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Note

Rapid and simple method for the routine determination of dobutamine in human plasma by high-performance liquid chromatography

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Dobutamine (Fig. 1), (±)4-[2-[(3-(*p*-hydroxyphenyl)-1-methylpropyl)-amino]ethyl]pyrocatechol, is a synthetic catecholamine developed as a β1-mimetic substance with predominantly inotropic and mild chronotropic effects [1, 2]. Dobutamine is structurally similar to the catecholamines dopamine and norepinephrine. In order to avoid the vasoconstrictory effects common to all endogenous catecholamines, it was necessary to modify the chemical structure of the pyrocatechol molecule (i.e. substitution of the amino-hydrogen by an elongated alkyl chain).

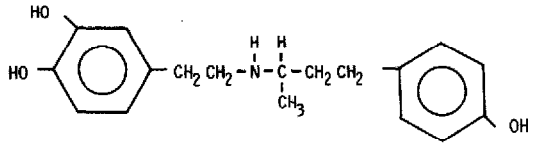

Fig. 1. Chemical structure of dobutamine.

Although dobutamine seems to be an ideal catecholamine, clinical experience shows that unpredictable secondary effects are possible. It is, thus, sometimes difficult to manage critically ill patients in septic shock. Even with accurate dosing (i.e. μg/kg/min), plasma levels triggering tachycardia may be attained. The reason for this effect seems to be a wide variation in the intravascular fluid volume and the complex pharmacokinetics of dobutamine [3].

Dobutamine, like all endogenous catecholamines, may lower arterial pO_2 by increasing the intrapulmonary right-to-left shunt. Therefore, arterial oxygen transport capacity may be decreased, although cardiac output has been

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improved [3]. This shunt seems to correlate with the plasma level of dobutamine. In such cases, it might be useful to monitor the plasma levels.

El-Kommos [4] described a spectrophotometric method for determination of dobutamine, but without a description of how to extract dobutamine from biological fluids. After a reaction with thiosemicarbazide and sodium hydroxide, the samples were incubated for 30 min and measured at 510 nm. The detection limit was only 1 μg/ml.

Schwartz et al. [5] described a sensitive method for catecholamines and dobutamine with an electrochemical detector, but needed a gradient technique in order to achieve rapid resolution of all catecholamines. Extraction was done with alumina at pH 8.6.

Hardee and Lai [6] described an extraction procedure that has no increase in the concentration of dobutamine in the extract, so needed to use an electrochemical detector to make it sensitive enough for pharmacological studies. McKennon and Kates [7] described a method using a fluorescence detector with a runtime of more than 20 min. Extraction was done by shaking the buffered samples twice with ethyl acetate and evaporating the organic layer to dryness.

We tried to develop a method for the determination of dobutamine in human plasma that is sensitive enough for clinical plasma samples. For routine determination of plasma levels, the extraction procedure should be rapid and practical.

EXPERIMENTAL

Chemicals

Dobutamine · HCl was obtained from Eli Lilly (Bad Homburg, F.R.G.) and the internal standard buphenine (Fig. 2), *p*-hydroxy-N-(1-methyl-3-phenylpropyl)norephedrine, from Tropon (Köln, F.R.G.). Dipotassium hydrogen phosphate was supplied by Serva (Heidelberg, F.R.G.), methanol by Roth (Karlsruhe, F.R.G.), phosphoric acid, sodium hydroxide and dibutylamine by Merck (Darmstadt, F.R.G.). Ethylene glycol bis(3-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) and glutathione were purchased from Sigma (Taufkirchen, F.R.G.).

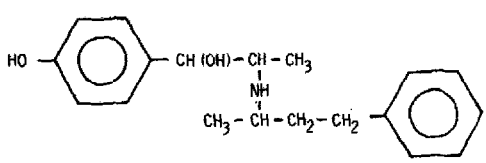

Fig. 2. Chemical structure of buphenine, the internal standard.

Samples

Arterial and mixed venous blood samples were obtained from surgical intensive-care patients and filled into heparinized, precooled tubes, and centrifuged immediately at 2000 *g* at 4°C for 10 min. The plasma was immediately mixed with antioxidants (i.e. 95 mg of EGTA + 60 mg of glutathione per ml of water) and frozen until assayed.

Extraction of dobutamine

Bond-Elut CN columns (Analytichem International, Harbor City, CA, U.S.A.) were rinsed once with 1 ml of methanol and twice with 1 ml of water. The plasma samples were thawed, centrifuged for 3 min at 3000 *g* and 1 ml was added to the extraction column together with 100 ng of the internal standard (100 μl out of 1 μg/ml standard, dissolved in 50% mobile phase and 50% methanol). The columns were rinsed twice with 1 ml of water under vacuum.

With 300 μl of a solution of 50% mobile phase and 50% methanol (adjusted to pH 2.3 with phosphoric acid), dobutamine and buphenine were eluted from the columns in a centrifuge at 1000 *g*, at 4°C for 5 min. The extracts were filtered through a 0.45-μm HV-filter from Millipore (Bedford, MA, U.S.A.) and stored at 4°C until injection.

High-performance liquid chromatographic (HPLC) system

The HPLC system consisted of a Kontron LC 410 pump (Kontron Analytik, Eching, F.R.G.) and an RP-18 column (150 × 3.9 mm I.D., Resolve®, 5 μm spherical) from Waters Assoc. (Milford, MA, U.S.A.). A 20-μl aliquot of extracted sample was injected via a Rheodyne 7125 valve (Berkeley, CA, U.S.A.). Detection was made using a GM 970 fluorescence detector from Kratos (Ramsey, NJ, U.S.A.), with an excitation wavelength of 195 nm and a cut-off filter of 330 nm. The signals were recorded and integrated automatically on a Chromatopac C-R1A from Shimadzu (Kyoto, Japan).

The eluent was 0.1 *M* dipotassium hydrogen phosphate in water—methanol (80:20) with an additional 9 ml of dibutylamine per l. The pH was adjusted to 2.6 with phosphoric acid. The eluent was degassed in an ultrasonic water bath under vacuum and recycled under helium atmosphere at a flow-rate of 1.5 ml/min (290 bar).

RESULTS AND DISCUSSION

Sample preparation and stability

A major advantage of this assay is the ease of sample preparation which reduces the assay time and the possibility of technical error. A second advantage is the high stability of the extracts. Even at room temperature (22°C), the extracts are stable for at least 72 h. Nevertheless, it is very important to centrifuge the blood samples immediately after collection, in order to mix the plasma with the antioxidants, and to freeze at −20°C to avoid a loss of dobutamine. On storing the samples with antioxidants at room temperature, we found a loss of 40% within 2 h, and at 4°C a loss of 50% within 24 h. At −20°C, the plasma samples were stable for at least two weeks.

Chromatography

The retention time for dobutamine was 2.6 min and for the internal standard 9.9 min (Fig. 3). The method is selective for dobutamine. There was never any interference with unknown peaks in the human plasma samples we investigated, neither with dobutamine nor with the internal standard (Fig. 3).

The recovery for dobutamine was 67.5 ± 6.2%, and for the internal standard 77.6 ± 6.7%. Recovery was determined by extraction of 28 dobutamine-free

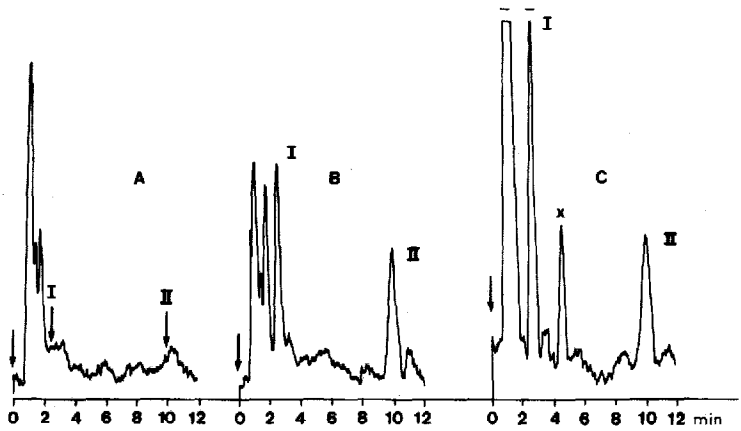

Fig. 3. Chromatograms of dobutamine HPLC assay of human plasma. (A) Extract of blank; (B) extract of blank plasma spiked with 10 ng of dobutamine (I) and 100 ng of internal standard (II); (C) plasma sample of a patient (I) with 75 ng/ml dobutamine; (x) unknown peak. Conditions: RP-18 column 5 μm spherical; eluent: 0.1 *M* dipotassium hydrogen phosphate in water—methanol (80:20) with 9 ml of dibutylamine per l, pH 2.6, flow-rate 1.5 ml/min; detector: fluorescence, excitation wavelength 196 nm, emission wavelength 330 nm.

plasma samples, spiked with a known amount of dobutamine and internal standard. The coefficient of variation (C.V.) of dobutamine for within-day extractions (intra-assay) was 6.2 and for day-to-day extractions (inter-assay) 7.9.

The calibration curve was determined by spiking dobutamine-free plasma with a known amount of dobutamine and internal standard. The assay was linear ($y = 1.07\ x - 4.2$) from 5 to 300 ng/ml of plasma ($r = 0.999$) and the sensitivity was 100 pg, using an injection volume of 20 μl (signal-to-noise ratio = 3). The sensitivity can be increased by increasing the injection volume and by reducing the volume of the extract.

CONCLUSIONS

We have described a method for the determination of dobutamine with a very simple extraction procedure. The method is sensitive and practical enough for routine determination of plasma levels within a reasonable time. We observed plasma levels from 15 to 200 ng/ml in critically ill patients. The results of our investigations in patients with septic shocks and increased intrapulmonary right-to-left shunts will be published elsewhere. Special attention will be paid to possible correlations between plasma levels of dobutamine and endogenous catecholamines and changes in cardiac output, systemic and pulmonary vascular resistance, and oxygen utilization.

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