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Short Communication

Rapid and sensitive assay of dobutamine in plasma by high-performance liquid chromatography and electrochemical detection

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ABSTRACT

A sensitive and specific high-performance liquid chromatographic method with electrochemical detection was developed for measuring dobutamine in human plasma samples. Following an external standard method, 0.1 ml of EDTA–glutathione plasma was diluted on ice with 0.2 ml of a 5% trichloracetic acid solution. The mixture was centrifuged, filtered, and 30 μl were injected. Assessment was done by electrochemical detection. The assay was linear from 1 to 400 ng/ml plasma. For determination of dobutamine we also used a liquid–liquid extraction method routinely applied for plasma catecholamines. Liquid–liquid extraction requires application of 100–1000 μl of plasma. The standard curve was linear from 0.1 to 600 ng/ml. Absolute recovery of dobutamine was 90 ± 3% with the liquid–liquid extraction procedure and 91 ± 3% with the protein precipitation method. For both methods dobutamine was separated on Nova-Pak C_{18} columns. The mobile phase used was 0.1 molar phosphate buffer–acetonitrile (80:20, v/v) with 1-octanesulfonic acid and triethylamine as ion-pair reagents. The pH was adjusted to 2.7.

INTRODUCTION

Dobutamine, ± 4-[2-[(3-*p*-hydroxyphenyl)-1-methylpropyl)-amino]ethyl]pyrocatechol, is a synthetic catecholamine with structural resemblance to isoprenaline and dopamine. Dobutamine acts primarily by stimulation of β_1-receptors, but also, although to a lesser degree, by stimulation of β_2- and α-receptors. With dobutamine not binding to the dopamine receptor, a dopamine-equivalent positive inotropic effect occurs but no selective vasodilation of renal arterioles. Due to the vasodilation caused by β_2-receptors there is no significant increase of systemic blood pressures following dobutamine infusion.

To investigate the pharmacokinetics and plasma levels of dobutamine infusion in patients hospitalized at our clinic, it was necessary to develop a rapid, specific and sensitive analytical method to measure dobutamine concentrations in human plasma.

Most of the methods described in the literature are either insensitive or require time-consuming and complicated preparation. McKennon and Kates [1] described a chromatographic method

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using fluorescence detection and nylidrin as internal standard. Sample preparation was performed by extracting the buffered samples twice with ethyl acetate and evaporating the organic layer to dryness. The run-time of this analysis was more than 20 min and the sensitivity 10 ng/ml dobutamine.

El-Kommos [2] described a complicated spectrophotometric method for the determination of dobutamine. After reaction with thiosemicarbazide and sodium hydroxide, the samples were incubated for 30 min and measured at 510 nm. The detection limit was 1 μg/ml dobutamine.

Hardee and Lai [3] described a chromatographic method with electrochemical detection. After the addition of an internal standard to the plasma of anesthetized horses, the samples were extracted using disposable, prepacked reverse-phase columns. Knoll and Brand [4] used a chromatographic method with Bondapack columns for the extraction of dobutamine and the internal standard. Detection was by fluorescence at an excitation wavelength of 195 nm and a cut-off filter of 330 nm. Detection of catecholamines and dobutamine was performed by Schwartz *et al.* [5] using a chromatographic method with electrochemical detection. Alberts *et al.* [6] used HPLC and fluorometric detection. This method is based on liquid–liquid extraction and derivatization with the fluorogenic agent 1,2-diphenylethylenediamine added prior to chromatography [7].

A modified radioenzymatic assay method allowing simultaneous measurement of plasma dobutamine and endogenous catecholamine concentrations was used by Martinez *et al.* [8].

At our laboratory, we developed a sensitive and rapid method for determining dobutamine in plasma samples during dobutamine infusion over a period of 48 h in patients with heart failure in NYHA (New York Heart Association) class III–IV. For the assessment of dobutamine in plasma, we used a liquid–liquid extraction method [9], which is routinely used at our laboratory for plasma catecholamine determination.

EXPERIMENTAL

Chemicals and reagents

Dobutamine · HCl was provided by Kali-Chemie Pharma (Hannover, Germany). Trichloracetic acid (TCA), sodium dihydrogenphosphate, phosphoric acid, ethylenediaminetetraacetic acid (disodium EDTA), octanol, heptane, methanol and acetonitrile (HPLC grade) were purchased from E. Merck (Darmstadt, Germany); octanesulfonic acid sodium salt (OSS) was obtained from Sigma (Steinheim, Germany). Reduced glutathione was purchased from Serva (Heidelberg, Germany); triethylamine (TEA) from Fluka (Buchs, Switzerland). Deionized water used in preparing the mobile phase and all other solutions and reagents was Milli-Q water prepared by the Millipore-reagent water system (Eschborn, Germany).

Instrumentation

The HPLC system consisted of a WISP 712 autosampler with a temperature-controlled sample tray (Waters, Milford, MA, USA), a Beckman pump (model 126; Beckmann, Munich, Germany), an electrochemical detector (ESA Coulochem model 5100A) with a high-sensitivity analytical cell (model 5100; ESA, Bedford, MA, USA), and the Beckman chromatography software version 3.1 or 6.1 (System Gold Chromatography; Beckman, Berkeley, CA, USA) for analysis of the assay. The mobile phase was degassed in the ERC.3520 degassere (ERC, Alteglofsheim, Germany). The chromatograms obtained were plotted either with the Epson LQ-850 printer or with a laser printer (Brother HL-4V).

Chromatography

The mobile phase was prepared by combining 0.1 *M* $NaH_2PO_4 \cdot H_2O$, EDTA (0.1 m*M*), OSS (3.0 m*M*), TEA (1.5 ml/l) solution and acetonitrile (80:20, v/v). The pH was adjusted to 2.7 with phosphoric acid. The solution was vacuum-filtered through a 0.45-μm membrane filter and degassed in the ERC degasser. Chromatographic separation was achieved on a Nova Pak C_{18} steel column (15 cm × 3.9 mm I.D., 4 μm particle size)

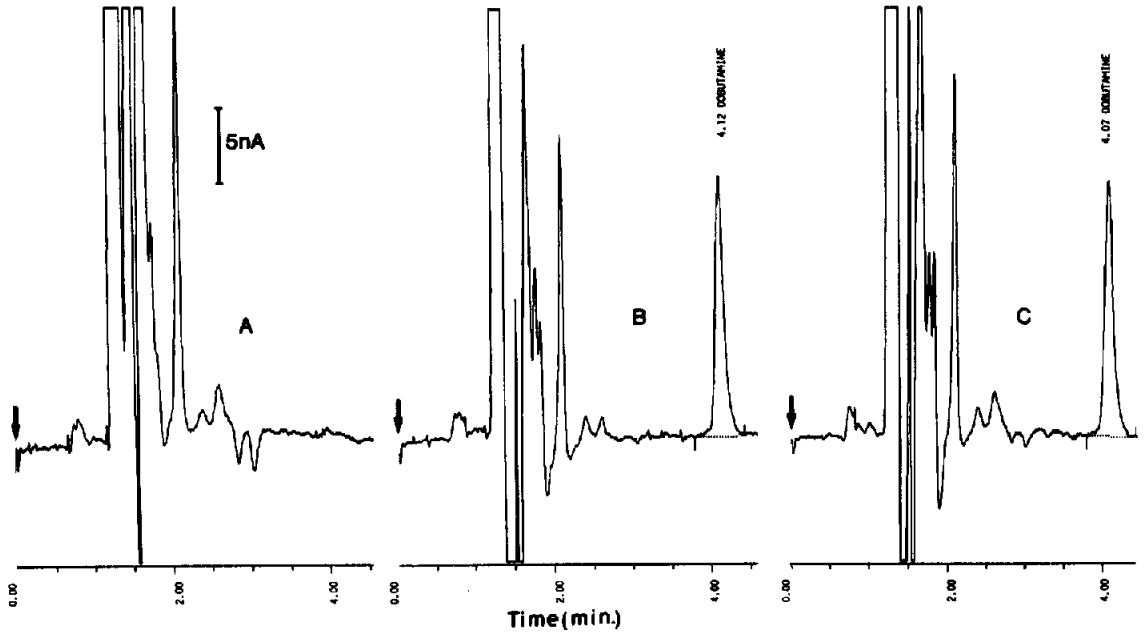

Fig. 1. Chromatograms of dobutamine HPLC assay according to Procedure 1. (A) Chromatogram of blank plasma; (B) Chromatogram of blank plasma samples spiked with 30 ng/ml of dobutamine; (C) Plasma sample of a patient treated with dobutamine (2.5 μg/kg/min) 20 min after starting the infusion. Dobutamine concentration: 33 ng/ml. Conditions: Nova-pak C_{18} reveresed-phase column (15 cm × 3.9 mm I.D., 4 μm particles) (Waters) was used. Eluent: 0.1 *M* sodium dihydrogenphosphate in water–acetonitrile (80:20, v/v) with 0.1 m*M* EDTA, 3 m*M* OSS, 1.5 ml/l TEA, pH 2.7, flow-rate 0.8 ml/min, temperature 50°C. Detector: Coulochem EC detector with high-sensitive analytical cell (Model 5011). Electrode 1:350 mV. Electrode 2: −260 mV.

at 42°C or 50°C. The flow-rate ranged between 0.8 and 1.2 ml/min.

Sample preparation

Blood samples of patients were collected in 12-ml polyethylene tubes coated with EDTA and containing 200 μl of a glutathione solution (600 mg in 10 ml of deionized water). After cold centrifugation at 1300 *g* for 10 min, the plasma was immediately stored at −20°C until assay after 1–6 weeks.

RESULTS AND DISCUSSION

For dobutamine assessment the following two procedures were used according to the external standard method:

Procedure 1 (protein precipitation). To 0.1 ml of EDTA–glutathione plasma on ice, 0.2 ml of a 5% TCA solution was added. The mixture was then centrifuged at 1500 *g* for 10 min, the supernatant decanted and filtered via a 0.45-μm filter; 30 μ of the filtered samples were injected. The calibration curve was determined by spiking dobutamine-free plasma with a known amount of dobutamine. The assay was linear ($y = 0.005\,x + 0.009$) from 1 to 400 ng/ml plasma ($r = 0.9996$). Sensitivity could be increased by either increasing the injection volume and/or detector sensitivity, and/or reducing the volume of TCA.

Procedure 2. For assessment of dobutamine concentration in plasma, we used a liquid–liquid extraction method [9], which is routinely used in our laboratory for plasma catecholamine determination; 100 μl of plasma were required. The standard curve was linear from 0.1 to 600 ng/ml, the correlation coefficient was 0.9946 ($y = 0.004\,x + 0.036$). Absolute recovery of dobutamine was 91 ± 3%. The sensitivity of this method can be increased as described above, and by extracting 1.0 ml instead of 0.1 ml of plasma.

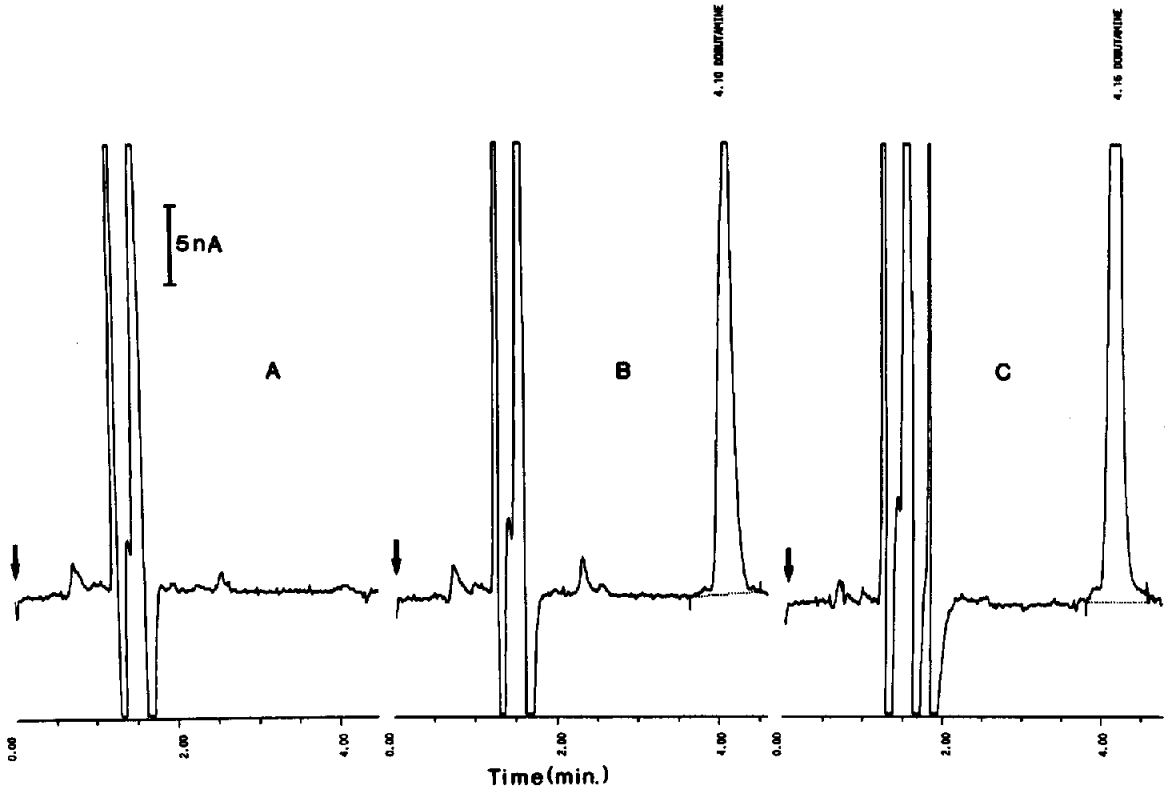

Fig. 2. HPLC assay of dobutamine according to Procedure 2. (A) Chromatogram of blank plasma extraction; (B) Extract of blank plasma spiked with 100 ng/ml dobutamine; (C) Plasma sample of a patient treated with dobutamine (10 μg/kg/min) 20 min after starting the infusion. Dobutamine concentration: 120 ng/ml. Chromatographic conditions as described in Fig. 1.

TABLE I

REPRODUCIBILITY OF THE ASSAY

Plasma pools 1, 2, and 3 were spiked with 625, 5000 and 10 000 pg/ml dobutamine for Procedure 1. Plasma pools 1, 2, and 3 were spiked with 375, 1500, and 3000 pg/ml dobutamine for Procedure 2. C.V. = coefficient of variation. M.C. = mean concentration.

Pool	Concentration (pg/ml)	Intra-assay ($n=6$)		Inter-assay ($n=6$)	
		M.C. (pg/ml)	C.V. (%)	M.C. (pg/ml)	C.V. (%)
Procedure 1					
1	625	591.2	3.4	613.2	4.1
2	5000	4728.7	5	4757.8	5.1
3	10 000	9536.5	4	9331.3	5.1
Procedure 2					
1	375	332.2	3.1	324.2	4.4
2	1500	1381.0	3.5	1376.0	4
3	3000	2905.3	5.2	2804.0	5.9

Characteristics of the assay

The linearity of the assay was tested by measuring pooled plasma to which incremental amounts of dobutamine were added. Linearity was excellent up to 400 ng/ml plasma for Procedure 1, and 600 ng/ml plasma for Procedure 2. The detection limit was 10 pg of dobutamine injected. Under standard assay conditions the detection limit was 1 ng/ml plasma for Procedure 1 and 100 pg/ml plasma for Procedure 2. Pooled plasma spiked with 625, 5000 and 10 000 pg/ml dobutamine for Procedure 1, and 375, 1500 and 3000 pg/ml for Procedure 2 was used to determine intra-assay as well as inter-assay precision (on 6 consecutive days). The results showed good reproducibility (Table I).

Sample stability

Catechol-O-methyltransferase and phenolsulfotransferase, the enzymes primarily responsible for the metabolism of dobutamine, are present in the plasma. The addition of antioxidant, centrifugation of the blood samples immediately after collection, and storage of the samples at −20°C are absolutely essential in order to avoid loss of dobutamine. At these conditions, the samples remained stable for at least 6 weeks.

CONCLUSION

In summary, we have described two methods for determining the dobutamine content of plasma samples. Both methods, in particular Procedure 1, are being used routinely at our laboratory to analyze patient samples containing dobutamine. At higher plasma dobutamine concentrations (1–400 ng/ml), Procedure 1 is the ideal method for rapid determination. Total time for preparation and assessment was *ca.* 30 min. Due to the low amount of 0.1 ml of plasma needed for determination and the low injection volumes of 20–30 μl, the increase in column backpressure could be retained minimal (0.750 MPa) for up to 500 injections. Procedure 2 is recommended for lower dobutamine concentrations (100 pg/ml). The results of the investigation and the plasma levels found for dobutamine following infusion in patients hospitalized in our clinic will be published elsewhere.

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