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QUANTITATION OF ADRENALINE AND NORADRENALINE FROM HUMAN PLASMA BY COMBINED GAS CHROMATOGRAPHY—HIGH-RESOLUTION MASS FRAGMENTOGRAPHY*

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SUMMARY

A gas chromatographic—high-resolution mass fragmentographic method for the simultaneous determination of adrenaline and noradrenaline from human plasma is presented. The catecholamines are separated by adsorption on alumina and converted by a selective, two-step procedure to the corresponding N-trifluoroacetyl-N-trimethylsilyl derivatives. The benzylic fragment $C_{16}H_{31}O_3Si_2$ (*m/e* 355.1568) of these derivatives is detected at a mass spectrometric resolving power of 5000. This high resolution detection was necessary to differentiate this fragment from others with the same nominal mass of 355 originating from the biological matrix and/or the bleeding from column and septum.

INTRODUCTION

There exists an increasing interest in the accurate and sensitive quantitative determination of the catecholamines adrenaline and noradrenaline in human plasma. Estimation of the concentration of circulating catecholamines is of importance in consideration of the pathogenesis of essential hypertension and the evaluation of stress factors in relation to cardiovascular diseases. Detection and localization of catecholamine-producing tumours such as neuroblastoma and pheochromocytoma can be achieved by means of plasma catecholamine measurements.

Analytical procedures for these estimations demand methods with a high

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degree of sensitivity and specificity since the plasma concentrations are extremely low. Among the detection methods which have been described for plasma catecholamines are fluorimetry, radiometry, gas chromatography (GC) and mass fragmentography. Fluorimetric procedures normally show a certain lack of specificity [1, 2], or need relatively large plasma samples [3]. The radioenzymatic double-isotope assay affords good reliability and specificity [4]. An increased sensitivity was obtained with tritiated S-adenosyl-methionine as methyl donor [5]. However, the experimental effort required for these methods is considerable. GC procedures with electron capture detection allow the required sensitivity [6], whereas the specificity is inadequate for the small amounts present in plasma. A GC method with a dual hydrogen flame detector has been reported, but the structure of the employed derivatives was not clarified [7].

Wang et al. [8] have published a low-resolution mass fragmentographic method using per-trifluoroacetyl derivatives. Drawbacks to this procedure, in addition to the low mass numbers of the detected fragments, are the instability and the poor GC separation of these derivatives.

Due to the high reliability and sensitivity of mass fragmentography we have developed another, improved method for plasma catecholamines using high-resolution mass fragmentography, which offers an outstanding degree of specificity.

EXPERIMENTAL

Materials

All reagents and solvents were of analytical grade and were obtained from E. Merck (Darmstadt, G.F.R.), unless otherwise specified. Noradrenaline was purchased from Fluka (Buchs, Switzerland), isoprenaline was a gift from Boehringer Ingelheim (Ingelheim, G.F.R.). Alumina (Woelm, neutral, activity grade I) was supplied by ICN Pharmaceuticals (Eschwege, G.F.R.) and treated as described by Von Studnitz [9]. N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) and N-methyl-bis-(trifluoroacetamide) (MBTFA) were obtained from Macherey-Nagel (Düren, G.F.R.).

Procedure

Blood samples were collected (after a short rest) from the brachial vein, into 10-ml Li-heparinized tubes which each contained 5 mg sodium dithionite as antioxidant. The blood was immediately cooled with ice-water and centrifuged (4°, 15 min, 1000 *g*). A 2-ml volume of plasma was deproteinized with 0.16 ml of 70% perchloric acid and again centrifuged (4°, 20 min, 15000 *g*). The supernatant was transferred, 20 ng isoprenaline and 1 ml of 0.2 *M* Na_2-EDTA were added, and the pH was adjusted to 6 with 4 *M* NH_4OH. A 300-mg amount of alumina was neutralized with 0.1 *M* ammonium acetate, both the adsorption and elution of the catecholamines were performed as described by Wang et al. [8]. A 10-μl volume of a 0.1% solution of methyl orange in methanol was added to the eluate, which was concentrated at 30° by vacuum evaporation and dried in a vacuum desiccator over potassium hydroxide—phosphorus pentoxide for at least 8 h. The residue was derivatized in a 1-ml glass-

stoppered tube following the procedure of Donike [10, 11] by dissolving in 20 μl of trifluoroacetic acid and treating with 45—55 μl MSTFA (the exact amount depends on the colour change of the indicator from red to yellow) for 30 min at 80°. A 10-μl volume of MBTFA was added and after a 10-min treatment at ambient temperature the mixture was heated for 5 min at 80°. Of this solution 2 μl were used for mass fragmentography. The solutions can be stored in a desiccator for several days, provided that a possible colour change of the indicator is titrated with a slight excess of MSTFA.

Gas chromatography—mass spectrometry

GC—mass spectrometry (MS) analyses were performed on a double focusing mass spectrometer MAT 311 A, which was coupled to a gas chromatograph Varian 1440 (Varian, Bremen, G.F.R.). An all-glass coupling system with a one-stage glass frit separator was used. A device for removal of large quantities of the solvent was installed between the GC column and the separator. The peak matching decade served for the exact monitoring of the desired mass using perfluorokerosene as reference substance. GC—MS conditions are as follows: column, 2 m × 2 mm I.D., 4% OV-17 on Chromosorb W AW DMCS; carrier gas, He, 30 ml/min; injector 250°; column temperature 200°; connection capillary 240°; separator 250°; line-of-sight 160°; ion source 160°; ionization energy 70 eV; emission current 3 mA; electron multiplier 2.0—2.4 KV; resolving power 5000; detected mass 355.1568.

RESULTS AND DISCUSSION

Due to their high stability against oxidation and excellent GC properties we chose the N-trifluoroacetyl-O-trimethylsilyl (N-TFA-O-TMS) derivatives [10, 11]. They can be well separated on silicon phases and do not show adsorption effects either on the GC column or in the GC—MS coupling system. The derivatization has to be carefully performed in a two-step procedure under controlled silylation conditions as outlined by Donike [10, 11].

The mass spectra of the N-TFA-O-TMS derivatives exhibit weak molecular ions, whereas the base peaks are formed by very intense benzylic fragments at *m/e* 355, which we used for mass fragmentography. Detection of the fragment ion at *m/e* 355, and not the molecular ion, leads to the loss of important information concerning the molecular structure. However, this mutual ion allows the simultaneous detection of both catecholamines and the internal standard isoprenaline.

The N-TFA-O-TMS derivatives have been successfully applied to the low-resolution mass fragmentographic determination of catecholamines from tissues [10]. Hitherto plasma samples could not be assayed using this type of derivative because of the low content of catecholamines and the large amounts of interfering substances from the biological matrix.

The sensitive and selective low-resolution monitoring of the benzylic fragment is excluded by the presence of ions with the same nominal mass number (Fig. 1). These undesirable ions originate either from the silicon phases of the column and the septum or from the biological material. They are particularly intensified in the analyses of plasma samples, where sometimes even negative

Fig. 1. Detection of N-TFA-O-TMS catecholamines in plasma. Sweep from mass 354.5 to 355.5 (R=5000). The expected signal of the benzylic fragment $C_{16}H_{31}O_3Si_3$ (*m/e* 355.1568) is indicated by an arrow (No. 3) and corresponds to the isoprenaline peak in the chromatogram.

peaks are observed. However, these difficulties could be overcome by increasing the resolving power of the mass spectrometer to 5000. Thus we were able to separate completely the monitored signal of the benzylic fragment $C_{16}H_{31}O_3Si_3$, with the precise mass of 355.1568, from the background. The impressive specificity of high-resolution mass fragmentography was previously shown in the detection of steroids by Millington et al. [12].

The comparison of the analyses of the same plasma sample with low-resolution detection (Fig. 2a, R = 1000, *m/e* 355) and high-resolution detection (Fig. 2b, R = 5000, *m/e* 355.1568) clearly demonstrates the superior specificity of high-resolution mass fragmentography. The low-resolution fragmentogram results in totally incorrect intensity ratios.

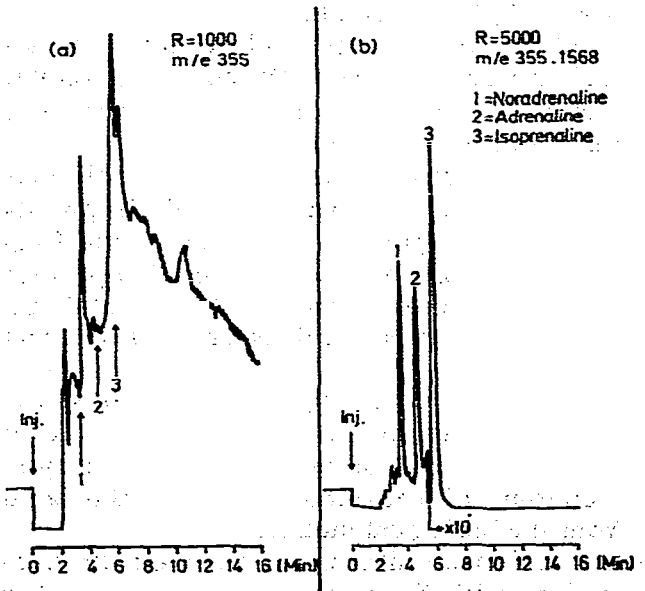

Fig. 2. Comparison of the detection of plasma catecholamines with low-resolution mass fragmentography, (a) R = 1000, *m/e* 355, and high-resolution mass fragmentography, (b) R = 5000, *m/e* 355.1568. The content of this sample was 0.2 ng/ml adrenaline and 0.7 ng/ml noradrenaline.

The detection limit was 2 pg of injected pure sample with a signal-to-noise ratio of 2 to 1. The coefficient of variation of the method is 16% (n = 6) at a catecholamine level of 0.2 to 0.7 ng/ml plasma. The standard curve for adrenaline shows a correlation coefficient of 0.9901 (Fig. 3). Plasma levels of healthy volunteers lay at 0.1 ng/ml for adrenaline and from 0.2 to 0.4 ng/ml for noradrenaline. These values correspond well with those obtained from the literature as determined by radioenzymatic assays.

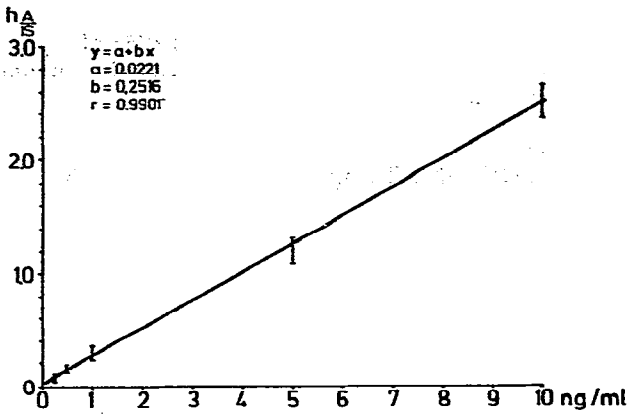

Fig. 3. Standard curve for adrenaline (N-TFA-O-TMS derivative).

By our method a pheochromocytoma could be diagnosed and localized before confirmation by surgical excision of the tumour. The determination of the catecholamine concentration in the vena cava inferior gave the following values.

TABLE I

VENOUS CATECHOLAMINE CONCENTRATIONS

Sample site	Noradrenaline (ng/ml)	Adrenaline (ng/ml)
V. cava inferior (superior part)	10.0	6.0
V. suprarenalis dextra	> 15.0	6.5
Abouchement of vv. renales	7.5	1.9
V. cava inferior (bifurcation)	4.0	1.5
V. iliaca communis dextra	1.0	1.0

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