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Dose Response Relationship Between Plasma ACTH and Cortisol After the Infusion of $ACTH_{1-24}$

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Summary. The authors examined the dose response relationship between plasma ACTH and cortisol concentrations after the administration of various doses of $ACTH_{1-24}$ (0.025 μg, 0.125 μg, 0.25 μg, 1 μg, 250 μg) in dexamethasone-suppressed normal volunteers. A logarithmic dose-response relationship between the dose of ACTH administered and plasma cortisol concentration was found. Although there was considerable variability in plasma ACTH concentrations, there was, however, a definite correlation between area under the curve for ACTH and area under the curve for cortisol after the various doses of ACTH.

Key words: ACTH cortisol and dose response – ACTH infusion

Introduction

It is well established that adrenocorticotropin (ACTH) stimulates the synthesis and release of glucocorticoids, such as cortisol, from the adrenal cortex. However, the relationship between plasma ACTH concentration and plasma cortisol concentration has been surprisingly little studied. Krieger et al. (1987) noted some instances of apparent dissociation between cortisol and ACTH pulses. Recently, Fehm and his collaborators (1984a) have also demonstrated that the occurrence of cortisol peaks without concomitant ACTH peaks may be more common than previously suspected. In their study (Fehm et al. 1984b), the early morning secretion of cortisol often was not preceded by a rise in plasma ACTH concentrations. We have also reported similar results (Krishnan et al. 1988a). One possible explanation for the occurrence of cortisol peaks in the absence of change in ACTH secretion could be related to the low sensitivity of the ACTH assay employed. The assay that we use in our studies is one of the most sensitive and specific assays available (Krishnan et al. 1988a). Using this assay, we recently documented, in dexamethasone-suppressed subjects, that infusions of very low doses of $ACTH_{1-24}$ result in less than 0.22 pmol/l changes in plasma ACTH. This could, however, produce significant changes in plasma cortisol concentrations (Krishnan et al. 1988b). These studies indicate the need for a critical re-evaluation of the dose response relationship between plasma ACTH and cortisol. Understanding the relationship between plasma ACTH and cortisol is essential to understand the pathophysiology of hypercortisolemia in depression. In this report, we have examined the dose-response relationship between plasma ACTH and plasma cortisol after the administration of various doses of $ACTH_{1-24}$ in normal healthy volunteers.

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Materials and Methods

Subject and Protocol

The study was approved by the Institutional Review Board at Duke University Medical Center. Six normal male volunteers were studied after obtaining informed consent. The volunteers were recruited by advertisement, and were carefully evaluated by history, physical examination and laboratory evaluation to rule out any medical or psychiatric disorders and substance abuse.

Subjects were studied in six test sessions, with an interval of 1 week between sessions. This interval (1 week) was chosen to allow the pituitary-adrenal axis to recover, and the adequacy of this interval has been documented in earlier studies (Kolanowski et al. 1975). In the first test session, the subjects were given 1 mg of dexamethasone p.o. at 11 p.m. Blood was obtained for plasma cortisol determination at 8 a.m., 4 p.m. and 10 p.m. the next day. For the remaining five test sessions, the subjects were given 4 mg dexamethasone p.o. at midnight. The next morning at 8 a.m., two intravenous catheters were placed, one in each forearm. The veins were kept open with a slow, normal saline drip. An hour later, through one vein, $ACTH_{1-24}$ was administered as a continuous infusion at a constant rate for 30 min using an infusion pump (IVAC Corporation, San Diego, CA). Five doses (one for each session were administered): 25 ng/70 kg body weight, 125 ng/70 kg body weight, 250 ng/70 kg body weight, 1 μg/70 kg body weight, and 250 μg/70 kg body weight, respectively. The dosage administered for an individual session was chosen using a randomized schedule in a double-blind manner. Blood was collected through the contra-

lateral arm at −15, 0, 5, 10, 15, 20, 30, 45, 45, 60, 90, and 120 min (0 being the time the infusion was started).

Preparation of ACTH Infusion

The $ACTH_{1-24}$ solution was prepared under sterile conditions as follows: 2.5 ml of sterile 1 *N* HCl was substituted for 2.5 ml of normal saline in a 250 ml saline infusion bag to produce a 0.01 *N* solution of HCl saline. 250 μg of $ACTH_{1-24}$ was dissolved in 2 ml of sterile acid albumin solution (0.1% solution of recrystallized human serum albumin brought to a pH of 3 with 30% HCl). This preparation was diluted in the 0.01 solution of HCl in saline to produce the requisite concentration for each dose in a 50 ml volume (bag volume could vary by ± 10%, as stated by Abbott Labs) which was administered over the 30-min period. Each dose was administered in the same volume. This preparation led to no loss of ACTH secondary to absorption to any of the surfaces used in the study, such as to the saline bag or tubing (the mean overall recovery after high-dose and low-dose ACTH run through the catheters was 106%).

Assays

ACTH was measured by a radioimmunoassay method (Krishnan et al. 1988a). The plasma for the ACTH assay was extracted by using C18 SepPak cartridges (Waters Associates, Milford, MA).

Details of this assay have been previously published (Krishnan et al. 1988a). The antibody binds to $ACTH_{1-24}$ more easily than to $ACTH_{1-39}$; therefore, different standards were used for measuring $ACTGH_{1-24}$. The plasma ACTH concentration data obtained reflect primarily $ACTH_{1-24}$. The intra-assay coefficient of variation (C.V.) was 7%; the interassay C.V. was 12%. Cortisol was measured by the Abbott TDX TM cortisol assay. Intra-assay C.V. was 8%, interassay C.V. was 8% (Ritchie et al. 1990). Samples from each subject were measured on the same assay.

Statistical Methods

The measured values of circulating ACTH and cortisol were transformed to their natural logarithms, prior to parametric analysis, to approximate normal distributions. Because of the repeated measurements on individuals, a blocked (repeated measures) design was used in the analyses with the randomized doses constituting one of the independent variables, along with the blocking factor of the subject ID. The repeated measurements across time, within a dose, were treated as multivariate dependent variables to avoid the problem of colinearity, because they had to be measured sequentially. The baseline value of the measured variable was used as a covariate in each case. The analyses allowed us to test for the effects of time and dose, as well as the interaction between these two factors. Further, pair-wise testing of mean values of the measured variable between different dose levels was made possible at each time point, using the estimates of error provided by each of the univariate tests. Bonferroni adjustment of significance was used to control for overall significance of the multiple pair-wise testing.

Additonal parameters also derived from individual patient time curves included the area under the curve (calculated using the method of trapezoids), the maximum level, and the time to maximum level. These variables, because of marked non-normal distributions in several cases, were subjected to Page's test (Hollander et al. 1973) (a blocked distribution-free test against ordered alternatives), to test for directional differences related to increases in dose.

As mentioned above, because of the non-normal distribution of many of these derived variables, Spearman's (non-parametric) correlation coefficient was calculated to obtain the relationship between variables within individuals. It was assumed that the relationship was monotonic, but not necessarily linear (as in Pearson's coefficient). Finally, an overall estimate of the correlation coefficient across subjects was calculated using Fisher's transformation and was tested for significance (Hollander et al. 1973).

Results

None of the normal volunteers were nonsuppressors on the 1 mg dexamethasone suppression test, i.e., cortisol <5 μg/dl at all timepoints following dexamethasone. Figure 1 illustrates the changes in log prisma cortisol concentration over time as a function of the dose administered. Our data indicate that there is a progressive increase in plasma cortisol concentrations as the dose of ACTH increases. The mean ± (standard deviation) maximum change from baseline (Δ Max) in plasma cortisol and ACTH concentration and the area under the curve (AUC) for cortisol and ACTH (for each of the five doses) are listed in Table 1. It is seen that for the smaller doses of ACTH, the large variability in the measured ACTH concentrations (Fig. 2) obscures the relationship between the administered dose of ACTH and the measured ACTH concentration when individuals are grouped together. The time to peak for the cortisol response following the 250 μg ACTH dose is greater than that following the other doses of ACTH.

The multivariate repeated measures of log-(ACTH) by dose and time resulted in significant multivariate F-tests for the effects of both dose ($P \leqq 0.0001$), time ($P \leqq 0.0001$) and the interaction of dose and time ($P \leqq 0.0001$).

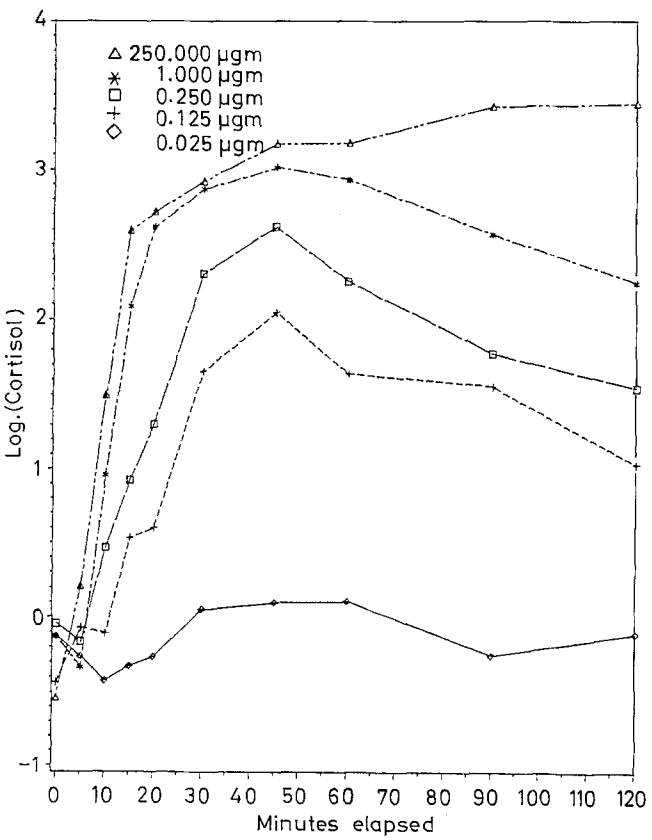

Fig. 1. Illustrates the changes in log plasma cortisol concentration (μg/dl) over time as a function of the ACTH dose administered

Table 1. Mean/(standard deviation)/ summed ranks for maximum change area under the curve and time to maximum change for ACTH and cortisol, by dose of ACTH

Dose ACTH	Max cortisol	Max ACTH	AUC cortisol	AUC ACTH	Time to max	
					Cortisol[a]	ACTH[a]
0.025 μgm	1.51	88.38	114.83	1,500.44		
	(125.3)	(32.6)	(2,075.0)			
	6	16	6	14	13	11
0.125 μgm	10.37	16.53	599.53	410.13		
	(5.9)	(7.8)	(318.4)	(285.5)		
	13	15	12	10	17	19
0.250 μgm	15.42	9.21	974.75	399.25		
	(4.7)	(5.8)	(296.7)	(332.9)		
	17	12	18	12	15	23
1.000 μgm	21.44	69.08	1,634.95	1,847.49		
	(2.9)	(108.5)	(237.1)	(2,908.7)		
	24	17	24	14	16	20
250.000 μgm	32.99	11,973.87	2,796.85	158,495.68		
	(3.7)	(24,061.5)	(366.4)	(155,584.9)		
	30	30	30	25	29	18

Cortisol is expressed in μg/dl, ACTH in fmol/ml

[a] Time in min

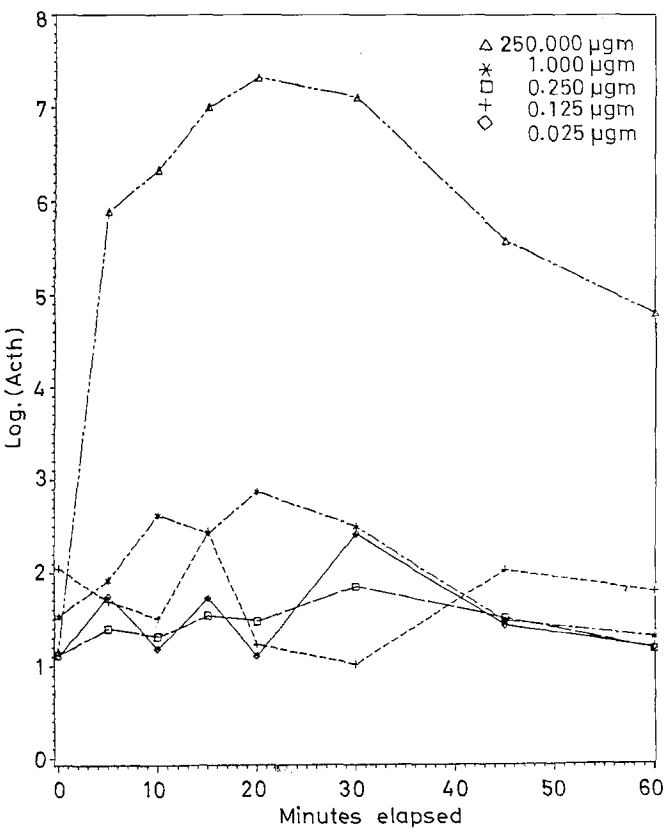

Fig. 2. Illustrates changes in log plasma ACTH concentrations (fmol/ml) time as a function of the ACTH dose administered

Because of the significant interaction, evaluations of the effect of dose were then done using the univariate ANOVAS at each time point. These univariate F-tests were highly significant for dose at every time point except the first, and multiple comparisons revealed that this effect was due solely to the elevated levels at the maximum dose of ACTH.

The multivariate test probabilities for log-(Cortisol) were similar in that all three tests for dose, time and their interaction were significant at $P \leqq 0.0001$. Again, investigation of the effect of dose was done separately at each time point because of the significant interaction, revealing highly significant univariate F-tests for dose at all time points except 0 and 5 min. Pair-wise comparisons of means by dose revealed a consistent pattern of increasing mean levels with dose at all time points beyond 5 min with most pair-wise comparisons proving significant even with significance criteria appropriately modified by Bonferroni adjustment. All of the derived variables demonstrated a significant increasing relationship to dose by Page's test. Significant results in this text, when the treatment is the ordered level of dose, thus implies a significant monotonic dose relationship.

Spearman's correlation coefficients between dose and the derived variables also proved significant for all the derived variables, ranging from a low of 0.167 for maximum change in circulating ACTH to exactly 1.000 for the change to maximum cortisol and the area under the cortisol time curves. These results, too, imply a significant increasing monotonic dose response.

Discussion

In this study, we report a logarithmic dose-response relationship between the dose of ACTH administered and plasma cortisol concentrations. This is in agreement with that reported by Landon et al. (1967) and Le Clerq et al. (1972) in humans. However, unlike their study, we also

found that the overall duration of the response and the peak levels reached continued to rise as doses of ACTH were increased. This finding may be explained by the fact that the peak cortisol concentrations were only reached long after the time of administration of the higher doses of ACTH and often at time points beyond those measured by Landon et al. (1967) and LeClerq et al. (1972). Although there was considerable variability between individuals in plasma ACTH concentrations after various doses of ACTH within individuals, there was, however, a definite correlation between AUC for ACTH and AUC cortisol (after the various doses of $ACTH_{1-24}$).

This finding demonstrates that the adrenals respond to the integral of the dose of ACTH, and therefore the integral of plasma ACTH concentration over time. These results are similar to those reported in dogs by Wood et al. (1982). At the lower doses of ACTH, in some individuals, the ACTH concentrations were close to or below the detection limit of the ACTH assay. In other, there were erratic values. The reason for this discrepancy is unclear.

It must be noted that similar discrepancies have also been reported previously (Krieger et al. 1975; Krishnan et al. 1988a). Further, in individual subjects, the rise in plasma cortisol concentration at lower ACTH doses occurred without a significant rise in ACTH, consistent with prior reports of the apparent dissociation between plasma cortisol and ACTH concentrations (Krieger et al. 1975; Fehm et al. 1984a; Fehm et al. 1984b; Krishnan et al. 1988a).

This report, together with our earlier study and the recent work of Wood et al. (1982), suggests that in some instances the occurrence of cortisol peaks without ACTH peaks may be due to lack of sensitivity of the ACTH assay in measuring peripheral venous plasma ACTH concentration. Further work is needed to clarify the pharmacokinetic/pharmacodynamic relationship between ACTH and cortisol under basal conditions. This understanding will help us to evaluate better the pathophysiology of hypercortisolemia in conditions such as depression, Cushing's syndrome, etc.

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