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Note

Gas chromatographic detection of N-methyl-2-phenylethylamine: a new component of human urine

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2-Phenylethylamine (PE) may well play an important role in normal [1,2] and abnormal [2,3] brain function. Thus the novel detection of its biologically active N-methyl derivative in human urine is of considerable interest. In this note we report the identification of this compound by gas chromatography (GC) and the confirmation of its presence using GC—mass spectrometry.

METHODS

The phenylethylamines were first concentrated by a solvent extraction procedure that has been published in detail elsewhere [4]. Briefly, they were extracted from NaCl-saturated, alkaline urine with diethyl ether and back extracted into acid. This was taken to dryness, made alkaline and re-extracted with diethyl ether. Benzylamine was added to the urine before extraction to serve as an internal standard.

The extracted phenylethylamines were reacted with trifluoroacetic anhydride and separated by GC as their trifluoroacetyl (TFA) derivatives [4]. Samples (2 μl) of a hexane solution of the derivatives, corresponding to 1—2 ml of urine, were chromatographed on a 6 ft × 4 mm I.D. column of 3% QF-1 on Diatomite C 'Q' (100—120 mesh) at 140°. The carrier gas was nitrogen (50 ml/min) and a hydrogen/air flame ionization detector was used.

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RESULTS AND DISCUSSION

Chromatograms of the phenylethylamines from two healthy individuals are shown in Fig. 1. Both urine samples had initially been hydrolysed with acid (pH 1) for 20 min at 100° to release conjugated amines. Peak 3 is coincident with that of the TFA derivative of authentic N-methyl-PE. Fig. 2 shows the mass spectrum of peak 3; the major ions at *m/e* 104 and 140 are consistent with a compound containing phenylethyl and N-methyl-trifluoroacetamide moieties, respectively. Comparison with the mass spectrum of genuine N-methyl-PE-TFA confirms the presence of N-methyl-PE in hydrolysed human urine and shows that, for the sample investigated, its derivative is the major component of peak 3. There were no cases of a peak being detected which co-chromatographed with, but could not be identified as, this compound.

Thus N-methyl-PE excretion can be estimated from the height of peak 3 although accurate quantification, using the ratio of the peak heights of the N-methyl-PE and the benzylamine (internal standard) derivatives, is made difficult due to the low concentrations of the former compound. However, significant quantities (0.2—1.0 μg/24 h) have been demonstrated in eleven out of fifteen hydrolysed normal urine samples, but in no case did excretion exceed 1 μg/24 h. Reproducibility of the method was approximately ± 0.2

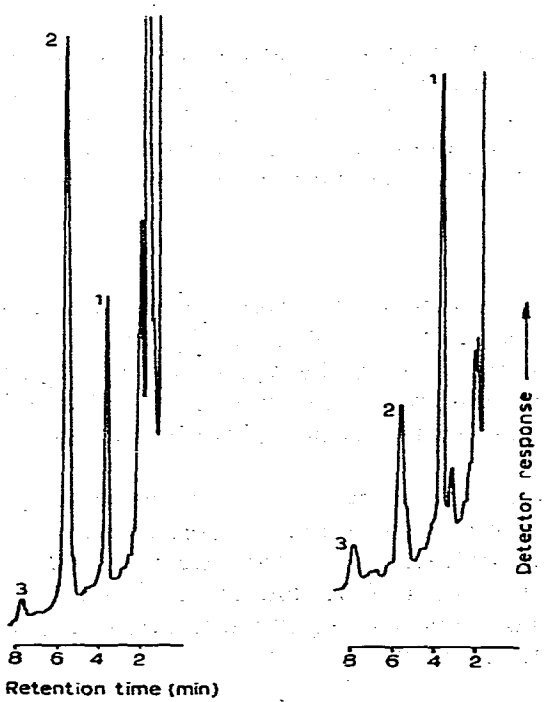

Fig.1. GC traces of hydrolysed urine extracts from normal individuals with peaks corresponding to benzylamine internal standard (1), PE (2) and N-methyl-PE (3). Gas chromatograph: Pye Unicam 104 Series 2; nitrogen flow-rate 50 ml/min; oven temperature, 140°. Column: 6 ft × 4 mm I.D. Diatomite C 'Q' (100—120 mesh) coated with 3% QF-1. Detector: hydrogen/air flame ionization.

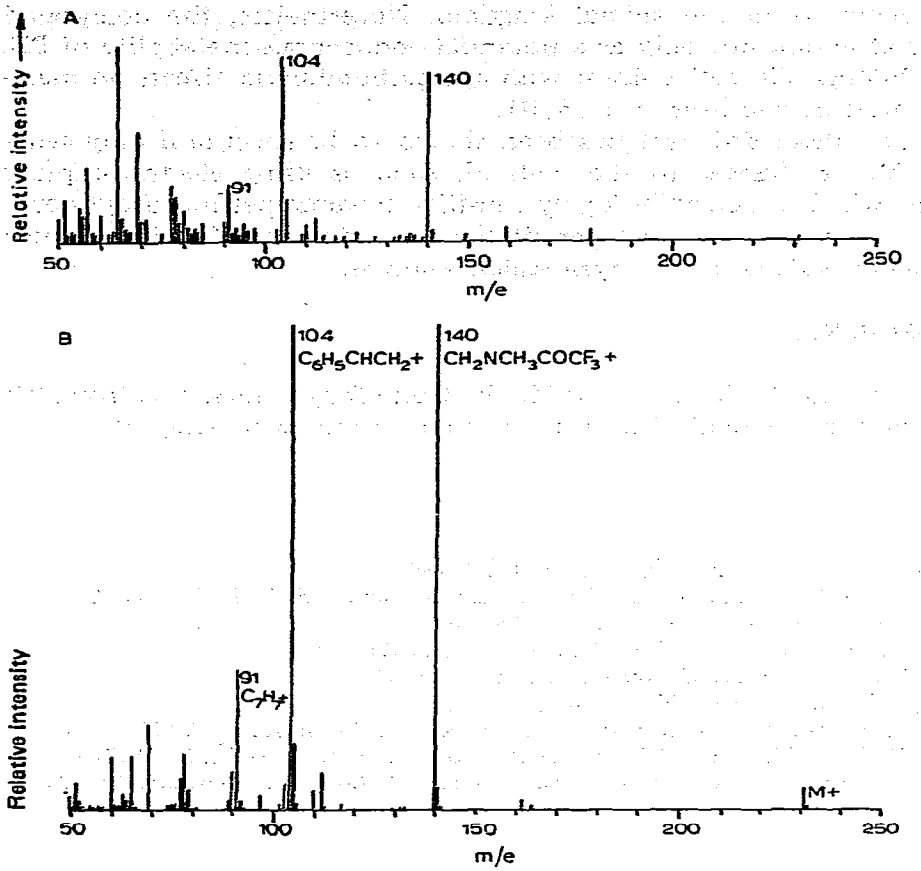

Fig.2. Mass spectra of peak 3 (A) and authentic N-methyl-PE-TFA (B). Mass spectrometer: LKB 9000S GC—MS. Column: 9 ft × 4 mm I.D. Diatomite C 'Q' (100—120 mesh) coated with 3% QF-1. Conditions: helium carrier gas flow-rate, 30 ml/min; oven temperature, 160°.

μg/24 h standard deviation, while variations in recovery were compensated for by the presence of the internal standard [4].

The compound is normally found only as an acid-labile conjugate. However, the corresponding GC peak has been obtained from a single unhydrolysed urine sample excreted by a normal individual who also exhibited a transient and anomalously large increase in urinary PE [5]. A peak corresponding to N-methyl-PE-TFA was also observed in some extracts of unhydrolysed urine from phenylketonuric patients, who normally excrete increased amounts of PE [5].

Urinary N-methyl-PE may perhaps be synthesized endogenously; a mammalian enzyme which methylates PE has already been described [6]. However, its variable excretion rate and presence as a conjugate could suggest that it is at least partially dietary in origin [7]. N-methyl-PE is known to occur in vegetable foodstuffs [8] in quantities up to 6.6 ppm, but it has not prev-

iously been reported in the animal kingdom. Nevertheless, the compound justifies some attention not only as a potential endogenous metabolite of PE, but also as a biologically active agent with sympathomimetic effects on mammalian blood pressure and heart rate [8, 9].

The procedure described here has been shown to be specific if only semi-quantitative. Minor changes to the method, such as using electron-capture GC detection (which is generally highly sensitive towards perfluoroacyl derivatives), should overcome this and permit the accurate quantification of such PE derivatives in a wide range of physiological samples.

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