AN IMPROVED METHOD FOR ANALYSIS OF CATECHOLAMINES
— GAS-LIQUID CHROMATOGRAPHY (GLC) EQUIPPED
WITH ELECTRON-CAPTURE DETECTOR

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Different groups of workers have reported analysis of small amounts of catecholamines (CA) in animal tissues by a gas-liquid chromatograph equipped with electron-capture detector (GC-ECD) (1, 2, 3). This method allows for adequate separations of a large range of CA and their metabolites and sensitive detection at the picogram level.

For the quantitative assay of biogenic CA, the choice of suitable stationary phase, internal standards and acylating agents plays a definite role in the accuracy of the findings. We obtained a good separation and sensitive detection of CA and their metabolites with the use of OV-105 on Chromosorb W as a stationary phase, alpha-methyl dopamine (α-MDA) and alpha-methylnorepinephrine (α-MNE) as internal standards for dopamine (DA) and norepinephrine (NE) respectively, and pentafluoropropionic acid anhydride (PFPA) as a fluorocylating agent.

A Packard Model 7400 gas chromatograph equipped with a dual ECDs (63Ni 15 mci)
was used. Siliconized glass columns (2 mm i.d. × 3 m) were packed with Chromosorb W-AW-DMCS (alcoholic treatment, 60–80 mesh) coated with 2% OV-105. Injection and detector chamber temperature were maintained at 225°C. Column temperature was 175°C. At this temperature, the carrier gas N₂ (99.999%) flow rate was 20 ml/min.

For the initial characterization, authentic CA prepared by dissolving 100–500 ng in 0.4N acetic acid-methanol was lyophylized in Riacti-Vials (5 ml) prior to fluoroacyl derivative formation. The lyophylized residues were dissolved in 50 µl acetonitrile and 10 µl of PFPA was added. The vials were tightly capped and placed for 30 min in a heating block maintained at 60°C. The acylating agent was removed under a stream of dry nitrogen and the residues were dissolved in an appropriate volume (50–500 µl) of benzene containing Aldrin 100 ng/ml as the internal standard for epinephrine (E) and 1.0 µl was then injected into the GLC-ECD.

Fig. 1-a shows a typical gas chromatogram of authentic E (retention time: 5.7 min), DA (7.9 min), NE (9.4 min), α-MDA (6.4 min), α-MNE (6.8 min) and Aldrin (13.6 min).

To estimate the biogenic CA, tissues and organs were removed from 250–300 g male Wistar rats. CA was extracted according to the method of Wang et al. (4). Adsorbed CA on alumina (200 mg) at pH 8.6 was eluated with 3 ml of 0.4N acetic acid-methanol. These eluates were lyphophilized and pentafluoroacylated as mentioned above. The internal standards, α-MDA and α-MNE, were added as an aqueous solution to the samples prior to homogenization.

Fig. 1-b shows a gas chromatogram of CA from the hypothalamus dissected according to the method of Glowinski and Iversen (5). The CA contents in various rat tissues are summarized in Table 1, and the results are in good agreement with the reported data (6).

Catecholamine metabolites and other amines were also studied using this stationary
phase. We obtained a good separation of PFPA derivative of authentic tyramine (retention time: 3.8 min), octopamine (5.3 min), metanephrine (7.9 min), normetanephrine (9.7 min) which were reacted at 60°C for 30 min for volatile derivatives formation. Alpha-methylated CA as the internal standard is advantageous, because the catechol base is quantitatively adsorbed onto alumina and can be added to the sample prior to adsorption and carried through the entire procedure. These properties facilitate the quantitative assay of CA and eliminate interference in the calculation by errors in each sample during analytical procedures. Alpha-methylated CA as the internal standard has been used in GC-MS analysis of CA (7), but the analysis by electron capture detector has not been reported.

The possibility of interference with tyramine, octopamine, metanephrine and normetanephrine to DA, NE and E is negligible because they are not adsorbed on alumina.

In the present study, α-methylepinephrine could not be used as an internal standard but, by utilizing a comparative value to Aldrin, it was possible to estimate the epinephrine content of a minute amount of brain epinephrine as well as peripheral epinephrine.

An enzymic double-labelled isotope method and GC-MS analytical method have been reported for the determination of small amounts of CA (8, 9), however, both methods are cumbersome and therefore, not popular. We found GC-ECD to be a powerful tool for the simultaneous determination of small amounts of CA. Moreover, gas chromatographic quantitative analysis of α-methylated compounds may offer a new approach to the metabolic studies of α-methyldopa, clinically widely used as an antihypertensive drug.

REFERENCES


RENNIN INHIBITORY EFFECT OF N-ACETYL-PEPSSTATIN

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It has been demonstrated that pepstatins isolated from streptmyces inhibit the renin activity and among pepstatins and other renin inhibitory substances, pepstatin A, isovaleryl pentapeptide is known as a most potent renin inhibitor (1, 2, 3). The solubility of this compound limits application in many studies. In the present work, the renin inhibitory activity of a soluble acid protease inhibitor, N-acetyl-pepsstatin, was demonstrated both in vitro and in vivo. N-acetyl-pepsstatin was found in a culture filtrate of streptmyces naniaensis by Murao and Satoi (4). The chemical structure is N-acetyl-valyl-valyl-4-amino-3-amino-hydroxy-6-methylheptanly-alanyl-4-amino-3-amino-hydroxy-6-methylheptanic acid; M.W. 644 (5). The same acetyl pentapeptide was also found from strain MD494-A1 of streptmyces parvisporogenes by Aoyagi et al (6). N-acetyl-pepsstatin is soluble in phosphate buffer at pH 7.4 in concentrations higher than 20 mg/ml.

In vitro experiments were carried out using partially purified dog renin and dog renin substrate as described in previous papers (7, 8). One tenth ml of 50 ng angiotensin I equivalent per hour (Ang-I Eq/hr) of renin and 0.2 ml of 200 ng Ang-I Eq of renin substrate were incubated for 1 hr at 37°C with N-acetyl-pepsstatin dissolved in 1/3M phosphate buffer (0.2 ml, pH 7.4) containing 8 mM EDTA and 0.02% Neomycin. The angiotensin I formed in this mixture was determined by radioimmunocassay. Inhibitory activity of N-acetyl-pepsstatin was expressed as percentage reduction in the production rate of angiotensin I. Average inhibitions of the renin activity by N-acetyl-pepsstatin in a concentration of 10⁻⁶ and 10⁻⁵ M were 30.9±7.2% and 64.8±3.2%, respectively (n=8), while those with the same concentration of pepstatin A were 49.7±7.8% and 89.2±3.7%, respectively (n=6).

Renin inhibitory effect of N-acetyl-pepsstatin in vivo was determined by inhibition of