The Effect of Small Doses of Human ACTH on Serum Corticosteroid Levels in Man* (34045)

ROGER K. MCDONALD,[1] ARNE R. SOLLBERGER, PETER S. MUELLER, AND MICHAEL H. SHEARD

Department of Psychiatry, Yale University School of Medicine, Connecticut Mental Health Center, New Haven, Connecticut 06508

The basic information which is needed to correlate events such as electroencephalographic data in temporal relationship to ACTH release, as evidenced by a rise in plasma 11-hydroxycorticosteroid (11-OHCS) levels, is lacking. This information is first, the rate of increase in serum 11-OHCS in relationship to the dose of ACTH; second, the maximum rise in serum 11-OHCS in relationship to the dose of ACTH administered; and third, the latency period between ACTH release into the bloodstream and a demonstrable rise in the circulating 11-OHCS.

Although no studies to supply all of these data have been carried out using human ACTH, Landon *et al.* (1) have studied the effect of very small doses of synthetic ACTH (Synachten) on plasma 11-OHCS levels in man. Their observations suggest that differences in physiological levels of circulating 11-OHCS normally reflect exceedingly small quantities of ACTH. In the present study, healthy male subjects were employed to study the relationship of adrenocortical secretory responsiveness to intravenously administered human ACTH. All subjects were pretreated with dexamethasone to suppress endogenous ACTH release.

Methods. Seven healthy male volunteers were studied at the Connecticut State Prison. The average age was 30.4 years (range 21–46 years) and their mean body weight was 85.1 kg (range 61–139 Kg). Each subject was given 2.25 mg of dexamethasone at 10 PM the day prior to the study. When the subjects were studied both the morning and afternoon, a second dose of 0.75 mg of dexamethasone was administered at the end of the morning study. The research design was the same in all cases: 5 or 6 subjects participated in each experiment receiving the same dose of ACTH. After the placement of a Cournand needle in a forearm vein, a blood sample was drawn and 1 ml of saline was injected. Thirty minutes later a second blood sample was drawn and 1 ml of a solution containing human ACTH[2] was injected intravenously, either 0.01 U, 0.10 U, 0.25 U, 0.50 U, or 1.0 U. Successive blood samples were then drawn at approximately 5, 10, 15, 20, 25, 30, 45, and 60 min, after which time the study was terminated. When a second study was carried out on the same day, at least 60 min elapsed before the study was repeated beginning with the saline injection as previously described. The 0.01 and 0.10 U dose, as well as the 0.25 and 1.00 U dose, experiments were performed on a single day. The 0.50 U dose study was done at 8:00 AM and 2:00 PM on the same day. Each blood sample was immediately placed in ice, and after clotting the serum was separated and frozen until it was analyzed within a 2-day period. The 11-OHCS were measured fluorometrically by the method of Mattingly (3).

Average 11-OHCS curves, both for group and for individual curves, were obtained through polynomial curve fitting over the ACTH postinjection time range (Yale Com-

* Supported by USPHS Grant MH 13917.

[1] Present address: Saint Elizabeths Hospital, National Institute of Mental Health, Washington, D. C. 20032.

[2] We are indebted to Dr. A. B. Lerner, Professor of Medicine (Dermatology), Yale University School of Medicine, for providing the human ACTH (Fraction 8B) (2) used in this study. This fraction has an ACTH activity of 139 U /mg.

puter Center Program 16S), because of small variations in actual assay timing. This procedure also yields a smooth curve estimate and made it possible to estimate individual latency periods. A fourth degree polynomial was judged to yield a satisfactory fit. The presence of a drug effect was assured by the presence of significant improvement of curve fit up to the third (almost always the second, occasionally the fourth) degree at probability levels of 0.05 – 0.001. An exception was the lowest-dose group (0.01 U) where no nonlinear fits were significant.

Results. Baseline serum 11-OHCS levels. With dexamethasone suppression of endogenous ACTH secretion, the morning baseline serum 11-OHCS levels in all subjects averaged 2.57 ± 0.22 μg/100 ml (SD = 0.57). Thirty minutes after the injection of saline the serum levels averaged 2.87 ± 0.33 μg/100 ml (SD = 0.87). Thus, the mechanics of the experimental procedure had no discernible effect on the level of circulating 11-OH CS. When two tests were performed on the same day, the mean baseline 11-OHCS levels were uniformly low in the test situation in which 0.10 U of ACTH was tested 120 min after the injection of 0.01 U ACTH (3.30 ± 0.31 vs. 3.50 ± 0.39 μg/100 ml). In the test situation where a comparison was made between 0.50 U ACTH in the morning (8:00 AM) and afternoon (2:00 PM) the average baseline values did not differ (2.62 ± 0.26 vs. 2.73 ± 0.17 μg/100 ml).

Studies were also carried out for the 1.0 U ACTH dose in six subjects 30 min after the 0.25 U AM study. Although the average baseline serum 11-OHCS level was 7.75 ± 0.80 μg/100 ml (SD = 1.96), its measurements during the first 20 min permitted the estimation of the initial rate of rise and the latency time.

ACTH dose-serum 11-OHCS response. The average serum 11-OHCS response curve for each dose of ACTH shown in Fig. 1 provides data on the rate of rise, the maximum level attained, and the time to reach maximum for three different doses of ACTH. The rate of rise in serum 11-OHCS is practically identical during the first 13 min after

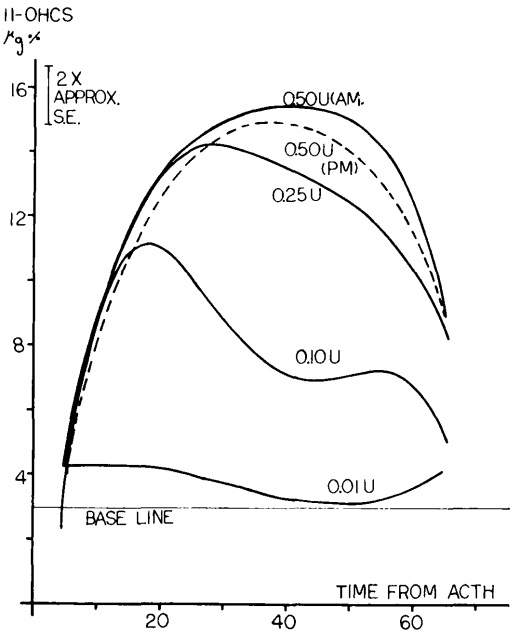

FIG. 1. The average 11-OHCS response curves for each dose of ACTH. Broken line is PM replication of 0.50 U AM curve. Average standard deviation of the points around the curves was 2.02 μg/100 ml; the approximate standard error in a curve point is 0.9 μg/100 ml; time in minutes.

ACTH administration at the 0.10, 0.25, and 0.50 doses (Fig. 1). This observation indicates that once a dose of ACTH is sufficient to elicit a clear adrenocortical response it produces a maximum response for about this period of time. Studies carried out with 1.00 U ACTH in six subjects for only 20 min produced the same initial (13-min) response curve as the lower doses. Thus, over a tenfold dose schedule, practically identical initial rates of rise of serum 11-OHCS occur.

A direct relationship exists between ACTH dose and maximum serum 11-OHCS levels attained. The average serum 11-OHCS maxima after doses of 0.10, 0.25, and 0.50 U of ACTH were 11.2, 14.7, and 15.9 μg/100 ml respectively. A scatter plot of the individual dose-response data for log ACTH dose per unit of body weight and maximum serum 11-OHCS levels (calculated from fitted curves) shows a highly significant correlation ($r = 0.83$, $p < 0.001$; Fig. 2).

The time required for the serum 11-OHCS

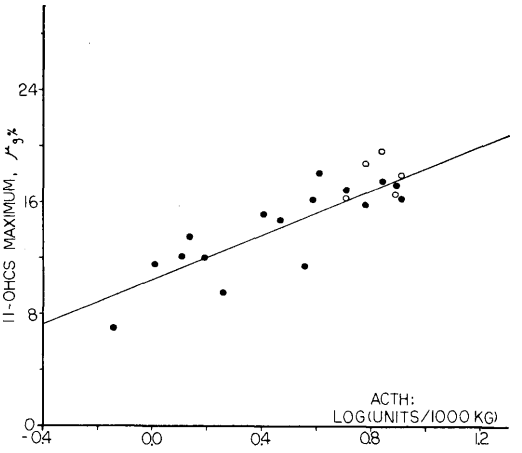

FIG. 2. Correlation between estimated serum 11-OHCS maxima and log dose ACTH ($r = 0.83$, $p < .001$, regr.: Max. = 10.389 + 8.020 log dose/1000 kg. Circles are PM replication of 0.50-U dose. These values were not included in regression calculations.

levels to reach maximum is clearly related to the dose of ACTH employed (Fig. 1). The average time to maximum 11-OHCS levels is for 0.10 U, 0.25 U, and 0.50 U (AM) 17.0, ± 1.3, 26.1 ± 2.4, and 37.4 ± 4.4 min, respectively.

Within the range of ACTH dosage schedules employed, latency period times can be demonstrated between time of injection of ACTH and rise in serum 11-OHCS levels which varied between 2.9 and 6.7 min. They were not significantly correlated with the ACTH log-doses per unit of body weight, (Fig. 3) ($r = -0.17$, $p > .10$). The mean latency times were for 0.10 U, 4.70 ± 0.23 min. (SD = 0.45); for 0.25 U, 4.93 ± 0.43 min (SD = 1.06); for 0.50 U, 4.38 ± 0.75 min (SD = 1.68); and for 1.0 U, 5.07 ± 0.44 min (SD = 1.09). The overall mean latency time of the obtained values for doses 0.10, 0.25, 0.50 U (AM) and 1.0 U was 4.80 min (SD = 1.11). Thus, the time between ACTH injection and observable rise in serum 11-OHCS should not be less than about 2.5 min nor greater than about 7.0 min in 95% of cases. Although neither the latency period nor the initial rate of rise in serum 11-OHCS differs appreciably for different doses of AC TH employed, it is evident that the larger the dose of ACTH, the longer the rise time and the higher the maximum levels of serum 11-OHCS levels attained.

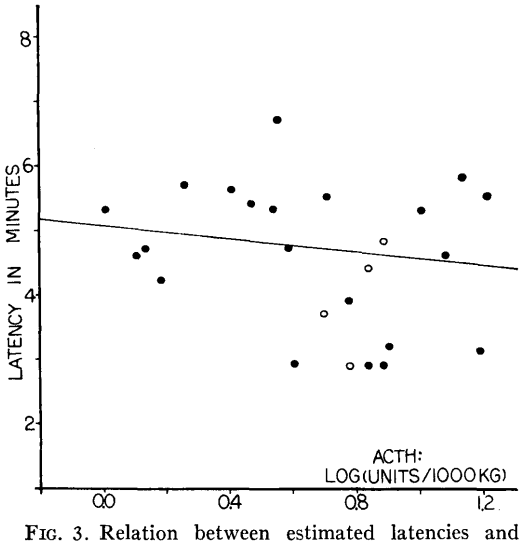

FIG. 3. Relation between estimated latencies and log dose ($r = -0.17$, $p > .10$). Circles are PM replication of 0.50-U dose. These values were not included in regression calculations.

Discussion. A highly consistent interindividual and reproducible intraindividual suppression of endogenous plasma 11-OHCS levels can be obtained on the dexamethasone schedule used. The suppression was considerably greater and more consistent than that observed by Danowski *et al.* (4) who used the same dose of dexamethasone and also worked with prison volunteers. The better suppression in the present study is probably due to the time interval between administration of the dexamethasone and measurement of the plasma 11-OHCS which was 10 hr in our study and 24 hr in that cited. Also noteworthy is the intraindividual response to this dose schedule of dexamethasone in which the individual baseline 11-OHCS levels were almost identical on four different occasions. The low values observed probably represent complete suppression of ACTH since values of this order after dexamethasone treatment have been shown to be partly the result of spurious fluorescence. In a similar study Landon *et al.* (1) reported that plasma hydrocortisone levels, when suppressed to similarly low levels by dexamethasone and determined by a double isotope procedure, were less than

a third of the total corticosteroid level measured by fluorometry.

The present studies show that a highly reproducible dose-response behavior for the control of compound F production by ACTH can be elicited in human subjects. The general shape of the average response curve to a single dose is a rapid rise starting at about 4.8 min after ACTH injection. This rise decelerates toward a peak which is a function of the dose of ACTH both in terms of the absolute value attained and the time required to attain it. The larger the dose of ACTH the higher the peak and the longer the time required for this peak to be attained.

The administration of 0.5 U of ACTH at 8:00 AM and 2:00 PM produces similar serum 11-OHCS response curves. This observation not only indicates that time of day *per se* is not an important determinant in the adrenocortical responsiveness to ACTH, but supports the observation that diurnal plasma 11-OHCS levels are best explained by parallel plasma ACTH levels (5).

The apparent potency of the human ACTH preparation used in this study is slightly less than that of the synthetic ACTH used by Landon (1). At doses of approximately 1.0 ng of human ACTH per kilogram of body weight two of three subjects (in the 0.01 U dose group) responded with a numerical rise in serum 11-OHCS which agreed with the values predicted from the regression of the maximum 11-OHCS responses on the ACTH dose. The minimum dose of synthetic ACTH noted by Landon *et al.* required to produce an observable rise in plasma 11-OHCS was approximately 31 ng, or about 0.5 ng/kg of body weight. The difference in observed responses may be due to the fact that saline was used in preparing the human ACTH onto glass, which occurs in non-acidified saline solutions, may account for losses from 36–70% (1). Assuming a correction factor within this range (50%) gives the same ACTH dose 11-OHCS response relationship for both studies.

Summary. The serum corticosteroid response to small doses of human ACTH was studied in a group of healthy male volunteers whose endogenous ACTH production was suppressed with dexamethasone. Whereas the initial rate of rise and the time required for onset of rise of serum 11-OHCS were independent of the dose of ACTH over a tenfold range (approximately 10–100 ng/kg of body weight), the maximum levels attained and the time required to reach these maxima were a direct function of the dose of ACTH employed.

The authors thank Mr. Kenneth B. Giddings for assistance in these studies.

1. Landon, J., James, V. H. T., Wharton, M. J., and Friedman, M. Lancet, **2**, 697 (1967).

2. Lerner, A. B., Upton, V. U., and Lande, S., *in* "Pharmacology of Hormonal Polypeptides and Proteins" (N. Back, L. Martini, and R. Paoletti, Eds.), p. 203. Plenum Press, New York (1968).

3. Mattingly, D., J. Clin. Pathol. **15**, 374 (1962).

4. Danowski, T. S., Cohn, R. E., Limaye, N. R., Sunder, J. H., and Moses, C., Metabolsim **15**, 304 (1966).

5. Graber, A. L., Givens, J. R., Nicholson, W. E., Island, D. P., and Liddle, G. W., Metabolism **14**, 804 (1965).

Received Feb. 19, 1969. P.S.E.B.M., 1969, Vol. 131.