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RADIOIMMUNOASSAY FOR STEROID HORMONES V. RADIOIMMUNOASSAY FOR PLASMA 11-DEOXYCORTISOL

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Abstract

A sensitive and reliable radioimmunoassay for human plasma 11-deoxycortisol has been developed. Antiserum against 11-deoxycortisol was produced in rabbits by immunizing with a 11-deoxycortisol-3-CMO-BSA conjugate. The antiserum cross-reacted with 17-OH-progesterone, estriol, cortisone, progesterone and estradiol less than 10%. 11-Deoxycortisol in 0.2 ml plasma was extracted with ethylether and separated from cross-reacting steroids by microcolumn chromatography (Sephadex LH-20), with methylene chloride: benzene: methanol (12:7:1, v/v/v) solvent. The intra-assay and the inter-assay precisions were 8.8 and 4.3%, respectively. The mean plasma 11-deoxycortisol concentration at 9:00 a.m. was 1.11±0.1 ng/ml (M ± SEM) in twenty healthy controls. Plasma 11-deoxycortisol levels at 4 hours after a single oral administration of Metyrapone (1 g) markedly increased to 113.5 ± 5.6 ng/ml in six healthy men (M ± SEM).

INTRODUCTION

11-Deoxycortisol is the precursor of cortisol and has no corticoid activity. Metyrapone inhibits the 11-β-hydroxylation in the biosynthesis of cortisol and results in the increased secretions of ACTH and 11-deoxycortisol via feedback mechanism. The metyrapone test is useful for evaluating the pituitary ACTH reserve.

Plasma levels of 11-deoxycortisol were determined by a competitive protein binding assay (CPBA)[1,2] and a radioimmunoassay[3] following metyrapone administration. But none of these methods was satisfactory for their sensitivity and separation of 11-deoxycortisol. A sensitive and accurate radioimmunoassay

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method with chromatography for the determination of 11-deoxycortisol in human plasma was described in this paper.

MATERIALS AND METHODS

Chemicals :

11-Deoxycortisol-1,2-^{3}H, (58.5 ci/mM; New England Nuclear Corp., Boston, Mass., U.S.A.) was purified by a thin layer chromatography. All other chemicals were the same as previously reported from this laboratory[4].

Antiserum :

The preparation of 11-deoxycortisol-3-CMO-BSA and the immunization of rabbits with the antigen were done according to the previously reported method[4]. 11-Deoxycortisol-3-CMO was kindly donated by Dr. Kanbegawa (Teikoku Zoki Pharmaceutical Co., Japan).

Extraction and Chromatographic Separation :

Plasma samples (0.2 ml) and 2,000 dpm of dried ^{3}H-11-deoxycortisol were extracted together with 2 ml ethylether. The extract was dissolved with 0.2 ml of the developing solvent [methylene chloride : benzene : methanol (12 : 7 : 1, v/v/v)] after evaporation by N_2 gas and subjected to the microcolumn (Sephadex LH-20, 8×130 mm) with the same solvent. The fraction containing 11-deoxycortisol, from 6.5 to 8.0 ml, was collected and divided into two parts, one for the radioimmunoassay, the other for recovery study.

Assay Procedure :

Non-radioactive 11-deoxycortisol standards were prepared in the concentrations of 100, 200, 500, 1000, 2000, 5000 pg/ml of ethanol. Labeled steroid (^{3}H-11-deoxycortisol, 10,000 dpm) were added to each chromatographed samples or standard solution (0.2 ml), and evaporated to dryness with N_2 gas. Diluted antisera (0.25 ml) with 0.05 M borate buffer (pH 8.0) to 1 : 60,000 were pipetted into all assay tubes. Following 30 min incubation at room temperature, bound was separated from free 11-deoxycortisol by 0.2 ml of saturated ammonium sulfate. The radioactivity was counted in a Model 3385 Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

1. Recovery : Recovery of labeled 11-deoxycortisol was $63.5 \pm 1.3\%$ in 50 assays, after extraction and separation on LH-20 Sephadex microcolumn.
2. Standard curve and sensitivity : A typical standard curve is shown in Figure 1. The average ^{3}H-steroid bound, determined at zero concentration of unlabeled steroid, was 70.5% in most assays. Lower limits of detection (20 pg) were estimated from standard curves.
3. Dilution test : A plasma, obtained from a normal healthy subject whose

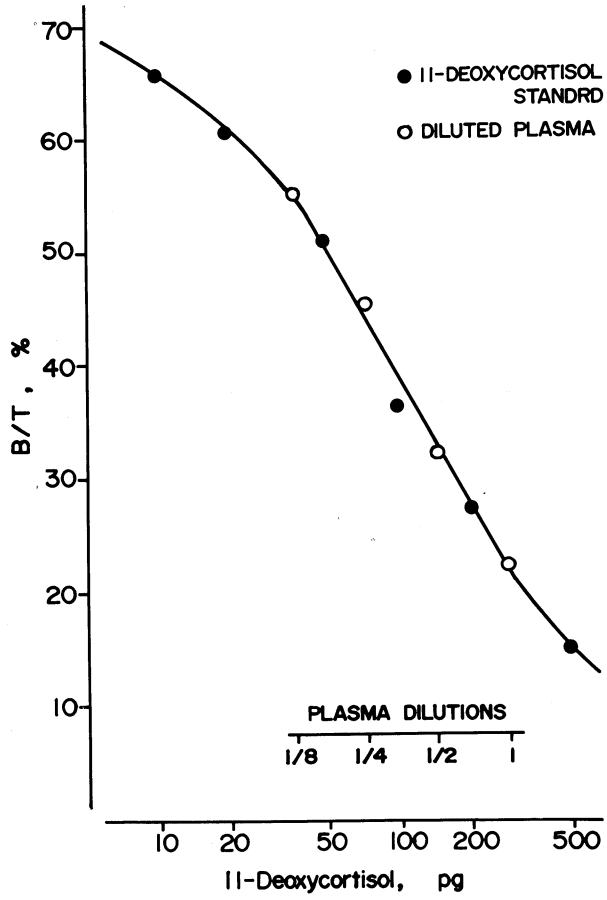

Figure 1. A typical standard curve and dilution test on semi-logarithmic scale. Standard curve ; each closed circle represents from four determinations. Dilution test ; each open circle represents from four determinations.

original plasma concentration of 11-deoxycortisol was 5.76 ng/ml, was diluted to 1 : 2, 1 : 4, 1 : 8. Diluted plasma samples were paralleled with the standard curve (Figure 1).

4. Precision and interassay variability : The intra-assay and inter-assay precisions were shown in Table 1. An average coefficient of the intra-assay precision was 8.8% among four different plasma samples. The average coefficient of the inter-assay precision was 4.3% among four different samples.
5. The accuracy of recovery : The mean recovery of added nonradioactive 11-deoxycortisol to dexamethasone-suppressed plasma was 107.8% (Table 2).

TABLE. 1

Precision of Plasma 11-deoxycortisol radioimmunoassay

Sample	11-deoxycortisol, ng/ml	CV,%
Within assay (N=6)		
A	0.64	9.4
B	1.68	4.8
C	2.51	13.1
D	4.29	7.9
		8.8 (average)
Between assay (3 different occasions)		
E	0.65	2.4
F	1.70	1.2
G	2.32	2.6
H	3.11	10.9
		4.3 (average)

6. Column separation : 11-Deoxycortisol, cortisone, and corticosterone were separated satisfactorily by the LH-20 Sephadex microcolumn (8 × 130 mm) with solvent of methylene chloride : benzene : methanol (12 : 7 : 1, v/v/v). The fractions containing the steroids are shown in Figure 2. 11-Deoxycortisol was obtained from 6.5 ml to 8.0 ml of eluate.

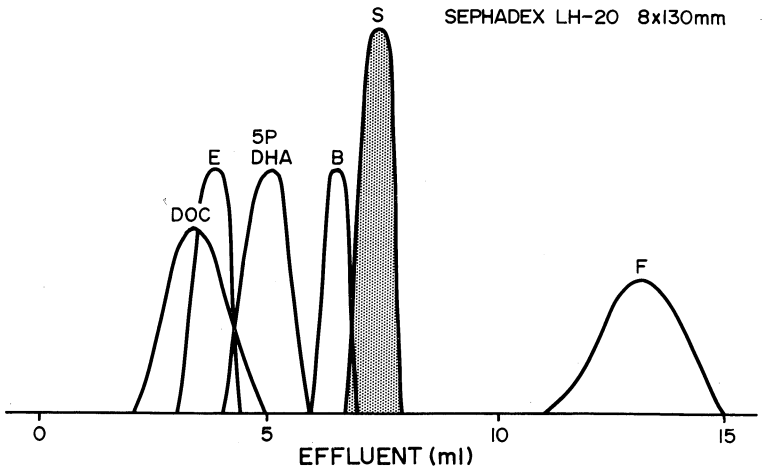

Figure 2. Elution patterns of labeled steroids on Sephadex LH-20 microcolumn. DOC ; deoxycorticosterone, E ; cortisone, DHA ; dehydroepiandrogen, 5P ; pregnenolone, B ; corticosterone, S ; 11-deoxycortisol, F ; cortisol. Estriol, estradiol and 17-OH-progesterone were eluted beyond 15 ml by the column and eluting positions of these steroids were not shown.

TABLE. 2

Recovery of added 11-deoxycortisol

11-deoxycortisol added (pg)	11-deoxycortisol determined (pg/ml)	11-deoxycortisol recoverd (pg/ml)	Recovery (%)
0	0.67		
20	0.87	0.97	111.5
50	1.17	1.28	109.4
100	1.67	1.82	109.0
200	3.67	3.36	91.6
500	5.67	6.66	117.5
			107.8 (average)

Plasma sample : plasma 0.2ml
11-deoxycortisol determined ; mean from four determinations.

7. Specificity of the assay : The cross-reactivities of the anti-11-deoxycortisol serum with various steroids are shown in Table 3. Cross-reactivities of 17-OH-progesterone, estriol, cortisone, estradiol, progesterone and cortisol were 9.2, 7.5, 6.0, 2.8, 1.6 and 1.6%, respectively. But the other steroids showed relatively low cross-reactivities of less than 1.0% in this assay.

TABLE. 3

The relative cross reaction of 11-deoxycortisol antisera

Steroids	Cross-reactivity, %
11-DEOXYCORTISOL (S)	100
17-OH-PROGESTERONE	9.2
ESTRIOL (E_3)	7.5
CORTISONE (E)	6.0
ESTRADIOL(E_2)	2.8
PROGESTERONE	1.6
CORTISOL (F)	1.6
DEHYDROCORTICOSTERONE (A)	0.4
ANDROTESTOSTERONE	0.3
DEHYDROTESTOSTERONE	0.2
CORTICOSTERONE	0.2
TESTOSTERONE	0.1
PREGNENOLONE	0.1
17-OH-PREGNENOLONE	0.1
DEHYDROEPIANDROSTERONE	0.1

8. Mean plasma concentration of 11-deoxycortisol : The mean plasma 11-deoxycortisol level at 9 : 00 a.m. was 1.11 ± 0.1 ng/ml in 20 normal healthy subjects.
9. Metyrapone test : Metyrapone (1 g) was taken orally by 6 normal subjects at 9:00 a.m. Blood samples were obtained at 4 hours following metyrapone load. Plasma levels of 11-deoxycortisol were markedly increased from 0.97 ± 0.09 to 113.5 ± 5.6 ng/ml after metyrapone.

DISCUSSION

The metyrapone-induced high concentration of plasma 11-deoxycortisol might be measured without chromatographic separation because of its prominent increase following an administration of the drug,[5,6] the level of which after metyrapone was shown to be as much as normal 9:00 a.m. cortisol level.

However, the basal level of plasma 11-deoxycortisol is very low comparing with cortisol or cortisone, which showed small but significant cross-reaction with the antiserum to 11-deoxycortisol.

Therefore, chromatographic separation should be done in order to determine the basal or suppressed level of 11-deoxycortisol.

The classical metyrapone test by Liddle[7] for detecting the disturbances of pituitary ACTH reserve is judged by an increase of urinary 17-OHCS excretion after metyrapone administration. The metyrapone-induced increasement of urinary 17-OHCS excretion is only 2 to 3 fold as much as basal value in normal subjects, and the measurement of urinary 17-OHCS is not specific for 11-deoxycortisol.

The single dose metyrapone test with determination of plasma 11-deoxycortisol allows precise evaluation for the pituitary ACTH reserve.

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