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Note

Rapid quantitative assay of plasma 11-deoxycortisol and cortisol by high-performance liquid chromatography for use in the metyrapone test

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Metyrapone stimulates corticotropin secretion through inhibition of the negative feedback mechanism by blocking the adrenal 11β-hydroxylase, i.e. the conversion of 11-deoxycortisol to cortisol. The single-dose version of the metyrapone test, first introduced by Jubiz et al. [1] forms a convenient substitute for the time-consuming classical metyrapone test [2]. In a study on a large number of patients Spiger et al. [3] reported that the estimation of plasma 11-deoxycortisol after a single dose of metyrapone is sufficient for testing the integrity of the pituitary-adrenal axis. In case of subnormal plasma 11-deoxycortisol levels these workers recommend the measurement of plasma cortisol levels to assure that the administered dose of metyrapone effectively blocked the 11β-hydroxylase.

Plasma levels of cortisol and 11-deoxycortisol are usually determined by a competitive protein binding (CPB) [1, 4—7] or a radioimmunoassay (RIA) method [3, 8—12]. The specificity of these methods is limited by the presence of cross-reacting material. Levels of interfering material can increase after administration of metyrapone. High-performance liquid chromatography (HPLC) does not suffer from this disadvantage. It has been demonstrated that HPLC is suitable for the determination of plasma cortisol [13—16]. Recently a method has been reported for the determination of cortisol and 11-deoxycortisol by means of reversed-phase HPLC [17]. However the accuracy of this method is questionable because no internal standard is used for quantification. We describe a rapid quantitative assay of 11-deoxycortisol and cortisol by means of HPLC on a silica gel column. A simple extraction procedure and the possibility of immediate quantification make this method very useful in the single-dose metyrapone test.

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EXPERIMENTAL

Apparatus and materials

A Varian liquid chromatograph Model 8500 was used, equipped with a stop-flow injector, a LDC Model 1203 254-nm single-wavelength UV detector and a 250 × 3 mm SI-10 column (silica gel, particle size 10 μm, Chrompack, Middelburg, The Netherlands). Samples were injected with a 25-μl Hamilton syringe. All solvents were of analytical reagent grade.

Standard stock solutions of 1 mg steroid per ml ethanol were diluted to working solutions of 1 μg steroid per ml ethanol.

Charcoal-stripped plasma was prepared by mixing 5 g charcoal with 100 ml plasma pool for 1 h at room temperature. Charcoal was then removed by centrifugation.

Administration of metyrapone

Twenty healthy subjects without evidence of pituitary-adrenal disease were studied by the single-dose metyrapone test. All subjects received approximately 30 mg of metyrapone per kg body weight at midnight. Blood samples were taken at 8 a.m. on two consecutive days, before and after metyrapone administration. Plasma was separated by centrifugation and stored at −20°C.

Determination of steroids

11-Deoxycortisol. To 0.6 ml plasma 0.05 ml 3 *N* sodium hydroxide solution and 100 ng prednisone as internal standard were added. Extraction was carried out by mixing for 5 min with 4 ml dichloromethane. After centrifugation the water phase was discarded and the extract was washed once with 0.1 *N* hydrochloric acid and once with water. After evaporation to dryness of 3 ml of the extract the residue was dissolved in 50 μl chloroform and 20 μl of this solution were injected onto the column. The eluent used in HPLC was chloroform—isooctane—methanol—water (48.5 : 48.5 : 2.9 : 0.12, v/v). The flow-rate was 65 ml/h. The ratio of peak heights of 11-deoxycortisol and prednisone was used as the basis for quantification.

Cortisol. For the determination of cortisol the method as described for 11-deoxycortisol was modified by using prednisolone as internal standard and chloroform—isooctane—methanol—water (71 : 25 : 3.75 : 0.25, v/v) as eluent.

Estimation of the recovery

The recovery of 11-deoxycortisol was estimated as follows: 100 ng 11-deoxycortisol was added to 0.6 ml charcoal-stripped plasma. Samples were then analyzed as described, except that prednisone was added after the extraction procedure. Recovery was calculated by comparing the ratio of peak heights to the ratio of peak heights of a reference mixture of the same amounts of 11-deoxycortisol and prednisone.

For the estimation of the recoveries of cortisol, prednisone and prednisolone the same procedure was carried out on the understanding that instead of prednisone, prednisolone, 11-deoxycortisol and cortisol respectively were used.

RESULTS

Plasma concentrations of 11-deoxycortisol were determined in 20 subjects. The amount of 11-deoxycortisol in all plasma samples before administration of metyrapone was below the detection limit of 25 nmol/l. Mean values of 11-deoxycortisol in plasma after metyrapone were 290 nmol/l ± 78 (S.D.), range 190—450 nmol/l.

In addition, in pre- and postmetyrapone plasma samples of six unselected subjects cortisol was also measured. Values of 11-deoxycortisol and cortisol of these samples are given in Table I.

Chromatograms of plasma extracts before and after metyrapone are shown in Figs. 1 and 2.

Sensitivity

The minimal detectable amount for both cortisol and 11-deoxycortisol was 4.5 pmol. This amount corresponds to a concentration of 25 nmol/l when 0.6 ml plasma is analyzed according to our method.

Reproducibility

The precision was estimated from duplicate assays, the relative standard deviation was 8.0% for 11-deoxycortisol (n=20) and 4.6% for cortisol (n=12).

Linearity

To 0.6 ml of a charcoal-stripped plasma pool 11-deoxycortisol was added in amounts equivalent to 55—1100 nmol/l. A constant amount of 100 ng prednisone as internal standard was added to each sample, which was then processed as described. The same procedure was followed for cortisol with prednisolone as internal standard. Concentrations and peak height ratios were linearly related over this whole range for 11-deoxycortisol as well as cortisol.

Recovery

Recoveries of cortisol, 11-deoxycortisol, prednisone and prednisolone were estimated as described. Results are shown in Table II.

TABLE I

CONCENTRATIONS OF 11-DEOXYCORTISOL AND CORTISOL IN PLASMA SAMPLES OF 6 NORMAL SUBJECTS BEFORE AND AFTER ADMINISTRATION OF METYRAPONE

Subject	11-Deoxycortisol (nmol/l)		Cortisol (nmol/l)	
	Before metyrapone	After metyrapone	Before metyrapone	After metyrapone
1	< 25	410	285	45
2	< 25	210	325	80
3	< 25	200	420	215
4	< 25	210	550	145
5	< 25	270	325	150
6	< 25	280	385	85
mean ± S.D.	< 25	265 ± 72	380 ± 87	120 ± 56

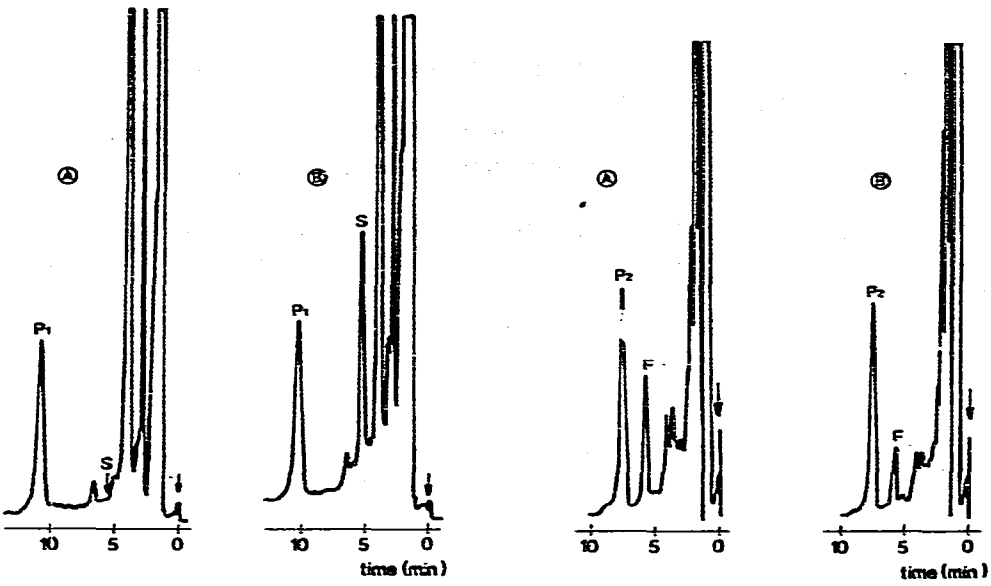

Fig. 1. Chromatograms of plasma extracts before (A) and after (B) administration of metyrapone. S = 11-deoxycortisol, P_1 = prednisone (internal standard). Column SI-10, eluent chloroform—isooctane—methanol—water (48.5 : 48.5 : 2.9 : 0.12, v/v). Flow-rate 65 ml/h; UV detection 254 nm, 0.008 a.u.f.s.

Fig. 2. Chromatograms of plasma extracts before (A) and after (B) administration of metyrapone. F = cortisol, P_2 = prednisolone (internal standard). Column SI-10, eluent chloroform—isooctane—methanol—water (71 : 25 : 3.75 : 0.25, v/v). Flow-rate 65 ml/h; UV detection 254 nm, 0.008 a.u.f.s.

TABLE II

RECOVERIES OF 100 ng STEROIDS ADDED TO CHARCOAL-STRIPPED PLASMA PRIOR TO EXTRACTION

Steroid	Recovery (%)	*n*
Cortisol	95.2 ± 0.8	4
11-Deoxycortisol	97.6 ± 0.7	4
Prednisone	100.7 ± 1.9	4
Prednisolone	96.2 ± 1.2	4

DISCUSSION

Concentrations of 11-deoxycortisol in plasma before and after a single dose of metyrapone as determined by our HPLC method are in good agreement with those estimated by CPB or RIA [4, 7, 11, 12]. The possibility of a rapid quantification of 11-deoxycortisol however is in favour of the HPLC method.

Concentrations of cortisol in plasma before metyrapone as determined by our HPLC method correlate well with those estimated by CPB or RIA [4, 11]. After a single dose of metyrapone however there is less agreement. For in-

stance, Schöneshöfer et al. [11] and Spark [4] reported postmetyrapone cortisol values of 248 ± 100 nmol/l and 236 ± 83 nmol/l respectively. These values are significantly higher than our postmetyrapone cortisol values of 120 ± 56 nmol/l. The difference is probably due to non-specificity of CPB and RIA [17]. It is obvious that levels of cortisol after metyrapone cannot be assessed correctly by means of CPB or RIA without additional purification. In our opinion HPLC is the advisable method to determine postmetyrapone concentrations of cortisol.

In conclusion: the method described offers a simple, rapid and reliable determination of 11-deoxycortisol and cortisol for use in the single-dose metyrapone test.

REFERENCES

1 W. Jubiz, A. Wayne Meikle, C.D. West and F.H. Tyler, Arch. Int. Med., 125 (1970) 472.
2 C.W. Liddle, H.L. Estep, J.W. Wendall, W.C. Williams, Jr., and A.W. Townes, J. Clin. Endocrinol. Metab., 19 (1959) 875.
3 M. Spiger, W. Jubiz, A. Wayne Meikle, C.D. West and F.H. Tyler, Arch. Int. Med., 135 (1975) 698.
4 R.F. Spark, Ann. Int. Med., 75 (1971) 717.
5 H.H. Kley and H.L. Krüskemper, Klin. Wochenschr., 50 (1972) 1049.
6 J. Kolenowski, J. Steroid Biochem., 5 (1974) 55.
7 B. Zieger, W. Hubl and M. Büchner, Zeitschr. Inn. Med., 30 (1975) 539.
8 D.K. Mahajan, J.D. Wahlen, F.H. Tyler and C.D. West, Steroids, 20 (1972) 609.
9 A. Wayne Meikle, S.C. West, J.A. Weed and F.H. Tyler, J. Clin. Endocrinol. Metab., 40 (1975) 290.
10 L.M.Y. Lee and R.S. Schiller, Clin. Chem., 21 (1975) 719.
11 M. Schöneshöfer, M. L'age and W. Oelkers, Acta Endocrinol., 85 (1977) 107.
12 W. Hubl, M. Büchner, K. Haussig, W. Rohde and F. Stahl, Endokrinologie, 71 (1978) 60.
13 F.K. Trefz, D.J. Byrd and W. Kochen, J. Chromatogr., 107 (1975) 181.
14 J.H.M. van den Berg, Ch. R. Mol, R.S. Deelder and J.H.H. Thijssen, Clin. Chim. Acta, 78 (1977) 165.
15 T. Kawasaki, M. Maeda and A. Tsuji, J. Chromatogr., 163 (1979) 143.
16 P.M. Kabra, L.L. Tsai and L.J. Marton, Clin. Chem., 25 (1979) 1293.
17 G.E. Reardon, A.M. Caldavella and E. Canalis, Clin. Chem., 25 (1979) 122.