Physiological dosing of exogenous ACTH

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Abstract. We evaluated the ACTH and cortisol responses to several doses of exogenous ACTH, and compared these values to the physiologic responses obtained in normal subjects undergoing insulin-induced hypoglycaemia. We determined that a cosyntrophin dose of 0.2 µg/kg body weight produced both ACTH and cortisol levels indistinguishable from the ‘physiologic’ stress-induced values. Since this dose is approximately 4 per cent of the standard 250 µg dose employed in tests of adrenal function, our findings suggest a need for caution in the interpretation of such tests.

For three decades iv ACTH infusions have been employed as a test of adrenal secretory capacity (Renold et al. 1952). With the synthesis of (1–24) ACTH (cosyntrophin), iv injection of 250 µg of this peptide has become a common screening test for adrenal suppression or atrophy (Lindholm et al. 1978; Kehlet & Binder 1973; Speckart et al. 1971; Kehlet et al. 1976). This dose has also been used as a standardized form of adrenal stimulation when assessing the suppressive effects of glucocorticoids on the adrenal itself (Saito et al. 1979). To help determine the appropriateness of such a large amount of ACTH when used in these tests, we first analyzed ACTH levels in normal subjects during insulin hypoglycaemia. We then administered graded doses of (1–24) ACTH in an attempt to mimic the physiologic pattern seen during ‘stress’.

Materials and Methods

Subjects

Healthy volunteers were screened to rule out any unrecognized abnormality of the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, none used any medications, and none had evidence of diabetes, hypertension, hirsutism, galactorrhoea, oligomenorrhea, or psychiatric disorder. The subjects were between the ages of 21 and 35 (mean, 24.6 years). Informed consent was obtained before each study, and the protocols were approved by the Clinical Investigations Committee of the University of Chicago.

Insulin hypoglycaemia

Five men and 5 women came to the Clinical Research Center (CRC) fasting, and an iv line was inserted at 08.00 h and maintained with saline at a minimal rate (30 ml/h). Baseline blood samples were obtained via the side arm of a three-way stopcock after clearing the tubing of saline at 08.00 h and at 08.30 h (0 min). The subjects then received regular insulin (0.15 U/kg body weight) iv. Additional blood samples were collected every 15 min for 2 h and placed into chilled tubes containing EGTA and glutathione. Plasma was separated and frozen until the hormones were assayed. ACTH and cortisol levels were determined for all subjects in single assay runs. Plasma glucose levels were determined at the time of each sampling (YSI Model 23 Glucose Analyzer, Yellow Springs Instrument Co., Yellow Springs, Ohio).

ACTH infusions

The subjects in this study came to the CRC at 08.00 h while fasting and an iv line was inserted. Baseline samples were obtained at 08.30 and 09.00 h (0 min). Beginning at 0 min, the subjects received infusions of (1–24) ACTH (Cortrosyn, Organon), 1.0 µg/kg body weight over 10 min. For these studies, 250 µg of cosyntrophin was reconstituted in 1 ml of saline then diluted in 30 ml. The appropriate dose was then further diluted in 50 ml of saline. The final preparation was infused over 10 min by means of a roller type infusion pump (Holter Roller Pump Model 911, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa). Further blood specimens were
obtained at 10, 15, 20, 30, 45, 60, 75, and 90 min and handled as described above. When the results had been analyzed the testing was repeated with reduced doses (0.2 µg/kg body weight, then 0.05 µg/kg body weight) using the same protocol.

ACTH assay

Plasma ACTH was measured by double antibody technique without extraction. The assay has a sensitivity of 5 pg/ml and intra- and inter-assay variations of <5% and <15%, respectively. It measures (1–39) ACTH and (1–24) ACTH on an equimolar basis.

Cortisol assay

Plasma cortisol was measured by competitive protein binding assay. The intra- and inter-assay variations in this system are <2.7% and <7.0%, respectively.

Statistical analysis

Hormonal responses were analyzed by calculating the area under the curve by sequential rhomboids (Elashoff 1979), then comparing groups by Student's t-test.

Results

Insulin hypoglycaemia

All subjects became hypoglycaemic (mean nadir, 26.9 mg/dl; range, 21–35 mg/dl) and exhibited diaphoresis and tachycardia. The mean ACTH response to hypoglycaemia is shown in Fig. 1. Peak ACTH levels ranged from 186 to 780 pg/ml (mean

![Mean ACTH levels during insulin hypoglycaemia (ITT) and ACTH infusion studies. Abscissa shows times for ITT (lower scale) and infusion studies (upper scale). Peak values have been superimposed for purposes of comparison.](image-url)

Fig. 1.
Mean cortisol responses during ITT and ACTH infusion studies. Abscissa shows times for ITT (lower scale) and infusion studies (upper scale). Peak values have been superimposed for purposes of comparison. (○ = ITT; □ = 1 µg/kg; ▢ = 0.2 µg/kg; ■ = 0.05 µg/kg).

408 pg/ml. ACTH levels began to rise by 30 min after insulin injection, peaked by 45 min, and returned to baseline by 120 min. The mean cortisol response is shown in Fig. 2. Cortisol levels plateaued within 30 min after the ACTH peak and began to decline, but remained well above baseline by the end of the study. The relationship between peak ACTH levels attained and peak cortisol levels appears in Fig. 3. Levels of ACTH above approximately 200 pg/ml appear to have resulted in maximal stimulation of adrenal secretion during the study period.

**Cosyntrophin infusions**

ACTH values after cosyntrophin infusion appear in Fig. 1. The protocol that was used produced reliable and reproducible results. The initial dose employed (1.0 µg/kg body weight) resulted in ACTH levels clearly in excess of those observed after hypoglycaemia ($P < 0.001$). When the dose was reduced to 0.2 µg/kg, the integrated ACTH response (area under the curve, see Table 1) was comparable to that seen with hypoglycaemia. Although recorded peak values at the end of the 10 min infusion (mean 1094; range 560–1500) were somewhat higher than peak levels after insulin hypoglycaemia (mean 375; range 102–780), the 15 min values (following the rapid redistribution phase) were quite comparable (mean 482; range 214–760) to the peaks following hypoglycaemic stress. Infusions of 0.05 µg/kg body weight of cosyntrophin resulted in an integrated ACTH response below endogenous ACTH secretion during the insulin studies, though this difference did not reach statistical significance (Table 1).

During the study period, the integrated cortisol response to 1.0 µg/kg of ACTH was the same as that observed after ITT ($P = NS$). The mean cortisol level was still rising, however, when the infusion study ended, while that for the ITT studies was already falling toward baseline (Fig. 2).

The pattern of cortisol secretion after 0.2 µg/kg of ACTH was indistinguishable from the cortisol response to hypoglycaemia ($P = NS$; see Fig. 2 and Table 1). After 0.05 µg/kg of ACTH, the integrated cortisol response was significantly less than in any of the other three groups ($P < 0.02$ vs ITT; $P < 0.05$ vs 1.0 µg/kg; $P < 0.05$ vs 0.2 µg/kg). There were no significant differences between the baseline cortisol values (–30 min for ACTH infusions and 0 min for ITT) among any of the groups ($P = NS$ for all combinations).
Fig. 3.
Comparison of peak ACTH levels and maximum cortisol levels achieved during the four studies.
(○ = ITT; ● = 1 µg/kg; □ = 0.2 µg/kg; ■ = 0.05 µg/kg).

Discussion
The iv injection of 250 µg of cosyntrophin has been advocated as a safe and reliable screening test for competency of the hypothalamic-pituitary-adrenal system in patients on chronic exogenous glucocorticoids, or in patients with suspected hypopituitarism (Lindholm et al. 1978; Kehlet & Binder 1973; Speckart et al. 1971; Kehlet et al. 1976). Results are generally felt to be normal if cortisol levels exceed 18 µg/dl at 30 min (Lindholm et al. 1978; Speckart et al. 1971; Borst et al. 1982a,b). Testing of patients with hypopituitarism has the theoretical potential for falsely normal results due to retained adrenal ability to respond to ACTH despite protracted ACTH deficiency (Borst et al. 1982a,b). Recently numerous investigators have reported normal responses to cosyntrophin in patients with inadequate HPA response to insulin hypoglycaemia (Borst et al. 1982a; Copeland & Ladenson 1982; Delaloye et al. 1982; Reschini et al. 1982), resulting in considerable controversy over

Table 1.
Mean integrated ACTH and cortisol responses (area under the curve ± SE) for the four study groups.

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<th>ITT</th>
<th>Dose (µg/kg)</th>
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<tr>
<td></td>
<td></td>
<td>1.0</td>
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<tr>
<td>ACTH (area)</td>
<td>282.6 ± 55.0</td>
<td>1383.7 ± 133.4</td>
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<tr>
<td>Cortisol (area)</td>
<td>27.3 ± 0.8**</td>
<td>26.6 ± 1.5**</td>
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* P < 0.001 vs 1.0 µg/kg group. ** P < 0.05 vs 0.05 µg/kg group. *** P < 0.02 vs 0.05 µg/kg group.
the value of the so-called 'short' ACTH test as a test for secondary hypoadrenalism. This question is of particular interest because of the discomfort and potential danger inherent to insulin hypoglycaemia in hypopituitary patients.

It has been reported by several investigators that the dose of cosyntrophin administered in the standard short stimulation test was far in excess of that required to induce maximum steroidogenesis (Kehlet & Binder 1973; Landon et al. 1964). By direct measurements of ACTH levels, we have confirmed this and have been able to determine a dose of cosyntrophin which clearly mimics the 'physiologic' response to hypoglycaemic stress. This dose is approximately 4–6% of the standard 250 µg used in most reports. Further, we have shown that ACTH levels in the range of 275–1000 pg/ml during these brief periods of stimulation do not result in any further increment in the immediate cortisol response. Higher levels resulted in prolongation of the cortisol secretion. The latter effect may reflect a prolongation of elevated ACTH levels with the larger doses administered (Fig. 1). It is possible that a 250 µg dose of cosyntrophin administered as a bolus, rather than over 10 min as we have done, would show relatively less of this prolongation. The elevated ACTH levels after 1.0 µg/kg, however, persisted more than 30 min beyond the infusion. It seems likely that a 250 µg iv would last at least as long, and an im dose, as reported by some investigators (Pham-Huu-Trung et al. 1978; Liddle 1981), would last even longer.

Among the available methods to test HPA integrity, insulin hypoglycaemia is probably the most valuable (Kehlet et al. 1976; Borst et al. 1982a,b; Copeland & Ladenson 1982; Delaloye et al. 1982; Reschini et al. 1982). This procedure tests hypothalamic responsiveness to a controlled stress as well as pituitary and adrenal secretory capacities. The short ACTH test assesses only the last of these functions, and, as we have shown, employs a remarkably excessive stimulus, compared to the best reference standard. We suggest that such large pharmacologic doses of ACTH may contribute to the difficulties noted in the interpretation of test results. The development and standardization of an ACTH test involving physiologic amounts of cosyntrophin may increase the diagnostic value.

Similar considerations may apply to studies of normal adrenal physiology, both in vivo and in vitro. Saito et al. (1979) have employed the standard dose of 250 µg of cosyntrophin, preceded by either placebo or 10 mg of dexamethasone, in an attempt to demonstrate whether so-called 'short loop' feedback of glucocorticoid on the adrenal exists. The current study indicates that the use of such a large dose is inappropriate in the evaluation of normal physiologic control mechanisms. Future studies should involve lower ACTH doses more compatible with the relatively small amounts required to fully stimulate adrenal function.

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References


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