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Technical note

Stability of 3,4-dihydroxyphenylacetic acid in plasma extracts assayed by high-performance liquid chromatography with electrochemical detection

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Abstract

3,4-Dihydroxyphenylacetic acid (DOPAC) can be easily assayed by high-performance liquid chromatography (HPLC) with electrochemical detection at the same time as norepinephrine (NE), epinephrine (E), and dopamine (DA). The latter catecholamines are stable in perchloric acid extracts for over 6 h at 4°C in the dark whereas DOPAC levels drop rapidly by more than 50% in 6 h at 4°C in the dark. This study investigated the effects of reducing agents [ascorbic acid, dithiothreitol (DTT), reduced glutathione with or without a metal chelating agent (diethylenetriaminepentaacetic acid or ethylenediaminetetraacetic acid)] on DOPAC. Extracted with alumina using 0.65 mmol/l DTT prior to HPLC and electrochemical detection, DOPAC remained stable in the perchloric acid extract for 2 h at 4°C in the dark.

1. Introduction

High-performance liquid chromatography (HPLC) with electrochemical detection is well-adapted to assay of plasma catecholamines norepinephrine (NE), epinephrine (E), and dopamine (DA) [1]. Several authors also advocate the use of HPLC for the assay of the product of oxidative deamination of DA, 3,4-dihydroxyphenylacetic acid (DOPAC) [2]. As plasma free DA levels are often under the limit of sensitivity of the detector, DOPAC might be useful for biological diagnosis of neurogenic tumours because it is a good indicator of peripheral secretion. The catecholamines are extracted from plasma, concentrated on alumina, and eluted with 0.1 *M* perchloric acid; NE, E, and DA remain stable for over 6 h at 4°C in the dark, whereas DOPAC drops rapidly (>50% decrease in 6 h at 4°C in the dark). This study investigated the effects of reducing agents with or without addition of a metal chelating agent (metals catalyze oxidative reactions) on the stability of DOPAC in perchloric acid extracts.

2. Experimental

We recently described the extraction method used in our laboratory [3]. In brief, for aqueous

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standards, 2 ml of triple-distilled water spiked with the compounds under study are extracted with alumina. For plasma standards, 2 ml of human plasma with and without spiking (10 nmol/l) are extracted. Spiking is performed using a 10 μmol/l solution of the four catecholamines (NE, E, DA, and DOPAC) to obtain a 10-nmol/l solution. Assays are performed with a 12 nmol/l solution of internal standard, dihydroxybenzylamine (DHBA). DOPAC, obtained from Sigma (Coger, Paris, France), is added to the plasma or aqueous standards before extraction.

To evaluate the stability of DOPAC in extracts, various reducing agents with or without a metal chelating agent [diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid disodium salt (EDTA)], were added before extraction to water (aqueous standards) and plasma (plasma standards): (a) 1 μmol/l ascorbic acid; (b) 50 μmol/l reduced glutathione (GH); (c) 0.65 mmol/l dithiothreitol (DTT) alone, or (d) with addition of 0.25 mmol/l DTPA; (e) 0.65 mmol/l dithioerythreitol (DTE) alone, or (f) with 0.25 mmol/l DTPA; (g) 0.50 mmol/l EDTA. DTPA, DTT, and EDTA were obtained from Sigma, DTE from Fluka (Coger), and GSH from Boehringer (Meylan, France). Assays were performed in triplicate.

Assays were performed by reversed-phase high-performance liquid chromatography on a column of Ultrasphere ODS (250 × 4.6 mm I.D., average particle size 5 μm). The mobile phase consisted in a 92:8 (v/v) mixture of 0.1 *M* sodium acetate, 0.05 *M* citric acid containing 50 mg EDTA Na$_2$, 125 mg/l of octane sulphonic acid sodium salt, and methanol. The Coulochem Esa analyzer (D1 +0.00 V; D2 +0.22 V; sensitivity 2000) used for electrochemical detection was connected to a Linseis 10-mV recorder.

3. Results and discussion

The best stability of DOPAC was observed with DTT alone (c); it was not improved by the addition of DTPA (d) or EDTA (g). Under condition (c), DOPAC remained stable in the

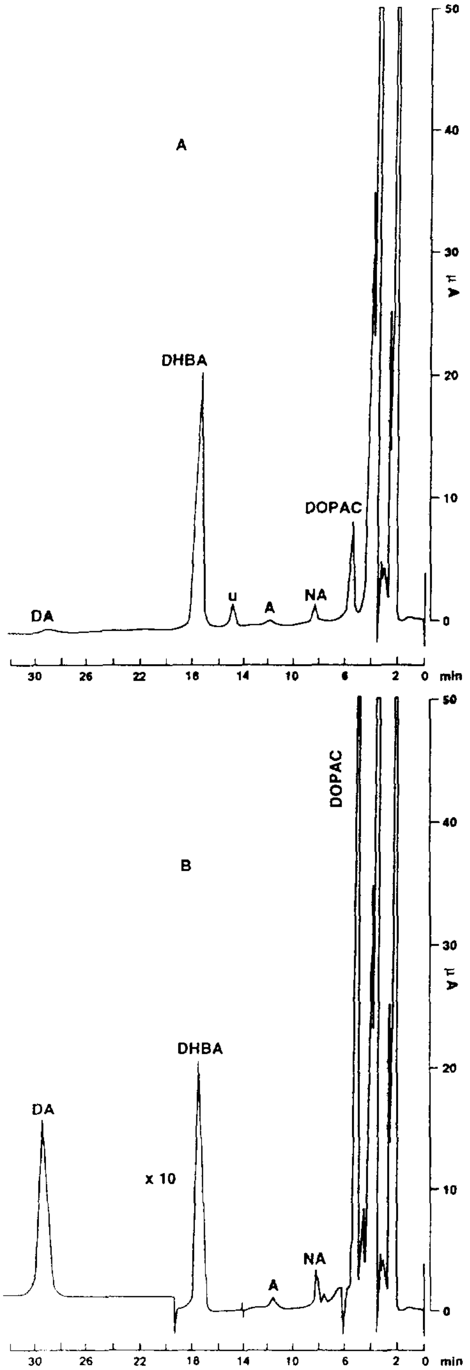

Fig. 1. Chromatograms of (A) extract of human plasma from a healthy subject, NE 0.55 nmol/l, E 0.30 nmol/l, DA approx. 0.15 nmol/l, DOPAC 8.6 nmol/l, u = unknown, and (B) extract of human plasma with very high DA and DOPAC concentrations (respectively 140 and 410 nmol/l).

perchloric acid extract for 2 h at 4°C in the dark (97% to 104% in the three assays compared with the first injection), then dropped by 7% compared to the initial level (91% to 94%). The same was true under condition (d) (DTT + DTPA), whereas a decrease of around 12% (85% to 90%) was noted under conditions (a), (e) and (f). Under condition (b) with GH, the conservation of DOPAC was also improved, although only for plasma standards: DOPAC decreased by only 3% (96% to 98%) in the 2 h after extraction. In contrast, in the aqueous standard under the same conditions, DOPAC dropped by 15% in 2 h (79% to 87%). NE, E and DA were stable in all cases (97% to 102%). Storage tubes reportedly have an effect on the stability of DOPAC [4], but the borosilicate glass tubes we used did not prevent the decrease in DOPAC.

When the extraction conditions (c) were repeated every 2 h, the mean DOPAC concentration (± S.D.) in samples from 12 healthy controls aged 30 to 45 year (median 39 year) was 23 ± 6 nmol/l, whereas DA was undetectable (below 0.15 nmol/l) (Fig. 1A). In one patient, enormous paroxysmal secretion of DA (140 nmol/l of free plasma DA) [5] was confirmed on the same graph by the elevated DOPAC concentration (410 nmol/l) (Fig. 1B). In view of the results obtained in this study, it appears advisable to perform DOPAC assays before extraction on alumina using DTT.

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