Review Article

Evaluation of the hypothalamo–pituitary–adrenal axis: The insulin tolerance test and beyond

NIHAL THOMAS

ABSTRACT

Assessment of the hypothalamo–pituitary–adrenal axis is essential to determine the strength of the adrenocorticotrophic hormone (ACTH) reserve in relation to clinical and subclinical hypocortisolaemia. The insulin tolerance test has been established as the gold standard; however, the time and the potential hazards involved have led to a search for more viable alternatives. Numerous tests have evolved, which include the 8 a.m. fasting plasma cortisol, standard short synacthen test, low dose (1 μg) synacthen test, metyrapone provocation and glucagon stimulation test. The low-dose (1 μg) synacthen stimulation test has emerged as a potential alternative but further well designed trials are required to establish this.

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INTRODUCTION

Testing the hypothalamo–pituitary–adrenal (HPA) axis is essential to determine if a patient requires therapy for underlying pituitary-dependent hypocortisolaemia. It also helps in determining whether glucocorticoid therapy can be withdrawn without harmful consequences. A patient who is unnecessarily treated with glucocorticoids runs the risk of potentially serious adverse effects; whereas at the other end of the spectrum, a cortisol-deficient patient when not supplemented with glucocorticoids is likely to develop life-threatening problems at the time of stress or illness.[1]

Over the last thirty years, various tests have been devised to assess the function of the HPA axis. These include the 8 a.m. fasting plasma cortisol, insulin tolerance test (ITT), standard (250 μg) short synacthen test (intravenous and intramuscular), lysine–vasopressin stress test, low-dose (1 μg) short synacthen test, metyrapone provocation and glucagon stimulation (subcutaneous and intramuscular test).

THE INSULIN TOLERANCE TEST (ITT)

It seems paradoxical that historically some of the most sound studies to determine the integrity of the HPA axis, both physiologically and statistically, were performed three decades ago.[2,3] Greenwood *et al.*[4] and Landon *et al.*[5] demonstrated how hypoglycaemia was a powerful stimulus for the release of adrenocorticotrophic hormone (ACTH). They assessed the integrity of the HPA axis in 38 normal subjects and demonstrated marked and sustained rise in plasma cortisol and growth hormone in response to hypoglycaemia. They established that this increase was either significantly attenuated or absent in patients with hypopituitarism[4,5] and preserved in those with anorexia nervosa.

The procedure of the ITT involves injecting 0.1 IU/kg of insulin intravenously and provoking hypoglycaemia (blood glucose <2.2 mmol/L), and then measuring plasma cortisol levels at 30, 60, 90 and 120 minutes after infusion. If all the measured plasma cortisols are less than the recommended reference interval, it indicates that the patient has significant hypocortisolaemia.

By the early 1970s, ITT was firmly entrenched in the endocrine diagnostic battery, and still remains the reference standard for determining the stability of the HPA axis.

The test is time-consuming (needs up to three hours for adequate testing), labour-intensive (the presence of a physician is mandatory during the course of the test), needs careful monitoring and is not without risk. It cannot be utilized in patients who have underlying epileptic disorders, cerebrovascular or ischaemic heart disease as hypoglycaemia can provoke a seizure or myocardial ischaemia. However, the test appears to be safe when executed with proper monitoring. An analysis of the data from previous studies shows that there has been no reported serious adverse consequence[2,4–12] amongst 228 subjects over the last 32 years in groups who were carefully selected prior to testing.

THE METYRAPONE STRESS TEST

Even before Greenwood and Landon published their work on the ITT, the metyrapone stress test[13,14] was described by Liddle. (He was the first to describe the precise methodology for the synacthen stimulation test, as well as the dexamethasone suppression test for Cushing's syndrome.) The subject is given 750 mg of metyrapone 4-hourly for 2 days. Subsequently, either plasma or urinary 11-deoxycorticosteroids or urinary 17-ketogenic steroids or plasma 17-hydrocorticosteroids are measured and compared to baseline values.[15–18]

Jubiz *et al.* described a simplified version of the test. Metyrapone could be administered as a single dose of 30 mg/kg at midnight and blood drawn for deoxycortisol between 8 a.m. and 9.30 a.m.[16]

Despite the apparent ease in performing this procedure, it became unpopular for many reasons. Physicians who utilize the test commonly record complaints of nausea from subjects. The drug itself is not freely available in many centres. Moreover, there is the added disadvantage of having to assay 11-deoxycortisol, an intermediary metabolite which normally accumulates when metyrapone inhibits the action of 11, ß-hydroxylase (and is therefore lower in patients with hypocortisolaemia).

Christian Medical College and Hospital, Vellore 632004, Tamil Nadu, India

NIHAL THOMAS Department of Medicine and Endocrinology

Correspondence to Diabetic Centre, Villa 1, Prince of Wales Hospital, Barker Street, Randwick, New South Wales, NSW 2031, Australia

This assay is not routinely performed in many laboratories, and infrequent utilization of any test may lead to technical problems and a greater interassay coefficient of variation.

Jacobs *et al.*[19] described the lowest incidence of side-effects with the metyrapone stimulation test as compared to ITT, lysine–vasopressin test and the standard short synacthen test.

More recently, Fiad *et al.* evaluated the overnight metyrapone stimulation test.[14] The study included 398 patients on whom 576 tests were performed. Of these, 87 were rechecked against a standard (250 μg) synacthen stimulation test and 17 against the ITT. Amongst the 17 patients who had secondary adrenal insufficiency, there was a discord between the ITT and metyrapone stimulation test in only one patient. They also reported a novel side-effect in the form of nocturnal nightmares.

Streeten *et al.*[20] suggested that inpatient monitoring is required since metyrapone could potentially worsen symptoms of adrenal insufficiency. However, Fiad *et al.* did not encounter this complication in the 576 metyrapone stimulation tests performed on an outpatient basis. Though the study by Fiad *et al.* was a retrospective one and there were no controls, it does suggest that the test could possibly be used more often in assessing the HPA axis.

THE LYSINE–VASOPRESSIN STIMULATION TEST

The lysine–vasopressin stimulation test was described in 1965 by Landon *et al.*[7] when the metyrapone stimulation test was in vogue. Vasopressin was thought to have corticotrophin-releasing hormone-like activity.

Based on this hypothesis, they proposed that stimulation with this compound may release cortisol indirectly and could be used in the assessment of the HPA axis. They tested their hypothesis in 12 patients with proven hypopituitarism,[21] 9 of whom had chromophobe adenomas, 2 had Sheehan's syndrome, 1 had a craniopharyngioma, 2 with probable hypothalamic dysfunction and 2 had had an adrenalectomy.

This study was conducted prior to the advent of recent imaging techniques and radiological diagnoses such as 'hypothalamic dysfunction' were based on a negative angiography.

Lysine–vasopressin was infused intravenously in isotonic saline at a rate of 3 to 5 pressor units per hour for 2 hours. The results of this study showed a strong correlation between ITT and the lysine–vasopressin test in all patients with proven chromophobe adenomas. In those subjects with prediagnosed hypothalamic disease and craniopharyngioma, there was a positive response to lysine–vasopressin, but none to the ITT. They also found a good correlation between these two tests and a porcine corticotrophin stimulation test (10 IU per hour for 2 hours) in patients with pituitary tumours.

In 1969, Jacobs and Nabarro compared the lysine–vasopressin test with ITT and metyrapone stimulation test.[19] In 20 patients subjected to ITT, there was no increase in plasma 11-hydroxycorticosteroid release. Eight of these 20 patients were subjected to a lysine–vasopressin stimulation test, and 5 of them showed a normal surge in 11-hydroxycorticosteroid levels. This was in contrast to the results of Landon *et al.*[21] Arguably, a different metabolite was being measured in this test (11-hydroxycorticosteroid, unlike cortisol in the previous study). However, following this study the lysine–vasopressin test fell out of use, especially with the easily available and cheaper synacthen.

THE SYNACTHEN STIMULATION TEST

ACTH is a polypeptide made up of 39 amino acids. The biological activity is associated with the N-terminal amino acid sequence. A polypeptide containing the first 24 amino acids ($ß^{1-24}$-cosyntropin or tetracosactrin) was introduced in the 1960s,[22] and is used extensively as a convenient test of HPA axis integrity and is called synacthen.

In the initial physiological study, a significant rise in plasma cortisol occurred when synacthen was infused at a 3 μg dose. Doses of 2 μg or less did not stimulate the normal adrenal gland. Prior to this, porcine ACTH had been used for diagnostic purposes, but allergic reactions have been described with this preparation.

The standard synacthen test (SST) involves 250 μg of cosyntropin being given intravenously or intramuscularly followed by measurement of plasma cortisol at 30 and 60 minutes after infusion. The initial study which reported the use of synacthen as a diagnostic test, was performed on 40 patients receiving corticosteroids for asthma, 22 patients who had stopped corticosteroid therapy previously (1 week to 12 months), 9 patients with Addison's disease and 66 controls.[23] The investigators demonstrated an inverse correlation between the dose of prednisolone received by the patients and plasma cortisol levels. Since in actual fact, patients on long-standing corticosteriods are ACTH-deficient, it inducted the SST as a new diagnostic test for assessing the HPA axis.

The above study utilized the intramuscular route for administration of synacthen. Subsequently, Speckhart *et al.*[24] demonstrated the use of the SST using the intravenous route, in a study with a small sample size, consisting of a heterogeneous group of patients with primary adrenal insufficiency and pituitary disease. By the 1970s further studies had established the SST for evaluation of the HPA axis.

The dose of ACTH administered has been criticized as supraphysiological, and the sensitivity of the test has now been questioned. A case report by Soule *et al.* aptly illustrated this, wherein a patient achieved a normal surge of cortisol following standard synacthen stimulation on two occasions, in spite of florid symptoms of hypocortisolaemia.[25]

There is concern that the SST may fail to detect hypocortisolaemia caused by partial ACTH deficiency. This has been demonstrated by Fiad *et al.*[14] who reported an unacceptably high (53%) discord between the metyrapone stimulation test and the SST (with a good correlation between ITT and the metyrapone stimulation test).

In contrast, other workers have argued in favour of the SST. Jackson *et al.*[9] have shown a strong positive correlation between the results of the SST and the ITT ($r=0.96$). However, the study was hampered by two defects: small sample size and an inadequate time interval between the SST and ITT; an ITT conducted shortly after an SST may potentially lead to synacthen causing an interference in the cortisol levels measured after the ITT. This problem should be avoided by conducting the ITT and the SST on separate days. Jackson argues that if the tests are held on separate days, day-to-day inter-assay variations may occur.

Hurel *et al.* have addressed the same aspect in a study with a large sample size.[10] However, the patients were studied retrospectively and were compared with prospective controls.

The above studies have been performed in patients with primary pituitary disease. In contrast, the SST seems to be oversensitive in patients with secondary ACTH deficiency due to glucocorticoid therapy. In a study by Hurel *et al.*, 36% of patients failed to respond to SST, while responding normally to ITT. Orme *et al.* demonstrated similar inefficacy in SST when compared to ITT and the intramuscular glucagon stimulation test.[36]

To search for an alternative, Dickstein and his colleagues used

250 µg, 5 µg and 1 µg doses of synacthen for adrenocortical stimulation, measured plasma cortisol at 30 minutes and compared these variable dose techniques against the ITT in normal subjects.[26] There was an equally good response at 30 minutes, following 1 µg of synacthen compared to 250 µg at 30 minutes.

Tordjman *et al.* attempted to substantiate these results in patients with hypothalamo–pituitary disorders.[27] The study had a small sample size, there was lack of uniformity with the reference standard (some patients had an ITT, whereas others had a metyrapone stimulation test) and an arbitrarily low cut-off value for diagnosis of hypocortisolaemia (497 nmol/L in contrast to 600 nmol/L used by most other researchers), which will naturally lower the specificity of the test.

Further studies[28–30] have shown that the 1 µg short synacthen test has promise, but a large prospective trial involving multiple patient groups is lacking. Precautions that have to be exercised with the 1 µg short synacthen test are ensuring that the entire dose is injected and avoiding it in patients with probable recent pituitary insufficiency.[31]

THE BASAL 8 a.m. CORTISOL

In 1986 Hagg *et al.*[32] demonstrated that 8.00 a.m. basal cortisol levels of more than 300 nmol/L may rule out a pituitary origin for hypocortisolaemia when compared to the maximal plasma cortisol concentration during an ITT. In this study, all but one patient with an 8 a.m. plasma cortisol >300 nmol/L had a normal response to insulin hypoglycaemia (sensitivity: 0.86; specificity: 0.94, if basal cortisol >200 nmol/L; sensitivity: 0.67; specificity: 0.94, if basal cortisol >300 nmol/L). They suggested that a basal plasma cortisol of >300 nmol/L might rule out the need for further evaluation of the HPA axis. This may be a useful screening test, but a low sensitivity of 67% is unacceptable when taking into account a potentially life-threatening complication like a hypoadrenal crisis.

THE GLUCAGON STIMULATION TEST

The glucagon stimulation test was initially used to evaluate growth hormone deficiency.[33] It involves injecting 1 mg of glucagon intramuscularly and drawing blood samples at 90, 120, 150 and 180 minutes after injection, for testing hormonal levels (growth hormone and cortisol).

Subsequently, its use in evaluation of the HPA axis was considered.[34] Rao *et al.* have described nausea as an adverse effect in 30% of subjects and vomiting in approximately 10%.[35] Very little work has been done to compare the ITT with the glucagon stimulation test. There was a good correlation (r=0.695) between ITT and the glucagon stimulation test in a study conducted on 16 patients by Omre *et al.*[36] The receiver operative characteristic plots in this study revealed that the glucagon stimulation test had a better diagnostic utility than SST.

THE CORTICOTROPIN-RELEASING HORMONE (CRH) STIMULATION TEST

Schlaghecke*et al.* attempted to use the CRH stimulation test in the assessment of the HPA axis in patients receiving prednisolone.[12] They administered 100 µg of CRH as a bolus and collected plasma for cortisol every 15 minutes for 2 hours thereafter. They performed an ITT in 61 of these subjects. There was a good correlation between ITT and the CRH stimulation test (r=0.82). However, CRH is expensive and not available in many centres.

FUTURE CONSIDERATIONS

In summary, ITT remains the reference standard for the assessment of function of the HPA axis and is safe when performed under careful monitoring. However, simplified tests are required for assessment of patients with probable ACTH deficiency on an outpatient basis. The standard short synacthen test appears to lack sensitivity in patients with primary pituitary disease. The low dose (1 µg) short synacthen test appears to hold promise; however, a large randomized trial needs to be performed to establish this. The metyrapone stimulation test which appears to have less adverse effects than initially perceived also requires a prospective assessment.

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