Simultaneous Mass Fragmentographic Determination of 3, 4-Dihydroxyphenylacetic Acid and 4-Hydroxy-3-Methoxyphenylacetic Acid in Brain Tissue*

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With 4 Figures

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Summary

A mass fragmentographic method for determination of 3, 4-dihydroxyphenylacetic acid (DOPAC) is described. Deuterium labelled DOPAC was used as internal standard. Derivatives suitable for GC-MS were prepared by reacting standard solutions and rat brain extracts with pentafluoropropanol and pentafluoropropionic anhydride. Fragments set into focus were m/e 592/597 (M+) and m/e 415/420 (M+ – 177). The sensitivity was in the pmole range with high specificity as tested by multiple ion analysis. The DOPAC level in rat striatum was 6.0 ± 1.1 nmoles/g with an experimental error below 8.5%. The recovery of 575 pmoles DOPAC added to brain extracts was 91%. Treatment of rats with pargyline (150 mg/kg i.p.) reduced the DOPAC level by about 95% whereas a slight elevation was obtained after administration of chlorpromazine (10 mg/kg i.p.). DOPAC and 4-hydroxy-3-methoxyphenylacetic acid (HVA) could be determined concomitantly in the same sample, by using the described technique for derivative preparation and an AVA allowing simultaneous recording of 4 mass numbers.

Introduction

3, 4-Dihydroxyphenylacetic acid (DOPAC) is a normal constituent of brain tissue (von Euler, 1958). It represents a major metabolite of dopamine (DA) from which it is formed by monoamine oxidase

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activity (Rosengren, 1960). Determination of DOPAC has so far been based on fluorimetric assay (Kügi et al., 1957; Murphy et al., 1969). To further explore the role of DOPAC in brain biochemistry more specific and sensitive methods for its determination are required. Massfragmentography (MF) combines three important qualifications of an analytical system, namely: separation power, sensitivity and specificity. The present paper describes the use of such a method for determination of DOPAC in small samples of brain tissue.

Methods

Male Sprague Dawley rats weighing about 150 g were used. Animals were killed with a guillotine. The brain was rapidly taken out and each pair of striata was dissected on ice and homogenized in 1+1 ml 0.1 M HCOOH containing 50 μM ascorbic acid. The homogenate was centrifuged at 26,000 g and the supernatant was frozen at −80 °C pending analysis.

Groups of three rats were treated with pargyline HC1 (Abbott lab., U.S.A., 150 mg/kg i.p.) or chlorpromazine (Hibernal LEO, 10 mg/kg i.p.) for 90 minutes.

2, 2-dideutero-2-(3, 4-dihydroxy-2, 5, 6-trideuterophenyl) acetic acid (DOPAC-ds) was prepared by K. Leander, Ph. D. (AB Syntab, Stockholm, Sweden) and used as internal standard. From the supernatant phase of brain homogenates DOPAC was extracted with ethyl acetate (4+2 ml) after addition of NaCl. The solvent was evaporated to dryness in a stream of N2. The residue was transferred with CH3OH to small tubes. Fifty μl of a mixture containing 20 % 3, 3, 3, 2, 2-pentafluoropropanol (Columbia Org. Chem. Do. Inc., U.S.A.) in pentafluoropropionic anhydride (PFPA) (Pierce Chemical Co., U.S.A.) were added and reacted for 15 minutes at 75 °C followed by evaporation (Wilk and Orlowski, 1973). Samples were dissolved in 30 μl PFPA and reacted for another 5 minutes at 75 °C followed by evaporation under reduced pressure. The residue was finally dissolved in 30 μl of 1% PFPA in ethyl acetate. Standard samples were prepared with a fixed amount of DOPAC-ds (3700 pmole) and different amounts of DOPAC (60—1730 pmole).

From derivatives of authentic and deuterium labelled DOPAC mass spectra were obtained using an LKB 9000 gas chromatograph-massspectrometer. The instrument was equipped with an accelerating voltage alternator (AVA) which allows simultaneous recording of 3 mass numbers. A silanized glass column 2 m × 3 mm (i.d.) packed with 3 % OV-17 on gas chrom Q was used. Flow rate of carrier gas (helium) was 15 ml per min. Temperatures were: column 150 °C, flash heater 250 °C, ion source 290 °C. Trap current was 60 μA and electron energy was 22.5 eV. Accelerating voltage was set to 3.5 kV at m/e 415 and 592. The base peaks (M+−177, m/e 415/420) and the molecular ions (M+, m/e 592/597) were chosen for massfragmentographic analysis (Fig. 1). Extracts from 10 striata were pooled and divided into