Immunoreactive Corticotrophin Levels in Adrenocortical Insufficiency

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Summary

Plasma concentrations of immunoreactive corticotrophin (ACTH) have been determined in 14 patients with untreated Addison's disease and in 42 patients with secondary adrenocortical insufficiency. Basal morning plasma ACTH levels were markedly raised in those with Addison's disease but were either in the normal range or undetectable in the group with secondary adrenocortical insufficiency. In the group with Addison's disease circulating ACTH values showed a definite nyctohemeral rhythm, a pronounced rise in response to insulin-induced hypoglycaemia, and an immediate fall following the intravenous injection of corticosteroids, with a half-life of between 13·5 and 44·2 minutes. When assays were performed with antisera directed against the portion of the ACTH molecule responsible for corticosteroidogenesis (the N-terminal portion) the apparent ACTH concentrations were lower than with antisera directed against the non-steroidogenic (C-terminal) portion of the molecule. This emphasizes that different antisera may give different apparent hormone concentrations, and that the ranges of values obtained in normal and abnormal states must be established for each antiserum.

Introduction

Primary adrenocortical insufficiency may result from a variety of disease processes involving the adrenal cortex, such as an autoimmune reaction or tuberculosis (Irvine *et al.*, 1967). It may also be due to a genetic enzyme defect, as in congenital adrenal hyperplasia, or follow the administration of drugs like metyrapone, which selectively inhibit adrenal enzymes necessary for cortisol synthesis. In addition, adrenocortical insufficiency may be secondary to pituitary or hypothalamic dysfunction, and the most common cause is the therapeutic administration of pharmacological amounts of corticosteroids. Both primary and secondary adrenocortical insufficiency are characterized by low corticosteroid concentrations in plasma and urine. As determined by bioassay, plasma corticotrophin (ACTH) levels are raised in the primary type (Addison's disease) whereas they are low or undetectable in secondary adrenocortical insufficiency (Liddle *et al.*, 1962; Vance *et al.*, 1962).

This paper reports the application of a radioimmunoassay for ACTH to studies on basal plasma levels in patients with primary and secondary adrenocortical insufficiency and to investigations into the integrity of the principal mechanisms controlling ACTH secretion in patients with Addison's disease (the nyctohemeral rhythm, the stress control mechanisms, and the negative feedback control). In addition, the hormone levels in the Addisonian patients have been measured by using two antisera with combining sites directed towards different parts of the ACTH molecule to establish whether both immunoassays give the same apparent hormone concentrations, since there is evidence that dissociation between biological and radioimmunological plasma ACTH concentrations may occur (Besser *et al.*, 1970).

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Patients and Methods

Of 56 patients studied 14 had untreated Addison's disease, 27 had hypothalamic and/or pituitary dysfunction (craniopharyngiomas or pituitary tumours), and 15 had received prednisolone (7·5-20 mg/day) for more than a year. In all cases the clinical diagnosis of adrenocortical insufficiency was confirmed by routine laboratory tests involving the use of exogenous ACTH, metyrapone, and insulin-induced hypoglycaemia (Landon *et al.*, 1966; Nieman *et al.*, 1967). The plasma and urine corticosteroid responses to maximum stimulation with ACTH over three days were used to differentiate between primary and secondary adrenocortical insufficiency. A further 50 normal adults were studied as control subjects.

Plasma levels of immunoreactive ACTH were measured by radioimmunoassay after prior extraction with Fuller's earth (Landon and Greenwood, 1968) or porous glass (Ratcliffe and Edwards, 1971). All concentrations quoted have been corrected for losses during the extraction. The coefficient of variation within an assay was 14% and between assays 16%. Human ACTH was used for iodination and as reference standard, either synthetic α_h^{1-39}* (Gideon Richter, Budapest) or natural (Lerner-Upton, Fraction 8B). Two rabbit antihuman ACTH sera were used for radioimmunoassay. One reacted with an antigenic determinant in the N-terminal part of the molecule; it is this portion (the N-terminal 24 amino-acids out of the 39) which is responsible for the corticosteroidogenic effects of ACTH. The other reacted with an antigenic determinant in the C-terminal part of the molecule (amino-acids 25-29) which has no steroidogenic activity. The C-terminal antibody reacted less than 1% with tetracosactrin (Synacthen, α^{1-24} ACTH) compared with the full molecule. In contrast, the N-terminal antibody reacted equally well with both the α^{1-24} and α_h^{1-39} ACTH sequences. Subsequently we shall refer in this paper to ACTH measured by the C-terminally directed antiserum as "C-terminal ACTH" and that measured by the N-terminally directed antiserum as "N-terminal ACTH." Twenty-eight plasmas were assayed with both antisera while the remaining samples were assayed with one or other antiserum.

The recovery of synthetic α^{1-24}, α_p^{17-39}, and α_p^{25-39} ACTH (Ciba Laboratories), added to dexamethasone-suppressed human plasma, was compared with that obtained with natural α_h^{1-39} ACTH by the porous glass method to establish whether dissociation in immunoreactive ACTH levels measured by different antisera could be due to preferential extraction of C-terminal fragments.

Plasma corticosteroid levels were determined by a fluorometric method (Mattingly, 1962). Data from different

* The nomenclature proposed by Li (1959) is used throughout this paper.

groups were compared, using Student's *t*-test (two-tailed), and half-times for disappearance of plasma ACTH were calculated from regression lines fitted by the method of least squares (Snedecor, 1956).

Results

Control Data.—The mean percentage recoveries from plasma of the synthetic ACTH fragments relative to natural α_h^{1-39} ACTH were: α^{1-24}, 86; α_p^{17-39}, 72; α_p^{25-39}, 17%. Thus the recovery of the N-terminal fragment was similar to that of the whole molecule, whereas the C-terminal peptide was poorly extracted. In samples obtained between 8 and 10 a.m. from 50 normal subjects plasma ACTH levels ranged from 15 to 70 pg/ml with either the N- or C-terminally directed antiserum. During insulin-induced hypoglycaemia tests performed in seven normal subjects the plasma N-terminal ACTH rose to maximum levels of between 130 and 400 pg/ml.

Addison's Disease: Basal N-terminal ACTH Levels and Nyctohemeral Rhythm.—All patients had raised N-terminal ACTH concentrations between 8 and 10 a.m. (range 320-1,200 pg/ml, mean 536 ± S.E. 51·9 pg/ml in 19 samples from 13 patients) (Fig. 1) and between 11 p.m. and midnight (range 110-500 pg/ml, mean 252 ± 42·8 pg/ml in 16 samples from six patients). In samples obtained from the same patient the night values were always lower than the morning values, by amounts which varied between 19 and 94%. An example is shown in Fig. 2. Nyctohemeral rhythmicity of ACTH secretion was thus maintained in the Addisonian patients.

Response to Hypoglycaemia.—Soluble insulin (0·1 unit/kg body weight) was given intravenously to two patients with Addison's disease. The blood sugar fell to less than 40 mg/100 ml and sweating occurred in both cases. The plasma ACTH rose from 400 to 960 pg/ml (N-terminal) in one case and from 1,330 to 1,740 pg/ml (C-terminal) in the other. Responsiveness to hypoglycaemia was thus maintained despite the high basal levels of ACTH.

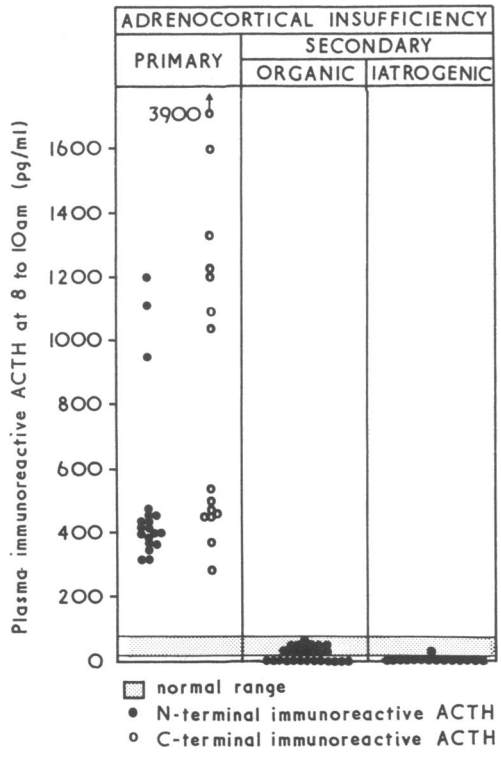

FIG. 1—Plasma immunoreactive ACTH levels between 8 and 10 a.m. in patients with adrenocortical insufficiency.

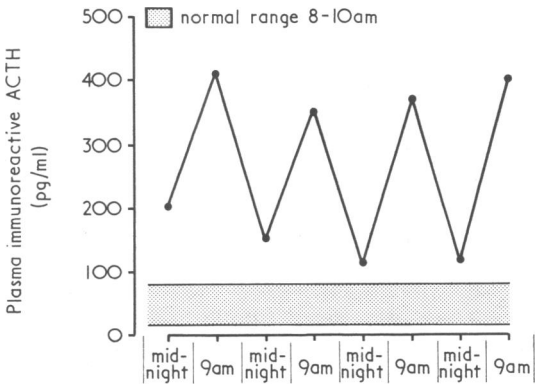

FIG. 2—Nyctohemeral rhythm of plasma N-terminal immunoreactive ACTH concentrations in a patient with untreated Addison's disease over four consecutive days.

Feedback Control.—Blood samples were obtained through indwelling forearm venous cannulae in four Addisonian patients at intervals for 60 minutes after 100 mg of cortisol or 2 mg of dexamethasone had been administered intravenously. Plasma levels had fallen in each case by the time of the first sample (either 5 or 10 minutes after the injection), showing that the feedback mechanism was intact and responded rapidly in these patients. The subsequent plasma ACTH levels fell exponentially with half-lives of between 13·5 and 44·2 minutes (see Table). In two subjects sampling was continued for a prolonged period following steroid suppression. ACTH levels became raised again 8 and 14 hours respectively after the cortisol injections.

Comparison of N-terminal and C-terminal Immunoreactivity.—Basal C-terminal levels in 15 samples from patients with Addison's disease taken between 8 and 10 a.m. ranged from 290 to 3,900 pg/ml (mean 1,001 ± 233·7) (Fig. 1) while at night the range was 61-3,150 pg/ml (mean 739 ± 312·1). These values were significantly higher than the N-terminal values shown above ($P<0.001$). Similarly, in the 28 plasmas assayed with both antisera from five patients with Addison's disease, C-terminal ACTH levels were significantly higher than the N-terminal levels ($P<0.001$). No significant difference in the rate of disappearance of plasma ACTH following intravenous corticosteroid was found with the two antisera.

Secondary Adrenocortical Insufficiency.—In 13 out of 27 patients with organic hypothalamic or pituitary disease basal plasma ACTH was undetectable (less than 15 pg/ml) between 8 and 10 a.m. but was normal in the remainder (Fig. 1). All but one patient on long-term corticosteroid therapy had undetectable levels of ACTH. The patients with normal plasma ACTH levels also had normal plasma fluorogenic corticosteroid concentrations, which failed to rise in response to hypoglycaemia.

Discussion

This investigation confirms the findings of bioassay that primary adrenocortical insufficiency is characterized by much higher basal levels of plasma ACTH than secondary adrenal insufficiency. Compared with bioassay procedures, however,

Half-time ($T\frac{1}{2}$ in Minutes) for Disappearance of Plasma Immunoreactive ACTH from the Circulation of four Addisonian Patients Given either 100 mg Cortisol or 2 mg Dexamethasone Intravenously. An N-terminal or C-terminal Anti-ACTH Antiserum was Used

	Case 1	Case 2	Case 3	Case 4
N-terminal $T\frac{1}{2}$	22·4	13·5	44·2	—
C-terminal $T\frac{1}{2}$	20·5	—	40·8	34·6

radioimmunoassay requires small volumes of plasma which facilitates dynamic studies. In patients with Addison's disease the functional integrity of the ACTH control mechanisms can be tested only by direct measurement of plasma ACTH since cortisol secretion cannot reflect circulating ACTH levels. We have found that despite the very high basal levels a significant nyctohemeral rhythm exists, the levels between 11 p.m. and midnight falling by amounts which varied between 19 and 94% of the morning ACTH concentrations. This confirms the bioassay findings of Graber *et al.* (1965).

An intact hypothalamic stress-responsive mechanism in Addison's disease was found for the first time by the pronounced increase in plasma ACTH levels which occurred in response to insulin-induced hypoglycaemia. This was studied in only two of the patients because, though no untoward side-effects were seen, the increased insulin sensitivity associated with Addison's disease made this test potentially hazardous. The very rapid fall of plasma ACTH, apparent at the time of the first sample (5-10 minutes) after intravenous corticosteroid administration, suggests that ACTH secretion ceased immediately and that the negative feedback control system was operating normally. Thus all three principal ACTH control mechanisms remain intact in primary adrenocortical insufficiency—the nyctohemeral rhythm, the response to stress, and the negative feedback.

The basal ACTH levels reported here in the Addisonian patients, using either an N- or C-terminally directed antiserum, fall within the range reported by Liddle *et al.* (1962) using bioassay. In four patients with untreated Addison's disease they found levels between 2 and 45 mU/100 ml (equivalent to 200-4,500 pg/ml in terms of the 3rd International Standard ACTH). Under certain circumstances, however, differences between biological and immunological ACTH activity have been found (Besser *et al.*, 1970). This may be due to the persistence in the circulation of biologically inactive, but immunologically reactive, fragments of ACTH following its degradation by tissue and plasma proteolytic enzymes. Though such dissociation between bioactivity and immunoreactivity may occur with an N-terminally directed antiserum, it is likely to be greater when the antibody is directed at the non-steroidogenic C-terminal portion, as suggested by our findings that C-terminal ACTH levels were higher than N-terminal levels when aliquots of the same plasma were assayed in both systems. This dissociation is not due to a preferential extraction of C-terminal fragments, since the recovery of a synthetic C-terminal peptide (α_P^{25-39} ACTH) was much lower than that of an N-terminal peptide (α^{1-24} ACTH). Indeed, the C-terminal levels may be underestimated in the presence of such fragments when the porous glass extraction technique is used.

These observations emphasize that each radioimmunoassay should be characterized by defining the combining site of the antiserum used—for example Orth *et al.* (1968) and Landon and Greenwood (1968)—and by establishing the ranges of ACTH levels in normal and abnormal states with that antiserum. When a measure of steroidogenically active ACTH is required, an N-terminally directed antiserum should be used, though unfortunately such antisera capable of measuring ACTH levels in the physiological range are more difficult to raise than comparable C-terminal antisera. Ideally, the relationship between the immunoreactive and biologically active ACTH should also be determined with a sensitive and specific bioassay.

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