Provocative Tests of the Hypothalamic-Anterior Pituitary-Target Organ Axis

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SUMMARY

Anterior pituitary peptide hormones which circulate at nanogram or picogram levels are readily measurable under basal conditions by radioligand assay. In addition, functional reserve may be further assessed by the use of specific provocative tests 'aimed' at either hypothalamus, anterior pituitary or the target organ. Some of these are briefly outlined.

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During the last decade the assessment of hypothalamic-anterior pituitary-target organ function has been revolutionised by the development of highly sensitive and specific assay techniques capable of measuring hormones in amounts as low as 10^{-9} to 10^{-12}g, the circulating levels in biological fluids. In addition, agents are now available capable of provoking anterior pituitary and peripheral hormone release by specific stimulation of the hypothalamus, pituitary or target organs. Thus, not only can basal hormone levels be accurately estimated, but the reserve of the secreting organ can be assessed, often in specific physiological terms. This paper deals in brief with the principle of the newer assays used to measure circulating hormone levels and then discusses these dynamic or provocative tests.

MEASUREMENT OF HORMONES IN BIOLOGICAL FLUID

When hormones circulate in relatively high concentrations, they are measured biochemically, e.g. the fluorometric assay for plasma cortisol. However, most hormones, particularly the polypeptides, circulate at nanogram (10^{-9} g) or picogram (10^{-12}g) levels and more sensitive techniques have to be employed. The traditional method of estimating these circulating hormones was by biological assay, which measured a biochemical or histological end-point of hormone function. These techniques were relatively insensitive, and specificity was often suspect as substances other than the hormone occasionally simulated the biological effects, as was found with the measurement of insulin-like activity. The probable existence of hormone antagonists in biological fluids also complicated this type of system.

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PRINCIPLES OF RADIOLIGAND ASSAY

$$\text{Hormone}^* \; + \; \text{Binding} \; \rightleftharpoons \; \text{Binding—Hormone}^*$$
$$\text{(F)} \qquad\qquad \text{agent} \qquad\qquad \text{agent} \qquad \text{(B)}$$
$$+$$
$$\text{Hormone}$$
$$\Updownarrow$$
$$\text{Binding — Hormone}$$
$$\text{agent}$$

Binding agent — Antibody — Radio-immunoassay; carrier
protein — Competitive protein binding assay;
Target tissue — Radioreceptor assay

Fig. 1. **Principles of radioligand assay for serum or plasma hormone levels. See text for details.**

Steroid or peptide hormones are currently measured by the radioligand technique (Fig. 1). This method depends on competition between radioactively labelled and unlabelled hormone for binding to any of a variety of binding agents. An increasing amount of unlabelled hormone displaces labelled hormone from the binding agent leading to a reduction in radioactive hormone binding (B) and an increase in free radioactive hormone (F). Various techniques exist for measuring the ratio of B to F. This proportionate drop of the radioactive bound fraction (expressed as B/F ratio) with increasing unlabelled hormone (standard) may be plotted graphically as in the luteinising hormone (LH) assay shown in Fig. 2. If an unknown plasma or serum is substituted for standards and the labelled hormone and binding agent kept constant, the

Fig. 2. **A typical radio-immunoassay standard curve for luteinising hormone. See text.**

B/F obtained will reflect the quantity of hormone therein. If the binding agent is an antibody to the appropriate hormone, the technique is known as radio-immunoassay. This is by far the most common type of radioligand system currently in use.

The competitive protein binding assay employs circulating hormone carrier proteins as binding agents, for example hormone-binding protein in the measurement of cortisol[1] or testosterone.[2] Recently, hormonal target tissue has been used as the binding agent; the so-called radio-receptor assay, as exemplified by the LH assay employing interstitial cell fractions of rat testis.[3] This latter technique is still experimental.

DYNAMIC PROVOCATIVE TESTS OF ANTERIOR PITUITARY-TARGET ORGAN FUNCTION

Fig. 3 illustrates the levels at which provocative function tests might be carried out in relation to the hypothalamus, anterior pituitary and target organ. The hypothalamus might be activated during sleep, by stress (e.g. insulin hypoglycaemia), by non-specific peptides such as arginine, biogenic amines like L-dopa or drugs influencing them such as chlorpromazine, or inactive analogues of peri-

pheral hormones which may interfere with the hypothalamic feedback mechanisms and so discharge the appropriate hypothalamic releasing factor, for example clomiphene citrate. Blockade of peripheral hormone synthesis may likewise release the hypothalamus and anterior pituitary from the negative feedback effects of that hormone, and so elevate its precursors, as exemplified by the effects of metyrapone on cortisol synthesis.

Provocative tests may stimulate the anterior pituitary directly to release its appropriate hormone. The recent discovery of the peptide sequence of thyrotrophin-releasing factor (TRF)[4] or the FSH/LH-releasing factor (FSH/LH-RF)[5] has enabled these peptides to be synthesised. Both are now available for provocative testing of pituitary function, respectively testing the release of TSH and pro-lactin, and the gonadotrophic hormones LH and FSH.

The administration of pituitary hormones may directly stimulate the target organ and so test that organ's responsiveness to the pituitary. Examples of such provocative tests include the administration of bovine TSH to elevate the thyroid ^{131}I uptake, the use of ACTH (or more recently, synacthen, the synthetic 1 - 24 peptide) to stimulate cortisol release from the adrenal, and the administration of human chorionic gonadotrophin (HCG), which has a biological action very similar to that of LH, to test testicular response as measured by plasma testosterone levels.

These tests will be considered in greater detail.

STIMULI TO HORMONE RELEASE   PROVOCATIVE TEST

Sleep
Biological rhythm   Psyche, stress   Sleep, hypoglycaemia, arginine, L-dopa, chlorpromazine, clomiphene

HYPOTHALAMUS

Releasing factor   Synthetic TRF, FSH/LH RF

ANT. PITUITARY

Trophic hormone   ACTH, TSH, HCG (LH)

TARGET ORGAN

Peripheral hormone   Metyrapone (blockade of cortisol synthesis)

Fig. 3. Provocative tests of the hypothalamic-pituitary-target organ axis.

effect of oestrogen feedback. As a consequence, levels of plasma LH rise gradually. Clomiphene citrate is given orally in a dose of 3 mg/kg/day (average 200 mg/day) for 10 days and blood is taken for LH immunoassay at the onset and during the course of the test. A typically normal response is shown in Fig. 6.
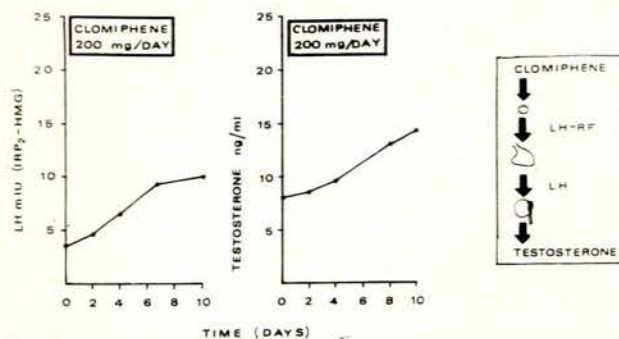
Fig. 6. Physiological rationale of, and a normal serum luteinising hormone (LH) and testosterone response to oral clomiphene citrate administration.

The demonstration of a simultaneous rise in plasma testosterone provides additional evidence of the testes' ability to respond to endogenous LH. The hypothalamic-pituitary-gonadal axis in males may therefore be conveniently assessed in a single test.

Metyrapone Test

Metyrapone, given orally (750 mg every 4 hours for 6 doses), blocks 11-hydroxylation of cortisol and lowers plasma cortisol levels. There is a slow rise of ACTH with an increase in the immediate precursor of cortisol, 11-deoxycortisol (11-DOC), which is metabolised to compounds measurable as urinary 17-hydroxycorticoids or 17-oxo(keto)-genic steroids. The increase in these metabolites implies the integrity of the hypothalamic-pituitary-adrenal axis. Twenty-four-hour urine collections are made two
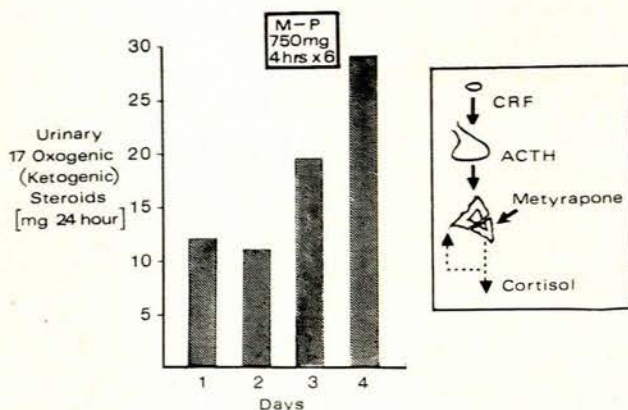
Fig. 7. Physiological rationale of, and a normal urinary 17-oxogenic steroid response to blockade of cortisol synthesis with oral metyrapone.

days preceding the day of, and the day succeeding, metyrapone administration. A typically normal test is shown in Fig. 7.

As urine collections are time-consuming and often inaccurate, and as completeness of metyrapone-induced blockade of cortisol synthesis is never certain, a modification of the test has recently been proposed[6] in which plasma cortisol and 11-DOC are measured simultaneously just before and 4 hours after completion of metyrapone administration. The response of 11-DOC can thus be assessed against the drop in plasma cortisol.

TRF-TSH Test

The hypothalamic thyrotrophin-releasing factor has recently been identified as a tripeptide, pyroglutamyl-histadyl-prolineamide.[4] This has subsequently been synthesised[7] and can be used for physiological studies and the clinical testing of pituitary-thyroid function in man, as it directly stimulates the anterior pituitary to release TSH. The stimulated thyroid subsequently releases T_3 and T_4, the latter rising inconsistently. TRF is usually given by the intravenous route in a single injection of 200 μg. Side-effects are minor and transient, and include flushing, nausea and a desire to micturate. Blood is taken basally for plasma immunoreactive TSH and tri-iodothyronine (T_3). Twenty, 40 and 60 minutes after administration of TRF, blood is sampled once more for TSH and again at 3 and 5 hours for T_3. A typically normal result is shown in Fig. 8. TSH rises acutely, peaks 20 minutes after TRF, and drops progressively towards basal levels. Plasma T_3 only rises after that time, achieving a peak between 3 and 5 hours.
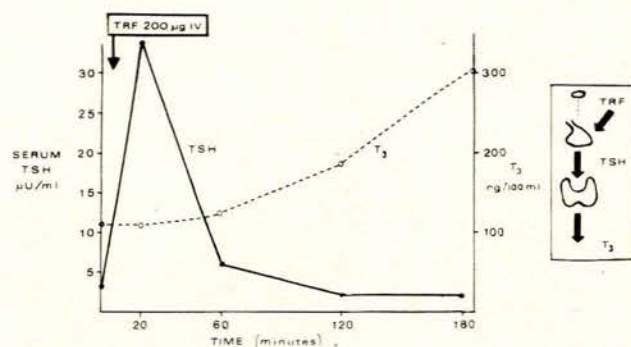
Fig. 8. Physiological rationale of, and a normal serum thyrotrophin (TSH) and tri-iodothyronine (T_3) response to 200 μg intravenous synthetic thyrotrophin-releasing factor (TRF).

The test is useful in the assessment of pituitary-thyroid reserve in patients with pituitary tumours or suspected hypopituitarism,[8] the responses being low. In hypothalamic hypothyroidism, TSH rise may be delayed but is otherwise normal.[9] In primary (thyroid) hypothyroidism, TSH is elevated with a hyper-response to TRF,[10] with low T_3 levels throughout the test. In hyperthyroidism, TSH is usually unmeasurably low, with a very poor response to TRF[20]

as its secretion is being maximally suppressed by the high endogenous T_3 and T_4 released by the hyperactive thyroid gland. This test has occasionally been helpful in subtle forms of hyperthyroidism where conventional studies may be negative, for example in the newly-recognised condition of tri-iodothyronine (T_3) thyrotoxicosis.

Quite unexpectedly, HPrl has been found to rise after TRF,[11] although the physiological relevance of this is not yet clear. The same blood samples used in the TSH estimation may be assayed for HPrl, providing yet another useful and sensitive test of anterior pituitary function. Basal HPrl is elevated, and the response to TRF exaggerated in many patients with the amenorrhoea-galactorrhoea syndrome, whether drug-induced or associated with hypothalamic or pituitary tumours.

FSH/LH-RF Test

The peptide sequence of FSH/LH-releasing factor (FSH/LH-RF) was recently announced[5] and the decapeptide has been synthesised.[12] Like TRF, it is a direct stimulus to the pituitary gland and may be diagnostically helpful in diseases of the hypothalamic-pituitary-gonadal axis. In combination with the clomiphene test, it should theoretically help to delineate the precise site of any lesion, as in hypothalamic diseases the response of the pituitary gonadotrophins to FSH/LH-RF should be normal (which in practice it rarely is), whereas clomiphene would be ineffective. FSH/LH-RF (100 μg) is given intravenously (25 μg in children) and blood taken basally and 20, 60 and 120 minutes after the injection for plasma immunoreactive LH and FSH. A typical result is shown in Fig. 9.
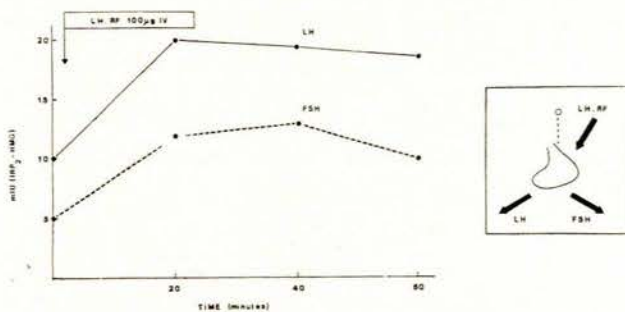
Fig. 9. Physiological rationale of, and a normal serum follicle-stimulating hormone (FSH) and LH response to 100 μg intravenous synthetic FSH/LH-RF.

LH and FSH rise abruptly at 20 minutes (LH responding to a much greater extent) and gradually return towards normal values.[33] Only one gonadotrophin-releasing factor may be present; its ability to stimulate either LH or FSH possibly varies with the circulating oestrogen level.[34] Very low oestrogen levels favour FSH and slightly higher levels favour LH response to the decapeptide. In adolescents or adults, therefore, LH is more elevated than FSH after the same dose of FSH/LH-RF, as illustrated, whereas in infants, FSH secretion is favoured.[35] LH and FSH responses may also vary with the stage in the menstrual cycle, being

somewhat elevated in the luteal phase, and greatest just before ovulation.[16]

This test is useful in children with delayed puberty.[13] If endocrine function is normal, there is nearly always a gonadotrophin response to FSH/LH-RF, whereas with organic hypothalamic or pituitary disease, the response is usually blunted or absent. It is of interest that even in the presence of hypothalamic disturbance, poor gonadotrophin release is often found. Presumably some prior activation by endogenous FSH/LH-RF is necessary in order to prime the pituitary to respond to the effect of the exogenously administered releasing factor.[13] Possibly the administration of repeated doses or of a long-acting FSH/LH-RF might distinguish between hypothalamic and pituitary disease, but as yet such studies have not been forthcoming.

Pituitary Trophic Hormone Stimulation Tests

The measurement of the thyroid ^{131}I uptake before and after bovine TSH (10 units intramuscularly per day for 4 days) is useful to differentiate primary from secondary hypothyroidism in patients with clinical and biochemical evidence of myxoedema. This test has largely been replaced by the immunoassay of a single plasma sample for TSH, which is elevated in primary (thyroid) hypothyroidism and low in hypothalamic or pituitary disease. A typically normal result is shown in Fig. 10.
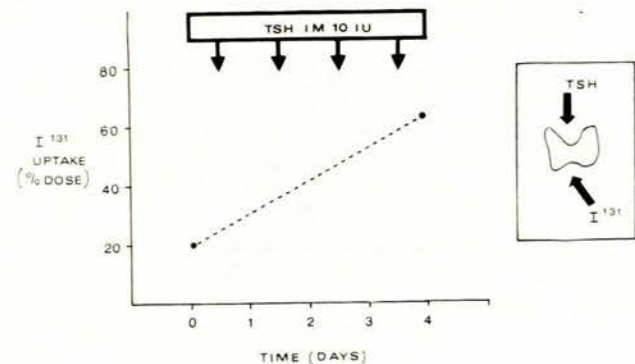

Fig. 10. Physiological rationale of, and a normal increase in thyroid ^{131}I uptake in response to, stimulation by bovine TSH 10 IU daily intramuscularly.

ACTH, given either intramuscularly or as a 4-6-hour intravenous infusion, has for long been used as a test of adrenal responsiveness. Recently ACTH extract has been replaced by the synthetic ACTH 1 - 24 amino acid peptide (synacthen), given intravenously or intramuscularly in a dose of 0,25 mg. Blood is taken basally and 30 and 60 minutes for cortisol estimation. A normal response is shown in Fig. 11. The test is simple and as the fluorometric assay for cortisol[17] is widely available, it is ideally suited to the screening of patients with suspected Addison's disease. It may be used as an outpatient procedure.

Human chorionic gonadotrophin (HCG) has a similar action to LH and can be used as a provocative test of
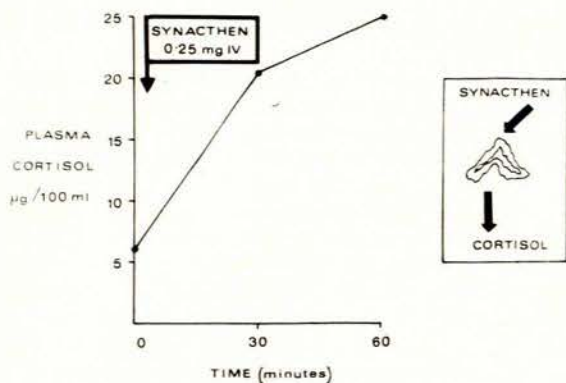
Fig. 11. Physiological rationale of, and plasma cortisol response to the stimulus of 0,25 mg synacthen intravenously.
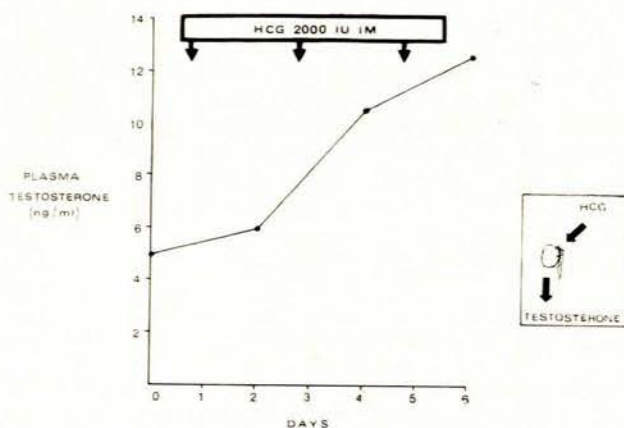

Fig. 12. Physiological rationale of, and normal plasma testosterone response to the stimulus of intramuscular HCG 2 000 IU on alternate days.

testicular function. There are many dose regimens recommended for this test and one is shown in Fig. 12, where HCG 2 000 units is given intramuscularly on days 1, 3

and 5 and blood taken for plasma testosterone on days 0, 2, 4 and 6. Basal testosterone levels should at least double if normal testicular Leydig cell responsiveness is present.

CONCLUSIONS

This paper is not comprehensive and deals exclusively with provocative tests of pituitary-target organ reserve. Other tests of the hypothalamic-pituitary pathway, for example assessment of the nyctohemeral rhythm of cortisol secretion, or tests of pituitary suppressibility in diseases of pituitary hyperfunction, may be very valuable. Indirect tests may be useful, for example the oral water load test and its response to hydrocortisone in the screening of suspected Addison's disease in areas where no biochemical facilities exist. Finally, radiology is crucial in the localisation of tumours of the pituitary or target organs, but falls outside the scope of this review.

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