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Research Article

PLASMA ADRENOCORTICOTROPHIC HORMONE IN ADDISON'S DISEASE AND ITS MODIFICATION BY THE ADMINISTRATION OF ADRENAL STEROIDS

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The depressant effect of adrenal steroids on the release of pituitary adrenocorticotrophic hormone (ACTH) was first demonstrated by Ingle in the experimental animal in 1937 (1). This observation has been confirmed by many investigators (2–7), but not by all (8). In man the clinical, morphological and biochemical evidence for steroidal inhibition of pituitary secretion is impressive but there are few actual measurements of blood ACTH reported. There is substantial evidence for an increased level of circulating ACTH in Addison's disease (9, 10) and postoperative bilaterally adrenalectomized patients (11), but an adequate quantitative evaluation of this activity has not been available. Evidence for an increased secretion of ACTH in the adreno-genital syndrome (12) is also convincing, but similar increases reported in rheumatic fever (13), Cushing's disease (14), pregnancy (14), the early post-partum period (15), and other conditions (16) have not been confirmed.

The present study was undertaken to determine the level of ACTH in peripheral blood in Addison's disease and to observe the effects of adrenal steroids on the alteration of this activity.

METHODS

The assay procedure followed in these studies has been previously described by Nelson and Hume (17) and utilizes the production of corticosteroids by the adrenal cortex of the hypophysectomized dog as a measure of ACTH activity in administered human plasma. It was performed as follows: 40 to 60 milliliters of blood were withdrawn from the patient into a heparinized syringe. The plasma was immediately separated by centrifugation and frozen. Within one to six days the plasma was injected into the assay animal. The assay was performed in a hypophysectomized dog in which a catheter had been placed in the right adrenal vein in such a way as to make intermittent, complete collections of the adrenal venous effluent possible. Physiological saline solution containing 0.01 N hydrochloric acid and 1 mU of U.S.P. Standard ACTH per ml. was used as a reference against which the plasma activity was measured. One, 2, 5 and 8 ml. of this standard, and 20 to 30 ml. of plasma were injected into the adrenal by the femoral vein during a two minute period. (The difference in volumes injected did not appear to affect the result significantly. Although use of whole plasma allows possibly toxic substances to be injected with the ACTH, acid-acetone, and oxycellulose purified extracts of duplicate samples of plasma always showed less ACTH activity than was present in the whole plasma.) One minute later the adrenal venous collection was begun and continued for exactly 10 minutes. A 5 ml. aliquot was removed and the remaining blood was returned to the venous circulation of the dog. The interval between collections was 30 minutes. In the performance of a typical assay, two control samples of 0.01 N HCl in saline and five unknown plasma samples were alternated with nine ACTH standard injections. Studies in this laboratory (18) and by Granirer (15) have shown that ACTH will retain its potency in frozen plasma for at least three or four weeks. The content of 17-hydroxycorticosteroids in the aliquot of whole blood removed from the adrenal vein of the dog was determined by a slight modification of the method of Nelson and Samuels (19).

Each determination required the withdrawal of at least 40 ml. of blood from the patient and it was therefore usually impossible to perform the determinations for ACTH in duplicate. Values of ACTH in this paper are expressed in mU per 100 ml. of plasma. The ACTH concentration of the plasma sample and its standard error were estimated in logarithmic terms by the use of the usual formulae from a comparison of the effect of an injected plasma sample on the release of 17-hydroxycorticosteroids with the response given by graded doses of U.S.P. Standard ACTH (20). By conversion to antilogarithms, the desired values are obtained in mU. Since the addition of logarithms implies multiplication of antilogarithms, the plus-minus symbols (±) usually used for standard errors are changed to the multiplication-division symbols $\left(\frac{\times}{\div}\right)$ for the mU (antilogarithms), as shown in

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the following example:

	Estimate of potency	Standard error
In logs	0.903	±0.114
In antilogs	8 mU	×1.30 ÷

In this example, the standard error may also be cited as 30 per cent of the potency estimate: (1.30 − 1.00) 100 equals 30 per cent. Squaring the antilogarithm of the standard error prior to multiplication and division will give the approximate 95 per cent confidence limits of each value. Differences between groups of values were evaluated by Student's "t" test; the two tail test being applied to the differences between groups of values in different patients and the one tail test for differences in the same patient (21). The lower limit of sensitivity of the assay varies somewhat depending on the individual responsiveness of the adrenal gland of the assay animal. In general, a significant increase in 17-hydroxycorticosteroid content of the adrenal venous blood was seen with 0.75 to 1.5 mU of ACTH. Therefore, depending on the amount of plasma administered, the minimal detectable level expressed per 100 ml. of plasma is about 2 to 5 mU per 100 ml. and values shown in the tables as "zero" could thus be somewhat elevated over normal if they should fall below this lower limit of sensitivity. Such values are more correctly expressed as "nondetectable" than as zero or negative values, but for convenience the zero symbol is retained in the tables. It was impossible to calculate satisfactorily an exact upper limit of these zero values.

TABLE I

Plasma ACTH in normal subjects, hospitalized patients with normal adrenal function, and patients with Addison's disease

Patient	ACTH *mU/100 ml.*	× and ÷ by antilog. S.E.M.	Patient	ACTH *mU/100 ml.*	× and ÷ by antilog. S.E.M.
		Normal subjects			
J. B.	0	*	D. M.	0	
J. F.	0		N. McW.	0	
D. N.	0		E. R.	0	
D. S.	0		R. F.	0	
		Hospitalized patients			
R. A.	0		H. B.	0	
M. S.	0		A. P.	0	
M. U.	0		J. G.	0	
E. U.	0		R. I.	0	
		Addisonian patients			
N. P.	7.5	2.44	J. G.	18.1	2.88
E. V.	2.9	1.29	A. R.	6.5	1.84
G. R.	0	*	D. McG.	10.2	1.44
D. R.	0		D. McG.	7.2	1.99
D. R.	10.2	1.43	D. McG.	6.0	1.66
W. L.	9.9	1.52	A. K.†	6.9	2.04
W. L.	14.1	1.46	A. K.	8.1	1.82
W. L.	30.0	1.39	A. K.	5.7	1.87
M. K.	12.4	1.87	W. D.	0	
M. K.	8.6	1.58	W. D.	3.5	1.07
M. K.	20.9	1.57	K. H.	0	
J. H.†	0		L. W.	12.5	1.55
J. H.	0		L. W.	24.8	1.99
J. H.	0		L. W.	26.9	2.02
J. H.	0		C. M.	16.6	2.31
J. C.	0		C. G.	7.2	1.56
J. C.	0		W. G.	18.0	1.74
L. H.	2.6	1.98	W. G.	19.1	1.63
L. H.	0		W. G.	17.1	1.41
E. C.	4.0	1.85	P. T.†	11.3	1.14
M. C.	16.7	2.35	J. S.	3.7	2.20
H. W.	0		G. S.	5.0	1.22
H. McC.	5.3	1.69	E. M.	6.1	1.38
H. McC.	6.7	1.34	J. O'S.	0	
H. McC.	3.3	1.37	M. L.	7.9	1.65
R. S.	24.7	1.58	L. L.	10.7	1.54
S. B.	6.8	2.02	L. L.	6.5	1.56

* Limits cannot be calculated for these values.
† Adrenal insufficiency due to bilateral adrenalectomy.

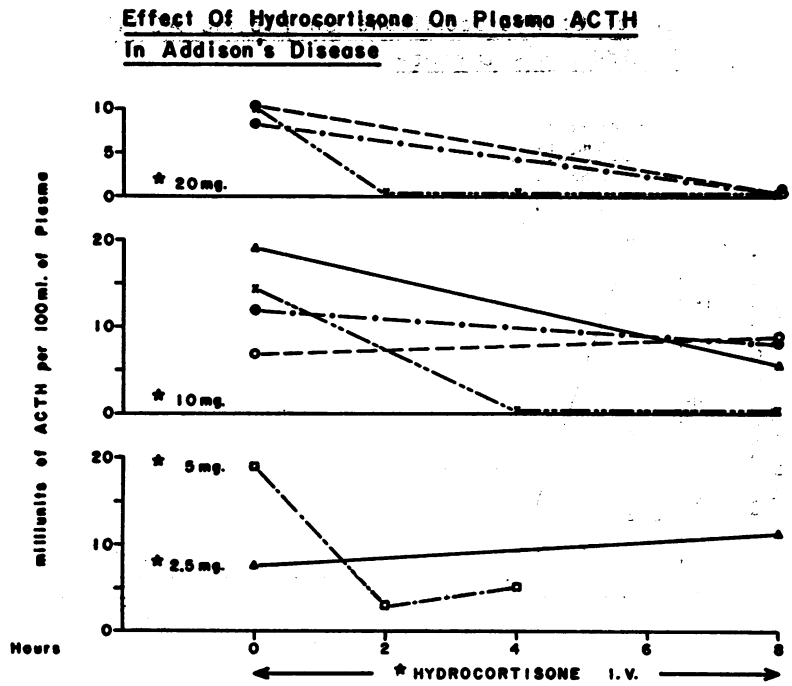

FIG. 1. INFUSION OF HYDROCORTISONE FOR EIGHT HOURS ON EIGHT OCCASIONS TO FOUR ADDISONIAN PATIENTS

The 5 mg. infusion was for four hours in a fifth patient. A distinctive line and symbol is shown for each patient, the symbol indicating the plasma level of ACTH expressed in mU per 100 ml. of plasma. Values at the zero line indicate activity was not detectable. Comparison of initial and final values by one tail "t" test gives a p of < 0.0025 for 20 mg. dose and < 0.20 for 10.0 mg. dose.

RESULTS

Table 1 shows the results of ACTH determinations performed on plasma samples from 29 patients with Addison's disease, three bilaterally adrenalectomized patients, eight hospitalized patients without adrenal dysfunction, and eight normal subjects. All samples were withdrawn in the fasting state between 7:30 and 9:00 A.M. The patients with adrenal insufficiency received no cortisone for at least 24 hours prior to the venous collection and most were on monthly injections of 25 to 50 mg. of desoxycorticosterone trimethylacetate. A few patients were given added salt during the period of study.

In agreement with most investigators, although in disagreement with some, no measurable level of ACTH was found in the normal subjects as well as in the hospitalized patients with no adrenal disease (12, 22, 23). In contrast to this group, only 2 of 28 patients with adrenal insufficiency failed to have ACTH activity if more than one plasma sample were assayed. The ACTH value for the 32 patients with adrenal insufficiency was found to be 8.4 ± 7.4 (S.D.) mU per 100 ml. This value was significantly different from the group of control subjects ($p < 0.001$). There were nine patients with Addison's disease in whom ACTH was not initially detectable. In three of these, repeat values showed detectable levels of activity, in four no repeat determinations could be obtained, and in two repetition gave a negative result on two and four occasions, respectively. One patient who had partial adrenal insufficiency had ACTH activity present on the three occasions on which it was measured.

Figures 1, 2, and 3 illustrate the ACTH values obtained in 10 patients with Addison's disease given 16 infusions of 2.5 to 20 mg. of free hydrocortisone in normal saline for periods varying from two minutes to eight hours. Samples of plasma for ACTH assay were obtained at intervals prior to, during, and up to 12 hours following the

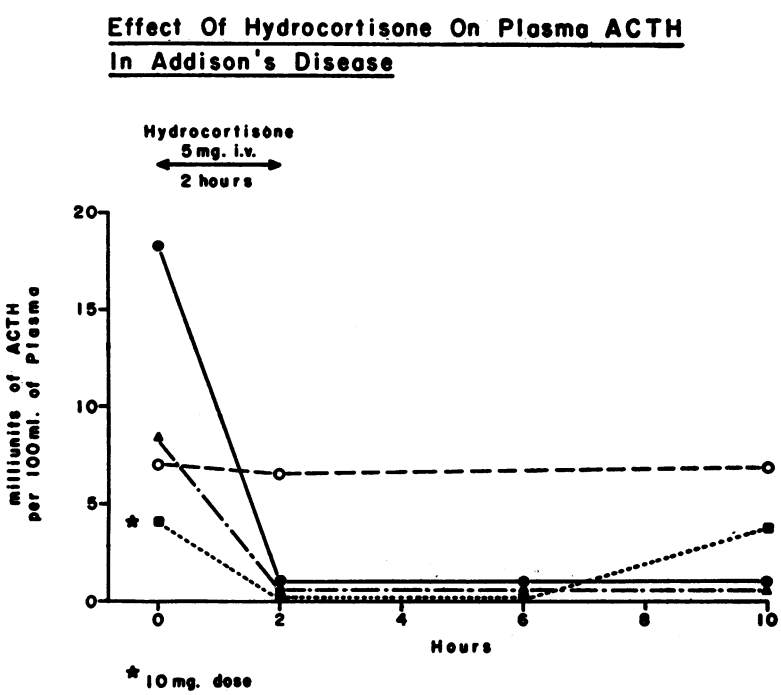

FIG. 2. INFUSION OF 5 MG. OF HYDROCORTISONE OVER A TWO HOUR PERIOD TO THREE PATIENTS AND 10 MG. TO A FOURTH PATIENT

Plasma was assayed up to eight hours following the end of the infusion. P for significance of difference between initial and lowest values is < 0.10.

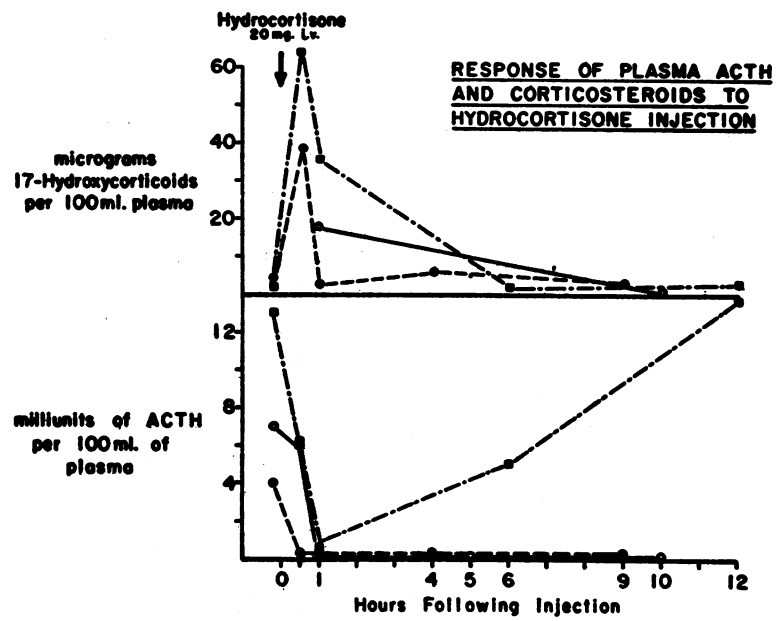

FIG. 3. TWENTY MG. OF HYDROCORTISONE GIVEN INTRAVENOUSLY TO THREE ADDISONIANS IN A TWO MINUTE PERIOD

Blood corticosteroids and ACTH activity were measured concurrently up to 12 hours following this infusion. P for significance of difference between initial and final values at one hour is < 0.10.

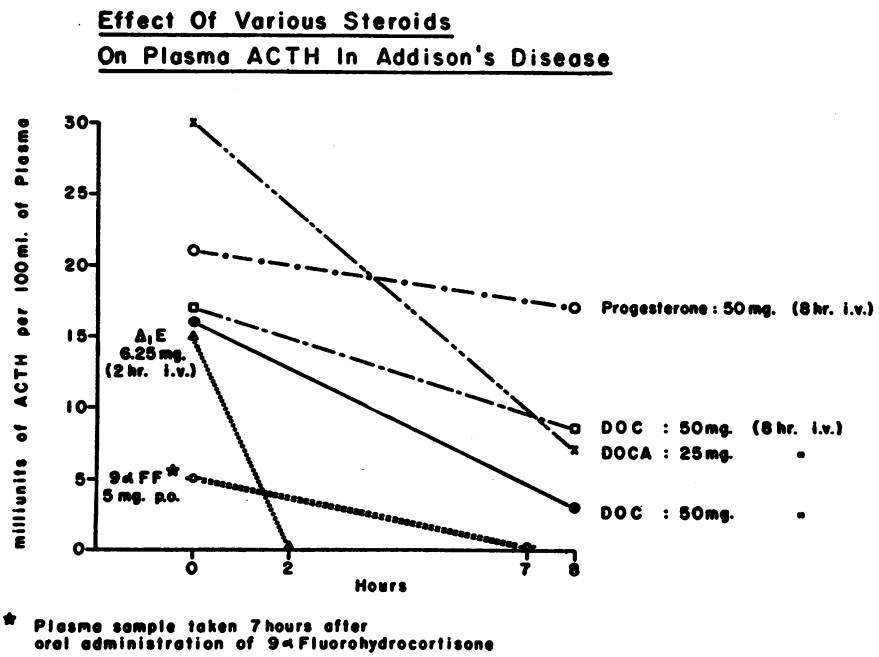

FIG. 4. INFUSION OF PROGESTERONE, FREE DESOXYCORTICOSTERONE (DOC), AND DESOXYCORTICOSTERONE ACETATE (DOCA) IN FOUR ADDISONIANS

Prednisone (Δ 1 E) was given intravenously in two hours and 9-α fluorohydrocortisone (9α FF) was given by mouth. P for significance of difference between initial and final values for DOC and DOCA is < 0.05.

beginning of the infusion. Plasma 17-hydroxycorticosteroids were determined at the same time as ACTH in three of these studies as shown in Figure 3. The infusions were maintained at a constant rate and, therefore, samples taken at intermediate points represent the effect of the fraction of the total steroid dose administered at that particular time. For example, one-fourth of the 20 mg. dose shown in Figure 1 had suppressed ACTH activity and represents the effect of 5 mg. given for two hours at the same infusion rate. Analysis of the ACTH values obtained prior to and at the lowest point during or following the infusion of hydrocortisone in the studies at 5, 10, and 20 mg. dose levels reveals that a significant fall in ACTH concentration had occurred ($p < 0.05 > 0.025$). Further analysis of the smaller groups separated by dosage and duration of infusion is given in the legend of each table. The groups in these smaller dose ranges were too small to lend significance to observed changes; however, it would appear that the smaller doses were less effective in suppressing ACTH than was the 20 mg. dose. The effect of intravenous desoxycorticosterone, prednisone, and oral 9-α fluorohydrocortisone on the suppression of plasma ACTH are shown in Figure 4.

DISCUSSION

Although attempts have been made to measure the levels of ACTH found in the plasma of patients with Addison's disease, the majority of reports deal only with the qualitative presence or absence of activity. Sydnor, Sayers, Brown, and Tyler have reported values of 2 to 4 mU ACTH per 100 ml. of whole blood in two Addisonian patients (24). Parrott has reported a similar patient with a level of 104 mU ACTH per 100 ml. of plasma while on treatment with 25 mg. of cortison (10). Values for circulating ACTH reported previously by Parrott for normal subjects, and particularly those found in patients with various diseases, have been criticized because they seemed unreasonably high (11). The methods used by these authors and others require the injection of at least 20 ml. of human plasma or its equivalent into the rat for a reliable estimation of ACTH potency in the administered plasma or plasma extract. The rat, the assay animal used in the ascorbic acid

depletion method, may be adversely affected if it receives more than 2 to 4 ml. of human plasma intravenously. Therefore, various procedures for the extraction or concentration of ACTH have been applied to the plasma prior to the actual assay by that method. Variations in recovery by the methods employed to extract ACTH before administration to the test animal may explain discrepancies in results found by different groups of workers (25, 11). The method used in this study allows the direct injection of 20 to 40 ml. of plasma into the assay animal and should obviate the difficulties in the extraction inherent in other procedures. Twenty-six of 28 patients with Addison's disease examined by this procedure had a measurable level of ACTH when adequate numbers of plasma samples were assayed. The mean value found for this group of 28 was 9.0 ± 7.6 mU per 100 ml. (this excludes those four patients on whom repeat determinations were not done and which if included give a value of 8.4 ± 7.4). This value is significantly different ($p < 0.001$) from the results found in the control group of 16 persons. A similar range of activity has been found in two previously reported patients (24) and in the adrenalectomized rat (26).

It has not been possible to correlate the presence or absence of ACTH activity with the degree of pigmentation or with the duration of the disease. However, the fact that all but one of the patients had been on cortisone treatment prior to the study would perhaps minimize differences which would have been otherwise apparent since it has been shown that steroid therapy decreases skin pigmentation in this disease (27). It was of interest that one of the patients with partial adrenal insufficiency had a level of ACTH comparable to the group as a whole. This patient had pigmentation typical of Addison's disease as did all the patients in this group of 32. Van Buchem and Arends have recently reported two patients with Addison's disease without pigmentation in whom they were unable to demonstrate the presence of either ACTH or melanophore stimulating hormone activity (28).

The depressant effect of adrenal steroids on plasma ACTH has been shown in the rat, the adreno-genital syndrome, and in the postoperative adrenalectomized patient. The present studies have shown that a similar reduction in plasma ACTH occurs in the human with Addison's disease upon the administration of hydrocortisone. The degree of reduction cannot be directly related to the dose of administered steroid, but it would seem that 5 mg. of hydrocortisone given intravenously during two hours is the smallest dose which will usually produce a fall in plasma ACTH. Infusion of hydrocortisone at the same rate (2.5 mg. per hour) over longer periods of time (4 to 8 hours) will effect more predictable results, and a larger dose (10 to 20 mg.) given over a shorter period will also produce a more constant reduction in ACTH concentration. The suppression of ACTH activity persisted for 8 to 10 hours following the infusion of steroid in four of six patients during a time when there was almost complete disappearance of 17-hydroxycorticosteroids from the plasma. Two of the six had a return of ACTH activity at six and eight hours following the end of the hydrocortisone infusion at a time when the steroid levels were equally low. There appears to be a variability in the responsiveness of the ACTH regulating mechanism in regard to both the ease of suppression and the rapidity of reappearance of plasma activity following suppression. Factors other than the *plasma* level of 17-hydroxycorticosteroids probably are required to account for the differences noted in the behavior of plasma ACTH in these patients.

Most steroids which have a hydrocortisone-like activity suppress pituitary ACTH to varying degrees and in general this has been shown to correlate with their carbohydrate regulating activity rather than with their salt retaining effects (4). The relative ACTH suppressing activity of progesterone and desoxycorticosterone found in this study in man are in approximate agreement with those observed in the rat (4). The relatively potent synthetic steroids 9-α fluorohydrocortisone and prednisolone also appeared to be inhibitors of ACTH secretion in man.

SUMMARY

A bioassay utilizing the adrenal venous blood content of 17-hydroxycorticosteroids in the hypophysectomized dog as a measure of ACTH activity has been applied to a study of the ACTH content of human plasma. Fifty-one determinations in 32 patients with adrenal insufficiency revealed a mean activity of 8.4 ± 7.4 mU ACTH per 100 ml. of plasma. This was significantly ele-

vated over that found for 16 control plasma samples obtained from eight normal subjects and from eight hospitalized patients without endocrine disease in whom no demonstrable activity was found. The elevated level of ACTH found in a group of 10 of the Addisonian patients was significantly reduced by the intravenous infusion of hydrocortisone. The extent of ACTH suppression was dependent upon the amount of steroid administered, although individual exceptions were noted. Delta-1-hydrocortisone and 9-α fluorohydrocortisone also appeared to be potent inhibitors of human ACTH secretion, whereas desoxycorticosterone had little and progesterone no such effect in one subject.

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