ADVANCED MEDICINE 21

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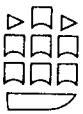

CHURCHILL LIVINGSTONE
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FOREWORD

When I accepted the invitation and privilege of organising the 1985 Advanced Medicine Conference at the Royal College of Physicians, I little imagined that that task and the subsequent mission of editing this volume could be so enjoyable. That this has been so is almost entirely attributable to the help in the first place that I have had from Dr Gwyn Williams and Miss Gillian Andrew at the College and from my secretaries Miss Dorothy Boland and Miss Bernadette Edinborough; and, above all, to the uniform excellence of the talks and the richness of the papers which followed. It was an unusual pleasure to sit through four days at a conference without a single dull moment – even if some speakers followed the measured delivery of their talks with an equally measured delivery of their manuscripts!

The conference is traditionally general but not comprehensive in the areas which are covered and this permits the organiser to indulge some of his prejudices. This will be most apparent in the omissions; but on the positive side I attempted, as a clinical pharmacologist, to place some emphasis in most sessions on current therapeutic innovations in each speciality.

Morris J Brown
Royal Postgraduate Medical School
May 1985

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THE IMPACT OF HYPOTHALAMIC REGULATORY HORMONES ON CLINICAL MEDICINE

A Grossman, G M Besser

Introduction

The discovery of the structures of the hypothalamic releasing hormones, thyrotrophin releasing hormone (TRH) and gonadotrophin releasing hormone (GnRH), in the early 1970s led to a rapid increase in our knowledge of the regulation of pituitary hormone secretion in man in health and disease. Gradually the diagnostic and therapeutic impact of these discoveries has become apparent. The realisation that the principal prolactin inhibiting factor released into the portal blood vessels was dopamine, together with the development of dopamine receptor agonists such as bromocriptine, has transformed the management of pituitary tumours and hyperprolactinaemic hypogonadism and infertility. Somatostatin has provided a unique tool for study of integrative mechanisms in the hypothalamus and gut and recently introduced long-acting analogues will be of value in management of acromegaly. Finally, over the last three years the identification and synthesis of corticotrophin releasing factor (CRF) and growth hormone-releasing hormone (GHRH) has greatly increased our ability to diagnose specific defects in the hypothalamo-pituitary axis. GHRH may also have considerable therapeutic potential in the management of growth hormone deficiency.

Thyrotrophin releasing hormone

TRH, a tripeptide originally isolated from ovine and porcine hypothalami, is able to stimulate the release of both TSH and prolactin in man. Although it is now known to be present both in the brain, where it is thought to function as a classical neurotransmitter, and in the gut, where it probably has a paracrine function, the principal use of TRH in clinical medicine is as a diagnostic test for disorders of thyroid function, the circulating TSH response to TRH reflecting the degree of pituitary TSH control of the thyroid. In normal subjects, the intravenous administration of 200μg of TRH is followed by a rapid rise in serum

TSH which peaks at 20 minutes, and thereafter falls at 60 minutes. The response is greater in women than in men and is much exaggerated in patients with primary hypothyroidism. However, such patients will usually have an elevated serum TSH basally, such that the TRH test does not usually add useful clinical information. An absent TSH response to TRH is seen in patients with thyrotoxicosis, as the elevated levels of circulating thyroid hormones directly inhibit the thyrotroph and prevent its response to TRH. The test is useful when basal levels of thyroid hormones are equivocal in confirming a diagnosis of thyrotoxicosis. However, flat TSH responses to TRH may also be seen in euthyroid patients with multinodular goitres and ophthalmic Graves' disease, since such a response simply indicates that the thyroid is not under pituitary TSH control. In patients with nodular goitres the gland is autonomous, and in euthyroid Graves' disease the level of circulating thyroid stimulating antibodies is simply insufficient to result in excess thyroid hormone secretion. It is possible that new super-sensitive immunoradiometric assays for serum TSH will be able to distinguish the suppressed basal levels of serum TSH in thyrotoxicosis from those in normal subjects, and may thus render the test of lesser clinical relevance. However, these are not yet generally available. Attenuated TSH responses to TRH are seen in patients with Cushing's syndrome, and in some patients with depressive illness. A 'delayed' TSH response to TRH, with the 60-minute value greater than that at 20 minutes, is often indicative of a hypothalamic disorder, but may also be seen in pituitary diseases when these cause stalk compression [1].

The prolactin response to TRH is occasionally helpful; a very low serum prolactin that fails to rise after TRH may indicate prolactin deficiency, which may arise post-hypophysectomy or in the rare Sheehan's syndrome. An absent prolactin response to TRH in hyperprolactinaemic patients is said to indicate the presence of a pituitary tumour, but there are so many false positives and false negatives that this test is not diagnostically useful. Serum growth hormone (GH) may rise after TRH in two-thirds of patients with acromegaly, but this response is also seen in patients with renal failure, hepatic failure, anorexia nervosa and depressive illness.

The gonatrophin releasing hormone

The acute administration of GnRH leads to a rise in serum LH, and, to a lesser extent, in serum FSH in normal subjects. The test does not release all the pituitary content of the pituitary, but merely a portion of it, the readily-releasable pool; a poor or absent response does not reliably differentiate between pituitary and hypothalamic causes of gonadotrophin deficiency. Some patients have a hypothalamic defect in endogenous GnRH synthesis or release, often in association with colour-blindness, anosmia or other midline defects. Early studies in such patients with hypogonadotrophic hypogonadism, or Kallman's syndrome, demonstrated that full pubertal maturation and subsequent fertility could be induced by regular frequent subcutaneous injections of synthetic GnRH [2]. It was hoped that long-acting analogues of GnRH would simplify the administration of such therapy, and many such analogues were subsequently

developed. However, gonadotrophins are secreted in a pulsatile fashion, with approximately one pulse every 1½ to 2 hours in normal men, and it was soon discovered that, unless GnRH was also given in a pulsatile mode, the pituitary rapidly failed to respond. Indeed, the circulating gonadotrophins soon fall to below basal levels following treatment with long-acting, highly potent analogues of GnRH, and normal subjects are rendered hypogonadal. This finding of pituitary 'desensitisation' has had two important clinical corollaries. In the first instance, the treatment of hypogonadotrophic states can only be treated sucessfully if GnRH is given in a pulsatile fashion similar to that secreted by the normal hypothalamus. Secondly, superactive analogues can be used when it is desirable to switch off the pituitary-gonadal axis. The most outstanding clinical application of these superactive analogues has been in the treatment of men with prostatic cancer [3]. Although the ameliorative effects of oestrogen therapy in this condition have been known for many years, in conventional dosage this treatment is associated with increased cardiovascular morbidity, and overall mortality does not improve.

Castration removes the majority of endogenous androgens and prolongs life in this hormone-sensitive cancer, but it is not always acceptable to the patient. Regular treatment with GnRH analogues, either subcutaneously or intranasally, is equivalent to a medical orchidectomy; serum testosterone falls to low levels over a matter of weeks, and stays low as long as treatment is continued. Considerable improvement in both clinical and biochemical indices of disease has been reported in many patients and tumour masses often shrink [3]. Most recently, depot injections of GnRH analogues have been found to be equally effective in suppressing the pituitary-gonadal axis, and are more convenient [4]. Although GnRH may interact with receptors directly on prostatic or testicular tissue in the rat, there is no evidence that this is also true in man, and it is thought that the effects of GnRH are entirely mediated by desensitisation of the pituitary, with associated reduction in secretion of LH and FSH.

In women, GnRH analogues have been used to cause temporary gonadal suppression in patients with endometriosis, and lower doses have been used as a form of contraception. However, in this case it is desirable to block merely the periovulatory surge of serum LH while leaving basal secretion unchanged, otherwise menstrual irregularity and oestrogen deficiency will result [5]. Trials are also in progress to assess the utility of GnRH analogues in the treatment of women with carcinoma of the breast – such tumours have been shown to diminish in size in response to GnRH in vitro. GnRH therapy does not appear to prevent the sterilising effects of chemotherapy in patients with malignant disease.

Children with precocious puberty may also respond to therapy with GnRH analogues, with a regression of the prematurely advanced, secondary sexual characteristics and their associated psychosocial problems. Equally important, the rate of advance of bone-age over chronological age is decelerated, such that predicted final height is increased [6]. Such therapy may soon become the treatment of choice in these children.

In patients with congenital or acquired GnRH deficiency, characterised by infrequent spontaneous gonadotrophin pulses, the delivery of GnRH by portable

pulsatile infusion pumps has led to the acquisition of secondary sexual characteristics as well as fertility. Subcutaneous administration is safer and more convenient than intravenous therapy, although not all groups are agreed that it is equally effective. Recent studies have defined the important parameters of dose size and frequency, and have led to many pregnancies, especially in women with menstrual disorders and infertility associated with low weight [7]. Although conception in such women can be diagnosed by ultrasound well before the first missed menstrual period, GnRH therapy is continued during the luteal phase to support the corpus luteum, but can then be discontinued. Unfortunately, the high success rate in these patients is not seen in women with the polycystic ovary syndrome, in whom the characteristic pituitary abnormality is one of increased LH amplitude and frequency. Hypogonadotrophic men can also be taken through puberty to normal potency and fertility with similar therapeutic regimens.

Dopamine: the prolactin inhibiting hormone

The predominant hypothalamic prolactin regulating hormone is inhibitory, dopamine being secreted from the hypothalamic neurones into the pituitary stalk portal capillaries to act on the pituitary prolactin-secreting cells. The discovery that the semi-synthetic ergot alkaloid, bromocriptine, was able to lower serum prolactin in experimental animals and man was rapidly followed by the realisation that this occurred via activation of the pituitary dopamine receptors. The utility of bromocriptine in the treatment of all forms of hyperprolactinaemia is now well established. In addition, some two-thirds of pituitary tumours associated with hyperprolactinaemia shrink on bromocriptine treatment, with improvement in pituitary function and a decrease in local mass effects of visual field impairment and headache. However, it now appears that in truly 'functionless' tumours, where the tumour does not itself secrete prolactin, a clinically significant change in tumour size occurs only rarely with dopamine agonist therapy [8]. Such tumours may cause hyperprolactinaemia by compressing or disrupting the portal vasculature and interfering with the supply of dopamine from hypothalamus to pituitary. Such 'pseudoprolactinomas' are usually associated with only mild hyperprolactinaemia (serum prolactin less than 1000mU/l), but serum prolactin values up to 6000–8000mU/l may occur. However, these levels usually indicate a true prolactinoma, and a patient with a prolactin level above 8000mU/l almost always has a prolactinoma. Marked and clinically valuable shrinkage of true prolactinomas with bromocriptine can be expected, even when such tumours are massive.

Recent data suggest that additional radiotherapy given once the tumour has been shrunk back into the pituitary fossa, or immediately if no extrasellar extension is present, delivered in small fractions (less than 180cGy/day) over a period of 5–6 weeks to a total dose of 4500cGy via 3 portals, is safe and leads to a progressive ablation of the tumour. Thus many patients eventually become normoprolactinaemic off all treatment, and can stop their bromocriptine [9]. New dopamine agonists on clinical trial, such as pergolide, have the advantage

of requiring only once-daily therapy, and there is evidence that occasional patients intolerant of one dopamine agonist may find an alternative drug more acceptable [10].

Somatostatin

While searching for the identity of the growth hormone releasing factor, Brazeau and his colleagues isolated from the hypothalamus a 14-aminoacid peptide that inhibited secretion of GH from the pituitary. It was subsequently found that this peptide, now known as somatostatin, is also able to inhibit the release of many of the enteropancreatic hormones. Somatostatin is also found within the gut, where it may act to inhibit gastrin release and gastrin-stimulated release of gastric acid in response to intraduodenal fatty acids [11]. Pancreatic somatostatin is found within the D cells of the pancreatic islets and inhibits both glucagon and insulin release. Somatostatin has to be administered parenterally to be biologically effective, and the natural material has a very short duration of action. However, infusions in man have been used to inhibit gastroduodenal haemorrhage and acute pancreatitis, although the evidence is not convincing that somatostatin is therapeutically useful in these situations as yet.

In addition to being present in the pituitary stalk capillaries, somatostatin circulates in man at low concentrations in the systemic circulation, and probably originates from the gut and pancreas. Islet cell tumours occasionally secrete somatostatin in sufficient quantities to produce a clinical syndrome characterised by hyperglycaemia (due to inhibition of insulin secretion), hypochlorhydria (inhibition of gastric acid), steatorrhoea (inhibition of pancreatic exocrine secretion) and cholelithiasis (inhibition of gall-bladder motility). Somatostatin has also been isolated from a variety of other tumours including carcinoids, medullary carcinoma of the thyroid, and phaechromocytomas.

Recently, an octapeptide analogue of somatostatin has been developed which is relatively long-acting, especially against GH as compared to insulin. Although this analogue still has to be administered subcutaneously to be effective, studies have shown that twice-daily administration can greatly decrease the elevated GH levels characteristic of acromegaly [12] (Figure 1). This improvement is additional to any therapeutic improvement mediated by bromocriptine, and suggests that with its introduction a more effective medical treatment of acromegaly is likely to become available in the near future.

Corticotrophin releasing factor

Although a factor in the hypothalamus capable of stimulating the release of ACTH had been described by both Guillemin and Schally many years ago, a further quarter of a century passed before the structure of CRF was elucidated.

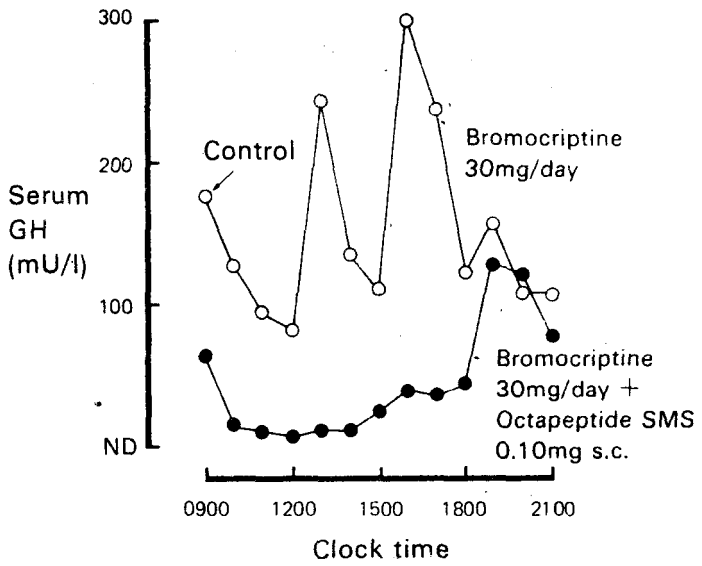

Figure 1. The effect of the octapeptide somatostatin analogue on the elevated GH levels in a patient with acromegaly. The patient was already on the maximally-effective dose of bromocriptine. (J A H Wass and G M Besser, unpublished observations)

Vale and co-workers demonstrated that ovine CRF was a 41-residue popypeptide that resembled a family of certain other previously described peptides, including angiotensinogen and some amphibian hormones [13]. This structural homology may indicate a common evolutionary origin. Ovine CRF-41 stimulates the release of ACTH both in vitro and in vivo in many animal species, including man, in whom the rapid rise in plasma ACTH is followed by a more gradual increase in circulating cortisol. Apart from mild flushing, the administration of the CRF at doses up to 100μg is without side-effects, although occasional studies have noted marked hypotension at higher doses. No change is seen in the levels of other pituitary hormones [14]. The administration of CRF is therefore a safe and specific test of the pituitary-adrenal axis, and depends on the amount of ACTH in the pituitary which is ready for release. It may be used to define the site of the abnormality in patients with ACTH deficiency. The standard test of ACTH reserve measures the cortisol response to insulin-induced hypoglycaemia, which depends on an intact hypothalamic-pituitary-adrenal axis since the glucostat is in the hypothalamus. Patients who show a deficient cortisol response to hypoglycaemia, but a normal response to CRF-41, must have a defect in the ability of the hypothalamus to synthesise or release CRF, or in its access to the pituitary via the pituitary stalk vessels. We originally described six such patients, four of whom had large pituitary tumours disrupting the portal vessels, one a hypothalamic metastasis from carcinoma of the breast, and one child with 'idiopathic hypopituitarism' [15] (Figure 2). Other studies have used CRF to demonstrate acquired deficiencies in endogenous CRF in patients with

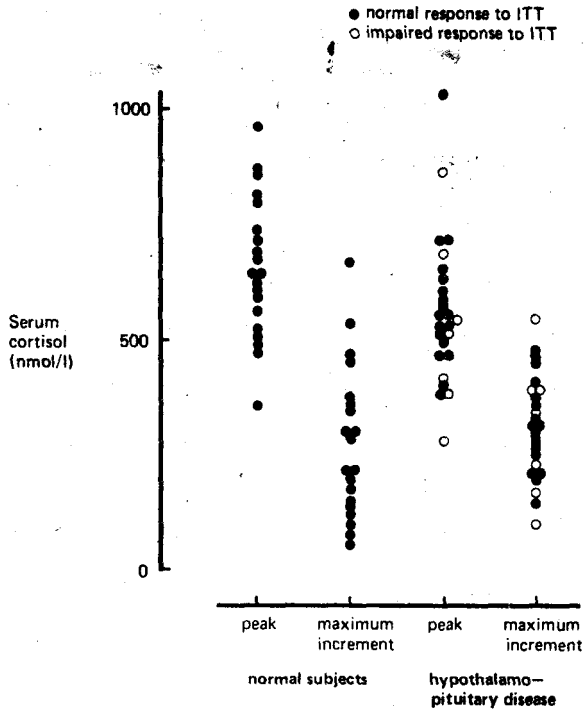

Figure 2. Peak and maximum incremental responses of serum cortisol to CRF-41 in 20 normal subjects, and 25 patients with disorders of the hypothalamo-pituitary axis. Seven patients had impaired cortisol responses to an insulin tolerance test (ITT), but six of these had normal responses to CRF-41. (Reprinted from Lytras et al [15], by courtesy of the Editors of *Clinical Endocrinology*)

hypothalamic tumours, especially suprasellar germinomas. However, it is important to test such patients before treatment with hydrocortisone is started, as secondary pituitary and adrenal atrophy may render the response to CRF-41 difficult to interpret. Since acute corticosteroid administration inhibits the ACTH-cortisol response to CRF-41 [15], and the ACTH response is increased when cortisol levels are low, the site of the negative feedback control of cortisol on ACTH secretion must be, at least in part, at the pituitary level modulating the ACTH response to CRF. There is, probably, an hypothalamic feedback site as well, varying secretion of CRF itself.

CRF-41 has also been used to investigate patients with suspected Cushing's syndrome. In patients with pituitary-dependent Cushing's disease the corticotroph is less sensitive to steroidal feedback and there is an enhanced ACTH and cortisol response to CRF, despite the supranormal basal plasma levels [15, 16]. Patients with adrenal tumours or the ectopic ACTH syndrome generally show little or no response to CRF-41. However, as with all tests for the differential diagnosis of the causes of Cushing's syndrome exceptions occur, especially in

the ectopic ACTH syndrome [17]. The usefulness of the CRF test in the diagnosis and differential diagnosis of Cushing's syndrome remains to be fully assessed.

Animal studies have demonstrated that CRF-41 is released into the portal blood during stress, and that neutralisation of endogenous CRF-41 with specific antiserum blocks most, but not all, of the stress-induced rise in ACTH. This suggests that other substances with CRF-like activity may exist, and there is considerable evidence that arginine vasopressin can potentiate the ability of CRF-41 to stimulate ACTH release, both in the rat [18] and in man [19]. It is likely that there are a series of compounds with ACTH-releasing activity, and that CRF-41 is only one of these, albeit the most important.

Recent studies of the human hypothalamus have demonstrated that CRF-like immunoreactivity is localised principally to the paraventricular nucleus, and the techniques of molecular biology have been used to sequence human CRF. This has been shown to be of similar length to ovine CRF, from which it differs by seven aminoacid residues. Human CRF is identical to that of the rat. Synthetic human CRF has similar effects to ovine CRF when administered to man, although it is shorter-acting as it is more rapidly cleared from the circulation.

CRF-like immunoreactivity is also located in other brain regions, where it may serve as a neural mediator of stress-induced changes in arousal and adaptation. CRF is also found in the gut, and there is preliminary evidence that it may modulate pancreatic function. One tumour ectopically secreting CRF-41 has so far been described [20].

Growth hormone releasing hormone

For many years, the structure of GHRH has evaded elucidation by investigators extracting large numbers of animal hypothalami. However, it had been realised for some time that occasional patients presented with acromegaly because of the coexistence of a carcinoid tumour, and it was suspected that in such cases the tumour was stimulating the pituitary by secreting a GH releasing factor (GRF). In 1982, Thorner and his colleagues described such a patient in whom the acromegaly was cured on removal of a pancreatic tumour. Extraction of the pancreatic tumour by Vale and co-workers demonstrated a 40-aminoacid GRF (human pancreatic GRF or hpGRF (1–40)) [21]. Guillemin's group isolated three peptides from a similar tumour from another patient; one peptide was identical to the Thorner hpGRF (1–40), but shorter (1–37) and extended (1–44) sequences were also found [22]. Both hpGRF (1–40) and hpGRF (1–44) have since been shown to specifically stimulate the release of GH in man; DNA hybridisation techniques and high-performance liquid chromatography have suggested that peptides identical or similar to hpGRF (1–40) and (1–44) exist in the human hypothalamus.

Stimulation by hypoglycaemia is a powerful stimulus to growth hormone (GH) secretion; it leads to the mobilisation of the hypothalamic releasing factor (GHRH) followed by release of the pituitary hormone. It was soon noted that many patients with little or no GH response to hypoglycaemia nevertheless responded to GRF with a rise in circulating GH, and most of these patients had

evidence of hypothalamic tumours. However, it was also found that many children with idiopathic isolated growth hormone deficiency or panhypopituitarism also responded to GRF with a rise in GH and somatomedin-C [23, 24]. This suggested that these children had a hypothalamic deficiency of endogenous GHRH synthesis or release. In older patients with idiopathic growth hormone deficiency, acute administration of GRF may not produce a rise in serum GH, but repeated 'priming' for several days may eventually induce a response [25]. It appears that GHRH is necessary for both the synthesis, as well as the acute release, of GH; all the known hypothalamic regulating hormones have similar trophic functions.

Analogues of GHRH with modified structures are also able to stimulate GH release; loss of the residues between aminoacids 30 and 44 does not affect activity, but analogues with less than 29 aminoacids rapidly lose potency.

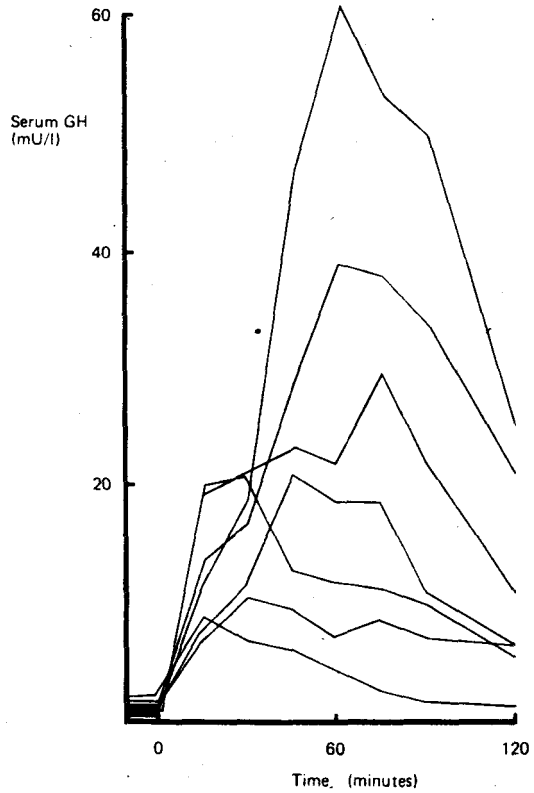

Figure 3. The serum GH responses to GHRH (1–29)NH2 200μg i.v. in seven children with cerebral tumours treated by cranial irradiation. All seven children had deficient GH responses to insulin-induced hypoglycaemia. (A Grossman, A Blacklay M O Savage, G M Besser, unpublished observations)

GHRH (1–29)NH2 has been used as a pituitary function test to demonstrate the failure of endogenous GHRH secretion in patients subjected to cranial irradiation [26]. Children with GH deficiency following cranial irradiation fail to grow and this appears to be due to deficiency of GHRH [27] (Figure 3).

Human GH has been in short supply in recent years, so that the discovery that many short children were deficient in GHRH rather than GH has led to speculation that analogues of GRF could be used in the treatment of short stature. Although there are as yet few long-term data, provisional results suggest that therapy with GRF is likely to play a major role in the treatment of such children. Whether this will be best given by pulsatile administration [28] or regular subcutaneous injections [29] (Figure 4) remains to be seen. The introduction of GRF therapy, together with trials on bioengineered human GH, will undoubtedly transform the management of short stature.

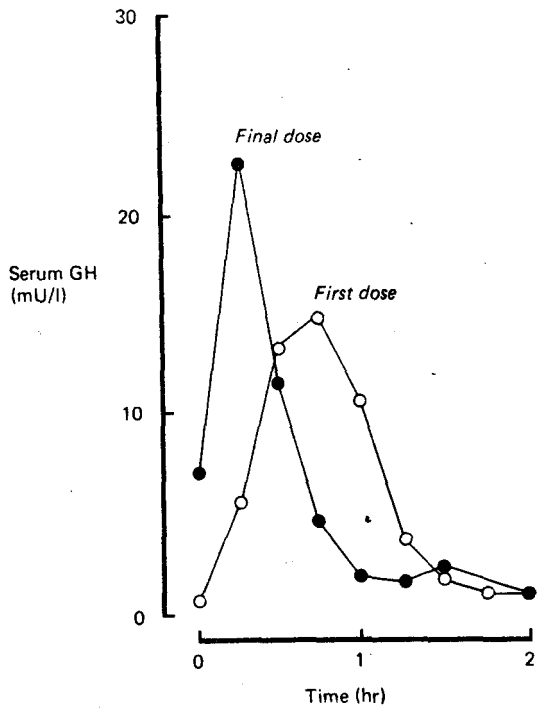

Figure 4. Serum GH response to GHRH (1–29)NH2 100μg subcutaneously before and after one week's treatment with GHRH (1–29)NH2 100μg s.c. twice daily in a child with short stature.