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ELECTRON-CAPTURE GAS CHROMATOGRAPHIC ANALYSIS OF β-PHENYLETHYLAMINE IN TISSUES AND BODY FLUIDS USING PENTAFLUOROBENZENESULFONYL CHLORIDE FOR DERIVATIZATION

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SUMMARY

A gas chromatographic procedure is described for the analysis of β-phenylethylamine (PEA) in tissues and body fluids. The method involves the use of pentafluorobenzenesulfonyl chloride for extraction and derivatization of PEA. This is followed by separation and analysis of the derivatized amine on a gas chromatograph equipped with a fused-silica capillary column and an electron-capture detector. The procedure is rapid, provides a stable and sensitive derivative, and has been applied to analysis of PEA in brain, heart, kidney, liver, lung, spleen and blood from the rat and urine from human subjects.

INTRODUCTION

β-Phenylethylamine (PEA) is a so-called "trace amine" [1] which has received a great deal of attention in recent years because of its possible involvement in the etiology of a number of psychiatric and neurological disorders [2]. The normally low levels of PEA in tissues and body fluids meant that highly sensitive and specific methods had to be developed in order to accurately quantitate this amine. Mass spectrometric (MS) procedures have predominated in this regard [3—7] although other techniques, including a radioenzymatic method [8], gas chromatography (GC) with electron-capture detection (ECD) [9] and a fluorescence method [10] have also been shown to give values in tissues similar to those given by MS. We report here a novel, rapid GC—ECD procedure for analysis of PEA which is readily applicable to quantitative analysis in both tissues and body fluids.

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EXPERIMENTAL

Animals

Male Sprague—Dawley rats (175—220 g) were obtained from Bio-Science Animal Services (Ellerslie, Alberta, Canada). The animals were housed in plastic cages on cedar chip bedding in a temperature-controlled room (21°C) with alternate 12 h light and dark schedule. Food and water were provided ad libitum. Animals were fed with Lab-Blox Feed from Wayne Food Division (Continental Grain, Chicago, IL, U.S.A.) composed of 4.0% crude fat (minimum), 4.5% crude fibre (maximum) and 24% crude protein (minimum).

Collection of urine samples from humans

Urine samples (24 h) were collected from twelve normal subjects (six males, six females). Collections were made in plastic receptacles containing 20 ml of EDTA (2%, w/v). At the end of the collection period, aliquots of the urine samples were retained and frozen at −20°C until the time of analysis.

Drugs and chemicals

β-Phenylethylamine hydrochloride, 2-(4-chlorophenyl)ethylamine (CPEA), pentafluorobenzenesulfonyl chloride (PFBSC), di-(2-ethylhexyl)phosphate (DEHPA), and DL-tranylcypromine (TCP) hydrochloride were obtained from Sigma (St. Louis, MO, U.S.A.). The hydrochloride salt of CPEA was prepared by Drs. T.W. Hall and R.G. Micetich in our laboratories. All solvents employed were of highest purity grade commercially available. Water was double distilled in a Corning AG-3 all-glass distillation apparatus.

Extraction and derivatization

Analysis of PEA in tissues of the rat. Groups of control rats or rats administered the monoamine oxidase inhibitor TCP (in isotonic saline solution) intraperitoneally (i.p.) at a 0.1 mmol/kg dose 30, 90, 180 or 240 min before death were used. Rats were sacrificed by cervical dislocation and brain, liver, heart, lungs, kidneys and spleen were dissected out immediately and frozen solid in isopentane on solid carbon dioxide. Blood was collected into vials containing 100 μl of a saturated disodium EDTA solution. All the tissues were stored at −60°C until the time of analysis. Partially thawed tissues were cut into small pieces, weighed and homogenized in 5 vols. of 0.4 *M* perchloric acid (containing 10 mg% of disodium EDTA) using a Tri-R Stir-R (Model S 63C) homogenizer and a Teflon pestle and glass mortar (clearance 0.1—0.15 mm). The homogenates were centrifuged (IEC, B-20 centrifuge) for 15 min at 12 000 *g*. Aliquots (3 ml) of supernatant were transferred to a set of tubes and 2000 ng of CPEA were added as internal standard to all tubes. A set of standards was also included in each assay run and carried through the entire procedure. In the case of blood from the rats, frozen samples were allowed to thaw out completely, and 1-g portions were weighed into tubes to which 2 vols. of 0.4 *M* perchloric acid were added. The tubes were vortexed and the contents were sonicated for 5 min in a sonicator bath, after which they were centrifuged at 1000 *g* for 5 min. The resultant clear supernatants were used in the assay procedure.

The perchloric acid supernatants were neutralized by addition of solid potassium carbonate and the precipitate was removed by centrifugation (1000 *g* for 3 min). The resultant supernatants were transferred to another set of tubes. The supernatants were basified by further addition of solid potassium carbonate, and a 3-ml mixture of toluene–acetonitrile–PFBSC (9:1:0.01) was added to each tube. The tubes were shaken vigorously for 2 min, and following a brief centrifugation, the top organic layer was transferred to a set of clean tubes and taken to dryness under a stream of nitrogen. The residue was taken up in 300 μl of toluene and an aliquot (1 μl) was used for GC analysis.

Analysis of PEA in human urine samples. After thawing, portions (4 ml) of urine were transferred to clean tubes. The internal standard CPEA (500 ng) was added to each tube and the samples were basified by addition of solid potassium hydrogen carbonate and centrifuged briefly at 1000 *g* for 3 min. The clear supernatants were transferred to another set of tubes, 400 μl of sodium phosphate buffer (pH 7.8) were added, and the urines were extracted by shaking with 4 ml of chloroform containing the liquid ion-pairing agent DEHPA (2.5%, v/v). Following a brief centrifugation, the top aqueous layer was aspirated off and the chloroform layer was vigorously shaken with 2.5 ml of 0.5 *M* hydrochloric acid solution for 5 min. The aqueous acid layer was transferred to another set of tubes following a brief centrifugation. The subsequent derivatization procedure was essentially the same as described for the analysis of PEA in the tissues of rat except that 4 ml of the derivatizing mixture were used.

Gas chromatography

A Hewlett-Packard (HP) 5890 gas chromatograph fitted with a 15 mCi ^{63}Ni linear electron-capture detector and a narrow-bore SE-54 fused-silica capillary column (dimensions 15 m × 0.255 mm, 0.25-μm film of 5% phenylmethyl silicone + 1% vinyl as stationary phase) obtained from J. & W. Scientific (Rancho Cordova, CA, U.S.A.) was employed. The carrier gas was helium at a flow-rate of 2 ml/min, and the makeup gas was methane–argon (5:95) at a flow-rate of 35 ml/min. The injection port and detector temperatures were 200°C and 300°C, respectively. A two-level oven temperature programme was used: the initial temperature of 105°C was maintained for 0.5 min, then the oven was heated at 30°C/min to 255°C, at which temperature it was held for 1 min; this was follwed by an increase (at 25°C/min) to 280°C, and the final temperature was maintained for 10 min. An HP 3392 A integrator was used to measure the peak areas. The retention times of the derivatives of PEA and the internal standard were 5.1 and 5.9 min, respectively.

Gas chromatography–mass spectrometry

Although GC–ECD was used for routine analysis, the structure of the derivative was first confirmed using a gas chromatograph equipped with a 15-m SE-54 column and coupled to a VG 7070-E mass spectrometer in the electron-impact (EI) mode. A PDP-11/73 computer was used to acquire the data. The operating conditions of the mass spectrometer system were as follows: ion source temperature, 250°C; interface temperature, 290°C; column pressure, 1.035 bar; accelerating voltage, 6 kV; ionization voltage, 70 eV; scan speed, 0.5 s/decade; dwell time, 0.5 s.

RESULTS AND DISCUSSION

The procedure is rapid and the derivative produced is stable, sensitive and has good chromatographic properties (see Fig. 1 for typical GC traces). The standard curves are linear from 1 to 1000 ng of PEA ($r > 0.99$) and the on-column sensitivity is < 5 pg. By decreasing the volume of toluene in which the derivative was dissolved to about 40—50 μl instead of the usual 300 μl, the sensitivity could be increased five- to six-fold. The mean overall recovery as determined using the procedure which utilizes the DEHPA procedure for the urines (20 ng PEA standards) was 93%. The proposed electron-impact MS fragmentation of the pentafluorobenzenesulfonyl (PFBS) derivative of PEA is shown in Fig. 2. Further confirmation of structure was obtained by conducting chemical-ionization (CI) MS (using methane), which yielded *m/z* 352 ($[M+H]^+$) as the base peak.

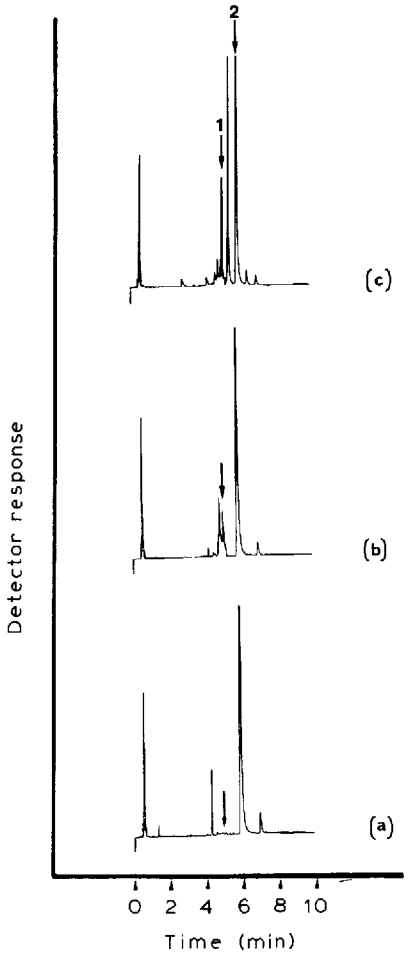

Fig. 1. Typical gas chromatograms from extracts of: (a) 0.1 *M* perchloric acid extract blank; (b) a control rat brain; and (c) a brain from a rat treated with tranylcypromine (0.1 mmol/kg) 0 5 h previously. Peaks: 1 = derivatized PEA, 2 = derivatized CPEA, internal standard. The portion of the gas chromatogram corresponding to peak 1 was run at attenuation values of 2↑5 in traces a and b and 2↑7 in trace c.

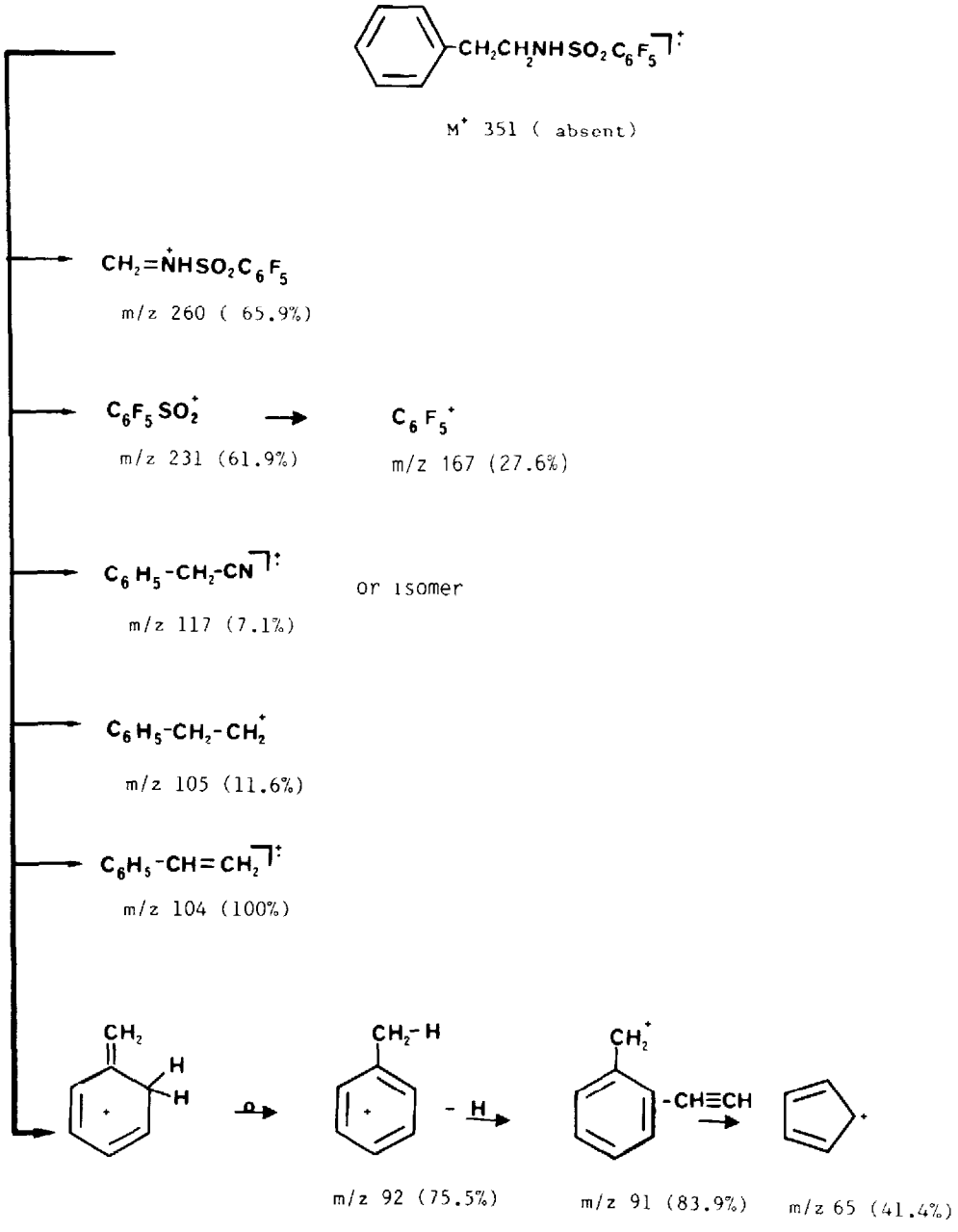

Fig. 2. Proposed electron-impact mass spectrometric fragmentation of derivatized PEA

The mean PEA levels in tissues from control rats and from those receiving TCP 30 min before death are shown in Table I. The control values are compared to those determined by high-resolution (HR) MS following dansylation and thin-layer chromatographic (TLC) separation [11]. The control rat brain levels using the present method are in close agreement with earlier reported values [mean ± standard error of the mean (S.E.M.), ng/g] of 1.8 ± 0.15 (TLC—HRMS) [3], 1.75 ± 0.63 (GC—EI-MS) [4], 1.5 ± 1.0 (radio-

TABLE I

LEVELS OF PEA IN THE TISSUES OF CONTROL RATS AND IN RATS 30 min FOLLOWING THE ADMINISTRATION OF TCP (0.1 mmol/kg i.p.)

Tissue	PEA level (mean ± S.E.M., n = 4—8) (ng/g)		
	Controls	TCP treated	Controls using TLC—HRMS*
Blood	2.49 ± 0.31	77.65 ± 7.84	1.9
Brain	1.88 ± 0.36	28.49 ± 2.88	1.8
Liver	3.20 ± 0.48	54.59 ± 8.37	2.0
Lung	5.09 ± 1.21	21.29 ± 3.13	4.0
Kidney	22.00 ± 1.58	141.23 ± 31.27	20.5
Heart	3.42 ± 1.7	17.78 ± 2.53	5.7
Spleen	3.83 ± 1.08	18.84 ± 1.31	4.7

*Data from Philips [11].

TABLE II

PEA LEVELS IN RAT BRAIN, LIVER AND BLOOD AFTER TCP ADMINISTRATION (0.1 mmol/kg i.p.)

Time (h)	PEA level (mean ± S.E.M., n = 6) (ng/g)		
	Brain	Liver	Blood
1.5	36.99 ± 3.30	65.61 ± 4.86	10.71 ± 0 70
3.0	47.57 ± 4.05	77.64 ± 8.41	13.93 ± 1 84
6.0	52.52 ± 19.78	118.75 ± 32.83	8.20 ± 0.46

enzymatic assay) [8], 1.1 ± 0.2 (GC—ECD) [9], 1.4 ± 0.8 (GC—EI-MS) [6], and 2.37 ± 0.03 (GC—positive-ion CI-MS) [7]. The levels of PEA rise dramatically in all tissues within 30 min of administration of TCP. Table II represents the rat brain, liver and blood PEA levels 1.5, 3.0, and 6.0 h after the TCP administration. PEA levels increase in brain and liver and continue to increase at 6 h, whereas in blood the levels have begun to fall by this time.

The 24-h urinary levels of free PEA in twelve normal human controls (six males, six females) were 3.40 ± 0.35 μg/g of creatinine (mean ± S.E.M.). These values are in good agreement with those reported using TLC—HRMS [12]. We previously reported a lengthier GC—ECD method [13] which provided for simultaneous analysis of the amines PEA, m- and p-tyramine, normetanephrine and 3-metanephrine, but gave values of PEA which were higher than those found by TLC—HRMS [12].

The method described here, involving shaking alkaline tissue homogenates or body fluids with an organic mixture of PFBSC, combines both derivatization and extraction in a single step, thereby providing a simple technique for rapid analysis of samples. In the case of analysis of PEA in urine samples it was necessary to include an extraction step with the liquid ion-pairing agent DEHPA to provide cleaner extracts. This process is simple and rapid compared to purifications involving conventional ion-exchange columns.

Many of the methods of analysis previously employed for PEA analysis

involve time-consuming and tedious extraction procedures. MS procedures, although highly sensitive and specific, are often outside the financial limits of small laboratories and require highly trained personnel. Our procedure involving GC—ECD with a capillary column offers an alternative method of analysis with accuracy and sensitivity sufficient for routine analysis.

It has recently been reported that PFBSC is a useful reagent for derivatization of standards of tyrosyl peptides [14] and nucleic acid pyrimidine bases [15] for GC—ECD analysis. In the present paper, we have demonstrated that it is readily applicable to analysis of PEA. Preliminary results in our laboratories indicate that PFBSC may also be a useful reagent for measurement of a number of other biogenic amines and important amine-containing drugs in tissues and body fluids.

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