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A Radioimmunoassay for Rat Serum Corticosterone

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ABSTRACT A reliable radioimmunoassay for rat serum corticosterone has been developed. 25 μl of diluted serum (1 : 100) was assayed with a specific antiserum raised against corticosterone-21-hemisuccinate conjugated to bovine serum albumin. The within-assay and between-assay coefficients of variation were 7.1% and 13.9%, respectively. The mean serum corticosterone concentration was 65.3 ± 6.0 ng/ml (n=10). The corticosterone level increased to 208.4 ± 23.7 ng/ml after ACTH administration, and was suppressed to the limit of assay sensitivity after dexamethasone administration.

Key words : radioimmunoassay — rat corticosterone — corticosterone

In the rat, corticosterone is the principal glucocorticoid secreted by the adrenal cortex. The determination of corticosterone in rat blood has been estimated by the fluorometric method[1] or protein binding assay[2]. Recently, a radioimmunoassay for the measurement of corticosterone, which was specific and easy to perform, was reported[3]. In the present study a specific and sensitive radioimmunoassay for rat corticosterone without the chromatographic step is described.

MATERIALS AND METHODS

Reagents. Radioactive 1,2,6,7-^{3}H-corticosterone (New England Nuclear corporation, specific activity, 105.0 Ci/m mol) was used after purification with thin-layer chromatography. All other chemicals were prepared as previously described[4-9]. The same antiserum used for human corticosterone radioimmunoassay[5] was utilized in this assay.

Standard curve. The standards were prepared in duplicate by adding 0, 10, 20, 50, 100, 200 and 500 pg of corticosterone to 0.1 ml of ethanol.

Samples. Serum samples were diluted to 1:100 in distilled water, and the corticosterone in 25 μl of the diluted sample was extracted with 1 ml ethylether.

Radioimmunoassay. ^{3}H-corticosterone (10,000 dpm) was added to tubes containing either standard or sample. After drying with N_2 gas, 250 μl of

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antiserum, diluted to 1 : 35,000 in 0.05M borate buffer (PH 8.0) containing 0.05% BSA and 0.075% bovine gamma globulin, was added to each tube. The two were mixed and incubated for 30 minutes at room temperature. Separating of bound and free hormones and radiocounting were performed as previously reported[4-9].

Animal preparations. Adult male Wistar rats (250-400g) were housed in wire-bottomed cages, and were permitted free access to food and water.

Rat blood was obtained by decapitation. The serum was separated and stored in a freezer at -20°C. The effects of synthetic ACTH and dexamethasone on serum corticosterone were examined. Synthetic $_{1-24}$ACTH, 10 μg/g body weight, or dexamethasone, 40 μg/g body were injected peritoneally, and blood samples were collected 2 hrs after drug administrations.

The results are expressed as mean±SEM.

RESULTS

Standard line and dilution test. A typical dose-response curve and a result of a dilution test are shown in Fig. 1. Practical sensitivity of the assay in the curve was 10 pg of corticosterone. Original serum, of which the concentration of corticosterone was 960 ng/ml, was diluted to 1:2, 1:4, 1:8 and 1:16, and these diluted sera showed a curve parallel to the authentic standard curve.

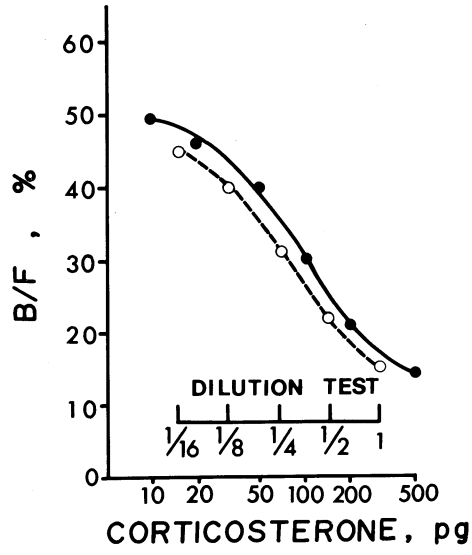

Fig. 1. A typical dose response curve (●—●) and dilution test (○—○) ; original serum was diluted to 1:2, 1:4, 1:8 1:16.

Precision. The intra-assay coefficient of variation for a pooled serum containing 49.9 ng/ml of corticosterone was 3.3% (n=8) and for a pooled

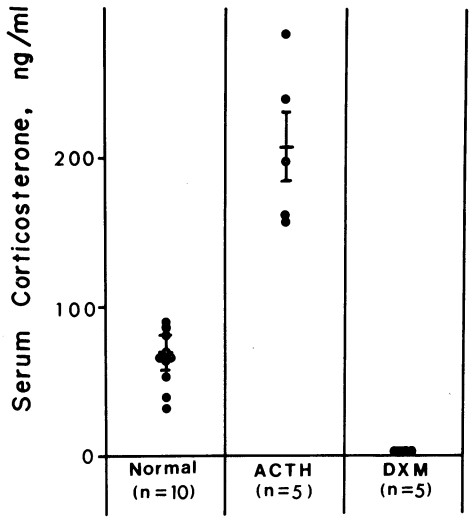

Fig. 2. Serum corticosterone levels of normal rats, and in the ACTH test and dexamethasone suppression test. ACTH : ACTH test, DXM; dexamethasone suppression test. Blood samples were obtained 2 hrs after the administration of drugs.

serum containing 195.5 ng/ml of the hormone, it was 10.8% (n=8). The inter-assay variability for the former serum was 13.9% (3 different occasions).

The specificity of antiserum. The cross-reactivity of the anti-corticosterone serum with various steroids has been previously reported[5].

Accuracy. This was examined by adding of 20, 50, 100 and 200 ng corticosterone to serum already containing 36 ng/ml. The mean recovery of added corticosterone was 92.6% (Table 1.).

TABLE 1. The accuracy of recovery of added corticosterone.

Corticosterone added ng	Corticosterone determined ng/ml	Recovery %
0	36	
20	60	93.3
50	98	88.8
100	146	93.2
200	248	95.2
	average	92.6%

Normal corticosterone level, ACTH test and dexamethasone test. The normal level of corticosterone in rats is 65.3±6.0 ng/ml (n=10). The concentration of corticosterone 2 hrs after ACTH stimulation was 208.4±23.7 ng/ml, and

the level 2 hrs after dexamethasone administration was below the assay limit.

DISCUSSION

The concentration of corticosterone in rat blood is 65.3±6.0 ng/ml, which is markedly higher than that in human plasma (7.1±3.2 ng/ml)[5]. Corticosterone is the principal glucocorticoid secreted by rats, and rat serum contains an approximate 10 : 1 ratio of corticosterone to cortisol. Therefore, although a prior chromatographic separation has been required for assaying human plasma corticosterone, this step is not required in the rat. The chromatographic step tends to introduce loss of the steroid. An emphasis is laid on the elimination of chromatography in this assay.

In the ACTH test, serum corticosterone increased to 3 times the basal level after ACTH injection, and it was suppressed by dexamethasone administration. These findings suggest that this assay system is useful for the evaluation of rat corticosterone secretion.

Acknowledgment

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