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RADIOIMMUNOASSAY FOR STEROID HORMONES
II. RADIOIMMUNOASSAY FOR PLASMA CORTICOSTERONE

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Abstract

A radioimmunoassay for human plasma corticosterone has been developed. Antiserum against corticosterone was raised in rabbits immunized with corticosterone-21-hemisuccinate conjugated to bovine serum albumin. The mean coefficient of variation between assays was 7.7 % and within assays was 8.6 %. Human plasma corticosterone is measured readily by assaying aliquots of an ether extract of 0.05 to 0.1 ml of plasma after microcolumn chromatography. The mean plasma corticosterone concentration at 9 a.m. was 7.1 ± 3.2 ng/ml in 45 normal subjccts. Plasma corticosterone increased 5.2 times as much as basal values after ACTH stimulation, whereas in radioimmunoassay cortisol increased as much as 2.4 times. On the other hand, plasma corticosterone decreased to 22.6 % of basal values at four hours after the administration of 1 mg dexamethasone, whereas by the radioimmunoassay cortisol decreased to 12.3 % of basal values.

INTRODUCTION

Corticosterone is the principal glucocorticoid secreted by the adrenal cortex in rats, rabbits and mice. In man, cortisol, corticosterone and aldosterone are the three major corticosteroids. However, little is known about the action of corticosterone in man and this is partly due to the lack of specific and sensitive methods for measurement of plasma corticosterone. In the present study, by using an antiserum raised against corticosterone-21-hemisuccinate-BSA, a sensitive and specific radioimmunoassay for corticosterone was developed for measurement of corticosterone in human plasma.

MATERIALS AND METHODS

1. Chemicals. Corticosterone-1, 2-^{3}H, 40 Ci/mM (New England Nuclear Corp.) was used after purification with thin-layer chromatography.

Sephadex LH-20 was purchased from Pharmacia Fine Chemicals. All other chemicals were prepared in the same way as previously reported about radioimmunoassay of cortisol[1].

2. Antigen and antiserum. The production of corticosterone-21-hemisuccinate-BSA and immunization of rabbits with the antigen was done according to the methods previously reported[1].

3. Sample preparation and chromatography. As seen in Table 1, 0.05 to 0.1 ml of plasma was transferred to assay tube containing dried 2,000 dpm of ^{3}H-corticosterone and the corticosterone was extracted with 1 ml of ether. After washing with 0.2 ml of water and dryness, the extract was transferred to microcolumn for chromatography, 2 ml syringe for Mantoux reaction with diameter of 8 mm. Sephadex LH-20 column height was 60 mm. The mixture of benzene; methanol (98 : 2) was used for developing solvent and the collected fraction (from 3.5 to 7.0 ml) was

TABLE 1.

Radioimmunoassay Procedures for Plasma Corticosterone

plasma 0.05 to 0.1 ml and ether 1 ml, with
dried 2,000 dpm of ^{3}H-corticosterone

↓ extract 30 seconds by Vortex mixer

microcolumn chromatography (benzene : methanol=98 : 2)

↓

after dryness, divide the corticosterone into two parts
with 0.4 ml of methanol

Recovery	Assay
counting vial containing 10 ml scintillator	^{3}H-corticosterone 10,000 dpm
	↓ evaporate in N_2 gas
	antiserum (1 : 35,000), 0.25 ml
	↓ mix 15 seconds, incubate 30 min. at room temperature
	saturated $(NH_4)_2SO_4$, 0.2 ml
	↓ stand still 10 min. at room temperature, centrifuge 10 min. 3,000 rpm
	0.2 ml supernatant into counting vial containing 10 ml scintillator

divided into two parts, one for assay, the other for recovery counting into counting vial.

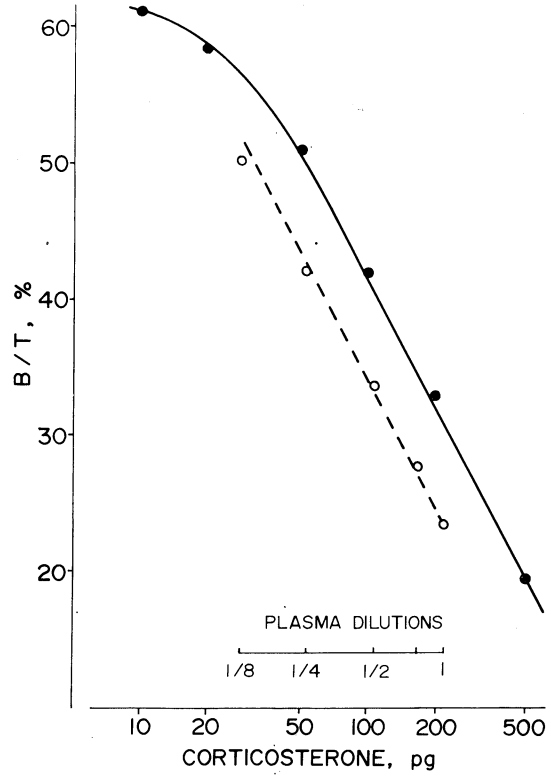
4. Assay procedure and radiocounting. The entire assay we carried out by the same methods previously reported[1] (Table 1).

5. Radioimmunoassay of cortisol and 11-OHCS determination. In the serial groups, radioimmunoassay of cortifol was performed by Nishida et al.'s method[1]. Also, in a serial group, plasma 11-hydroxycorticosteroid (11-OHCS) was determined by De Moor's method[2].

RESULTS

1. Recovery throughout the whole assay was 51.2±12.1 % in 50 assays.

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



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-corticosterone is 1: 35,000. Dilution test; original plasma, of which concentration of corticosterone was 37.8 ng/ml, was diluted to 1:2, 1:4 and 1:8. Each open circle represents the mean from four determinations in the dilution test.

62.5 % in duplicate at 0 pg of standard corticosterone and was 18.0 % at 500 pg of added corticosterone. Practical sensitivity is 20 pg in the assay.

3. Dilution test. The result of dilution test of the assay is shown in Figure. Original plasma, of which concentration of corticosterone was 37.8 ng/ml, was diluted to 1:2, 1:4 and 1:8, and these diluted plasma 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 2. Average precision as defined by the coefficient of variation was 8.6 % in four different plasma samples. The average coefficient of variation in between assay done in 3 different occasions was 7.7 % for five different plasmas.

5. The accuracy of recovery. The mean recovery of added corticosterone from 0.1 ml plasma was 116.3 % (Table 2).

TABLE 2.

The Accuracy of Plasma Corticosterone Radioimmunoassay

Within assay (N=6)		
	Corticosterone, ng/ml (average)	C.V., %
Sample A	1.26	7.5
B	4.44	9.2
C	19.47	7.2
D	25.10	10.5
		8.6 (average)

Between assay (3 different occasions)		
	Corticosterone, ng/ml (average)	C.V., %
Sample E	1.73	8.5
F	8.66	9.5
G	12.80	7.2
H	23.76	4.1
I	25.96	9.1
		7.7 (average)

Recovery		
Corticosterone added (pg)	Corticosterone determined (pg)	Recovery, %
0	309.2	
100	423.8	114.6
500	899.3	118.0
		116.3 (average)

Recovery; Corticosterone determined is expressed by the means from four determinations for 0.1 ml plasma.

6. Specificity. The cross-reactivity of the anti-corticosterone serum with various steroids is shown in Table 3, Progesterone, DOC and dehydrocorticosterone cross-reacted 27.4 %, 20.8 % and 20.4 %, respectively. However, according to their cross-reactivities and polarities on the chromatography, corticosterone should be separated chiefly from 11-deoxycortisol and cortisone.

TABLE 3.

The Crossreactivity of Anti-corticosterone-3-0 (1 : 35,000)

Steroids	Cross-reactivity, %
PROGESTERONE	27.4
DOC	20.8
DEHYDROCORTICOSTERONE (A)	20.4
PREGNENOLONE	19.2
5β-PREGNANEDIOL	12.3
5α-PREGNANEDIONE	11.7
ALDOSTERONE	10.3
TESTOSTERONE	8.5
ANDROSTENEDIONE	8.2
CORTISOL (F)	7.8
11-DESOXYCORTISOL (S)	6.5
CORTISONE (E)	5.1
DHEA	3.6
ETIOCHOLANOLONE	3.6
DIHYDROTESTOSTERONE	3.4
17-OH-PROGESTERONE	3.3
ESTRADIOL (E_2)	3.2
17-OH-PREGNENOLONE	2.4
ANDROSTERONE	2.2
ESTRONE (E_1)	1.5
ESTRIOL (E_3)	1.4
PREGNANETRIOL	1.4
EPIESTRIOL	0.9
TETRAHYDROCORTISOL (THF)	0.9
DEXAMETHASONE	0.0

7. Mean plasma corticosterone level at 9 a.m. was 7.1 ± 3.2 ng/ml in 45 normal adult subjects.

8. In Table 4, simultaneously determined plasma corticosterone, cortisol and 11-OHCS values before and after ACTH stimulation are listed. In 6 normal subjects, corticosterone increased 5.2 times in average as much as basal level after ACTH administration, whereas cortisol and 11-OHCS increased 2.4 and 2.3 times as much, respectively. Basal corticosterone/11-OHCS ratio was 3.7 % and after ACTH, it was 8.6 %, more

than 2 times as much as basal ratio. Basal corticosterone/cortisol ratio was 5.2 % and after ACTH the ratio increased 2 times as much. In four-hour 1.0 mg dexamethasone suppression test, corticosterone was suppressed to 22.6 % of basal level at 4 hours after dexamethasone, whereas cortisol was suppressed to 12.3 % of basal level (Table 4). Consequently, basal corticosterone/cortisol ratio of 6.6 % increased to 11.2 % at 4 hours after dexamethasone.

TABLE 4.

Rapid ACTH test and 1 mg dexamethasone four-hour suppression test in two different subject groups

Case	Rapid ACTH test[1]			1mg four hour test[2]		
	Before	30 min	60 min	Before	2 hours	4 hours
1	(203) 4.8 / 170	(305) 26.3 / 258	(339) 28.5 / 296	10.1 / 135	2.6 / 59	0.85 / 14
2	(212) 5.9 / 171	(440) 41.9 / 363	(550) 42.4 / 438	7.4 / 86	2.4 / 18	1.8 / 10
3	(252) 10.0 / 195	(450) 43.2 / 328	(504) 44.1 / 384	10.6 / 150	2.3 / 80	1.3 / 26
4	(140) 4.2 / 115	(321) 22.1 / 282	(371) 25.0 / 337	7.0 / 96	1.4 / 30	1.8 / 10
5	(190) 9.9 / 122	(310) 27.6 / 218	(370) 29.1 / 267	3.3 / 126	1.9 / 38	1.4 / 15
6	(150) 7.5 / 106	(359) 29.9 / 310	(441) 34.4 / 330			
B / Cortisol	5.2 %	10.8 %	9.9 %	6.6 %	6.1 %	11.2 %
B / 11-OHCS	3.7 %	8.6 %	7.9 %			

1) ; (11-OHCS) Corticosterone (B) / Cortisol, ng/ml, before, at 30 min. and 60 min. after Cortrosyn 0.25 mg injection.

2) ; Corticosterone (B) / Cortisol, ng/ml, before, at 2 hours and 4 hours after single dose 1 mg dexamethasone administration.

DISCUSSION

Fluorometric methods[3,4], competitive protein binding assays[5,6] or double isotope derivative procedures[7,8], are now used for measuring corticosterone. The fluorometric methods are simple and sensitive but not specific without eliminating interfering fluorogens which have never been positively indentified.

In the present study, corticosterone and cortisol togather occupied

79.8 % of 11-OHCS values (Table 4). This means that about 20 % of 11-OHCS values reflect the nonspecific interfering fluorogens. The double isotope derivative methods are too laborious for routine clinical use. As to competitive protein binding methods, Ruder et al.[9] pointed out radioimmunoassay's superiority to the competitive binding assay from the following reasons in their work about the radioimmunoassay of cortisol; i) the binding affinity of corticosteroid binding globulin (CBG) is only 1/100 that of cortisol antiserum, ii) the practical sensitivity of CBG-assay is limited to 1.0 ng, iii) CBG-assay has a standard curve with a small useful range, and iv) separation of bound and free has been a major problem with the CBG-assay.

The present study is presenting a radioimmunoassay of corticosterone in human plasma. So far only a few papers[10,11,12] are available about radioimmunoassay for corticosterone in human blood. The mean plasma radioimmunoassayed corticosterone level at 9 a.m. is 7.1 ± 3.2 ng/ml in 45 normal subjects, which is slightly higher than previously reported values of 3.2 ng/ml or 4.4 ng/ml[10,11].

Basal corticosterone/cortisol ratio is 5.9 % in average in the present study (Table 4), which agrees with the results previously obtained by double isotope derivative methods[7,8]. In ACTH test, basal corticosterone/cortisol ratio increased 2 times as much at 30 min. after ACTH stimulation, and this is mainly due to the larger increment of corticosterone. Also by 1.0 mg dexamethasone suppression test, basal corticosterone/cortisol ratio was found to have increased about 2 times as much at 4 hours after dexamethasone, and this is mainly due to the more effective suppression of control.

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