CLINICAL RESEARCH

Combined use of vasopressin and synthetic hypothalamic releasing factors as a new test of anterior pituitary function

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Abstract

Nine normal volunteers and 15 patients with pituitary disorders were given a combined test of anterior pituitary function using four hypothalamic releasing factors and arginine vasopressin. Rapid sequential intravenous infusions of human corticotrophin releasing factor 100 µg, growth hormone releasing factor 100 µg, luteinising hormone releasing hormone 100 µg, and thyrotrophin releasing hormone 200 µg were administered. Arginine vasopressin (10 pressor units) was given intramuscularly at the same time. Plasma or serum samples were assayed for concentrations of cortisol, growth hormone, luteinising hormone, follicle stimulating hormone, prolactin, and thyroid stimulating hormone at multiple times for 120 minutes. No troublesome side effects occurred. The results of the releasing factor combined test with arginine vasopressin were compared in the same subjects with a conventional combined test using insulin together with thyrotrophin releasing hormone and luteinising hormone releasing hormone. No difference was observed in the basal and peak concentrations of luteinising hormone, follicle stimulating hormone, thyroid stimulating hormone, and prolactin. Both cortisol and growth hormone responses to the releasing factors with arginine vasopressin were much greater than those seen with insulin induced hypoglycaemia or the combined releasing factors without arginine vasopressin. Patients with pituitary hypofunction were similarly recognised in both studies. There was a rapid increase in all hormone values with a peak usually by 60 minutes.

In most people adequate assessment of individual hormone reserves may be achieved using basal, 30 minute, and 60 minute samples. This new combined releasing factor test appears to be a safe, rapid, and useful test of anterior pituitary function.

Introduction

Several stressful stimuli have been used to assess pituitary growth hormone and adrenocorticotrophic hormone reserves, of which insulin induced hypoglycaemia is the most widely used. Implicit in the induction of hypoglycaemia is the necessity for continuous and close medical supervision. Furthermore, this test very often causes unpleasant effects. The test is also reported to give variable growth hormone responses under similar conditions.¹

Over the past two decades hypothalamic factors have been described which selectively stimulate specific cell types of the adenohypophysis. The isolation and characterisation of thyrotrophin releasing hormone² and luteinising hormone releasing hormone³ led to extensive clinical use of these factors combined with insulin hypoglycaemia as a combined test of anterior pituitary function.⁴ Two further hypothalamic peptides have now been characterised and synthesised: growth hormone releasing factor⁵ and corticotrophin releasing factor.⁶

Growth hormone releasing factor selectively stimulates the release of growth hormone in man⁷ and has been used in clinical studies in adults^{8,9} and children.^{10,11} Corticotrophin releasing factor is effective in rat pituitary in stimulating release of adrenocorticotrophic hormone both *in vitro*¹² and *in vivo*.¹³ Corticotrophin releasing factor also releases adrenocorticotrophic hormone with consequent increases in plasma cortisol in man,¹⁴⁻¹⁶ though it is generally thought to be less effective than insulin hypoglycaemia.¹⁷ In normal men the coadministration of the four release factors thyrotrophin releasing hormone, luteinising hormone releasing hormone, corticotrophin releasing factor, and growth hormone releasing factor results in increments in the plasma concentrations of their target anterior pituitary hormones indistinguishable from those seen after separate administration.¹⁸ Vasopressin is a potent stimulator of the secretion of adrenocorticotrophic hormone in isolated rat pituitaries¹⁹ and in human pituitary tumour cells.^{17,20} Both studies showed strong synergistic stimulation of adrenocorticotrophic hormone secretion by ovine corticotrophin releasing factor and synthetic vasopressin.

We report the combined use of the four releasing factors together with vasopressin as a combined test of anterior pituitary function (the releasing factor test) in normal volunteers and patients with pituitary disorders. The results were compared with those of the conventional combined test using insulin (the insulin combined test).

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Materials and methods

Synthetic luteinising hormone releasing hormone was purchased from Ayerst Laboratories, thyrotrophin releasing hormone from Roche Products Ltd, and arginine vasopressin from Parke-Davis (Pitressin). Soluble insulin was used in the insulin combined test.

Growth hormone releasing factor (1-29 amide) and human corticotrophin releasing factor were obtained from Bachem Laboratories, Torrance, USA. Each peptide was dissolved in sterile hydrochloric acid (1 nmol/l; 3.6 ng/100 ml) and sodium chloride (154 nmol/l; 9.0 ng/ml) containing 0.25% human serum albumin. The solution was sterilised by Millipore filtration and shown

TABLE I—Clinical effects of combined pituitary function tests

	Nausea	Mood change	Sweating	Pallor	Flushing	Palpitation
Insulin combined test (n=23)	16	18	23	21	0	21
Releasing factor test (n=24)	15	8	2	0	18	5

TABLE II—Hormone results in normal subjects during insulin combined test and releasing factor test (n=9 for both tests). Results expressed as mean (SEM) [range]

	Basal		Peak	
	Insulin combined test	Releasing factor test	Insulin combined test	Releasing factor test
Luteinising hormone (U/l)	4.5 (0.4) [2.7-6.5]	3.8 (0.7) [2.2-8.2]	27.0 (5.1) [11-50]	23.6 (5.1) [5.4-50.0]
Follicle stimulating hormone (U/l)	2.7 (0.4) [1.0-4.3]	2.4 (0.4) [1.0-4.5]	6.2 (1.2) [1.6-13.7]	6.3 (1.3) [1.7-17.9]
Thyroid stimulating hormone (mU/l)	<1.0 [all <1.0]	<1.0 [1.0-1.4]	10.2 (2.4) [2.1-25.0]	14.0 (2.8) [3.1-26.3]
Prolactin (µg/l)	13.2 (2.8) [8.8-29.0]	11.0 (2.8) [6.7-28.0]	62.2 (8.8) [31-81]	58.8 (9.8) [32-59]
Cortisol (nmol/l)	501 (46) [312-669]	332 (50) [93-619]	674 (42) [460-785]	740 (50) [569-1180]
Growth hormone (mU/l)	5.8 (1.4) [1.0-11.4]	6.7 (1.8) [1.1-22.0]	31.6 (8.8) [16-77]	47.8 (9.1)* [8.1-141.0]

*Compared with result of insulin combined test $p < 0.001$.

Conversion: SI to traditional units—Cortisol: 1 nmol/l $\approx$ 0.036 µg/100 ml.

by the limulus test to be non-pyrogenic. It was lyophilised under vacuum and stored in 100 µg aliquots at -20°C . Immediately before use each phial was brought to room temperature and made up for injection with sodium chloride (154 nmol/l) and hydrochloric acid (1 nmol/l).

Protocol—An intravenous cannula was inserted 30 minutes before the start of each study at 0800. The insulin combined test consisted of consecutive intravenous injections over two minutes of luteinising hormone (100 µg), thyrotrophin releasing hormone (200 µg), and soluble insulin (0.15 U/kg). The releasing factor test consisted of consecutive intravenous injections over two minutes of growth hormone releasing factor (100 µg), human corticotrophin releasing factor (100 µg), luteinising hormone (100 µg), and thyrotrophin releasing hormone (200 µg). An intramuscular injection of arginine vasopressin (10 pressor units) was also given at time zero. For both tests blood was drawn at -30, zero, 30, 60, 90, and 120 minutes. In nine subjects, including four normal volunteers, an identical study was undertaken using the four releasing factors but omitting vasopressin. Concentrations of growth hormone, luteinising hormone, follicle stimulating hormone, thyroid stimulating hormone, and prolactin were measured by double antibody radioimmunoassay.²¹ Cortisol was measured by a solid phase first antibody radioimmunoassay.²²

Subjects—Nine normal volunteers (six women) aged 22-28 years (mean 24) underwent both the insulin combined test and the releasing factor test in random order. In four women the releasing factor test was undertaken in the early follicular phase and in two in the mid-luteal phase. The insulin combined test was carried out within a few days before or after the releasing factor test. Fifteen patients aged 25-27 years (mean 36) were similarly studied with both tests: five had prolactinomas (two untreated, two in whom bromocriptine was stopped six weeks before the study, one given interstitial radiation), five acromegaly (two active, one posthypophysectomy, one given interstitial radiation, one given external irradiation); one Cushing's syndrome due to ectopic secretion of adrenocorticotrophic hormone; two non-functioning tumours; and two idiopathic isolated diabetes insipidus. No patient was having cortisone replacement therapy, but two were receiving thyroxine, one testosterone, and one oestrogen replacement therapy. All subjects fasted overnight and gave informed consent to the procedures.

Statistical methods—Values are presented as the mean and standard error

of the mean (SEM), except where mentioned otherwise. The Mann-Whitney test was used to evaluate statistical significance when comparing the results for the two protocols.

Results

Clinical effects—Table I summarises the observed effects of both the insulin combined test and the releasing factor test. No changes occurred in blood pressure or temperature after administration of the releasing factors. An increase in pulse rate of 5-10 beats/min and lasting up to 30 minutes was noted in most subjects undergoing the insulin combined test. With the releasing factor test transient nausea, flushing, and a feeling of warmth over the face and upper trunk occurred. Neither symptom persisted for more than 10 minutes in any subject and was in no case described as unpleasant. The mean nadir blood glucose value during the insulin combined test was 1.8 mmol/l (32.4 mg/100 ml; range 1.6-2.2 mmol/l (28.8-39.6 mg/100 ml)) which was enough to produce symptoms in all subjects.

Luteinising hormone, follicle stimulating hormone, thyroid stimulating hormone, and prolactin—There was no significant difference in the response of any of these hormones to either the insulin combined test or the releasing factor test in the patients with pituitary disease. Table II gives the results for

FIG 1—Comparison of plasma cortisol response to releasing factor test (●) and insulin combined test (▲) in 14 patients with pituitary disorders. Points are means. Bars are SEM. * $p < 0.02$, ** $p < 0.01$.

Conversion: SI to traditional units—Cortisol: 1 nmol/l $\approx$ 0.036 µg/100 ml.

normal volunteers. In these also there was no significant difference in the hormonal response between the two tests.

Cortisol—Figure 1 shows the changes in plasma cortisol values after both combined pituitary function tests in all patients except the one with Cushing's syndrome. The single patient with a history of external irradiation to the pituitary gland showed a considerably greater peak concentration (833 nmol/l; 30 µg/100 ml) in the releasing factor test than in the insulin combined test (360 nmol/l; 13 µg/100 ml). For all subjects combined the mean basal concentration was 346 (SEM 32) nmol/l (12.5 (1.2)

$\mu\text{g}/100\text{ ml}$) before the releasing factor test rising to a mean peak of 719 (40) nmol/l (26.0 (1.4) $\mu\text{g}/100\text{ ml}$) at 60 minutes, and 404 (33) nmol/l (14.6 (1.2) $\mu\text{g}/100\text{ ml}$) before the insulin combined test rising to 604 (58) nmol/l (21.9 (2.1) $\mu\text{g}/100\text{ ml}$) at 90 minutes ($p<0.001$). The mean maximal increment from basal was thus greater with the releasing factor test (373 (19) nmol/l;

FIG 2—Comparison of serum growth hormone response to releasing factor test (●) and insulin combined test (▲) in 13 patients with pituitary disorders. Points are means. Bars are SEM. ** $p<0.01$.

FIG 3—Comparison of plasma cortisol response to combined releasing factors with (●) and without (○) arginine vasopressin in nine subjects. Points are means. Bars SEM. * $p<0.05$.

Conversion: SI to traditional units—Cortisol: 1 nmol/l $\approx$ 0.036 $\mu\text{g}/100\text{ ml}$.

13.5 (0.7) $\mu\text{g}/100\text{ ml}$) than with the insulin combined test (200 (11) nmol/l; 7.2 (0.4) $\mu\text{g}/100\text{ ml}$) ($p<0.01$). In the nine normal volunteers the mean cortisol increment from basal was 408 nmol/l (14.8 $\mu\text{g}/100\text{ ml}$; range 104–705 nmol/l (3.8–25.5 $\mu\text{g}/100\text{ ml}$)) during the releasing factor test and 173 nmol/l (6.3 $\mu\text{g}/100\text{ ml}$; range 135–444 nmol/l (4.9–16.1 $\mu\text{g}/100\text{ ml}$)) after the insulin combined test ($p<0.01$) (table II).

Growth hormone—The growth hormone response to the two combined pituitary function tests in the patients was compared (fig 2) after excluding two patients with high circulating concentrations of growth hormone due to active acromegaly. Basal growth hormone concentrations were similar in the

two tests but the peak concentrations achieved were very different. The releasing factor test produced a peak of 35.7 (SEM 3.6) mU/l at 30 minutes compared with a peak of 22.0 (3.6) mU/l with the insulin combined test at 60 minutes ($p<0.01$). A greater growth hormone response to the releasing factor test was also seen in the nine normal subjects (table II).

Releasing factor test without vasopressin—The releasing factor test was repeated in nine subjects with the omission of arginine vasopressin. No person noted any difference in symptoms between the two tests. There was no significant difference between the basal and stimulated concentrations of luteinising hormone, follicle stimulating hormone, thyroid stimulating hormone, and prolactin. The cortisol and growth hormone responses, however, were significantly greater when the releasing factors were given with arginine vasopressin. With vasopressin cortisol rose from a basal concentration of 440 (SEM 57) nmol/l (15.9 (2.1) $\mu\text{g}/100\text{ ml}$) to a peak of 776 (45) nmol/l (28.1 (1.6) $\mu\text{g}/100\text{ ml}$) at 60 minutes, and without vasopressin from 428 (43) to 575 (40) nmol/l (15.5 (1.6) to 20.8 (1.4) $\mu\text{g}/100\text{ ml}$) at 60 minutes (peaks, $p<0.05$) (fig 3). Four subjects with normal cortisol reserve were given arginine vasopressin alone on separate occasions. Basal and peak cortisol values were 429 (58) and 439 (54) nmol/l (15.5 (2.1) and 15.9 (2.0) $\mu\text{g}/100\text{ ml}$). Their mean peak cortisol concentration achieved in the releasing factor test without vasopressin (780 nmol/l; 28.3 $\mu\text{g}/100\text{ ml}$) was not significantly different from that achieved with the insulin combined test (664 nmol/l; 24.1 $\mu\text{g}/100\text{ ml}$). The peak concentration of growth hormone in the releasing factor test with arginine vasopressin was 42.4 (11.2) mU/l at 60 minutes and without arginine vasopressin 18.9 (5.7) mU/l at 30 minutes ($p<0.001$) (fig 4). There was no change in growth hormone concentration in the four subjects given vasopressin alone. The peak growth hormone concentration seen in the releasing factor test without vasopressin (23.0 mU/l) was not significantly different from that in the insulin combined test (18.7 mU/l).

FIG 4—Comparison of serum growth hormone response to combined releasing factors with (●) and without (○) arginine vasopressin in eight subjects. Points are means. Bars are SEM. ** $p<0.001$.

Discussion

The usefulness of a combined test to assess anterior pituitary reserve has been clearly shown,^{4,23} but those studies used insulin induced hypoglycaemia as the stimulus for the release of growth hormone and cortisol. Growth hormone releasing factor and corticotrophin releasing factor specifically stimulate growth hormone and cortisol release respectively,^{7,16} and in our study the four releasing factors given in combination with arginine vasopressin did not produce significantly different release of thyroid stimulating hormone, luteinising hormone, follicle stimulating hormone, and prolactin from that with the conventional combined test using insulin. In the case of prolactin it might be expected that the stress of insulin induced hypoglycaemia in the classical test would cause a

greater response than the releasing factor test, but we did not find this. Nevertheless, both the cortisol and growth hormone responses were found to be significantly greater in both the group of normal subjects and the total group studied with the releasing factor test. This increased response appeared to result from the inclusion of arginine vasopressin, without vasopressin cortisol and growth hormone responses were similar to those seen with the insulin combined test. Although arginine vasopressin is well known to stimulate secretion of adrenocorticotrophic hormone and hence cortisol, its synergistic effect with secretion of growth hormone releasing factor in man has not been reported. Arginine vasopressin alone had no effect on growth hormone secretion, and more studies (that is, in animals and isolated tissues) are needed to explore the precise relation in this apparently pronounced synergistic action.

The insulin combined test assesses hypothalamic-pituitary reserve, while the releasing factor test measures the readily releasable pool of anterior pituitary hormones. Thus in some cases of tumours in the pituitary area where stalk or hypothalamic damage has occurred it would be expected that results of the releasing factor test would differ from those of the insulin combined test. Other forms of hypothalamic insufficiency—for example, after cranial irradiation—would also be expected to show a different response, and the releasing factor test in these circumstances would not assess the need for hormone replacement therapy. The releasing factor test, however, is a valid clinical test of pituitary disease alone, and absence of response has the same implication in these cases as the results of an insulin combined test. The releasing factor test appears to be particularly valuable in assessing the extent of damage to pituitary function after surgery for pituitary tumours and also the success of pituitary ablation used in the treatment of hormone dependent cancers. In practical terms it is often known that pituitary tumours or other pituitary diseases exist and what is needed is a test of pituitary function as such; the releasing factor test is eminently applicable in this setting. The current cost of the basic peptide components of this test is £65 and, when manpower is considered, it may therefore be cheaper overall than the insulin combined test when this new combined releasing factor test becomes available commercially.

In most subjects the peak growth hormone and cortisol responses reportedly occur within 30-60 minutes with the combined releasing factors.¹⁸ Our study confirms this and suggests that pituitary function may be evaluated with a 60 minute test rather than the 120 minutes required with the insulin combined test. The growth hormone response to growth hormone releasing factor has been reported to vary within the same subject^{7,9} and further study of individuals' variation in their response to the releasing factor test would be of interest.

Arginine vasopressin in the low dose used in this study has not been reported to have side effects,²⁰ and we observed no reactions in the subjects studied. It is worth noting that thyrotrophin releasing hormone, used in both tests, has rarely been recorded as associated with pituitary apoplexy.²⁴

Two studies using multiple releasing hormones without arginine vasopressin have been reported in normal subjects.^{18,25} Our study reports the combined use of the four releasing factors together with vasopressin as a powerful test of the readily releasable pool of pituitary hormones in both normal subjects and patients with pituitary disorders. The releasing factor test is quicker and more pleasant than the insulin combined test. It is more feasible for use on

an office basis (for example, not risking unconsciousness and not needing a supervised meal as required after insulin induced hypoglycaemia) and may well prove to be generally safer, reducing the level of professional attention required. This test of pituitary function therefore appears to be a very useful addition to the insulin combined test, and indeed in some circumstances may replace it. Further work is required to establish the particular indications for its clinical use.

LM is in receipt of a Hammersmith Special Health Authority grant.

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(Accepted 2 December 1985)