

SHORT REPORTS

Quantitative Analysis of Catecholamines in Skin and Serum

C. Hansson and E. Rosengren

Department of Pharmacology, University of Lund,
Lund, Sweden

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Abstract. A quantitative method for the analysis of noradrenaline and adrenaline by means of high performance liquid chromatography (HPLC) and electrochemical detection is described. The method is applicable to tissues as well as serum.

Key words: Catecholamine; High performance liquid chromatography; Biological sample

There is a great need for analytical methods for catecholamines permitting quantification of these compounds in serum and in small tissue specimens. Several studies on standard solutions and tissues with high concentrations of catecholamines have been performed to achieve chromatographic separation of these amines by High Performance Liquid Chromatography (HPLC) (4, 5). In earlier work on the catechol amino acids 5-S-cysteinyldopa and dopa, we elaborated a method for the analysis of normal serum levels of these compounds by means of ion-pair reverse-phase chromatography in combination with an electrochemical detector (3). The technique used in those studies has been further developed and applied to the chromatographically more difficult analysis of noradrenaline (NA) and adrenalin (A) in normal human tissues and body fluids.

MATERIAL AND METHODS

A Waters model 6000 A pump was used together with a Rheodyne Model 7120 sampling valve injector with a 500 μ l loop. Assay was performed by a thin-layer amperometric detector (Bioanalytical Systems Inc., Model LC-10). The chromatographic separations were performed by isocratic elution of a column (4.6 $\times$ 250 mm) packed with 5 μ m octadecyl-silica (Nucleosil C₁₈) with an aqueous eluent containing 0.25 g octane sulfonic acid and 6% methanol per litre. The pH of the eluent was adjusted

to 2.0 by means of 85% phosphoric acid. The flow rate was 1.4 ml/min and the working electrode potential was set at +0.75 V vs. Ag/AgCl as reference electrode.

Peak identities for NA and A were confirmed by changing the chromatographic system, whereupon the sample peaks behaved exactly as authentic samples. Addition of small amounts of authentic material to the sample showed no inhomogeneities in the peaks in any chromatographic system.

Procedure

20 ml human blood samples were collected in plastic centrifuge tubes containing 10 mg Na₂S₂O₅ and centrifuged at 4 500 rpm for 10 min. To 10 ml of serum, 2 ml of 10% EDTA, 1 mg of ascorbic acid and dihydroxybenzylamine (internal standard) were added. Proteins were precipitated by adding 1.0 ml of 4 M HClO₄, whereupon the sample was centrifuged for 10 min at 15 000 rpm and filtered. The pH of the filtrate was adjusted to 5.5 with K₂CO₃, centrifuged and then loaded onto a strong cation exchange column (Dowex 50W-X4, Na⁺-form, 5 $\times$ 50 mm). The ion exchange column was washed twice with 5 ml of water and with 20 ml of 0.05% EDTA solution. The catecholamines were then eluted with 15 ml of 1.2 M HCl. The eluate was transferred to a beaker containing 200 mg of activated alumina (1), 200 mg of EDTA and 10 mg of Na₂S₂O₅. The pH was adjusted to 8.6 with 4 M NaOH. The sample was stirred for 5 min, then transferred to a centrifuge tube and washed with water (4 $\times$ 10 ml) followed by centrifugation. After the final centrifugation and decanting of the supernatant the catechols adsorbed on alumina were eluted in the following way: 1.0 ml of water was added and the pH then adjusted to 1.75 with 35% HBF₄. The sample was finally stirred gently for 15 min and centrifuged. For analysis, 500 μ l of the supernatant was used.

Tissues to be analysed were homogenized in 0.4 M HClO₄ and centrifuged for 5 min at 14 000 rpm, whereupon the supernatant was treated as described for serum.

RESULTS AND DISCUSSION

In Fig. 1 a typical chromatogram of catecholamines obtained from rat skin is shown. The analysed eluent was obtained by purification on a strong cation exchange resin followed by adsorption on alumina and then elution by the non-nucleophilic acid tetrafluoroboric acid. This acid was found superior to the other eluents tested in that the recovery was high, only a small front effect appeared when injected onto the HPLC system, and it eluted no compounds from the alumina which disturbed the analysis.

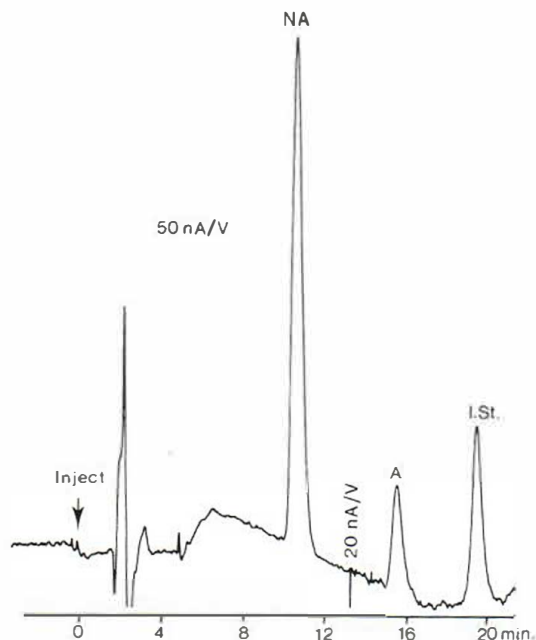


Fig. 1. Chromatogram of material obtained from a rat skin. The contents of NA and A were found to be 75 ng and 9 ng, respectively, per gram wet tissue. I.St. = internal standard.

The chromatographic separation of catecholamines has been performed on several types of column packing material. Ion exchange material has been used widely, as the retention for these compounds on a normal reverse-phase chromatographic system is too low to permit a satisfactory analysis. By addition of a hydrophobic counter ion to the mobile phase used to elute a C_{18} -reverse-phase column, an increased retention is achieved. This technique called ion-pair reverse-phase chromatography, makes it possible to alter the retention of catecholamines to any desired value by simply changing the hydrophobic properties and the concentration of the counter ion. Both theoretical and experimental studies by other workers have been made along similar lines (4, 5). However, these investigations have been performed exclusively on standard solutions or on urine containing high concentrations of catecholamines. The direct application of these techniques to samples with extremely low catecholamine concentrations—such as normal human serum—is not possible, since the purification steps used give the catecholamines in a solution not suitable for such analysis.

As the mobile phase was not suitable as eluent for

the catecholamines from alumina it was essential to have a chromatographic system which achieved equilibrium quickly after the sample to be assayed had been injected. The use of alkylsulphates as counter ion has been widely used in this type of analysis. In our experience, however, these compounds were unsuitable, since they resulted in a non-stable system. The alternative, the alkylsulphonates, gave a much superior stability of the chromatographic system. This is essential, as we found it necessary to use an injector with a large loop (500 μ l) in order to have a sufficient amount of substances injected.

A simpler purification of serum catecholamines, only on alumina, combined with a Nucleosil SA 5 column at 55°C, and with lithium phosphate buffer (pH 3) as eluent, gave very sharp and well-separated peaks.

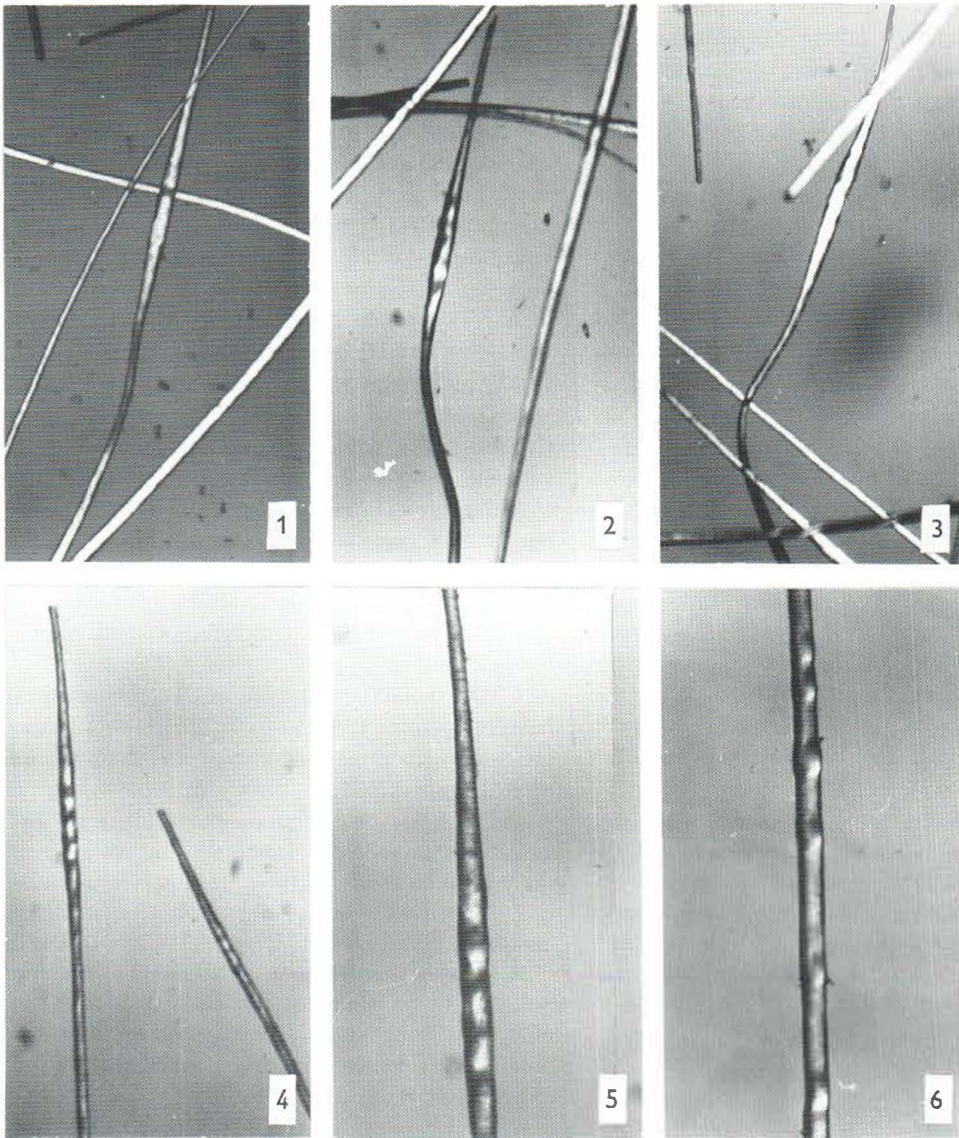
The present methods as described here have the same sensitivity as the radiometric method (2); they have given values of the same order as the radiometric one. The HPLC methods make it possible to analyse natural material, avoiding the uncertainty of the enzymatic step.

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The Hair in Acrodermatitis Enteropathica—a Disease Indicator?

A. Dupré, J. L. Bonafé and J. P. Carrière

Department of Dermatology Hôpital de La Grave,
Place Lange, Toulouse, France

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We have had under observation an infant boy, aged 9 months, with a typical acrodermatitis enteropathica (A. E.): eczematiform and psoriasiform skin

Figs. 1, 2 and 3. The ends of the hair are "spearhead-like" with a general swan-neck appearance.

Figs. 4, 5 and 6. The hair displays numerous striae, with a slight trichonodosis.

lesions around the body orifices, mucosal lesions, paronychia, widespread and almost total alopecia, association with gastrointestinal malfunction and low zinc levels. Zinc sulphate therapy was instituted and within 10 days the whole situation had improved and the hair had grown.