CLINICAL RESEARCH

Growth hormone releasing factor : comparison of two analogues and demonstration of hypothalamic defect in growth hormone release after radiotherapy

A GROSSMAN, N LYTRAS, M O SAVAGE, J A H WASS, D H COY, LESLEY H REES, A E JONES, G M BESSER

Abstract

Human pancreatic growth hormone releasing factor (hpGHRF(1-40)) stimulates the release of growth hormone in normal subjects and some patients with growth hormone deficiency. A study comparing the shorter chain amidated analogue hpGHRF(1-29) with an equivalent dose of hpGHRF(1-40) in seven normal subjects showed no significant difference in growth hormone response between the two preparations. Six patients with prolactinomas were also tested; these patients had received megavoltage radiotherapy previously but had developed growth hormone deficiency as shown by insulin induced hypoglycaemia. In all six patients 200 µg hpGHRF(1-40) or hpGHRF(1-29)NH₂ produced an increase in the serum growth hormone concentration.

These data suggest that hpGHRF(1-29)NH₂ may be useful for testing the readily releasable pool of growth hormone in the pituitary and that cases of hypothalamo-pituitary irradiation resulting in growth hormone deficiency may be due to failure of synthesis or delivery of endogenous GHRF from the hypothalamus to pituitary cells.

Introduction

Radiotherapy has been utilised for many years in the management of pituitary tumours, but why hypopituitarism develops in some patients, often years after treatment, is not clear. It may be an effect of the tumour itself—either ongoing changes from the effects of the original growth or continued tumour growth—but this seems unlikely, as tumour growth is almost invariably controlled. Possibly the hypopituitarism is a late expression of cellular depletion due to irradiation of the slow renewal system of the pituitary parenchyma. On the other hand, it may result from radiation damage to the hypothalamus, which receives the same radiation dose. Recently we reviewed a large group of women with prolactinomas treated by megavoltage radiotherapy followed by interim dopamine agonists and noted that the prevalence of deficient growth hormone responses to insulin induced hypoglycaemia rose from 24% before treatment to 79% a mean of 4.2 years after radiotherapy.¹ We were not clear, however, whether the acquired growth hormone deficiency was due to a defect in the ability of the hypothalamus to synthesise growth hormone releasing factor (GHRF) or deliver GHRF to the pituitary cells via the portal capillary vessels of the pituitary stalk, or both, or to damage to the pituitary cells themselves.

The structure of GHRF has been determined from extraction of human pancreatic tumours associated with ectopic production of GHRF.^{2,3} The principal activity appeared to reside in human pancreatic (hp) GHRF(1-44) in one tumour³ and in the slightly shortened hpGHRF(1-40) in the second,² but both forms were equipotent in growth hormone releasing activity. Synthetic hpGHRF(1-40) stimulates the release of growth hormone in normal subjects and some patients with hypothalamic lesions or idiopathic growth hormone deficiency,⁴⁻⁶ presumably by a direct action on pituitary somatotrophs.^{7,8} hpGHRF(1-29)NH₂ has also been shown to possess full intrinsic activity and potency in vitro.² We have therefore investigated the effects of injecting hpGHRF(1-29)NH₂ and hpGHRF(1-40) in normal subjects and patients with deficient growth hormone responses to hypoglycaemia after megavoltage radiotherapy. Our aim was to

St Bartholomew's Hospital, London EC1A 7BE

A GROSSMAN, BSC, MRCP, lecturer in endocrinology
N LYTRAS, MD, research registrar in endocrinology
M O SAVAGE, MD, MRCP, consultant paediatric endocrinologist
J A H WASS, MD, MRCP, senior lecturer in endocrinology
LESLEY H REES, MD, FRCP, professor of chemical endocrinology
A E JONES, FRCP, FRCP, professor of radiotherapy
G M BESSER, DSC, FRCP, professor of endocrinology

Department of Medicine, Tulane University, New Orleans, Louisiana, USA

D H COY, MD, professor of medicine

Correspondence to: Professor G M Besser, Department of Endocrinology, St Bartholomew's Hospital, London EC1A 7BE.

determine whether in patients with radiotherapy induced growth hormone deficiency there were normal or abnormal responses to GHRF.

Patients and methods

Seven normal subjects (four men, three women; age range 21-35 years) were given 200 µg hpGHRF(1-29)NH₂ and 200 µg hpGHRF(1-40) on separate occasions at least one week apart and in random order. Subjects were tested after an overnight fast, with an indwelling forearm venous cannula in place. After taking two basal blood samples (15 minute interval) we administered synthetic hpGHRF(1-40) or hpGHRF(1-29)NH₂ as a 200 µg intravenous bolus at 0900. Subjects

FIG 1—Changes in serum growth hormone concentrations in seven normal subjects given hpGHRF(1-40) or hpGHRF(1-29)NH₂. Points are means. Bars are SE.

remained supine throughout, and blood was sampled every 15 minutes for two hours. Pulse rate, blood pressure, and side effects were recorded with each blood sample.

Six patients with prolactinomas treated by megavoltage radiotherapy were also investigated. These patients (table) were chosen from our irradiated group of 36 patients as having low or undetectable concentrations of serum growth hormone in response to hypoglycaemia after radiotherapy. The radiotherapy had been delivered to give a tumour dose of 4500 cGy (rads) in 25 fractions over five weeks from a linear accelerator.¹ Pretreatment serum prolactin concentrations varied from 3000 to 16 000 mU/l; all except one patient (case 4) had radiological evidence of macroadenomas. The GHRF test was carried out as in the normal subjects; the first two patients tested (cases 1 and 5) were given 200 µg hpGHRF(1-40), while the other four were given 200 µg hpGHRF(1-29)NH₂. In addition, on a separate occasion each patient had an intravenous insulin tolerance test, conducted according to a standard protocol.⁹

Blood samples after injection of GHRF(1-29)NH₂ or GHRF(1-40) were assayed for serum growth hormone by radioimmunoassay using MRC standard 66-217. Interassay coefficients of variation were 7.1% at 11 mU/l and 9.5% at 5.8 mU/l; the level of detection for the assay is 1 mU/l. In the insulin tolerance test blood sugar values were measured by the neocuproine technique.

hpGHRF(1-40) and hpGHRF(1-29) (as the amidated derivative) were synthesised according to previously described structures by a solid phase technique similar to that used for the synthesis of corticotrophin releasing factor.¹⁰ Samples of 200 µg were dissolved in 2 ml acid-saline (0.02% hydrochloric acid in 0.9% saline) to minimise adsorption and stored at 4°C until immediately before use.

Peak growth hormone responses and growth hormone response areas in the normal subjects were analysed by the non-parametric Wilcoxon matched pairs test and by Student's paired *t* test after logarithmic transformation to normalise the data.

Each patient and normal volunteer gave written, informed consent, and the study was approved by the ethical committee of St Bartholomew's Hospital.

Results

The seven normal subjects showed no significant difference in peak responses to hpGHRF(1-40) and hpGHRF(1-29)NH₂; serum growth hormone concentrations rose to mean peaks of 67 (SE 22) mU/l (range 15-178 mU/l) and 57 (21) mU/l (range 15-180 mU/l) in the two tests respectively (fig 1). There was also no difference in the total amount of growth hormone secreted over 120 minutes (mean response areas after hpGHRH(1-40) and hpGHRH(1-29)NH₂ were 284 (SE 84) units and 260 (85) units respectively).

In the six patients the mean maximal growth hormone response to hypoglycaemia before radiotherapy was 38 (SE 7) mU/l (range 19-63). After megavoltage radiotherapy there was no growth hormone response to an equivalent degree of hypoglycaemia in five of the patients (table; fig 2), while in the sixth patient serum growth hormone rose subnormally to a peak concentration of 8 mU/l. In each case hpGHRF(1-40) or hpGHRF(1-29)NH₂ induced an increase in serum growth hormone that was greater than that seen after hypoglycaemia (table; fig 2). In three patients (cases 3, 4, and 6) serum growth hormone values rose to within the range established in our normal subjects.

Transient facial flushing for two to five minutes was noted in all normal subjects and patients given hpGHRF but no change in pulse rate or blood pressure or other side effects were noted.

Discussion

In this small study amidated hpGHRF(1-29) appeared to be equipotent to hpGHRF(1-40) at a dose of 200 µg, and both preparations caused mild facial flushing. hpGHRF(1-29)NH₂ is considerably simpler in structure and less expensive to synthesise. On present data it appears to be a useful form of GHRF for use in diagnostic and therapeutic trials.

Of the six patients irradiated for prolactinomas, five showed no growth hormone response to hypoglycaemia three to 11 years after radiotherapy, and in the sixth patient (tested only two years after treatment) there was a small but subnormal response to hypoglycaemia. Nevertheless, all six responded to hp-

Clinical details and peak growth hormone responses to growth hormone releasing factor (GHRF) and insulin induced hypoglycaemia in six women with prolactinomas treated by radiotherapy

Case No	Age (years)	Years since radiotherapy	Other hormone deficiencies	Minimum blood sugar (mmol/l)		Peak growth hormone response (mU/l) to:		
				Before radiotherapy	After radiotherapy	Hypoglycaemia		GHRF
						Before radiotherapy	After radiotherapy	
1	37	11	TSH	1.1	1.2	30	2	6
2	49	6	LH, FSH	1.2	0.7	19	<1	3
3	33	6	None	1.3	1.4	32	2	19
4	28	5	None	1.0	1.4	63	3	31
5	35	2	None	2.0	1.8	58	8	12
6	29	3	None	1.0	1.3	25	1	25

TSH = Thyroid stimulating hormone. LH = Luteinising hormone. FSH = Follicle stimulating hormone.

Conversion: SI to traditional units—Blood sugar: 1 mmol/l ≈ 18 mg/100 ml.

FIG 2—Changes in serum growth hormone concentrations after insulin induced hypoglycaemia and after 200 µg hpGHRF(1-40) or 200 µg hpGHRF(1-29)NH₂ given intravenously in six women with prolactinomas treated by radiotherapy.

GHRF. In three cases growth hormone responses were within the normal range, while in the remaining three growth hormone values rose to a greater extent after hpGHRF than after hypoglycaemia. Interestingly, the two patients with the lowest responses to hpGHRF were tested six and 11 years after radiotherapy. Other studies have shown an approximate correlation between responses to GHRF and hypoglycaemia in most subjects.⁵ As hpGHRF has an action directly at the pituitary level, our data suggest that radiotherapy to the hypothalamo-pituitary region causes a functional disconnection between the hypothalamus and pituitary. The megavoltage irradiation was delivered by a three field isodose technique and, although the field size rarely exceeded 4 × 4 cm (the smallest compatible with uniform irradiation of the tumours), the hypothalamus as well as the pituitary was inevitably irradiated in each instance.

The nature of the change in growth hormone responsiveness to hypoglycaemia is unknown, but a defect in the synthesis or release of endogenous GHRF or a disturbance in the portal capillary blood supply of the pituitary stalk appears possible. A similar defect in gonadotrophin releasing hormone has also been noted in occasional patients about eight years after this regimen of radiotherapy.¹ The different time course of these changes indicates that selective changes within the hypothalamus are more likely than generalised changes in the portal vasculature, although on present data both possibilities remain. Long term absence of endogenous GHRF may decrease the synthesis of growth hormone,¹¹ leading to a fall in that held intracellularly as a readily releasable pool. By analogy with the other hypothalamic releasing hormones this is likely to be the portion of growth hormone within the cell on which GHRF acts.

Children with idiopathic growth hormone deficiency reportedly respond frequently to hpGHRF,^{5 6 12} as do patients with hypothalamic, but not pituitary, lesions.^{6 13 14} This suggests

that such children may benefit from hpGHRF administered therapeutically long term. In addition, substantial numbers of children suffer from growth hormone deficiency after radiotherapy for intracranial tumours.¹⁵ Although our studies were of adults, in whom growth hormone deficiency is not clinically important, it seems possible that children with radiotherapy related deficiency of the hormone might also be treated with synthetic hpGHRF.

We acknowledge the help of Julia Williams, Anne Knights, and Sue Tree in these studies, which were supported by the Medical Research Council, Peel Medical Trust, and Joint Research Board of St Bartholomew's Hospital.

References

- Grossman A, Cohen BL, Charlesworth M, *et al*. The treatment of prolactinomas with megavoltage radiotherapy. *Br Med J* 1984;288:1105-9.
- Rivier J, Spiess J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* 1982;300:276-8.
- Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 1982;218:585-7.
- Thorner MO, Rivier J, Spiess J, *et al*. Human pancreatic growth-hormone-releasing factor selectively stimulates growth-hormone secretion in man. *Lancet* 1983;ii: 24-8.
- Borges JLC, Blizzard RM, Gelato MC, *et al*. Effects of human pancreatic tumour growth hormone releasing factor on growth hormone and somatomedin C levels in patients with idiopathic growth hormone deficiency. *Lancet* 1983;ii:119-24.
- Grossman A, Savage MO, Wass JAH, *et al*. Growth-hormone-releasing factor in growth hormone deficiency: demonstration of a hypothalamic defect in growth hormone release. *Lancet* 1983;ii:137-8.
- Adams EF, Winslow CLJ, Mashiter K. Pancreatic growth hormone releasing factor stimulates growth hormone secretion by pituitary cells. *Lancet* 1983;ii: 1100-1.
- Webb CB, Thominet JL, Frohman LA. Ectopic growth hormone releasing factor stimulates growth hormone release from human somatotroph adenomas in vitro. *J Clin Endocrinol Metab* 1983;56:417-9.
- Hall R, Anderson J, Smart GA, Besser GM. *Fundamentals of clinical endocrinology*. London: Pitman, 1980.
- Sueres-Diaz J, Coy DH, Vigh S, *et al*. Synthesis and biological properties of ovine corticotrophin-releasing factor. *Life Sci* 1982;31:429-35.
- Barinaga M, Yamanoto G, Rivier C, Vale W, Evans R, Rosenfeld MG. Transcriptional regulation of growth hormone gene expression by growth hormone-releasing factor. *Nature* 1983;306:84-5.
- Pintor C, Corda R, Puggioni R, Locatelli V, Cella SG, Muller EE. Prolactin-lowering effect of growth-hormone-releasing-factor in children with growth hormone deficiency. *Lancet* 1983;ii:1088-9.
- Sassolas G, Rousset H, Cohen R, *et al*. La somatocrine provoque la libération de somathormone dans un cas de déficit en somathormone d'origine hypothalamique chez l'enfant. *C R Seances Acad Sci (III)* 1983;296:527-9.
- Wood SM, Ch'ng JLC, Adams EF, *et al*. Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor (GRF(1-44)) in acromegaly and hypopituitarism. *Br Med J* 1983;286:1687-91.
- Shalet SM. Disorders of the endocrine system due to radiation and cytotoxic chemotherapy. *Clin Endocrinol (Oxf)* 1983;18:637-59.

(Accepted 24 April 1984)

ONE HUNDRED YEARS AGO Several daily papers have recently discussed a letter from Mr. H. P. Dunn, published in the *Standard*, on the use and abuse of gymnastics for young women. The letter, as might be expected, turns attention to the evils of excessive gymnastic training. As violent exercise produces bad results on weak youths, and as girls are, on the average, weaker than young men, it is natural to suppose that injuries to health after gymnastic training may be commoner amongst females than amongst males, and experience shows this to be the case. No doubt the best kind of physical training in youth is voluntary exercise, either in playing at the outdoor sports popular with English boys, or in rambles over fields and hedges. For certain social reasons, not entirely unconnected with physical matters, neither such sports, nor such congenial walking exercise, can be carried out in girls', as thoroughly as in boys' schools, and methodical gymnastics stand to cricket in the same relation as a walk with other fellow-pupils in line and in class does to a pleasant saunter with one or two school-fellows along a country lane. The aim of the authorities at a girls' school should be to allow as much congenial exercise as possible to every pupil, and to encourage physical activity as strongly as cleanliness and deportment; gymnastics are quite secondary to such conditions. Mr. Dunn is right in recommending frequent drilling, but in preferring dumb-bell practice to bar and ladder gymnastics, it must be remembered that severe palpitations are not unfrequently set up by the use of dumb-bells, even when that form of exercise is not disagreeable, and when the subject is apparently free from visceral disease. (*British Medical Journal* 1884;ii:574.)