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RADIOIMMUNOASSAY FOR STEROID HORMONES III. RADIOIMMUNOASSAY FOR PLASMA DEHYDROEPIANDROSTERONE

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Abstract

A specific, sensitive and reliable radioimmunoassay for plasma dehydroepiandrosterone (DHEA) has been developed. Anti-DHEA serum was obtained by immunizing rabbits with a DHEA-3-hemisuccinate-BSA conjugate. A useful range in the standard curve was from 10 pg to 500 pg. DHEA was separated from cross-reacting steroids by microcolumn chromatography. The coefficients of variation for within-assay and between-assay are 6.3 % and 7.7 %, respectively.

INTRODUCTION

It has been demonstrated that dehydroepiandrosterone (DHEA) is produced by both the adrenal cortex and the gonads[1,2]. Investigation of the physiological role of this steroid has been hampered by lack of a simple, sensitive method for the measurement of plasma levels of the steriod. In the present study, a sensitive and specific radioimmunoassay was developed for measurement of plasma DHEA in man.

MATERIALS AND METHODS

1. Chemicals. Dehydroepiandrosterone-7-^{3}H (10 Ci/mM, New England Nuclear Corp.) was used after purification with thin-layer chromatography. All other chemicals were prepared in the same way as previously reported[3].

2. Antigen and antiserum. Dehydroepiandrosterone-3-hemisuccinate was the gift from Dr. Kambegawa (Teikoku Zoki Pharmaceutical Co., Japan). The production of DHEA-3-hemisuccinate-BSA and immunization of rabbits with the antigen were done according to the methods reported[3].

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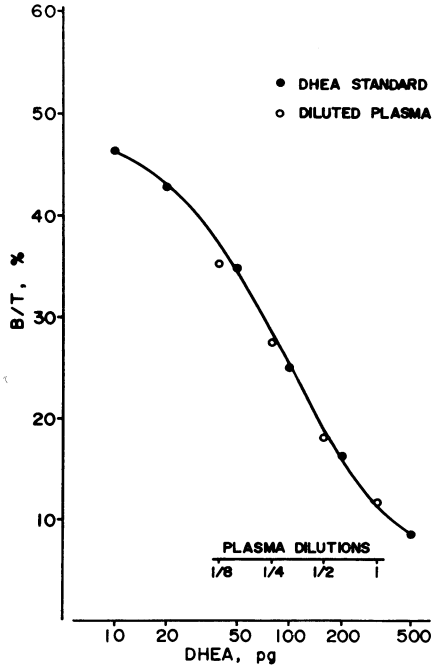
3. Sample preparation and chromatography. 0.05 ml to 0.1 ml of plasma was transferred to assay tube containing dried 2,000 dpm of ^{3}H-DHEA and the DHEA was extracted with 1 ml of ether. The extract was applied to a microcolumn for chromatography in the same way as reported about radioimmunoassay of corticosterone[4]. Benzene : methanol 85 : 15 solution was used as developing solvent and the collected fraction (from 1.5 ml to 2.5 ml) was divided into two parts, one for assay, the other for recovery counting.

4. Assay procedure and radiocounting. The entire assay was carried out by the same method reported[3]. The antiserum was diluted to 1 : 60,000.

RESULTS

1. The recovery of ^{3}H-DHEA from 50 samples was 73.7 $\pm$ 4.2 % (S.D.).

2. A typical dose response line and sensitivity. A typical dose response line is shown in Figure. Bound per cent for total ^{3}H-DHEA was 57 %



A typical dose response line and dilution test on semi-logarithmic scale. Dose response line; each closed circle represents the mean from duplicate determinations. The final dilution of anti-DHEA is 1:60,000.
Dilution test; original plasma was diluted to 1:2, 1:4 and 1:8, each open circle represents the mean from four determinations.

at 0 pg of standard DHEA and was 8.5 % at 500 pg of added DHEA. Practical sensitivity was 20 pg in the assay. Water blank value was not different from 0.

3. Dilution test. The result of dilution test of the assay is shown in Figure. Original plasma, of which concentration of DHEA was 6.4 ng/ml, was diluted to 1 : 2, 1 : 4 and 1 : 8, and the diluted plasmas showed the parallel curve with authentic standard curve.

4. Within assay and between assay. The results of within assay and between assay experiments are shown in Table 1. Average precision (within assay) as defined by the coefficient of variation was 6.3 % and the average coefficient of variation in between assay was 7.7 %.

5. The accuracy of recovery. The mean recovery of added DHEA from 0.1 ml plasma was 99.0 % for plasma I and 104.1 % for plasma II (Table 2).

TABLE 1.

Precision and Reproducibility of Plasma DHEA Radioimmunoassay

Samples	DHEA, ng/ml (Average)	CV, %
Within assay (N=6)		
A	2.49	5.6
B	5.34	5.9
C	9.29	6.5
D	16.11	6.2
		6.3 (Average)
Between assay (3 different occasions)		
E	3.11	9.3
F	6.85	6.9
G	10.85	8.1
H	13.45	6.6
		7.7 (Average)

6. Specificity. The cross-reactivities of the anti-DHEA serum with various steroids are shown in Table 3. Androstenedione, androsterone and testosterone cross-reacted 19.8 %, 11.4 % and 2.0 %, respectively. DHEA should be separated on chromatography from androstenedione and testosterone, which have the close polarities with DHEA and have some cross-reactivities with DHEA antiserum.

7. Mean plasma DHEA level at 9 am was 5.99 ± 1.42 ng/ml (range, 3.52-8.34 ng/ml) in 22 normal adult men and 5.66 ± 1.14 ng/ml (range, 4.17-7.78 ng/ml) in 16 normal adult women. Totally, it was 5.85 ± 1.32 ng/ml in 38 normal men and women.

TABLE 2.

Recovery of added DHEA from Plasma

Plasma sample	DHEA added (pg)	DHEA determined (pg)	Recovery (%)	Plasma sample	DHEA added (pg)	DHEA determined (pg)	Recovery (%)
I	0	231		II	0	376	
	20	259	103.2		20	403	101.8
	50	268	95.4		50	450	105.6
	100	347	104.8		100	459	96.4
	200	434	100.7		200	639	110.9
	500	664	90.8		500	927	105.8
			99.0 (Average)				104.1 (Average)

Plasma sample; plasma 0.1 ml. DHEA determined; means from four determinations.

TABLE 3.

The Cross-reactivity of Anti-DHEA-1-1 (1:60,000)

Steroids	Cross-reactivities, %
ANDROSTENEDIONE	19.8
ANDROSTERONE	11.4
ESTRONE (E_1)	3.7
ESTRADIOL (E_2)	3.4
TESTOSTERONE	2.0
ETIOCHOLANOLONE	1.8
PREGNENOLONE	1.6
PROGESTERONE	1.4
DIHYDROTESTOSTERONE	1.4
DEHYDROCORTICOSTERONE (A)	1.2
CORTISOL (F)	1.1
EPIESTRIOL	1.1
ALDOSTERONE	$<$1.0
CORTICOSTERONE (B)	〃
DOC	〃
TETRAHYDROCORTISOL (THF)	〃
11-DESOXYCORTISOL (S)	〃
CORTISONE (E)	〃
17-OH-PREGNENOLONE	〃
17-OH-PROGESTERONE	〃
5α-PREGNANEDIONE	〃
5β-PREGNANEDIOL	〃
PREGNANETRIOL	〃
ESTRIOL (E_3)	〃
DEXAMETHASONE	0.3

8. Rapid ACTH test and dexamethasone suppression test in normal subjects. In rapid ACTH test in 6 normal subjects, basal DHEA (5.85 ± 0.74 ng/ml) increased to 7.79 ± 0.88 ng/ml at 30 min and 8.83 ± 1.01 ng/ml at 60 min after intramuscular injection of 0.25 mg Cortrosyn (Table 4). And in dexamethasone suppression test, basal DHEA levels of 5 normal subjects (7.79 ± 0.73 ng/ml) decreased to the lowest level of 2.57 ± 0.21 ng/ml at the time between 4 hours and 6 hours after single dose administration of 3 mg dexamethasone.

TABLE 4.

Rapid ACTH Test and Dexamethasone Suppression Test in Normal Subjects

	Rapid ACTH Test			Dexamethasone Suppression Test		
Case	Basal	30 min	60 min	Basal	4 hrs	6 hrs
1	4.90	8.76	8.30	8.56	3.25	**2.80**
2	6.74	8.35	8.01	7.09	3.88	**2.77**
3	6.55	9.08	10.46	8.34	**2.60**	4.07
4	6.54	7.52	9.71	6.74	**2.39**	2.51
5	4.90	7.01	8.42	8.21	**2.28**	2.32
6	5.48	7.09	8.09			
	5.85	7.97	8.83	7.79	**2.57**	
	± 0.74	± 0.88	± 1.01	± 0.73	± **0.21**	

Plasma samples for determination of DHEA (ng/ml); before, at 30 min and at 60 min after i.m. injection of 0.25 mg Cortrosyn in ACTH test and before, at 4 hrs and at 6 hrs after p.o. administration of 3 mg dexamethasone in suppression test. The number in thic letter means the lowest level of plasma DHEA after dexamethasone.

DISCUSSION

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are together regarded as the major adrenal androgens. A reliable and practical radioimmunoassay for plasma DHEA has been developed at this laboratory. Plasma values for DHEA in normal men and women in the present study agree with data from the literatures[2,5]. The difference in mean plasma DHEA values between adult men and women was within 1 ng/ml in the present method or Nieschlag's method[2]. Furthermore, Abraham *et al.*[6] reported that plasma DHEA levels showed no significant difference between luteal phase and follicular phase during the menstrual cycle.

The response of plasma DHEA to intramuscular injection of ACTH (Cortrosyn) showed the highest value at 30 min or at 60 min after

administration, whereas i. v. infusion of ACTH caused an increase in DHEA and DHEAS secretion by 15-30 min in Nieschlag *et al's* report[2].

Concerning gonadal secretion of DHEA, Nieschlag *et al.*[2] and Laatikainen *et al.*[1] demonstrated that DHEA was found to be secreted by the normal gonad, and that exogenous human chorionic gonadotropin (HCG) caused an increase in plasma DHEA.

Dexamethasone suppressibility of plasma DHEA was reduced compared with that of cortisol[7]. This is explained by the contribution to DHEA by hydrolysis of the relatively abundant DHEAS[8].

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