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Short Communication

Measurement of plasma catecholamines by high-performance liquid chromatography with electrochemical detection in intensive care patients after dobutamine infusion

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ABSTRACT

A procedure for the determination of plasma catecholamine concentrations in critical care patients after dobutamine infusion is presented. A modified chromatographic system is required with an additional washing procedure to achieve maximum sensitivity and stable chromatographic conditions. The influence of storage time on the catecholamine concentrations of plasma samples is reported in detail. A time-dependent decrease in catecholamine concentrations of up to 12 and 39% was found within two and ten months, respectively.

INTRODUCTION

High-performance liquid chromatography (HPLC) with electrochemical detection (ED) has been used for the accurate and precise determination of plasma catecholamines in the low pg/ml range. From the literature it appears that, at present, reversed-phase columns [1–3] are employed more frequently for chromatographic separation than strong cation-exchange material. Because the sensitivity of the reversed-phase C_{18} column method is comparable with that of a radioenzymic assay and may exceed that of gas chromatography–mass spectrometry, many investigators give preference to HPLC–ED. This method is

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also relatively inexpensive and suitable for routine laboratory determination of catecholamines in plasma.

Preparation of samples by extraction with alumina [4,5] yields reliable results, and the procedure is available as a commercial kit. Although this extraction method combined with ED should be selective for catecholamines, Goldstein *et al.* [6] reported that dihydrocaffeic acid interferes with the catecholamines, while other investigators separated dihydrocaffeic acid from epinephrine and the internal standard. An additional peak in the chromatogram was found by Bouloux and Perrett [7] in patients receiving labetalol, an α- and β-adrenoreceptor-blocking drug, which does not interfere with quantification of the catecholamines in the chromatographic system of Koller [8].

Only limited information about the stability of catecholamines in stored plasma samples is found in the literature [9]. This serious problem is of interest in clinical trials, in which catecholamine determination is performed in plasma samples kept frozen for a long time. To evaluate the usefulness of catecholamine storage, frozen plasma samples were examined after up to a year of storage.

Additional peaks may be a problem in analysing catecholamines in intensive care patients, and the chromatographic system must be carefully checked for interfering peaks. This study attempts to elucidate the problems involved in the determination of catecholamines in intensive care patients after dobutamine infusion.

EXPERIMENTAL

Chemicals

The internal standard dihydroxybenzylamine (DHBA) and the catecholamines norepinephrine (NE), epinephrine (E) and dopamine (DA) were purchased from Sigma (Munich, Germany). The ion-pairing agent sodium 1-octanesulphonic acid was supplied by Fluka (Neu-Ulm, Germany) and the alumina by Woelm (Steinheim, Germany). Orthophosphoric acid, perchloric acid, Na_2-EDTA, citric acid, sodium acetate and Tris base, all of reagent grade, were obtained from Merck (Darmstadt, Germany).

Stock solutions of catecholamines

Standard solutions of NE (10 ng/ml), E (2 ng/ml), DA (2 ng/ml) and DHBA (10 ng/ml) as the internal standard were prepared by stepwise dilution in 0.05 *M* phosphoric acid. Concentrated stock solutions were kept at $-70°C$ and diluted every day.

Chromatography

All experiments were carried out using a high-pressure pump with a column dumping system (Gynkotek, Munich, Germany), a PE-ISS 100 autoinjector (Perkin-Elmer, Überlingen, Germany) with a cooling system and an M 20 ampero-

metric detector (Gynkotek, Munich, Germany). The glassy carbon working electrode was set at 650 mV against an Ag/AgCl reference electrode. The sensitivity of the electrochemical detector was maintained at 0.08 nA full scale. Separation was performed on a 125 mm × 4 mm I.D., 5-μm Hypersil ODS C_{18} column (Melz, Berlin, Germany). Quantification was achieved using peak-area ratios against standards processed by the extraction and assay procedure with a Shimadzu C-R4A integrator (Shimadzu, Tokyo, Japan) [10].

The mobile phase (pH 4.6) consisted of 50 m*M* sodium acetate, 10 m*M* citric acid, 0.2 m*M* EDTA, 1 m*M* octanesulphonic acid and 5% methanol (v/v). The solvent was filtered through a 0.22-μm membrane filter and degassed by ultrasonic agitation under vacuum. The flow-rate was 0.7 ml/min and the injection volume 50 μl.

Sample preparation

Blood samples were collected from intensive care patients under controlled conditions via the arterial catheter for direct blood-pressure monitoring. Prechilled EDTA tubes were used for sampling. After centrifugation, the plasma was divided into 1-ml aliquots and stored at −70°C, or the plasma was deproteinized with 0.6 *M* $HClO_4$, centrifuged and stored at −70°C. Plasma samples from ten intensive care patients who received no exogenous catecholamines were fractionated in ten aliquots. The decrease of catecholamine concentration was analysed over a ten-month period.

Catecholamines were extracted from plasma (1 ml or 2 ml in 0.06 *M* $HClO_4$) onto alumina as described by Eriksson and Persson [11], with desorbed catecholamines in 100 μl of 0.05 *M* H_3PO_4 being drawn through a capillary column containing a 35-μm porous polyethylene bed (Biorad, Munich, Germany) to remove alumina particles prior to injection.

RESULTS

The linearity of the detection system was demonstrated by analysing known amounts of catecholamines with 500 pg/ml DHBA as the internal standard. Responses to each catecholamine investigated were found to be linear over the range 25–1000 pg/ml. The linearity of the extraction procedure was established by extracting and assaying plasma with the physiological amount of catecholamines, and added amounts of catecholamines plus the internal standard. For each catecholamine investigated, peak-area ratios of exogenous catecholamines were found to be linear over the range 25–1000 pg/ml. The coefficients of correlation in the assay system were: NE, $r = 0.995$; E, $r = 0.988$; DA, $r = 0.986$.

Recovery from the extraction procedure was determined by comparing yields from a series of plasma extractions with known amounts of catecholamines (50–1000 pg/ml). The calculated recoveries of the control analyses were 71 ± 3.6% (NE), 65 ± 4.2% (E), 68 ± 3.8% (I.S.) and 60 ± 4.7% (DA). These values are within the range reported by other authors [12,13].

The limits of detection of the HPLC system were assessed according to the IUPAC definition [15] with a value of $k=3$ from a blank (extracted plasma) sample ($n=5$) at the 95% confidence level as 5.7–6.9 pg/ml for NE, 7.4–11.2 pg/ml for E and 10.6–16.8 pg/ml for DA.

The assay precision ($n=5$) was evaluated as the confidence interval at the 95% confidence level of each catecholamine concentration added (Table I). The performance of the assay system was monitored by analysing two standard solutions with concentrations of NE = 500 pg/ml, E = 100 pg/ml, DA = 100 pg/ml and NE = 250 pg/ml, E = 50 pg/ml, DA = 50 pg/ml, respectively, before starting sample measurements. The results of the control analyses had to be in agreement with the data in Table I.

TABLE I

ASSAY PRECISION IN THE DETERMINATION OF CATECHOLAMINES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

Assay precision in the catecholamine concentration range from 50 to 1000 pg/ml is presented as confidence intervals of norepinephrine (NE), epinephrine (E) and dopamine (DA) concentrations at a 95% confidence level ($n=5$).

Concentration added (pg/ml)	Concentration found (pg/ml)		
	NE	E	DA
50	43.9–55.2	42.5–57.2	38.7–61.1
100	90.8–112	85.5–112	84.5–123
200	180–225	180–216	176–221
300	280–320	267–319	264–335
500	451–549	447–555	446–550
1000	914–1087	904–1094	902–1068

To examine the problem of storage of catecholamines, plasma and deproteinized plasma samples obtained from ten surgical patients were stored at −70°C and analysed in detail at one-month intervals over a period of ten months. The data obtained (Fig. 1) clearly show that the catecholamine concentrations decrease by up to 17% after four months and up to 39% after ten months. The decrease over ten months seems to be similar in both the plasma and the deproteinized samples. This decrease was independent of the basal levels of NE and E, which ranged from 120 to 1000 and 60 to 160 pg/ml, respectively. We found no difference between the catecholamine concentrations of plasma samples with and without metabisulphate.

Analysis of plasma catecholamine levels in patients given dobutamine infusion yielded ranges of 200–1500 pg/ml for NE, 25–150 pg/ml for E and 20–40 pg/ml for DA. DA was not clearly detectable in some patients' plasma (Fig. 3).

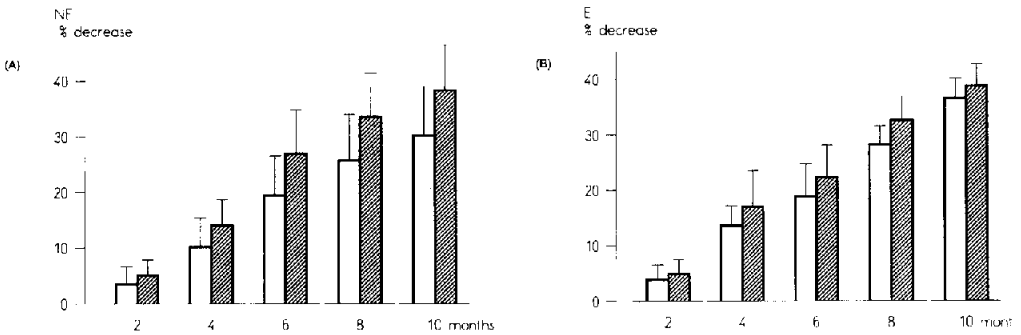

Fig. 1. Decrease of catecholamine concentrations in plasma samples (▨) and deproteinized samples (□). (A) Decrease of norepinephrine (NE); (B) decrease of epinephrine (E). Values are presented as mean and standard deviation ($n = 10$).

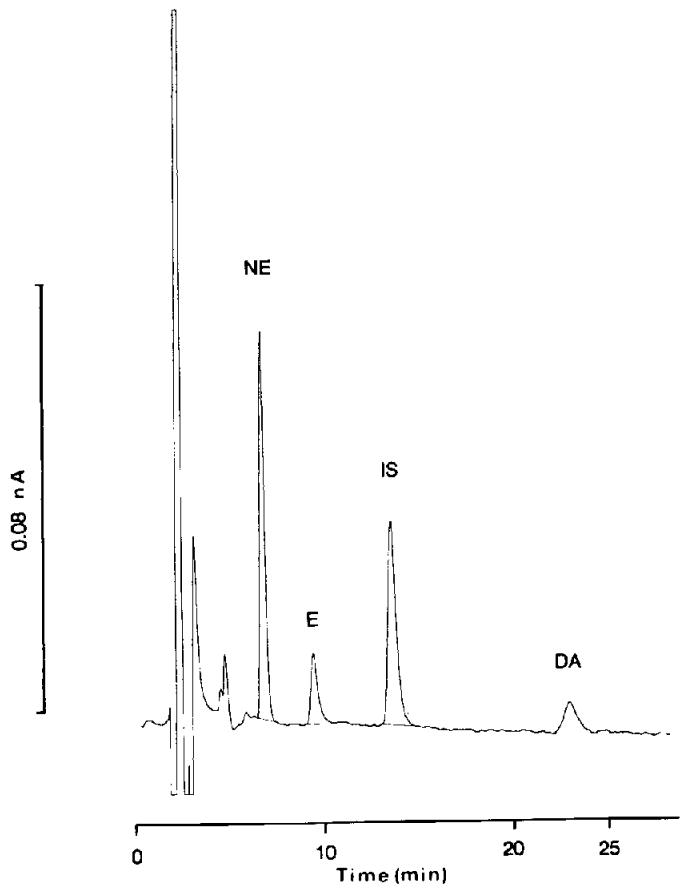

Fig. 2. Chromatogram of a standard solution containing 5 ng/ml NE, 1 ng/ml E, 5 ng/ml DHBA (internal standard, IS) and 1 ng/ml DA. The retention times were 6.1 min for NE, 9.1 min for E, 13.4 min for IS and 22.7 min for DA. Chromatographic conditions as described in Experimental.

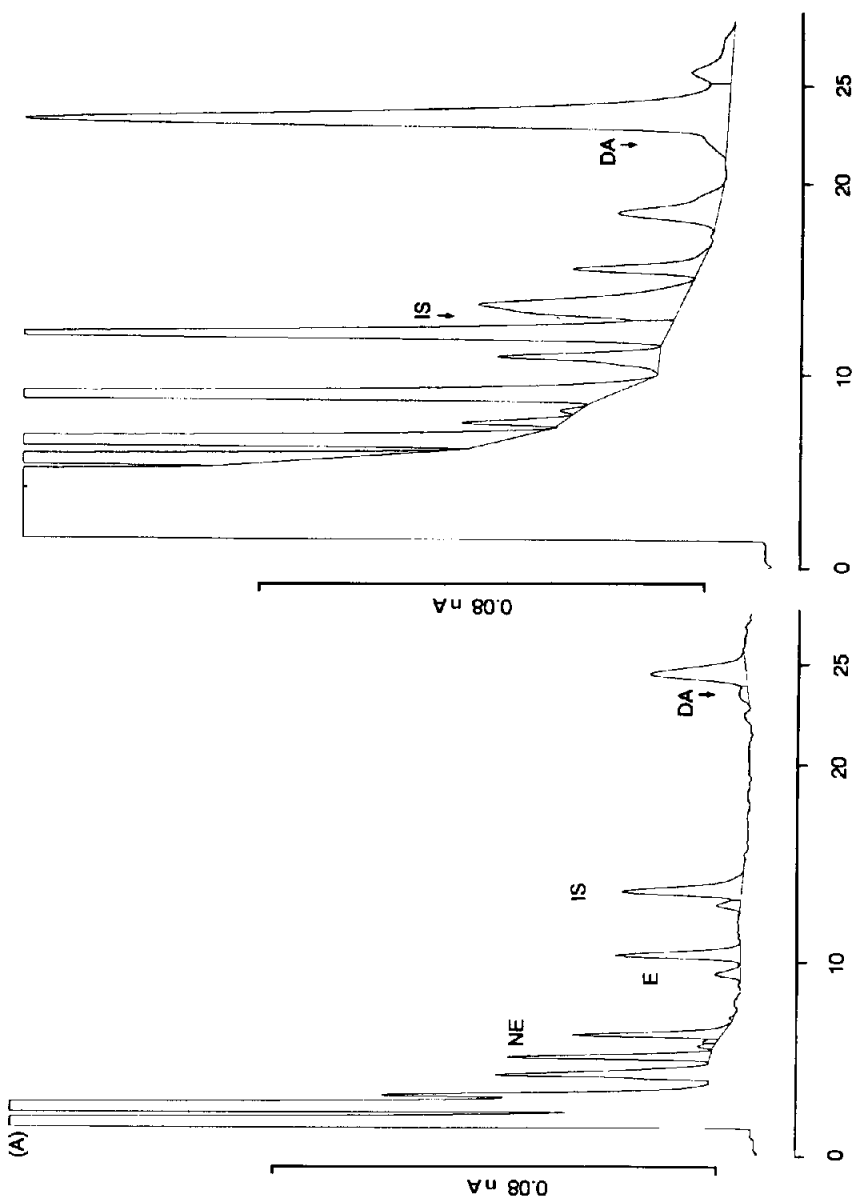

Fig. 3. Plasma catecholamine chromatograms of patients undergoing dobutamine therapy: (A) 5 μg/kg min; (B) 10 μg/kg/min. Chromatographic conditions as described in Experimental.

The long retention times of the catecholamines in Fig. 2 are advantageous in that the interfering peaks seen more often in the plasma of intensive care patients are clearly separated from the catecholamine peaks.

The chromatogram in Fig. 3 shows many additional peaks. However, these unidentified peaks were restricted to the chromatographic traces of all the plasma samples from patients with dobutamine administration (> 5 μg/kg/min). It was not possible to identify E and DA in this chromatogram. After a retention time of more than 1 h, another peak was detectable; this may be related to dobutamine, since no similar peak was found in samples without dobutamine.
Interfering peaks associated with dobutamine metabolites could be avoided by analysing another plasma sample 2 h after the first.

DISCUSSION

Interfering non-catecholamine peaks reported by some investigators [11] are attributed to artefacts in chromatograms of standards or, like the dihydrocaffeic acid peak, could be separated from the catecholamines in different chromatographic systems [8].

The unidentified peaks reported here after dobutamine infusion seem to be related to a metabolite of dobutamine. Indeed, while measuring plasma dobutamine after alumina extraction, Noda *et al.* [14] detected an unidentified substance that may contribute to the pharmacological effect of dobutamine and is not related to 3-O-methyldobutamine, since none of the 3-methoxy metabolites react at 600 mV. This may correspond to the unidentified peak in Fig. 3, which is not only separated from the dopamine peak but also increases at higher dobutamine concentrations.

A modification of the chromatographic conditions made it possible to determine plasma catecholamines in intensive care patients after dobutamine infusion. Prolonged retention times provided better separation of the catecholamines from the interfering peaks. It was necessary to wash the column with acid after every analysis and to verify the chromatographic separation by analysing the standard solution. Only by using this time-consuming method was it possible to analyse catecholamine concentrations in patients after dobutamine infusion.

In contrast to the findings of Meineke *et al.* [9], who reported that frozen catecholamine samples are stable at -20°C for at least three months, our data clearly show that catecholamine concentrations decrease by up to 12% after two months and up to 39% after ten months in samples kept frozen at -70°C. The decrease was independent of the basal catecholamine concentrations. In view of the instability of stored plasma catecholamines, samples should be analysed within two months.

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