The one microgram adrenocorticotropin test in the assessment of hypothalamic–pituitary–adrenal function

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Abstract

The possibility of assessing hypothalamic–pituitary–adrenal (HPA) function by the standard ACTH test (250 µg) has been widely discussed in the past years and compared with the role of the insulin tolerance test (ITT). Recently, it was shown that low doses of ACTH, such as 1 µg i.v., induce a maximal adrenal response and, by reducing the discrepancies compared with the ITT also allow one to detect mild forms of secondary hypoadrenalism. In the present study the 1 µg ACTH test was performed in patients with hypothalamic–pituitary disease in order to assess adrenal function, and the results have been compared with those obtained after the insulin test. Fifty-seven patients (31 men and 26 women, aged 19–73 years) with hypothalamic–pituitary diseases were studied: 51 patients were affected with pituitary tumor and 6 patients had hypothalamic disorders. All these patients and 18 healthy volunteers (7 men and 11 women, aged 19–46 years) received 1 µg i.v. ACTH injection. In addition, the ITT (0.1–0.15 U/kg body weight) was performed in all patients. In normal subjects mean cortisol levels significantly ($P < 0.001$) increased from a baseline of 393 ± 43 nmol/l to a peak of 770 ± 41 nmol/l after 1 µg ACTH. In 44 patients with hypothalamic–pituitary disease 1 µg ACTH caused a cortisol rise similar to that of normal subjects (from 332 ± 17 to 769 ± 24 nmol/l; $P < 0.001$), while an impaired response (from 124 ± 23 to 312 ± 46 nmol/l) was observed in 13 cases (23%), 7 of them with low morning cortisol levels (10–127 nmol/l) and 6 with basal values at the lower limit of normality. The cortisol response to ITT was compared with that obtained after the 1 µg ACTH test: 10 patients failed both challenges, 4 patients who passed the ACTH test failed the ITT, while 3 patients who failed the ACTH test passed the ITT. The 23 out of 57 patients (40%) who showed a cortisol peak greater than 750 nmol/l after 1 µg ACTH had a normal response to ITT. A positive correlation between cortisol peaks after ACTH and after insulin was also found ($r = 0.68$, $P < 0.001$). Assuming a 100% accuracy of ITT, the low dose ACTH test yielded a 71% sensitivity and a 93% specificity.

In conclusion, the low-dose ACTH test is a useful, safe and inexpensive tool for the initial assessment of HPA function in patients with hypothalamic–pituitary disease. In fact, the ITT is unnecessary when cortisol peaks are greater than 750 nmol/l after 1 µg ACTH and also when very low cortisol basal levels indicate an overt hypoadrenalism. Within these limits the ITT is mandatory and its important role in the recognition of secondary adrenal failure is further confirmed.

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Introduction

The usefulness of the adrenocorticotropin (ACTH) test in the evaluation of the hypothalamic–pituitary–adrenal (HPA) axis in patients with suspected secondary hypoadrenalism has been widely discussed.

In the past, several studies have shown that the short Synacthen test (SST), performed with the standard dose of 250 µg, was able to replace the insulin tolerance test (ITT) in the detection of secondary hypoadrenalism (1–5). Although the SST only measures the functional integrity of the adrenal gland, it also provides an indirect assessment of the integrity of HPA function in conditions of chronic corticotropin deficiency, owing to

the correlation between peak cortisol levels after 250 µg ACTH and after ITT (1–5). However, it has been questioned whether the use of a supraphysiological dose, such as 250 µg, may give false positive results, particularly in conditions of partial ACTH deficiency, and it is not always correlated with the response to stress (6–9). Therefore, the ITT, despite its well-known contraindications, maintains its prominent role and is still regarded as the gold standard for the evaluation of the HPA axis.

Recently, it has been shown that in normal subjects a maximal adrenal response can be obtained with smaller doses of ACTH (0.5, 1, 5 µg) that may induce the release of immediately available cortisol, while the higher dose

of 250 µg probably causes further cortisol synthesis (10–12). Since the dose of 1 µg allows either the detection of mild forms of secondary hypoadrenalism that escape detection by the conventional SST, or the reduction of the discrepancies between ITT and SST, the challenge has been recommended as a preliminary screening procedure (13, 14). So far, the usefulness of a low dose ACTH test in the diagnosis of secondary hypoadrenalism has been evaluated in patients with hypothalamic–pituitary disorders (15–18) and in patients taking inhaled corticosteroids (19, 20).

Recent findings of a close association between cortisol responses to 1 µg ACTH and ITT in a small number of subjects with hypothalamic–pituitary disease (15–17), prompted us to study a large group of these patients. The aim of the present work was to evaluate the role of 1 µg ACTH in the assessment of adrenal function and to compare the results to those obtained after the insulin test in order to identify the proper role of each challenge.

Subjects and methods

Subjects

Fifty-seven patients (31 men and 26 women, aged 19–73 years) with hypothalamic–pituitary diseases were studied. Fifty-one patients had a pituitary tumor: 25 cases were affected with acromegaly, 15 with non-functioning adenoma, 10 patients had a prolactinoma and 1 had a follicle-stimulating hormone-secreting tumor. Six patients had hypothalamic disorders of various etiology: 3 cases had craniopharyngioma, 1 had an astrocytoma, 1 had a hamartoma, and 1 had a cyst of Rathke's pouch. Twelve patients were evaluated before treatment, while another forty-five cases were investigated after pituitary surgery (performed from 1 month up to 312 months) or radiation therapy. Patients with Cushing's disease were excluded. Fifteen patients were receiving steroid replacement treatment from 1–168 months (median 30 months); they tapered the dose and stopped the therapy at least 48 h before being investigated.

Eighteen healthy volunteers (7 men and 11 women, aged 19–46 years) recruited from the medical staff served as controls.

Informed consent was obtained from both patients and normal subjects.

Testing protocols

The low dose ACTH test was performed by the administration of 1 µg tetracosactrin (Synacthen, Ciba, Origgio, Italy) into an antecubital vein between 0800 h and 0900 h. Blood samples for cortisol determination were taken at 0, 30, 40 and 60 min after the injection. A stock solution of 5 µg/ml was kept at 4 °C in glass tubes. A concentration of 1 µg/ml was obtained by

Figure 1 Serum cortisol (means $\pm$ S.E.M.) modifications after ACTH (1 µg i.v.) in 44 responsive patients with hypothalamic–pituitary diseases (solid line, open squares), in 13 non-responsive patients (solid line, closed squares) and in 18 normal subjects (dotted line, closed circles). The asterisks indicate the significance between non-responsive patients and normal subjects ($P < 0.001$).

further dilution in a sterile saline solution, immediately before injection.

The insulin tolerance test was carried out by the i.v. administration of 0.1–0.15 U/kg body weight of short-acting human insulin after an overnight fast. Samples for cortisol and glucose determination were taken at 0, 30, 45, 60, 90 min. The hypoglycemia was considered as adequate when a nadir serum glucose lower than 2.2 mmol/l (40 mg/dl) was achieved. Criteria for normal responses to ITT were peak cortisol values greater than 500 nmol/l (2–4, 21, 22).

There were no serious adverse events during ACTH and insulin tests in any of the subjects. The tests were performed in a randomized order.

Hormone measurements

Serum cortisol (1 nmol/l = 0.0362 µg/l) levels were measured on unextracted samples by an RIA method (Diagnostic Products, Los Angeles, CA, USA). The intra- and interassay coefficients of variation were 7.3% and 5.4% respectively; the lower limit of sensitivity was 11 nmol/l. In our laboratory the limits of normality were 140–700 nmol/l. All samples from a given patient were measured in the same assay.

Statistical analysis

The data, expressed as means $\pm$ S.E., were analyzed using paired or unpaired *t*-test, as appropriate, and by the ANOVA test for repeated measures followed by Scheffe

Figure 2 Correlation between peak serum cortisol concentrations after ACTH (1 µg i.v.) and basal cortisol levels in 57 patients with hypothalamic–pituitary diseases ($r = 0.74$, $P < 0.001$). Dotted lines indicate normal ranges: the vertical line shows the lower limit of normality of basal cortisol levels and the horizontal line shows the cut-off value for normal cortisol responses after 1 µg ACTH.

Figure 3 Correlation between peak serum cortisol concentrations after ITT and basal cortisol levels in 57 patients with hypothalamic–pituitary diseases ($r = 0.64$, $P < 0.001$). Dotted lines indicate normal ranges: the vertical line shows the lower limit of normality of basal cortisol levels and the horizontal line shows the cut-off value for normal cortisol responses after ITT.

F-test. Correlations between basal and peak cortisol levels, and peak cortisol responses for each test were calculated by regression analysis. P values < 0.05 were considered significant.

Results

In normal subjects 1 µg ACTH injection (Fig. 1) significantly ($P < 0.001$) increased mean cortisol levels from a baseline of 393 ± 43 nmol/l to a peak of 770 ± 41 nmol/l (range 541–1215 nmol/l). All healthy volunteers showed a cortisol rise greater than 500 nmol/l: the peak level was obtained at 30 min in 10 subjects, at 40 min in 7 subjects and at 60 min in only 1 case.

Seven of the fifty-seven patients with hypothalamic–pituitary disorders (12%) showed basal cortisol levels (62 ± 19 nmol/l) below the normal limits. Following 1 µg ACTH (Fig. 1) 44 patients had a cortisol response similar to that in normal subjects, i.e. greater than 500 nmol/l: mean cortisol concentration rose from 332 ± 17 to 769 ± 24 nmol/l ($P < 0.001$; peak range 550–1304 nmol/l). On the other hand, in 13 patients (23%) serum cortisol response (from 124 ± 23 to 312 ± 46 nmol/l, peak range 10–484 nmol/l) was significantly lower than that in normal subjects ($P < 0.001$). The peak cortisol occurred at 30 min in 51% of the patients, at 40 min in 37% and at 60 min in the remaining patients.

An impaired response to ACTH was present in 7 patients with low morning cortisol levels (10–

127 nmol/l), and also in 6 patients with basal values at the lower limit of normality (155–243 nmol/l) (Fig. 2). A positive correlation between basal and peak cortisol levels was found ($r = 0.74$, $P < 0.001$).

The insulin test induced a normal cortisol rise (from 352 ± 21 nmol/l to 763 ± 32 nmol/l, $P < 0.001$; peak range 514–1303 nmol/l) in 43 out of 57 patients. An impaired cortisol response (from a baseline of 173 ± 37 nmol/l to 303 ± 48 nmol/l, peak range 10–487 nmol/l) was observed in the remaining 14 patients (25%).

Figure 3 shows that an impaired response to ITT was found in 6 patients with low basal cortisol levels (10–126 nmol/l), and also in 8 patients with low/normal or normal baseline values (165–365 nmol/l); only 1 patient with a reduced baseline cortisol (118 nmol/l) showed a normal response to ITT. A positive correlation between basal and peak cortisol levels was found ($r = 0.64$, $P < 0.001$).

The responsiveness to ITT was compared with that obtained after the 1 µg ACTH test (Fig. 4). Forty patients positively responded to both tests; ten patients failed both challenges, while four patients who passed the ACTH test failed the ITT. However, three patients who failed the ACTH test passed the ITT. It is worth noting that the 23 patients (out of 57 patients (40%)) who had a cortisol peak greater than 750 nmol/l after 1 µg ACTH always showed a normal responsiveness to ITT. Out of the 15 patients who were receiving steroid replacement therapy, 10 failed the 1 µg ACTH and 12 the insulin test. A positive correlation between peak cortisol levels after

Figure 4 Correlation between peak serum cortisol responses after ITT and after 1 µg ACTH in 57 patients with hypothalamic-pituitary diseases ($r = 0.68$, $P < 0.001$). Dotted lines indicate the cut-off value for normal cortisol responses after ACTH and ITT. The triangles show 7 patients with discrepant results, i.e. the 3 patients who failed the ACTH test and passed the ITT (closed triangles), and the 4 patients who passed the ACTH test but failed the ITT (open triangles).

ACTH and those after insulin was found ($r = 0.68$, $P < 0.001$).

Assuming a 100% accuracy of ITT, the 1 µg ACTH test yielded a 71% sensitivity and a 93% specificity. The positive predictive value was 77% and the negative predictive value was 91%.

Discussion

The evaluation of HPA function is of great importance in patients with hypothalamic-pituitary disease in order to establish the need for corticosteroid replacement therapy. It is known that the corticotropin challenge does not assess the entire axis and normal responses have been observed despite deficient responsiveness to insulin; moreover, metyrapone is not widely available owing to local restrictions, and even the reliability of the 250 µg ACTH test has recently been questioned (23). Since reported failures have been very rare (24), the ITT is considered the best method for detecting secondary hypoadrenalism, provided that the magnitude of cortisol release in response to stress is superimposable on that physiologically evoked by different stressful events. Recently, it has been suggested that stimulation with low doses of ACTH performs as well as the insulin test in the diagnosis of secondary adrenal insufficiency (14–16, 18).

As already reported (11, 12, 15), the present study confirms that the administration of 1 µg ACTH is able to induce a significant cortisol rise in healthy subjects after

30–40 min. In agreement with previous authors who defined the cut-off value for peak cortisol after 1 µg ACTH as 497–500 nmol/l (11, 15, 18, 25), all our normal controls showed a peak greater than 500 nmol/l and this limit was considered as the 'pass' criterion for the test.

An impairment of cortisol responsiveness to ACTH was observed in 23% of our patients with hypothalamic-pituitary disease. Interestingly, the ACTH stimulation also allowed the diagnosis of hypoadrenalism in 6 out of 57 patients (10.5%) with low/normal basal cortisol levels, indicating the poor value of a single morning determination in diagnosing adrenal failure. Indeed, when baseline cortisol is at the lower limits of normality, a stimulation test is mandatory, as confirmed by the finding of a positive responsiveness to ACTH in one woman with a low basal cortisol level.

In our series the insulin test revealed HPA dysfunction in 25% of patients, in agreement with previous data (5, 26, 27). In agreement with the response to the ACTH challenge, impaired responses were also found in patients with low/normal basal cortisol, confirming the necessity of further dynamic testing before instituting steroid replacement therapy in such cases.

Using the cut-off value of 500 nmol/l for acceptable cortisol responses to either ACTH or insulin (4, 11, 15, 21, 22), concordant results were present in 50 out of 57 patients (88%) who underwent both tests; moreover, there was a positive correlation between the responses after 1 µg ACTH and after insulin tests, as previously found (16). Contrary to the findings of Rasmuson *et al.* (16) who did not observe any discrepancy between tests, in our study some discordant results were obtained. In fact, 4 patients, operated on 5–48 months previously and without recent ACTH deprivation, passed the low dose test but failed the ITT, as occasionally reported by others (18): this finding militates against a good sensitivity of the 1 µg ACTH challenge. In contrast, only 3 patients who failed the low dose ACTH test positively responded to insulin, in agreement with similar findings in 2 patients reported by Tordjman *et al.* (15). This observation suggests that 1 µg ACTH is able to reveal a presumably mild hypoadrenalism that had been overcome by the more powerful stimulus of hypoglycemic stress, as described in steroid-treated patients (19).

Notably, the 23 out of 57 patients in whom 1 µg ACTH increased cortisol levels above 750 nmol/l, invariably passed the ITT. Since all patients here investigated had been operated on at least 1 month before testing and thus did not have any acute pituitary dysfunction, the unpleasant insulin challenge could have been avoided in a substantial number of cases (40%). On the whole, assuming the accuracy of the insulin test to be 100%, the 1 µg ACTH challenge had a sensitivity of 71% and a specificity of 93%.

Although a single morning cortisol measurement is of poor value in assessing the HPA axis, all patients with levels below 100 nmol/l failed both tests, confirming

that a very low baseline cortisol strongly suggests HPA insufficiency (26–28). In contrast, normal HPA function was recognized in all patients with basal cortisol above 400 nmol/l who showed positive responses to tests.

In conclusion, the present study confirms that in patients with hypothalamic–pituitary disease the 1 µg ACTH test is a useful, safe and inexpensive tool for the initial assessment of HPA function; particularly, it seems appropriate for exclusion of adrenal insufficiency in outpatients. In fact, it replaces the need for ITT when the peak cortisol response is greater than 750 nmol/l, strongly predicting a normal function of the axis. The finding of very low cortisol basal levels, indicating an overt hypoadrenalism, also makes the hypoglycemic stress unnecessary. In the grey zone within these limits the ITT is mandatory and its important role in the recognition of secondary adrenal failure is further confirmed.

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