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RADIOIMMUNOASSAY FOR STEROID HORMONES
IV. RADIOIMMUNOASSAY FOR PLASMA PREGNENOLONE

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Abstract

A convenient and reliable radioimmunoassay for plasma pregnenolone has been developed. Anti-pregnenolone serum was obtained by immunizing rabbits with a pregnenolone-3-hemisuccinate-BSA conjugate. A useful range in the standard curve was from 10 pg to 500 pg. Pregnenolone in 0.1 ml plasma was extracted with ether and was separated from cross-reacting steroids by microcolumn chromatography. Plasma concentrations of pregnenolone at 9 a.m. were: men, 0.91±0.17 ng/ml; women, 1.06±0.37 ng/ml.

INTRODUCTION

Pregnenolone is the first steroid formed from cholesterol in the biosynthesis of the steroid hormones of the adrenal cortex and gonads. Recently, it has been measured in human peripheral blood by protein binding assay[1] or radioimmunoassay[2,3].

To continue our study of steroid biosynthesis, we have developed a radioimmunoassay for the measurement of pregnenolone in human plasma, using an antiserum generated against a pregnenolone-3-hemisuccinate BSA conjugate.

MATERIALS AND METHODS

1. The solvents, reagents, assay buffer, and equipment were the same as previously described for the radioimmunoassay of plasma DHEA[4].
2. Steroids. Pregnenolone-7-^{3}H, 20 Ci/mM (New England Nuclear Corp.) was used after purification with thin layer chromatography. Non-radioactive pregnenolone was obtained from Sigma. A series of steroid hormones used for cross-reactivity study of assay were kindly donated by Teikoku Zoki Pharmaceutical Co., Japan.

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3. Preparation of antiserum. The production of pregnenolone-3-hemisuccinate BSA and immunization of rabbits with the antigen-BSA conjugate was done according to the methods previously reported[5].

4. Plasma extraction and chromatography. Pregnenolone in 0.2 ml plasma sample and 2,000 dpm of ^{3}H-pregnenolone, as internal standard, were extracted together with 1 ml of ether and the extract was transferred to a microcolumn for chromatography as previously described for corticosterone[6]. Benzene : methanol 85 : 15 (v/v) was used for elution and the fractions containing pregnenolone were collected from 1.4 to 2.2 ml.

5. Radioimmunoassay and radiocounting. The entire assay after chromatographic purification and counting of the radioactivity were performed as in the previous report about corticosterone[6]. The antiserum was diluted to 1 : 45,000. Three water blank samples were run with each assay. If pregnenolone levels in the blanks were above sensitivity, the mean of the three blanks was subtracted from the levels of pregnenolone measured in the unknown samples prior to correction for recovery.

RESULTS

1. Following ether extraction, and microcolum chromatography, the recovery of ^{3}H-pregnenolone was 69.25±5.53 % in 50 assays.

2. A typical dose response line and sensitivity. The bound per cent was plotted against the logarithm of the dose of pregnenolone. A typical standard curve is shown in the Figure. Bound per cent at 20 pg was significantly different from that of 0 pg. The sensitivity of the standard curve was 20 pg of pregnenolone.

3. Dilution test. The dilution test of the assay is shown in the Figure. Original plasma, of which concentration of pregnenolone was 1.77 ng/ml, was diluted to 1 : 2, 1 : 4 and 1 : 8, and these diluted plasmas showed the identical 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. The average coefficient of variation for within assay and between assay were 4.6 % and 3.7 %, respectively.

5. The accuracy of recovery. The mean recovery of added pregnenolone from 0.2 ml plasma were 111.8 % and 104.2 %, respectively for two different plasma samples (Table 2).

6. Specificity. The specificity of antiserum anti-pregnenolone-1 was tested by cross reaction studies with various steroids (Table 3). The

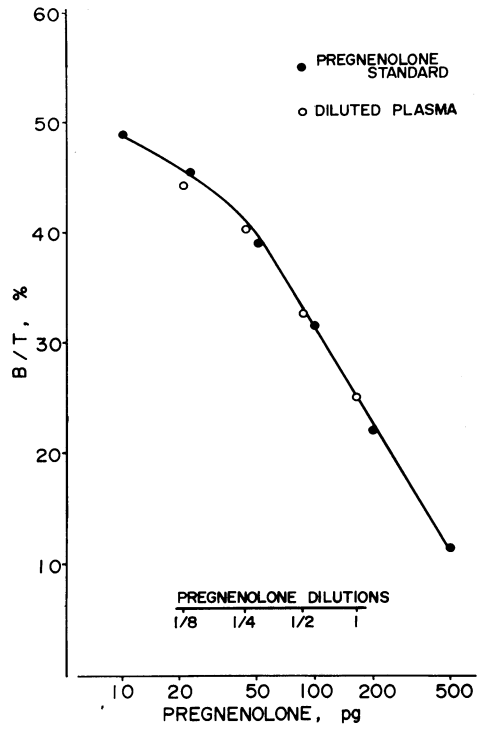

Fig. 1. A typical dose response line and dilution test on semilogarithmic scale. Dose response line; each closed circle represents the mean from duplicate determinations. The final dilution of anti-pregnenolone is 1:45,000. Dilution test; original plasma was diluted to 1:2, 1:4 and 1:8, Each open circle represents the mean from four determinations.

TABLE 1

Precision and Reproducibility of Plasma Pregnenolone Radioimmunoassay

Within assay (n=6)			Between assay (3 different occasions)		
Plasma	Pregnenolone, ng/ml (Average)	CV, %	Plasma	Pregnenolone, ng/ml (Average)	CV, %
A	0.56	6.6	D	0.98	3.4
B	1.03	4.1	E	1.38	4.0
C	1.70	3.1	F	2.33	3.8
		4.6 (Average)			3.7 (Average)

TABLE 2

Recovery of Added Pregnenolone from Plasma

	Pregnenolone added (pg)	Pregnenolone determined (pg)	Recovery (%)		Pregnenolone added (pg)	Pregnenolone determined (pg)	Recovery (%)
Plasma I	0	81		Plasma II	0	148	
	20	114	112.9		20	182	108.3
	50	146	111.5		50	202	102.0
	100	214	118.2		100	219	88.3
	200	302	107.5		200	393	112.9
	500	633	109.0		500	709	109.4
			111.8%				104.2%

Pregnenolone determined; means from four determinations in 0.2 ml plasma.

TABLE 3

The Cross-reactivity of Anti-Pregnenolone-1 (1:45,000)

Steroids	Cross-reactivities, %
PROGESTERONE	25.2
5β-PREGNANEDIOL	11.4
5α-PREGNANEDIONE	8.4
CORTISOL (F)	4.4
17-OH-PREGNENOLONE	4.1
DOC	2.9
ESTRADIOL (E_2)	2.7
17-OH-PROGESTERONE	2.6
ANDROSTENEDIONE	1.3
DIHYDROTESTOSTERONE	1.3
EPIESTRIOL	1.2
TETRAHYDROCORTISOL (THF)	1.1
ANDROSTERONE	1.0
DEHYDROCORTICOSTERONE (A)	<1.0
PREGNANETRIOL	〃
ETIOCHOLANOLONE	〃
11-DESOXYCORTISOL (S)	〃
CORTISONE (E)	〃
CORTICOSTERONE (B)	〃
DEHYDROEPIANDROSTERONE (DHEA)	〃
TESTOSTERONE	〃
ESTRONE (E_1)	〃
ESTRIOL (E_3)	〃
ALDOSTERONE	〃
DEXAMETHASONE	<0.1

steroids known to be present in near the pregnenolone fraction on the chromatography had minor or no detectable cross reaction.

7. Mean plasma pregnenolone levels at 9 a. m. were: 0.91±0.17 ng/ml for 22 normal adult males; 1.06±0.37 ng/ml for 10 normal adult females; 0.96±0.26 ng/ml for both of 32 normal subjects. These mean values were not significantly different from each other.

8. As shown in Table 4, in rapid ACTH test in normal subjects, pregnenolone increased about 3 times in average as much as basal level after ACTH. In 9 a. m. suppression test, basal pregnenolone decreased to 0.64 ng/ml after 1 mg and 0.56 ng/ml after 2 mg dexamethasone and those levels were equivalent to 60.9 % and 58.0 % of basal level, respectively.

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	3	n	60 min	120 min
1	1.09	3.84	5.50	0.89	0.90		0.85	**0.68***
2	0.48	0.80	1.49	1.22	**0.67***		0.80	1.18
3	1.59	1.18	2.94	1.09	**0.56***		0.80	0.72
4	1.27	2.72	3.65	0.82	**0.57****		0.60	0.58
5	1.01	3.01	3.37	0.94	**0.49****		0.62	0.61
6	2.01	3.46	3.06	1.20	0.89		**0.63****	0.77
	1.24 ±0.52	2.50 ±1.23	3.34 ±1.29					

Plasma samples for determination of pregnenolone (ng/ml); before, at 30 min and at 60 min after i. m. injection of 0.25 mg Crotrosyn in ACTH test and before, at 30 min, 60 min and 120 min after p. o. administration of 1 mg (*) or 2 mg (**) dexamethasone in suppression test. The numbers in thick letter mean the lowest levels of plasma pregnenolone after dexamethasone.

DISCUSSION

The present study describes a simple and reliable radiommunoassay of plasma pregnenolone. The plasma levels of pregnenolone obtained by the present method compared favorably with levels obtained by other published methods for pregnenolone[1-3,7-9]. Normal 9 a. m. plasma concentrations in ng/ml obtained with this assay were 0.91±0.17 for adult males and 1.06±0.37 for premenopausal female subjects. These mean values were not significantly different. Di Pietro *et al.*[2] also found no difference of plasma pregnenolone between men and women during folli-

cular phase or luteal phase. Bermudez *et al.*[1] reported no difference of pregnenolone level between men and women during follicular phase.

There are conflicting reports regarding the changes in plasma pregnenolone concentrations during the menstrual cycle. Bermudez *et al.*[1] and Abraham *et al.*[8] reported a significant rise of plasma pregnenolone in the luteal phase, whereas, Di Pietro *et al.*[2] and McKenna *et al.*[9] found no difference between the two phases.

In rapid ACTH test, plasma pregnenolone increased 3 times as much as basal level after ACTH. The increment was greater than that of DHEA[4] or cortisol[10] and less than that of corticosterone[10].

Dexamethasone suppressibility of plasma pregnenolone was reduced compared with that of cortisol which was suppressed to the limit of sensitivity of an assay at 4 hours after dexamethasone[11]. This may be explained by the contribution to pregnenolone by hydrolysis of pregnenolone sulfate as it is explained by the contribution to DHEA by hydrolysis of DHEA sulfate[12].

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REFERENCES

1) Bermudez, J. A., Doerr, P. and Lipsett, M. B.: Measurement of pregnenolone in blood. Steroids 16: 505-515, 1970
2) DiPietro, D. L., Brown, R. D. and Strott, C. A.: A pregnenolone radioimmunoassay utilizing a new fractionation technique for sheep antiserum. J. Clin. Endocrinol. Metab. 35: 729-735, 1972
3) Abraham, G. E., Buster, J. E.. Kyle, F. W., Corrales, P. C. and Teller, R. C.: Radioimmunossay of plasma pregnenolone. J. Clin. Endocrinol. Metab. 37: 40-45, 1973
4) Nishida, S., Matsumura, S., Horino, M., Oyama, H. and Tenku, A.: Radioimmunoassay for steroid hormones. III. Radioimmunoassay for plasma dehydroepiandrosterone. Kawasaki Med. J. 3: 179-184, 1977
5) Nishida, S., Matsumura, S., Horino, M., Oyama, H. and Tenku, A.: Radioimmunoassay for steroid hormones. I. Radioimmunoassay for plasma cortisol. Kawasaki Med. J. 2: 81-89, 1976
6) Nishida, S, Matsumura, S., Horino, M., Oyama, H. and Tenku, A.: Radioimmunoassay for steroid hormones. II. Radiommunoassay for plasma corticosterone. Kawasaki Med. J. 2: 135-142, 1976
7) Bermudez, J. A. and Lipsett, M. B.: Early adrenal response to ACTH: Plasma concentrations of pregnenolone, 17-hydroxypregnenolone, progesterone, and 17-hydroxyprogesterone. J. Clin. Endocrinol. Metab. 34: 241-243, 1972
8) Abraham, G. E., Buster, J. E., Kyle, F. W., Corrales, P. C. and Teller, R. C.: Radioimmunoassay of plasma pregnenolone, 17-hydroxypregnenolone and dehydroepi-

androsterone under various physiological conditions. J. Clin. Endocrinol. Metab. 37: 140-144, 1973

9) McKenna, T. J. and Brown, R. D.: Pregnenolone in man: Plasma levels in states of normal and abnormal steroidogenesis. J. Clin. Endocrinol Metab. 38: 480-485, 1974

10) Nishida, S., Matsumura, S., Horino, M., Oyama, H. and Tenku, A.: The variations of plasma corticosterone/cortisol ratios in normal men following ACTH stimulation or dexamethasone administration. J. Clin. Endocrinol. Metab. 45: 585-588, 1977

11) Nishida, S., Matsumura, S., Horino, M., Oyama, H. and Tenku, A.: Single dose four hour dexamethasone suppression test in normal men and its application for the diagnosis of Cushing's syndrome. Steroids 28: 411-421, 1976

12) Rosenfeld, R. S., Hellman, L., Roffwarg, H., Weitzman, E. D., Fukushima, D. K. and Gallagher, T. F.: DHEA is secreted episodically and synchronously with cortisol by normal man. J. Clin. Endocrinol. Metab. 33: 87-92, 1971