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A GAS–LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF 2-PHENYLETHYLAMINE IN RAT BRAIN TISSUE

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Abstract—A gas chromatographic method for the estimation of 2-phenylethylamine in rat brain is described. The method utilises ion exchange resin, acetylation in aqueous solution and solvent extraction procedures for purification prior to derivative formation and gas chromatography with electron capture detection. This method gives a value of 1.1 ± 0.2 ng/g (mean ± S.E.M.) for the endogenous concentration of 2-phenylethylamine in rat brain.

Several reports suggest that 2-phenylethylamine (PE) is excreted in abnormal quantities in phenylketonuria [1], schizophrenia [2] (though here there is some dispute [3]) and depression [4, 5], and this amine has also been implicated in the aetiology of migraine [6]. The systemic administration of PE to rodents is reported to reduce the concentration of brain catecholamines and 5-hydroxytryptamine 5-HT [7, 8], while *in vitro* experiments, using synaptosome-enriched fractions of rat brain, show that PE modifies the normal transport of noradrenaline, dopamine and, to a lesser extent, 5-HT[9–11], across synaptosomal membranes.

The apparent importance of PE has led to the development of several methods for its quantification [12–19]. However, with the exception of the radioenzymatic method of Saavedra [17], the gas chromatographic-mass spectrometric method of Willner *et al.* [19] and the mass spectrometric integrated ion current method of Durden *et al.* [18] the methods appear to lack specificity. We report here a specific and sensitive gas chromatographic method for the estimation of PE in rat brain tissue.

MATERIALS AND METHODS

Reagents. All reagents were Analar (B.D.H., Poole, Dorset) except where stated.

Water was double distilled in an all-glass apparatus. Methanol (spectroscopic grade; B.D.H.) was redistilled over potassium hydroxide, the fraction boiling between 64.5° and 65.5° was collected into an all-glass container and stored at 4° until required. Methanolic-HCl was prepared from this redistilled methanol and HCl gas.

Amberlyst 15 (80–120 mesh) was washed with 2 M-sodium hydroxide, water, 4 M-HCl and water. The procedure was repeated at least 4 times. The resin was then washed with redistilled methanol, 3 M methanolic-HCl, methanol and finally water.

Acetic anhydride was redistilled twice and the fraction boiling between 139° and 140° collected into an all-glass container and stored at 4° until required. Ethyl acetate, pentafluoropropionic anhydride (Sequanal grade; Pierce & Warriner Ltd., Chester, U.K.) and cyclohexane (laboratory reagent) were used without further purification.

Gas chromatography. The gas chromatograph used in this work was a Hewlett Packard 57 13 fitted with a 15-mCi ^{63}Ni source linear electron capture detector. The glass column (1.8 m × 4 mm i.d.) was packed with 3% OV-3 on 100–120 mesh Gas Chrom Q (Applied Science Laboratories). The carrier gas was 5% methane in argon at a flow rate of 40 ml/min. The injection port temperature was 250°, detector temperature 250° and the column temperature 130°.

Animals. Male Wistar rats, 180–220 g were obtained from the Biochemistry Department, University of Birmingham.

Methods

The rats were killed by cervical fracture and the brains removed rapidly at room temperature, the pineal and meninges being excluded. The tissue was immediately transferred to a glass vial, frozen on solid carbon dioxide and stored at −20° until the assay was carried out. The tissue, which had been allowed to partially thaw, was homogenised in 5 vol. of ice cold 0.4 N perchloric acid using a Potter–Elvejhem Homogeniser with a Teflon glass pestle (0.1–0.15 mm clearance). Internal standards were added to the appropriate tissue samples during the homogenisation and subsequently handled in parallel with the other brain homogenates.

Stage 1. The homogenates were centrifuged at 10,000 *g* for 20 min at 4° and the supernatant adjusted to a pH of 7.5 with 2 M-KOH; the insoluble potassium perchlorate was removed by centrifugation at room temperature for 5 min. The supernatant was then applied to the Amberlyst 15 columns (2.5 × 0.4 cm) and allowed to percolate through the resin under gravity (30–40 ml/hr). The resin was washed with 10 ml of water and the amine eluted with 10 ml 1.6 M-methanolic-HCl. The column eluate was evaporated to dryness at 40° in a rotary evaporator under vacuum.

Stage 2. The residue was taken up in 3.0 ml of water and a small amount of sodium bicarbonate was added followed by 0.3 ml acetic anhydride; the sample was shaken and further additions of sodium bicarbonate

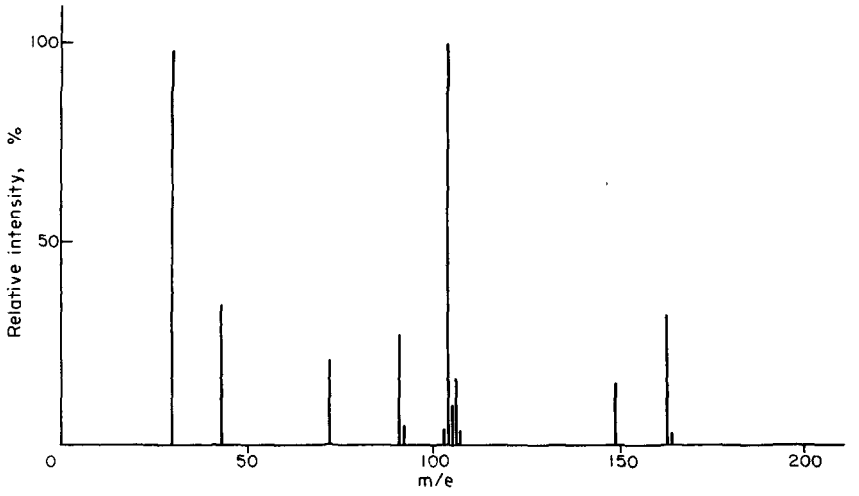

Fig. 1. Mass spectrum of *N*-acetyl, 2-phenylethylamine. The mass spectrum was obtained on an AEI MS 9 with an ionisation potential of 70 eV. The spectrum has been normalised. The spectrum shows a molecular ion at 163 and the major fragmentation appears to be due to cleavage α- to the nitrogen to give the base peak at 104.

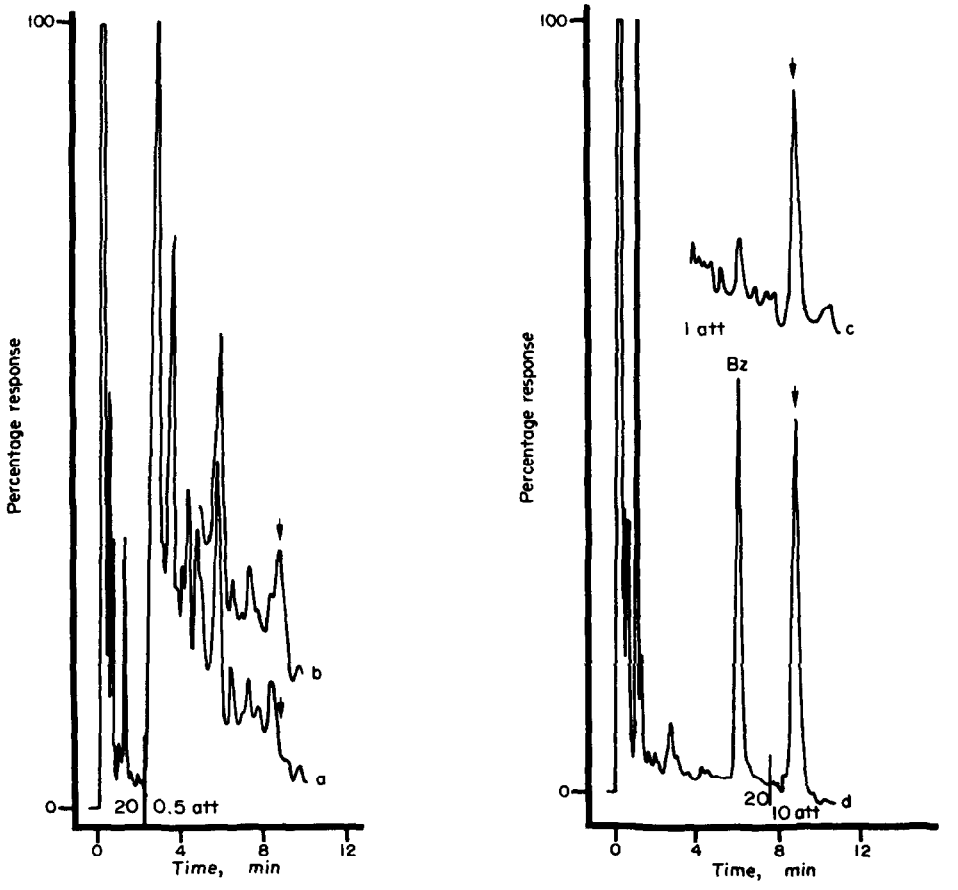

Fig. 2. Gas chromatograms of the 2-phenylethylamine derivative. (a) blank value obtained with perchloric acid; (b) from 4 g of brain tissue; (c) as (b) plus 20 ng PE as internal standard; (d) from 2 g of brain tissue from animal injected i.p. 4 hr before death with 75 mg/kg pargyline hydrochloride.

made until the effervescence ceased. The samples were then shaken for 5 min with 3 ml of ethyl acetate, centrifuged to separate the phases, and the organic layer evaporated to dryness at room temperature under a stream of nitrogen in 0.8 ml capacity reaction vials.

Stage 3. To each vial was added 20 μl of ethyl acetate and 100 μl of pentafluoropropionic anhydride; the reaction vials were then tightly capped with Teflon lined seals and heated at 60° for 30 min in an aluminium heating block. The samples were allowed to cool to room temperature and transferred to 4.0 ml of a saturated solution of sodium tetraborate on top of which was 400 μl of cyclohexane, the sample being added to the cyclohexane phase. The tubes were shaken for 15 sec, centrifuged briefly to separate the phases and an aliquot of the upper organic phase removed into a capped vial. A 1-μl sample was injected onto the gas chromatograph and the PE-derivative was eluted with a retention time of 9.0 min under the conditions described above.

RESULTS AND DISCUSSION

Since there is no evidence that PE in brain tissue is subject to any rapid post-mortem changes* it was unnecessary to take special precautions, other than those mentioned above, in killing the animal and removal of the brain. Further, we have found that brain tissue can be stored at −20° for periods of at least 1 month with no loss in PE. Internal standards were routinely added during the homogenisation stage.

Column chromatography. Amberlyst 15 was used in preference to the standard cation exchange resins for the chromatographic purification as it was found necessary to elute the amine from the resin with methanolic-HCl. This frequently resulted in poor flow characteristics with the standard cation exchange resins due to removal of the aqueous phase. However, Amberlyst 15, a macroreticular cation exchange resin, shows very little shrinkage on removal of the aqueous phase, with the result that optimal flow characteristics are maintained during the elution with methanolic-HCl. The recovery of 25 ng of PE from the resin under these conditions is 89.5 ± 1.0% (mean ± S.E.M., $n = 5$).

Acetylation. Under the conditions used PE is converted to *N*-acetyl-2-phenylethylamine, the structure of which has been confirmed by mass spectrometry (Fig. 1).

This acetylation stage confers several advantages in this assay procedure besides functioning as an additional purification stage. The derivative is readily soluble in ethyl acetate and can be recovered from the aqueous phase in excellent yield (95+%) without contamination from inorganic salts which interfere with the subsequent reaction. Further, the compound is insufficiently volatile to be lost on evaporation of the solvent ethyl acetate.

Preparation of pentafluoropropionate derivative. The structure of the product obtained from the reaction of *N*-acetyl-2-phenylethylamine with pentafluoropropionic anhydride was investigated with nuclear magnetic resonance spectroscopy.

Proton magnetic resonance spectra of this compound show a singlet due to 5 aromatic protons at 7.17 ppm, a singlet from the protons of the acetyl group at 2.39 while the side chain protons α- and β- to the nitrogen gave complex multiplets centred at 3.90 and 2.82 ppm; the lower field resonance being tentatively assigned to the protons α- to the nitrogen in agreement with previous work [20]. Fluorine magnetic resonance spectra showed two singlets at 116.4 ppm (due to CF_2) and 82.1 ppm (due to CF_3) using CCl_3F as reference. No coupling was observed which is typical of the C_2F_5 group. This data is consistent with the structure *N*-acetyl, *N*-pentafluoropropionyl-2-phenylethylamine.

Removal of excess reagent. The normal method of removal of excess pentafluoropropionic anhydride prior to gas chromatography is to evaporate the reagent under a stream of nitrogen; this, however, results in a considerable loss of the derivative due to its volatility. We have found that it is possible to partition the derivative between cyclohexane and a saturated solution of sodium tetraborate, obtaining a complete separation of the pentafluoropropionic anhydride from the PE derivative which is quantitatively recovered in the organic phase. Such a separation is not possible with the *N*-pentafluoropropionyl-2-phenylethylamine as this derivative is hydrolysed on contact with aqueous solutions. The greater stability of the *N*-acetyl, *N*-pentafluoropropionyl-2-phenylethylamine is presumably due to the decrease in the basicity of the aliphatic nitrogen following acetylation.

Gas chromatography. The gas chromatographic characteristics of the derivative are good but it was found necessary to use injection port temperatures of 250° in order to allow continued use of the column over a period of time; presumably this prevented the deposition of involatile tissue components on the injection port. Under these conditions the PE derivative is resolved from the similar derivative formed from benzylamine (thought not to be present in the rat brain tissue [21]). Derivatives formed from phenylethanolamine, *p*-tyramine, octopamine, noradrenaline, dopamine and adrenaline do not co-chromatograph with the derivative formed by 2-phenylethylamine under the conditions used in this assay.

A typical GC record obtained from 4 g of rat brain tissue is shown in Fig. 2b and the value of 1.1 ± 0.2 ng/g (mean ± S.E.M., $n = 6$) obtained by this method for the endogenous concentration of PE in rat brain is in good agreement with other published work [17–19]. Administration of pargyline hydrochloride (75 mg/kg i.p.) 4 hr before death caused a dramatic increase in the brain levels of PE (Fig. 2d) to 96.9 ± 19.2 ng/g (mean ± S.E.M., $n = 6$). On the same GC record can be seen the peak due to benzylamine (Bz), a metabolite of pargyline [21], which under these conditions was found to have a concentration in whole brain of 221.6 ± 29.8 ng/g (mean ± S.E.M., $n = 6$).

The method described above was developed for the estimation of PE but it can be readily modified for the quantification of a number of structurally related cerebral amines and drug metabolites.

* Personal communication from A. A. Boulton and S. R. Philips.

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