. Riggin and Kissinger [12] employed a cation-exchange column for the HPLC system and determined the salsolinol level in human and rat urine and rat whole brain. The detection limit for salsolinol was reported to be ca. 2 ng/g of tissue (10 pmol/g of tissue). Odink et al. [13] determined the urinary salsolinol level, and their detection limit was 100 fmol (6 nmol/l of urine). The present method with an ODS column and a graphite electrode resulted in a significant improvement in the sensitivity, which was several times higher than the previous reports [12,13]. Generally, as a column support, ODS is known to be able to attain much higher separation of compounds than ion exchangers. In addition, it was revealed that the graphite electrode provides a much better sensitivity and stability for analysis of catechol compounds than the glassy carbon electrode generally used. These improvements make it possible to measure salsolinol in human brains.

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