CLINICAL STUDY

Is the plasma ACTH concentration a reliable parameter in the insulin tolerance test?

K Borm, M Slawik, I Selier, F Flohr, M Petrick 1, J Honegger 1 and M Reincke

Division of Endocrinology, Department of Medicine 2 and 1Department of Neurosurgery, University Hospital of Freiburg, Germany

(Correspondence should be addressed to K Borm, University of Freiburg, Department of Medicine 2, Hugstetter Str. 55, 79106 Freiburg, Germany; Email: borm@med1.ukl.uni-freiburg.de)

Abstract

Objective: The insulin tolerance test (ITT) is an established standardized test for the evaluation of the hypothalamic–pituitary–adrenal axis. While a peak cortisol value of >18 μg/dl is usually interpreted as a sufficient response to the ITT, the plasma ACTH response has not yet been standardized.

Methods: We evaluated retrospectively the peak plasma ACTH concentrations during 140 ITTs in 125 patients with suspected pituitary insufficiency and prospectively in 15 healthy subjects.

Results: All healthy subjects had a peak cortisol concentration ≥18 μg/dl; 32 of 125 tests in the patients showed an insufficient cortisol response (peak cortisol concentration <18 μg/dl). The peak stimulated ACTH concentration in patients with secondary adrenal insufficiency (SAI) was 49.2±37.2 pg/ml (mean±S.D.) vs 130.9±89.3 pg/ml in patients without SAI, and 110.9±55.4 pg/ml in normal subjects (P < 0.001). There was a weak, but significantly positive correlation between the peak ACTH and peak cortisol concentrations (rho = 0.446, P < 0.001), but there was also a very wide spread of the values. Defining a cut-off value for the peak plasma ACTH concentration with a sufficient sensitivity and specificity to identify patients with an impaired hypothalamic–pituitary–adrenal (HPA) axis was not possible. A peak plasma ACTH < 20 pg/ml as a cut-off value had a sensitivity of 25% and a specificity of 98% for SAI. A cut-off value of a peak plasma ACTH < 140 pg/ml had a sensitivity of 97% but a low specificity of 39%.

Conclusions: Although there is a significant positive correlation between the peak ACTH and the peak cortisol concentrations, we conclude that there is no additional benefit in determining the ACTH concentrations during an ITT. Because of the strong variations of the values, the peak ACTH concentration is a poor parameter for the evaluation of the HPA axis.

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Introduction

Isolated or multiple pituitary hormone deficiencies can result from various conditions such as pituitary tumors, pituitary surgery or radiation therapy of the pituitary region. The ability of the hypothalamic–pituitary–adrenal (HPA) axis to respond to stress must be carefully studied in any patient at risk for hypopituitarism in order to prevent complications from secondary adrenal insufficiency (SAI). Various stimulation tests are performed to evaluate the function of the pituitary. The insulin tolerance test (ITT) is widely accepted to be the gold standard for the evaluation of the HPA axis (1–8). Hypoglycemic stress (plasma glucose <2.2 mmol/l) is a powerful stimulus for the secretion of pituitary hormones (7, 9–11). While a peak cortisol value of ≥18 μg/dl or ≥500 nmol/dl is usually interpreted as a sufficient response to the ITT to exclude SAI (4, 9, 12–14), there are few data about the sufficient peak adrenocorticotropic hormone (ACTH) concentration during the ITT. Therefore, we evaluated 140 ITTs to study the peak stimulated ACTH concentrations in 125 patients at risk for SAI and in 15 healthy subjects.

Subjects and methods

Subjects and ITT

We evaluated retrospectively 125 ITTs in patients with suspected SAI which had been performed between March 1999 and December 2002 in our department (Table 1). Patients before or after transsphenoidal surgery for Cushing’s disease were excluded from the study. Additionally, we investigated prospectively the ACTH response during ITT in 15 healthy subjects. All patients and healthy subjects were tested with a bolus insulin dose of 0.1–0.15 IU/kg body weight i.v.
Reasons for suspected pituitary insufficiency

Number 125 15

Table 1 Characteristics of patients and healthy individuals.

<table>
<thead>
<tr>
<th>Reason for suspected pituitary insufficiency</th>
<th>Patients</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Operation of a pituitary tumor –&gt; 3 months prior to the ITT</td>
<td>65</td>
<td>—</td>
</tr>
<tr>
<td>Operation of a pituitary tumor &lt; 3 months prior to the ITT</td>
<td>33</td>
<td>—</td>
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<td>Operation and radiation therapy of a pituitary tumor</td>
<td>12</td>
<td>—</td>
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<tr>
<td>Other reasons (untreated tumors, cysts, sarcoidosis, cerebral trauma, Sheehan’s syndrome, idiopathic pituitary insufficiency)</td>
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The ITTs were started before 1000 h after an overnight fast for 10–14 h. Patients on chronic glucocorticoid replacement therapy took the last dose of hydrocortisone 20–24 h before the ITT. Blood samples were taken at −15, 0, 30, 45, 60 and 90 min for the determination of plasma glucose (sodium citrate-containing tubes) and plasma ACTH (EDTA-containing tubes), serum cortisol and serum growth hormone concentrations in a standardized procedure. A successful ITT was defined by a blood glucose concentration <2.2 mmol/l and symptoms of hypoglycemia, i.e. perspiration, fatigue, somnolence or hunger. A normal adrenal response to ITT represented a peak serum cortisol ≥18 μg/dl. The definition was based upon the usually accepted cut-off values for a sufficient cortisol response (4, 9, 12–14), which was revalidated in our clinic by 30 ITTs in normal subjects (mean peak cortisol±S.D.: 24.5±2.9 μg/dl, range: 18.4–29.3 μg/dl, 2.5–97.5 percentile: 18.5–26.6 μg/dl). The patients were followed up continuously after testing for at least 1 year, and the need for glucocorticoid replacement therapy was recorded. Some patients underwent additional testing (such as ACTH stimulation testing) or repeated ITT testing confirming the previous test result. All patients with a normal peak cortisol concentration >18 μg/dl during ITT did not require or receive glucocorticoid replacement therapy. Twenty-nine of 32 patients with an insufficient cortisol response during ITT (<18 μg/dl) were treated with chronic glucocorticoid replacement therapy. Three patients with mild impairment (peak cortisol between 17.0 and 17.9 μg/dl) did only receive glucocorticoids during stressful episodes, such as increased physical activity, surgery and acute illness. Written informed consent was obtained from every healthy subject. The study was approved by the ethics committee of the University of Freiburg.

**Statistical analysis**

All tests were retrospectively analyzed by one person. All values represent means±S.D., if not stated otherwise. The correlation of the maximum plasma ACTH and the maximum serum cortisol was calculated using the Spearman rank correlation coefficient. The sensitivity and the specificity were calculated for different cut-off levels of peak plasma ACTH concentrations. The statistical analyses were calculated using the Statview program (SAS Institute Inc., Cary, NC, USA).

**Results**

All healthy subjects had a peak cortisol concentration >18 μg/dl. In the patients, 108 tests showed a sufficient peak serum cortisol concentration (peak serum cortisol ≥18 μg/dl). In 32 patients, the peak stimulated cortisol concentration was <18 μg/dl, implying by definition an SAI. The peak serum cortisol concentrations in patients with SAI were significantly lower than those in patients with a normal cortisol response or in healthy subjects (10.6±5.6 μg/dl in patients with SAI vs 26.4±8.1 μg/dl in patients without SAI (P < 0.001), and 24.9±2.6 μg/dl in healthy subjects (P < 0.001) (Table 2)). The average peak plasma ACTH concentration was lower in patients with SAI than in patients with a normal cortisol responses (49.2±37.2 pg/ml in patients with SAI vs 130.9±89.3 pg/ml in patients without SAI and 110.9±55.4 pg/ml in healthy subjects). A significant positive correlation between the stimulated plasma ACTH concentrations and the stimulated serum cortisol concentrations during the ITT was observed (rho = 0.446, P < 0.001), but there was a wide variation of the values (range 6–497 pg/ml in patients and 25–209 pg/ml in healthy subjects; Fig. 1a, Table 2). To exclude possible effects resulting from an early postoperative condition, we analyzed the subgroup of patients who had the ITT more than 3 months after pituitary surgery (Fig. 1b). However, this had no impact on the results.

Low stimulated plasma ACTH concentrations occurred in all groups, high peak ACTH concentrations (ACTH > 200 pg/ml) occurred only in the patients without SAI and in the healthy subjects (Fig. 2). We tried to define a cut-off value of the maximum immediately centrifuged and measured. Plasma ACTH and serum cortisol concentrations were determined by chemiluminescence using an autoanalyzer (Nichols Advantage (Nichols Institute Diagnostics, San Clemente, CA, USA) with intra- and interassay variations of 1.2–4.2% and 6.4–8.4% respectively). Accuracy of the assay procedure over the study period was documented by daily calibration, by regular determination of reference plasma samples and by quarterly participation in national quality controls.
plasma ACTH concentration that would allow us to identify most patients with SAI. Aiming at a high specificity, a maximum plasma ACTH < 20 pg/ml as a cut-off value would have a sensitivity of 25% and a specificity of 98% for the diagnosis of SAI. A higher cut-off increases sensitivity but decreases specificity considerably. For example, setting the threshold for the diagnosis of SAI at <140 pg/ml, the sensitivity would be 97%, but the specificity would decrease to 39% (Fig. 3).

Discussion

For the evaluation of the HPA axis, the ITT is widely accepted as a gold standard to exclude SAI (1–6, 8, 11). A peak cortisol concentration of 18 μg/dl is defined as a sufficient stress response of the HPA axis (4, 9, 12–14). The plasma ACTH concentration is frequently also measured during the ITT. However, the response of the ACTH concentration following hypoglycemia has not yet been standardized and remains a matter of debate because of conflicting results (1, 4, 15–18).

Lindholm et al. (15) investigated the correlation between stimulated cortisol and ACTH values in 26 healthy subjects. They demonstrated that there was a wide spread of the stimulated plasma ACTH concentration and that there was no significant correlation between the ACTH and the cortisol values. Donald (17) observed in a smaller number of subjects (nine healthy controls and six patients) that there was only a poor correlation between the ACTH and cortisol concentration during the ITT. Staub et al. (19) did not find a significant correlation between plasma ACTH and serum cortisol; however, they studied only seven subjects. Nye et al. (18) investigated prospectively the reproducibility of the cortisol and ACTH responses to the ITT in ten healthy subjects, with repeated ITTs in eight subjects. They observed a better reproducibility of the cortisol response than of the ACTH response to hypoglycemic stress. Erturk et al. (4) reviewed retrospectively 193 ITTs in 120 female and 73 male patients with known or suspected hypothalamic–pituitary disease (age 43.4 ± 14.1 years, range 15–82 years) to study the various diagnostic criteria for pituitary insufficiency. They induced hypoglycemia by a bolus injection of 0.15–1 IU/kg body weight. One hundred and thirty-three patients had an intact HPA axis, 60 patients had an insufficient rise indicating SAI. A positive correlation between the stimulated cortisol concentrations and the logarithmically transformed stimulated ACTH concentrations could be demonstrated. Because the variation of the peak ACTH values was considerable (range 5–755 pg/ml), they concluded that the stimulated ACTH values are a poor criterion of the function of the pituitary (4). Tuchelt et al. (1) reported on the ITTs of 109 patients (age range 18–84 years, mean age 45 years, 62 female and 47 male patients) and 25 normal subjects (age range 21–50 years, seven women and 18 men). The ITTs were performed with 0.1–0.15 IU insulin/kg body weight. It was shown that 38 of 85 patients with a normal cortisol response had subnormal ACTH responses compared with healthy subjects. They speculated that these patients might have a decreased ACTH reserve and may be at risk for developing pituitary insufficiency during the course of the disease (1). Pre-analytical handling of the samples was only described by Erturk et al. (4) and Donald (17). Although they cooled and centrifuged the samples directly after collection and stored them deep frozen until assaying, there was still a very wide variation in the ACTH concentration.

The diagnosis of SAI is generally easy to establish in patients with complete ACTH deficiency who show clear test results during stimulation tests. However, more difficult is the distinction between mild forms of ACTH deficiency and normal ACTH secretion. In this context, a cut-off level of 18 μg/dl for cortisol is arbitrary, and interpretation of the test result has to take into account the appropriateness of the test procedure and the reliability of the cortisol assay.

In our study, diagnosis of secondary pituitary insufficiency was established if the peak cortisol response was <18 μg/dl after appropriate hypoglycemia had been
achieved. This cut-off has been validated in our center by 30 tests in normal subjects, with the lowest peak cortisol response being 18.4 μg/dl. Concerning the patients in our study, all subjects with a peak cortisol concentration >18 μg/dl did not require glucocorticoid replacement, and pituitary–adrenal function remained stable during follow-up. Three of 32 patients with SAI had a peak cortisol concentration below, but close to the predefined cut-off level of 18 μg/dl (peak cortisol concentration 17.0–17.9 μg/dl). In these subjects, SAI may be questioned, since they did not require chronic glucocorticoid replacement therapy during follow-up. However, SAI represents a spectrum ranging from complete loss of ACTH secretion to very mild impairment of ACTH secretion, which is still sufficient for everyday life but requires glucocorticoid substitution therapy during stressful episodes such as acute illness or surgery. We considered these three subjects to have mild impairment of adrenal reserve and advised them to take glucocorticoids only.

Figure 1 (a) Peak plasma ACTH and peak serum cortisol during the ITT in 125 patients and 15 healthy controls. There is a significant positive correlation, but with a strong variation of the values (Spearman rank correlation: \( \rho = 0.446, P < 0.001 \)). The horizontal line represents the threshold peak cortisol level (18 μg/dl) indicating SAI in the ITT. (b) Peak plasma ACTH and peak serum cortisol concentration during the ITT in the subgroup of patients who had surgery more than 3 months prior to the ITT.
during episodes of increased stress. Peak plasma ACTH concentrations during ITT in these three subjects did not help to classify these subjects, and sensitivity and specificity of peak plasma ACTH concentrations during ITT as predictors of adrenal insufficiency did not change after exclusion of these subjects (data not shown).

It has been described before by several authors that in healthy subjects the pituitary secretes more ACTH than necessary for a sufficient stimulation of cortisol

Figure 2 Maximal plasma ACTH concentrations in patients without SAI, in patients with SAI (stimulated serum cortisol concentration <18 μg/dl) and in healthy subjects. Low stimulated ACTH concentrations occurred in patients without SAI, in patients with SAI and in healthy subjects.

Figure 3 The sensitivity and specificity of different peak ACTH concentrations which could be arbitrarily defined as cut-off values for pituitary insufficiency.
secretion (1, 5, 14, 17, 20, 21). Therefore, patients with a decreased pituitary ACTH reserve may have a normal cortisol response to hypoglycemic stress (1). This is supported by Oelkers et al. (5) who described that plasma ACTH levels between 50 and 60 pg/ml (or 11–13 pmol/l) stimulated the serum cortisol concentrations to almost 80% of the maximal increment obtained with plasma ACTH levels above 300 ng/l.

Looking at the 140 ITTs performed at our department, it becomes clear that peak ACTH concentrations are not a sensitive index of SAI. There was strong variation of the stimulated cortisol values at any arbitrarily chosen stimulated ACTH concentration. Patients with the same stimulated ACTH concentration could have either severe SAI or completely normal pituitary function. Only in those ITTs with very high peak ACTH concentrations could an SAI be excluded, but at the cost of a low sensitivity. On the contrary, there are many patients who had similar peak cortisol concentrations but had completely different stimulated ACTH concentrations. This suggests that hypoglycemia-induced ACTH concentrations are not a reliable method for the evaluation of SAI. This can be explained by the fact that adrenocortical responsiveness in patients with suspected SAI is not only dependent on peak plasma ACTH levels, but also on structural changes of the adrenal cortex, such as a adrenocortical atrophy due to chronically reduced plasma ACTH concentrations.

Other factors may contribute to the wide variation of the peak plasma ACTH concentrations during ITT. For example, measurements of plasma ACTH concentrations are less reliable than those of cortisol under routine conditions. Even under optimized conditions the inter- and intra-assay variability of plasma ACTH determination by RIA, IRMA or ELISA remains higher than that of serum cortisol. In addition, as a peptide hormone, ACTH is more rapidly degraded (which may be a pre-analytical problem under clinical routine conditions), whereas a steroid hormone like cortisol will remain unchanged even after prolonged exposure to room temperature. Many investigators, therefore, suggest immediate centrifugation and processing of the samples after collection or frozen storage until they are assayed. However, direct processing of the samples is not stipulated by the manufacturer of the assay (Nichols Institute Diagnostics), and ACTH concentrations did not change in random samples stored at room temperature for up to 4 h in our setting (Borne M Reincke, unpublished observation).

Based on our findings, we propose that the stimulated plasma ACTH concentration is a poor parameter for SAI in the ITT. With regard to the additional costs of the determination of the ACTH concentrations, we conclude that there is no additional benefit in determining plasma ACTH concentrations. Investigators should be discouraged from measuring plasma ACTH concentrations routinely during the ITT as well as overinterpreting high or low ACTH concentrations during the ITT as predictors of pituitary function.

Acknowledgements
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References
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