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Note

Simultaneous assay of 3,4-dihydroxyphenylethylene glycol and norepinephrine in human plasma by high-performance liquid chromatography with electrochemical detection

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The ratio of norepinephrine (NE) to its deaminated metabolite free 3,4-dihydroxyphenylethylene glycol (DHPG) in plasma has recently been suggested to be of use in diagnosis of pheochromocytoma [1, 2]. However, only a few assays have been described which can simultaneously measure NE and DHPG [1—4]. These have either used radioenzymatic techniques [1, 2] or high-performance liquid chromatography (HPLC) with electrochemical detection (ED) [3, 4]. HPLC—ED assays have advantages over radioenzymatic techniques in that they are simpler and cheaper to perform. However, only one previously described HPLC—ED method [3] has measured DHPG and NE simultaneously in human plasma, and this method did not allow ideal resolution of NE from the solvent front. We have previously described a simultaneous method [4] for DHPG and catecholamines in rat brain and now report modifications to the technique that allow the simultaneous assay of DHPG and NE in human plasma.

EXPERIMENTAL

The chromatographic system consisted of a Pye-Unicam LC-XPS pump, a precolumn (40 × 1 mm) packed with LiChroprep (Merck), and a 25 cm × 4.0 mm I.D. reversed-phase column. The column was packed with Spherisorb 5-μm silica (Phase Separations). The mobile phase was 70 m*M* NaH_2PO_4 (pH 3.0) (Fisons) with 1.85 m*M* octanesulphonic acid (Fisons) and 13.4 m*M* EDTA (BDH, AnalaR). The flow-rate was 1 ml/min and the column effluent was passed through a TL3 cell (Bioanalytical Systems) with a glassy carbon

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electrode where the catechols were detected using an LC4 amperometric detector (Bioanalytical Systems). The applied potential was set at +0.66 V relative to the Ag/AgCl reference electrode. The sensitivity of the detector was set at 1.0 nA/V, and the background current was 1.2 nA.

Alpha-methyl norepinephrine (αMNE), (—)-NE bitartarte and DHPG used as standards in the assay were purchased from Sigma (St. Louis, MO, U.S.A.).

Fresh human blood was collected from normal volunteers after 5 min resting supine. Rabbit blood was obtained by ear artery cannulation. Blood samples were collected into lithium heparin tubes containing 1 mg sodium metabisulphite and centrifuged immediately at 1000 *g* for 5 min at 4°C to separate plasma. Plasma (2.5 ml) was placed in 10-ml conical polycarbonate tubes, then 4 ng of αMNE (internal standard) was added along with 500 μl of 1.5 *M* Tris (pH 8.6) to adjust the pH of the mixture to 8.6. Acid-washed alumina (50 mg) was added and the sample mixed gently for 30 min. The alumina was allowed to settle and the plasma aspirated. The alumina was then washed twice with 10 ml of twice distilled water. Catechols were eluted by vigorously mixing the alumina with 250 μl of 0.2 *M* perchloric acid. A 200-μl aliquot of the eluate was injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The sensitivity of the system (twice background) was 30 pg for DHPG, NE and αMNE. While this sensitivity was adequate for the routine measurement of DHPG and NE, it was inadequate to reliably detect epinephrine. The retention times were 4.9 min for DHPG, 11.8 min for NE, 19.6 min for αMNE and 23.6 min for epinephrine. Dopamine had a retention time of 57.0 min, but was not detected in plasma samples assayed. The usual run time was, therefore, 30 min per sample. The recoveries of 4 ng of each of the catechols from alumina were DHPG, 54.1 ± 2.1%; NE, 59.4 ± 3.1%; and αMNE 65.1 ± 2.5% (mean ± S.D., $n = 8$). The recovery of DHPG was significantly lower ($p < 0.001$, Student's *t*-test) than that of the internal standard, as has previously been reported [3]. However, over the concentration range usually assayed (0.5—5 ng) the recovery of DHPG correlated linearly with that of αMNE ($r = 0.9772$, $p < 0.001$, $n = 11$). The within-day coefficients of variation were 4.1% for DHPG and 3.4% for NE ($n = 8$) while the between-day coefficients of variation were 8.9% for DHPG and 4.6% for NE ($n = 6$).

A typical chromatogram from human plasma is presented in Fig. 1. Human plasma DHPG and NE levels were 5.16 ± 0.98 n*M* and 1.38 ± 0.35 n*M*, respectively (mean ± S.D., $n = 6$). These results are similar to those reported using previous techniques [1—3]. The identity of the DHPG peak was further validated by demonstrating that it was greatly reduced by pretreating rabbits with the monoamine oxidase inhibitor, pargyline, 100 mg/kg 5 h prior to blood sampling (Fig. 1).

The system described differs from the previously described method for brain tissue [4] in the column used, the lower concentration of ion-pairing reagent and the absence of methanol in the mobile phase. These changes were necessary to resolve DHPG from the solvent front and NE from uric acid. The internal standard (αMNE) was used in preference to dihydroxybenzylamine [3, 4]

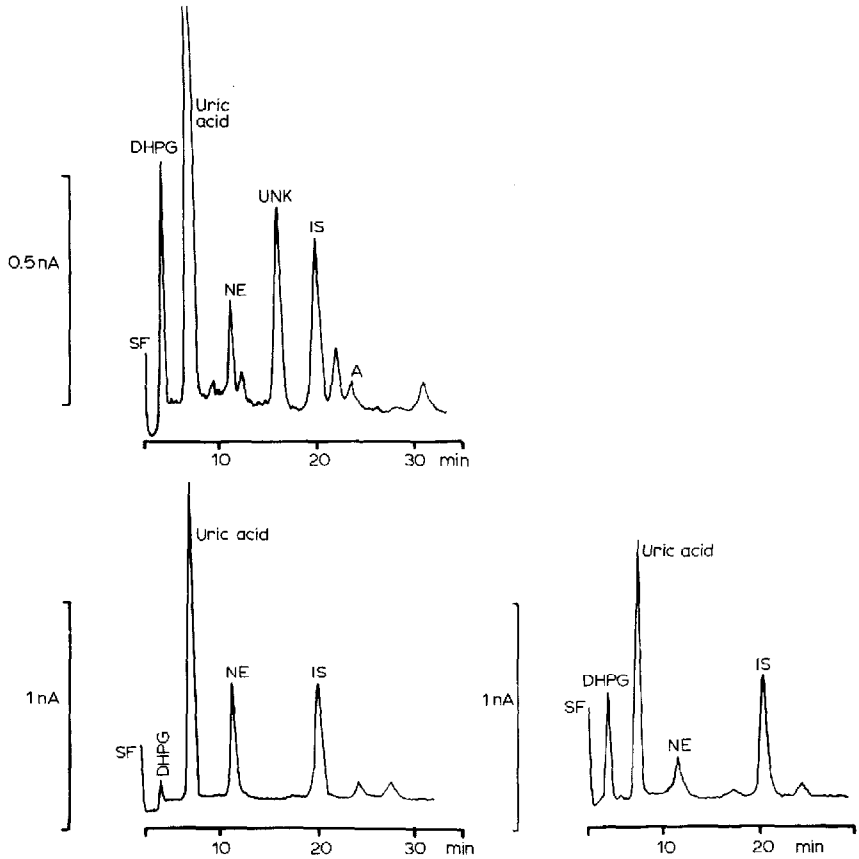

Fig. 1. Top: typical chromatogram of human plasma. Bottom, left: chromatogram of rabbit plasma 5 h after treatment with the monoamine oxidase inhibitor pargyline (100 mg/kg intraperitoneally) showing a marked reduction in the DHPG peak and associated increase in the NE peak compared to a chromatogram of normal rabbit plasma (bottom, right). Peaks: IS = internal standard (αMNE); UNK = unidentified peak; SF = solvent front; A = epinephrine.

because in the present system dihydroxybenzylamine co-chromatographed with epinephrine. This method allows the simple, simultaneous determination of NE and its major metabolite DHPG in human plasma by HPLC—ED. The technique will be useful for studies of sympathetic activity in man and possibly for the diagnosis of pheochromocytoma [1, 2].

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