Impaired growth hormone response to human pancreatic growth hormone releasing factor [GRF (1–44)] in Type 2 (non-insulin-dependent) diabetes

N. T. Richards, S. M. Wood, N. D. Christofides, S. C. Bhattacharji and S. R. Bloom

Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK

Summary. Human pancreatic growth hormone releasing factor [GRF(1–44)] is the largest molecule of several peptides recently isolated from pancreatic tumours associated with acromegaly. It has been shown to stimulate the release of growth hormone in normal subjects and provides a safe and reliable tool for examining growth hormone release. A study was conducted to examine the release of growth hormone in patients with Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes. GRF(1–44) stimulated the release of growth hormone in normal subjects and produced no side ef-

fects. A response of similar magnitude occurred in Type 1 diabetic patients despite their concomitant hyperglycaemia. In contrast, the response in Type 2 diabetes was significantly impaired compared with normal volunteers ($p < 0.05$) and Type 1 diabetic patients ($p < 0.02$). These findings may well indicate that there is a defect in central hormonal control in Type 2 diabetes.

Key words: Diabetes, growth hormone releasing factor.

Several studies have shown both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients to have abnormal basal and stimulated concentrations of growth hormone [1–4]; abnormal growth hormone metabolism has been suggested as a causative factor in the development of some of the complications of diabetes, namely proliferative retinopathy [5] and nephropathy [6].

The recent isolation of a number of growth hormone releasing factors from extra-pituitary tumours of patients presenting with acromegaly has provided a new tool for the investigation of growth hormone secretion [7–10]. The extraction, purification and sequencing of these growth hormone releasing peptides has now been successfully carried out [8, 9]. Figure 1 shows the primary structure of the largest of these peptides GRF(1–44). We have developed a radioimmunoassay for this peptide.

The effects of bolus doses of GRF(1–44) have been reported recently in normal man, acromegaly and patients with hypopituitarism [11] where it has been found specifically to stimulate growth hormone secretion, with no effect on other anterior pituitary hormones. A single injection of GRF(1–44) offers an easy, safe and reliable method of studying growth hormone release without subjecting patients to the rigours of an insulin tolerance test. We have used this technique to study growth hormone release in Type 1 and Type 2 diabetes.

Subjects and methods

The study was approved by the Ethical Committee of the Hammersmith Hospital and Royal Postgraduate Medical School. Each subject gave their fully informed consent before joining the study.

Normal subjects

These were divided into two groups as summarised in Table 1. Group 1 formed the control group for the Type 1 diabetic patients and Group 2 for the Type 2 diabetic patients. All normal subjects were in good health, had no personal or family history of diabetes, and were on no medication.

Fig. 1. Primary structure of human pancreatic growth hormone releasing factor GRF(1–44)

Table 1. Clinical and biochemical features of subjects studied

Subjects	Age (Years)	Sex (M:F)	Duration of disease (years)	Ideal body weight ^a (%)	Fasting blood glucose (mmol/l)	Haemoglobin A _{1c} (%)	Treatment
Normal subjects							
Group 1 (n=7)	23.0 ± 1.4	4:3	–	105 ± 3	4.2 ± 0.1	6.8 ± 1.1	None
Group 2 (n=4)	56.5 ± 2	1:3	–	108 ± 3	4.7 ± 0.3	7.1 ± 0.1	None
Type 1 diabetes (n=10)							
12	30	M	18	108	4.1	11.2	Insulin
13	45	M	17	98	15.0	–	Insulin
14	57	M	34	104	2.2	–	Insulin
15	38	M	12	104	6.0	8.0	Insulin
16	33	F	22	106	16.3	7.7	Insulin
17	20	F	3	137	16.6	12.8	Insulin
18	20	F	9	116	15.4	9.5	Insulin
19	24	F	18	114	14.0	13.3	Insulin
20	30	F	18	110	9.9	8.5	Insulin
21	49	F	38	112	6.2	8.0	Insulin
Mean ± SEM	34.6 ± 4	4:6	19 ± 3	111 ± 3.4	10.6 ± 1.8	9.9 ± 1.8	
Type 2 diabetes (n=8)							
22	54	M	3	111	6.6	7.1	Insulin
23	73	M	6	111	5.3	6.1	Insulin
24	35	M	3	119	4.1	6.1	Diet
25	64	M	12	116	6.7	7.0	Insulin
26	57	F	8	133	4.3	6.6	Glibendamide
27	50	F	18	121	8.7	10.1	Insulin
28	66	F	5	113	6.1	–	Insulin
29	40	F	2	153	11.6	10.3	Insulin
Mean ± SEM	54.8 ± 5	4:4	7.1 ± 2	122 ± 5	6.7 ± 0.9	7.6 ± 0.7	
Hypopituitarism (n=6)							
	46.0 ± 6	5:1	–	106 ± 4	4.9 ± 0.2	–	Hormonal replacement (see text)

Values are expressed as mean ± SEM. ^a Body weight expressed as percentage from Metropolitan Life Insurance Tables (1959)

Diabetic patients

The details of both the Type 1 and 2 diabetic patients are included in Table 1. The Type 1 diabetic patients were characterised by their acute onset of hyperglycaemia, presenting between the ages of 6 and 29 years (mean 15.9 ± 2.4 years). Either at presentation or subsequently, each had had episodes of ketoacidosis. All had required insulin therapy from the time of diagnosis. In contrast, the Type 2 diabetic patients had no such history of acute onset of disease, and had been diagnosed between the ages of 32 and 67 years (42 ± 7.4 years). None had had ketoacidosis, and all had been treated with dietary restriction or oral hypoglycaemic agents for several years. Six had recently been commenced on insulin because their blood glucose concentrations had been persistently above 10 mmol/l.

None of the diabetic patients had evidence of diabetic retinopathy on ophthalmoscopy, or nephropathy, as judged by their normal plasma creatinine and absence of proteinuria.

Patients with hypopituitarism

Details of these patients are shown in Table 1. All had grossly impaired anterior pituitary function as assessed by their hormonal responses to hypoglycaemia, gonadotrophin hormone releasing hormone and thyrotrophin releasing hormone. Four patients needed thyroxine replacement therapy, two of these also required steroid replacement. Three male patients required testosterone, and one of these had diabetes insipidus requiring therapy with Des-amino-D-arginine vasopressin. The cause of their pituitary failure was known in five of the six patients and included pituitary tumours treated by inter-

nal or external irradiation (2), hypothalamic sarcoidosis (1), congenital hypoplasia of the hypothalamus and pituitary (1) and trauma (1).

Experimental procedure and assays

Subjects reported to the metabolic unit at approximately 09.00 h, having fasted from midnight. Throughout the test period they lay supine in a relaxed environment. An indwelling intravenous cannula was placed in a forearm vein on arrival and kept patent with citrated saline (0.154 mol/l). Basal blood samples were taken at 15-min intervals over the subsequent 30 min. Pure human GRF(1–44), synthesised by solid phase techniques (Bachem, Torrance, California, USA), was used in this study. Before administration it was dissolved in 2.5 ml human serum albumin (Lister Blood Products Laboratories, Elstree, Herts, UK) and made up to 10 ml with isotonic saline. A dose of GRF(1–44) (100 µg) was given as a bolus intravenous injection over 15 s at time zero, and blood samples were taken at 2, 5, 10, 15, 30, 60, 90 and 120 min. Pulse and blood pressure were measured at each sampling time and patients were asked to report any side effects. Blood samples (10 ml) were taken into plain glass tubes, and glass tubes containing lithium, heparin and trasylol; the serum and plasma being removed after centrifugation (1600 g for 5 min) and stored at –20 °C for measurement of growth hormone, glucose and GRF(1–44).

Assays

Plasma glucose concentrations were measured by a glucose oxidase method using a Beckman glucose analyser (Beckman Instruments,

Fullerton, California, USA). Growth hormone was measured by a specific rapid radioimmunoassay procedure [12]. The World Health Organisation's first international reference preparation for human growth hormone (MRC 66/217) was used as standard. All assays were carried out in duplicate and at multiple dilutions, with parallelism between sample and standards. Haemoglobin A_{1c} was measured by ion exchange chromatography at 22 °C (Bio-Rad kit, Bio-Rad Laboratory, Watford, Herts, UK).

GRF was measured by a recently developed radioimmunoassay. Synthetic hpGRF₁₋₄₄ NH₂ (Bachem, Torrance, California, USA) was used for immunisation, iodination and as a standard. The antibody used (GRF 9-affinity constant 7.5×10^{11} l/mol) was produced in a rabbit immunised with GRF conjugated to bovine serum albumin using bis-diazotised benzidine coupling [13]. The label was prepared using the chloramine T method and was purified by high performance liquid chromatography on a Bondapak C18 column [14]. The antibody (used at a final dilution of 1/64,000) does not cross-react with any member of the glucagon-secretin family of peptides, but fully cross-reacts with synthetic hpGRF₁₋₄₀, hpGRF₁₋₃₇ and hpGRF₁₋₂₉ NH₂. Each sample was assayed in duplicate in a total reaction volume of 800 µl. Incubation was over 5 days at 4 °C. Charcoal adsorption was used to separate antibody-bound label from free label, for which 500 µl of a suspension containing 4 mg of charcoal was added to each tube. The assay was able to detect changes of 0.4 fmol/tube with 95% confidence. The intra-assay and inter-assay variations were less than 15%.

Statistical analysis

Results are expressed as mean $\pm$ SEM. Significance of results was assessed using Student's paired t-tests.

Results

None of the subjects studied showed any change in pulse or blood pressure and no subjective side effects were reported during the test.

Normal subjects

Figure 2 shows the normal responses of growth hormone to GRF(1-44) (100 µg). In all control subjects, the growth hormone response was rapid. The peak values varied between 22 and 225 mU/l (mean 97 ± 23 and 116 ± 46 mU/l) for groups 1 and 2, respectively. Values fell to 25 mU/l or below by 120 min.

Figure 2 also shows the decline in GRF(1-44) concentrations with time in the two normal groups. The peak concentration varied between 410 and 1300 pmol/l (mean values of 825 ± 135 and 677 ± 204 pmol/l) in groups 1 and 2, respectively. In four of group 1 and all in group 2 the level had returned to the basal value by 120 min.

Type 1 diabetic patients

The growth hormone response to GRF(1-44) (100 µg) in Type 1 diabetes is shown in Figure 2. In all 10 subjects the response was rapid. The peak value varied between 32 and 208 mU/l (mean 103 ± 27 mU/l) which was not significantly different from that for normal subjects.

Although the rate of rise of growth hormone appeared to be faster in the Type 1 diabetic patients compared to control subjects, there was no significant difference between the two on a Wilcoxon ranking test. The responses did not appear related to prevailing plasma glucose concentrations, since patients with low or normal plasma glucose concentrations had similar variations in response to those with hyperglycaemia (Table 2).

Figure 2 shows the decline in GRF(1-44) concentration with time. The peak concentration varied between 600 and 1600 pmol/l (mean 1008 ± 131 pmol/l) at 2 min which was not significantly different from that achieved in the corresponding normal group. In all subjects levels had returned to basal values by 120 min.

Type 2 diabetic patients

The growth hormone response to GRF(1-44) (100 µg) in Type 2 diabetes is shown in Figure 2. In all subjects the response was significantly smaller than that seen in Type 1 diabetic patients ($p < 0.02$) and in normal subjects of a similar age ($p < 0.05$). A measurable rise did not occur until 10 min after the bolus injection of GRF(1-44). The peak value was reached between 10 and 60 min and varied between 2.3 and 39 mU/l (mean 12 ± 5 mU/l) at 60 min. There was a tendency for pat-

Table 2. Individual peak growth hormone concentrations in response to GRF(1-44) in relation to the mean plasma glucose of each patient during the test

	Patient no.										
	1	2	3	4	5	6	7	8	9	10	11
<i>Normal subjects</i>											
Mean plasma glucose (mmol/l)	4.0	4.5	4.2	4.5	4.1	4.1	4.2	4.2	4.3	5.6	4.6
Peak growth hormone (mU/l)	98	85	100	25	234	147	95	106	149	22	200
<i>Type 1 diabetes</i>											
Mean plasma glucose (mmol/l)	12	13	14	15	16	17	18	19	20	21	
Peak growth hormone (mU/l)	4.1	15	2.7	6.2	16.4	16.6	15.3	14.2	9.8	6.2	
	118	163	122	43	35	38	153	208	14	33	
<i>Type 2 diabetes</i>											
Mean plasma glucose (mmol/l)	22	23	24	25	26	27	28	29			
Peak growth hormone (mU/l)	6.5	5.4	4.1	9.5	4.3	8.7	6.2	11.6			
	12	20	45	8.8	39	8.5	3.6	2.3			

NORMAL SUBJECTS <30 YEARS OLD

Fig. 3. Mean $\pm$ SEM serum growth hormone concentrations in six patients with hypopituitarism in response to GRF(1–44), 100 μ g given at 0 min

Fig. 2. Mean $\pm$ SEM serum growth hormone concentrations in normal subjects (<30 years); Type 1 diabetic patients; normal subjects (>50 years) and Type 2 diabetic patients in response to GRF(1–44), 100 μ g given intravenously at 0 min, are shown in the left panel. Their respective GRF concentrations ($\pm$ SEM) are shown in the righthand panel

ients with lower plasma glucose concentrations to have less impaired growth hormone response, but on comparison with normal subjects or Type 1 diabetic patients with similar glucose concentrations these responses were low (Table 2).

The corresponding decline in GRF(1–44) concentrations with time in the Type 2 diabetic patients is shown in Figure 2. The peak concentration varied between 520 and 1300 pmol/l (mean 916 ± 130 pmol/l) at 2 min. In all subjects the level had returned to basal values by 120 min. This was not significantly different from that seen in Type 1 diabetic patients or normal subjects of a similar age.

Subjects with hypopituitarism

The response to GRF(1–44) (100 μ g) in subjects with impaired pituitary function is shown in Figure 3. All members of the group had basal growth hormone levels below the lower limit of sensitivity of the assay (1 mU/l). One subject had no response to GRF(1–44); the remainder had very much smaller responses compared with normal subjects. The peak growth hormone levels varied between 1.0 and 6.8 mU/l (mean 2.6 ± 1.0 mU/l) at 30 min.

Plasma glucose

GRF(1–44) had no significant effect on plasma glucose in any of the subjects studied.

Discussion

A previous study has shown GRF(1–44) specifically to stimulate growth hormone release in normal man, acromegaly, and patients with impaired pituitary function [11]. The effects of GRF(1–44) in patients with diabetes mellitus have not been investigated previously.

Several studies have investigated the growth hormone response to a variety of stimuli in both Type 1 and 2 diabetes. Fasting growth hormone and its response to exercise have been reported to be greater in diabetic patients compared to control subjects [1, 2]. In

these studies a negative correlation was found between body weight and the magnitude of the growth hormone response to exercise, in both normal and diabetic subjects [1, 2]. Failure to take into account the influence of obesity on growth hormone responses has led to confusing anomalies in the literature [3, 4]. The contribution of body weight to the impaired growth hormone responses of the Type 2 diabetic patients in the present study was considered. As judged by their weight, expressed as a percentage of ideal body weight, the Type 2 diabetic patients, as a group, were more obese than their Type 1 counterparts or the control subjects. Although obesity therefore may have played some part in the lower growth hormone responses observed in these Type 2 diabetic patients, it was felt not to be a major factor in that the four patients in this group who were considered to be obese (ideal body weight > 115%) did not have lower responses compared to the other four non-obese patients (ideal body weight < 115%). Furthermore, comparison of the growth hormone responses of Type 2 diabetic patients of the same body weight as similarly aged control subjects clearly shows the former to have very impaired growth hormone responses.

An impaired growth hormone response to arginine infusion has been demonstrated in Type 2 diabetes [4, 15], while the response to hypoglycaemia has been reported to be normal in both Type 1 and 2 diabetes [16, 17]. L-dopa has also been used to investigate growth hormone release in diabetic patients; it has been suggested that the mechanism whereby L-dopa releases growth hormone is via conversion to dopamine, and stimulation of growth hormone releasing factor. Growth hormone responses to L-dopa have been found to be impaired in diabetes while hyperglycaemia inhibited the response in normal subjects [18]. Within the group of Type 2 diabetic patients of the present study, there was a tendency for those patients with lower blood glucose concentrations to have slightly better growth hormone responses. However when the responses of control subjects and Type 2 diabetic patients with comparable blood glucose concentrations were examined, the latter always had markedly lower growth hormone responses. This would suggest that elevated blood glucose per se was not responsible for the impaired growth hormone response in Type 2 diabetes. This is further supported by our findings in Type 1 diabetes; most of these patients had marked hyperglycaemia yet had growth hormone responses to GRF which fell within the normal range. The lack of effect of blood glucose on these responses was well illustrated by two patients, whose blood glucose concentrations were 2.2–2.7 mmol/l and 15.3–15.8 mmol/l during the study, while their respective peak growth hormone responses were 122 and 153 mU/l. This may indicate that, in diabetes, the pituitary growth hormone cells become unresponsive to the normal inhibitory influence of glucose.

It has recently been reported that the growth hormone response to GRF(1–44) falls off with age [19].

These findings are somewhat biased by the unusually large responses of four of the younger patients; in the absence of these responses there would be no significant age-dependent impairment of the growth hormone response to GRF. Our data in elderly control subjects and in Type 1 diabetic patients of similar age do not support a diminishing growth hormone response to GRF with increasing age, and this is unlikely to be the explanation for the impaired response by comparison with age-matched controls in the Type 2 diabetic patients in our study.

Abnormally high concentrations of somatostatin within the hypothalami of Type 2 diabetic patients may provide an explanation for their impaired response to GRF. Evidence from rat studies suggests that there is a dynamic interaction between GRF and somatostatin in the regulation of growth hormone secretion [20]. Enhanced secretion of somatostatin has been reported in animal models of Type 2 diabetes [21, 22], and high plasma concentrations have been reported in Type 2 diabetes [23].

In conclusion the use of GRF revealed marked differences in the growth hormone responses between Type 1 and Type 2 diabetic patients, and Type 2 diabetic patients and normal subjects. These cannot readily be explained by differences in the age, sex, weight, severity of disease or current medication of these patients. Nor can they be explained by altered handling of the peptide GRF(1–44) as shown by the concentration-time curves.

These findings may well indicate a defect in central hormonal control in diabetes, such that the sensitivity of the growth hormone secreting cells of the pituitary to a variety of regulatory factors including GRF, circulating glucose, amino-acids, and as yet unknown modulators, is abnormal. This clearly warrants further investigation.

Acknowledgements. The present studies were supported by a grant from the British Diabetic Association.

References

1. Hansen AP (1973) Abnormal serum growth hormone response to exercise in maturity onset diabetes. *Diabetes* 22: 619–28
2. Hansen AP (1970) Abnormal serum growth hormone response to exercise in juvenile diabetics. *J Clin Invest* 49: 1467–1478
3. Burday SZ, Fine PH, Schalch DS (1968) Growth hormone secretion in response to arginine infusion in normal and diabetic subjects: Relationship to blood glucose levels. *J Lab Clin Med* 71: 897–911
4. Merimee TJ, Burgess JA, Rabinowitz D (1966) Arginine infusion in maturity onset diabetes mellitus. *Lancet* 1: 1300–1301
5. Lundbaek K, Christensen NJ, Jensen VA, Johansen K, Olsen TS, Hansen AP, Orskov H, Osterby R (1971) The pathogenesis of diabetic angiopathy and growth hormone. *Dan Med Bull* 18: 1–7
6. Lundbaek K, Margensen CE, Anderson MJE (1974) Increased kidney size and glomerular filtration rate in untreated juvenile diabetes normalization by strict insulin treatment. *Diabetologia* 10: 378 (Abstract)
7. Frohman LA, Szabo M, Berelowitz M, Stachura ME (1980) Partial purification and characterisation of a peptide with growth hor-

- mone releasing activity from extrapituitary tumours in patients with acromegaly. *J Clin Invest* 65: 43–54
8. Rivier J, Spiess J, Thorner M, Vale W (1983) Characterisation of a growth hormone releasing factor from a human pancreatic tumour. *Nature* 300: 276–278
 9. Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johnson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia, successful treatment of acromegaly by removal of a pancreatic islet tumour secreting a growth hormone releasing factor. *J Clin Invest* 70: 965–977
 10. Guilleman R, Brazeau P, Bohlen PE, Esch F, Ling N, Wehrenberg W (1982) Growth hormone releasing factor from a human pancreatic tumour that caused acromegaly. *Science* 218: 585–587
 11. Wood SM, Ch'ng JLC, Adams EF, Webster JD, Joplin GF, Mashiter K, Bloom SR (1983) Abnormalities of growth hormone releasing factor GRF (1–44) in acromegaly and hypopituitarism. *Br Med J* 286: 1687–1691
 12. Adams EF, Brajkovich IE, Mashiter K (1981) Growth hormone and prolactin secretion by dispersed cell cultures of human pituitary adenomas: long term effects of hydrocortisone, oestradiol, insulin, 3,5,3 tri-iodo thyronine and thyroxine. *J Clin Endocrinol Metab* 53: 381–386
 13. O'Shaughnessy DJ (1982) Antibodies. In: Bloom SR, Long RG (eds) *Radioimmunoassay of gut regulatory peptides*. WB Saunders, London, pp 11–20
 14. Hunter WM, Greenwood FC (1982) Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 194: 495–496
 15. Tchobroutsky G, Rosselin G, Assan R, Derot M (1968) Arginine infusion in diabetes mellitus. *Lancet* 2: 498–499
 16. Tchobroutsky G, Assan R, Rosselin G, Derot M (1967) Taux plasmatiques de l'hormone somatrophe chez de diabétiques premiers résultats de déterminations a jeun, au repos et après administration d'arginine ou d'insuline. *Ann Endocrinol Paris* 28: 766–775
 17. Fatourech V, Molnar GD, Service FJ, Ackerman E, Rosevear JW, Moxness KE, Taylor WF (1969) Growth hormone and glucose interrelationships in diabetes: studies with insulin infusion during continuous blood glucose analysis. *J Clin Endocrinol* 29: 319–327
 18. Mims RB, Scott CL, Modebe OM, Bethune JE (1973) Prevention of L-dopa induced growth hormone stimulation by hyperglycaemia. *J Clin Endocrinol Metab* 37: 660–663
 19. Shibasaki T, Shizume K, Nakahara M, Masuda A, Jibiki K, Demura H, Wakabayashi I, Ling N (1984) Age related changes in plasma growth hormone response to growth hormone releasing factor in man. *J Clin Endocrinol Metab* 58: 212–214
 20. Wehrenberg WB, Ling N, Bohlen P, Esch F, Brazeau P, Guilleman R (1982) Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. *Biochem Biophys Res Commun* 109: 562–567
 21. Kadowaki S, Iaminato T, Chiba I, Goto Y, Nozawa M, Seino Y, Matsukura S, Fujita T (1980) Somatostatin release from the isolated perfused diabetic rat pancreas. *Diabetes* 29: 960–963
 22. Weir GC, Clore ET, Zmachinski CJ, Bonner Weir S (1981) Islet cell secretion in a new experimental model for non-insulin dependent diabetes. *Diabetes* 30: 590–595
 23. Wood SM, Webster J, Bloom SR (1982) Regulatory peptides in Type 2 (non-insulin-dependent) diabetes. *Diabetologia* 22: 18 (Abstract)

Received: 10 October 1983
and in final form: 16 August 1984

Dr. S. M. Wood
Department of Medicine
Royal Postgraduate Medical School
Du Cane Road
London W12 0HS
UK