Circulating growth hormone forms in Type 1 diabetic subjects: comparison with normal subjects and acromegals

I. A. MacFarlane, Susan Stafford and A. D. Wright

Diabetic Clinic, The General Hospital, Birmingham, UK

Abstract. The molecular forms of growth hormone (GH) in serum from 18 Type 1 diabetic patients with poor metabolic control were analysed using sephadex G-100 chromatography. The profiles obtained were compared with those from normal subjects whose GH secretion was stimulated by exercise and hypoglycaemia and eight acromegalic patients. In the three groups three distinct GH forms were found: little (monomeric), big and big-big-GH. Samples from normal subjects contained 45% little-GH which was less than samples from the diabetics and acromegals (53% and 65%, respectively, $P < 0.01$). Further samples from normal subjects after the onset of hypoglycaemia showed an increase in little-GH. In the three groups, the higher the proportion of little-GH, the lower the proportion of big-big-GH, while the proportion of big-GH remained similar. In the acromegals the proportion of little-GH was strongly correlated with the log concentration of serum GH. As little-GH is cleared from the circulation quicker than the larger forms these data indicate that the main component of the frequent surges of GH secretion in poorly controlled Type 1 diabetic subjects is little-GH (monomeric forms). The sustained release of GH found in acromegaly is composed largely of monomeric forms.

With poor metabolic control often show large spontaneous GH peaks during 24 h (Johansen & Hansen 1971; Hansen et al. 1981). When serum or plasma is fractionated by sephadex chromatography and the resultant fractions assayed by RIA, circulating GH can be shown to consist of three distinct forms (Gorden et al. 1976). These are little or monomeric GH (approximately mol. wt. 20000-daltons), big or dimeric GH (approximate mol. wt. 40000-daltons) and big-big or oligomeric GH (mol. wt. greater than 60 000) (Stolar et al. 1984a).

In healthy subjects, approximately half of the GH secreted after stimulation by hypoglycaemia or arginine circulates in the little form. In acromegalic patients little GH constitutes a larger proportion of circulating GH (60–80%) (Gorden et al. 1976). There are no data however, on the forms of GH circulating in insulin-dependent diabetic subjects. We thought it important to study the circulating GH forms in insulin-dependent diabetic subjects as there has been considerable interest in the role of excessive GH secretion in the development and progression of diabetic microvascular complications (Gerich 1984).

Patients and Methods

a) Normal subjects

Eleven healthy control subjects were studied who were known not to have diabetes and whose fasting blood glucose was less than 5 mmol/l. Eight were male. The
median age was 25 years (range 21–35 years). Hypoglycaemia (blood glucose < 2 mmol/l) was induced by iv insulin in eight subjects (0.2 U/kg). Venous blood samples were taken at the onset of hypoglycaemia and in three subjects 20–30 min later. Blood samples were taken from eight subjects after 20–30 min of vigorous exercise.

b) Diabetic patients
Eighteen insulin-dependent diabetic patients were studied during admission for stabilisation. Fourteen were male, and the median age was 30 years (range 16–45 years). All were within 15% of ideal body weight, and all had a normal serum creatinine, median 69 µmol/l (range 56–101 µmol/l). The diagnosis of diabetes had been made one week to 28 years previously (median 14 years). Multiple venous blood samples were taken in the rested, fasted state between 09.00 and 12.00 h after omitting the morning insulin injections. A serum sample from each patient which contained a GH concentration > 10 mU/l was run on the sephadex column. All had poor diabetic control: random blood glucose concentrations > 15 mmol/l, marked glycosuria and glycosylated haemoglobin (HbA1) > 12% (Menard et al. 1980).

c) Acromegalic patients
Eight patients with excessive GH secretion due to acromegaly were studied. None had diabetes. Two were untreated, six had had previous radiotherapy or pituitary surgery, and none was taking bromocriptine. Five were male, and the median age was 53 years (range 30–64 years). Venous blood samples were taken in the rested, non-fasted state.

Venous blood was allowed to clot at room temperature, the serum separated and stored at −20°C before chromatography.

Radioimmunoassays
1) Measurement of GH in serum. A double antibody RIA was used. Standards were prepared from hGH, NIBSC 66/217, diluted in 0.05 m sodium phosphate buffer. One international unit (IU) is equivalent to 0.5 mg of hGH. [125I]hGH was prepared using chloramine-T and hGH NIBSC, 69/46. Rabbit anti-hGH antisemur (M153) raised against monomeric hGH was donated by the Endocrine Laboratory, Birmingham and Midland Hospital for Women. The sensitivity of the assay was 0.6 mU/l. The intra-assay and the inter-assay coefficients of variations, estimated from repeat assays of a serum pool giving 50% displacement of tracer, were 5.7% and 11.0%, respectively (Stafford 1984).

2) Measurement of GH in chromatography fractions. In order to measure the lower concentrations of GH in the column fractions, the RIA was modified by increasing the volume of standards and samples in the assay to improve the sensitivity to 0.2 mU/l (Stafford 1984).

Chromatography
Serum samples (0.5–2 ml) were applied to the top of a 1.5 × 80 cm column of sephadex G-100 at 4°C. The column was run with 0.05 m ammonium carbonate solution, pH 8.6, containing 0.9% NaCl, 0.1% bovine serum albumin and 0.1% NaN3 at a constant flow rate of 16.2 ml/h.

Serum samples were stored at −20°C for varying lengths of time before being applied to the column. Aliquots of sera from four subjects (1 normal, 1 diabetic, 2 acromegalic) were applied twice, 3–6 months apart. There was no significant difference between the elution profiles on the two occasions indicating no interconversion of components after storage at −20°C. There was no significant difference between the profiles obtained from aliquots of samples from two subjects stored at −20°C and 4°C.

Statistical methods
To analyse the column chromatography data, block diagrams were constructed and the area under the curve for each of the three components (little, big and big-big-GH) were calculated as a percentage of the total area. The mean of the percentage of each component was taken for the three groups of subjects. These percentages were compared using unpaired t-tests. The serum GH concentrations were log10 transformed to produce a normal distribution.

Results
The serum GH concentrations in the sample applied to the column were not significantly different in the three groups. The median GH value in the 16 samples from the 11 normal subjects (8 at onset of hypoglycaemia and 8 after exercise) was 43 mU/l (range 6.2–125); the median value in the 18 samples from 18 diabetics was 22.5 mU/l (range 12–74); the median value in the 8 samples from the 8 acromegalics was 33 mU/l (range 9–110). A typical elution profile from one of the normal subjects is shown in Fig. 1.

In the normal subjects there was no significant difference between the percentage of each GH component found in sera after exercise or at the onset of clinical and biochemical hypoglycaemia. The percentages of the three GH components in the three groups are shown in Table 1. The percentage of little GH was increased in the acromegalic patients compared to the normals (P < 0.001). The diabetic sera also had a higher percentage of little GH circulating than normal.
Fig. 1.
Representative Sephadex G-100 profile (1.5 x 80 cm column) of serum GH: normal subject after insulin induced hypoglycaemia. V₀, void volume (dextran 2000); Vᵣ, free sodium iodide-125. The shaded area denotes the undetectable range.

(P < 0.01) though this was less than sera from the acromegals (P < 0.01). Conversely the big-big component in the acromegals and the diabetics was lower than in the normals (P < 0.02). The mean percentage of big GH in the three groups were similar.

In the acromegal patients there was a significant positive correlation between log serum GH concentration and the percentage of the little component (r = 0.83, P < 0.02) and a significant negative correlation with the percentage of the big-big component (r = 0.94, P < 0.001). How-

Table 1.
Percentages (mean and SEM) of the GH components after chromatography.

<table>
<thead>
<tr>
<th></th>
<th>Big-big</th>
<th>Big</th>
<th>Little</th>
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<tbody>
<tr>
<td>a) Normal subjects</td>
<td>34.4 (1.8)</td>
<td>19.9 (0.8)</td>
<td>45.6 (2.8)</td>
</tr>
<tr>
<td>(n = 16)*</td>
<td></td>
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<tr>
<td>b) Diabetic patients</td>
<td>28.2 (1.6)</td>
<td>18.3 (0.9)</td>
<td>53.4 (2.0)</td>
</tr>
<tr>
<td>(n = 18)</td>
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<tr>
<td>c) Acromegalic patients</td>
<td>20.2 (2.8)</td>
<td>15.3 (1.5)</td>
<td>64.5 (3.4)</td>
</tr>
<tr>
<td>(n = 8)</td>
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<tr>
<td>a vs b</td>
<td>P &lt; 0.02</td>
<td>ns</td>
<td>P &lt; 0.01</td>
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<tr>
<td>a vs c</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.001</td>
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<tr>
<td>b vs c</td>
<td>P &lt; 0.02</td>
<td>ns</td>
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ns: not significant.
* 16 sera from 11 normal subjects: 8 after exercise, 8 at onset of hypoglycaemia.
ever, there was no correlation between log serum GH concentration and the percentage of the big component in the acromegalics. There were no significant correlations between log serum GH concentration and any of the components in the normal subjects or the diabetic patients.

In three normal subjects the median serum GH concentration was 31 mU/l at the onset of hypoglycaemia. This had increased to 94 mU/l in serum samples taken 20–30 min later. Comparing the GH forms in the samples taken at the onset of hypoglycaemia with those 20–30 min later, little-GH increased (median 43% rose to 56%), big-big-GH decreased (median 37% fell to 26%) and big-GH was unchanged (median 20% and 18%).

Discussion

The antibody used in the RIA’s to measure GH in the serum and chromatography fractions was raised in rabbits against monomeric GH and therefore may not recognise all the circulating GH fragments and higher molecular weight components. Therefore the results cannot be considered to be absolute quantities of GH. However, this limitation does not preclude an interpretation of the relative preponderance of the large fractions of GH in the normal subjects and the two groups of patients.

Chromatography of serum samples on sephadex G-100 confirmed the results of other workers that normal subjects and acromegalic patients have three distinctive immunoreactive GH components circulating (Gorden et al. 1976). Three GH components were also separated from the sera of diabetic patients. It was necessary for some serum samples to be stored before application to the column, but the results showed that there was no interconversion of GH components after storage at −20°C for several months or at 4°C for several weeks. Similar results were obtained by Goodman et al. (1972) and Gorden et al. (1973a).

Serum samples from the controls after exercise or immediately following insulin-induced hypoglycaemia showed no significant difference in the gel elution profile of GH, and these samples were therefore considered as one group in the analysis of data. Our data demonstrate a significantly higher proportion of little-GH and a significantly lower proportion of big-big-GH in the acromegalis compared with the diabetics and controls. The diabetics also showed higher little and lower big-big-GH proportions compared with the normal subjects. The proportion of big-GH in the three groups was similar. Gorden et al. (1976) also found a higher proportion of little-GH in acromegalis compared with normal subjects. When further samples were taken 20–30 min after hypoglycaemia in the normal subjects, the proportion of little-GH had increased at the expense of big-big-GH.

The larger the GH component, the longer it takes it takes to be cleared from the circulation (Hendricks et al. 1985). It is unlikely that there are differences in clearance of the GH components between the three groups. Therefore it is possible to speculate that different patterns of GH secretion are responsible for our findings i.e., frequent and marked GH secretion is associated with an increased release of monomeric forms. This explains the higher proportion of little-GH in the diabetics and the later hypoglycaemic samples from the normals. The sustained, excessive GH release in acromegaly is mainly monomeric GH. The strong positive correlation found between the serum GH level and the proportion of little-GH in the acromegalic patients supports this hypothesis. Also our findings suggest that an increase in little-GH secretion is at the expense of big-big-GH secretion.

Recent studies on sera from stimulated normal subjects and acromegalis using polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulphate-PAGE and iso-electric focussing have shown that plasma big and big-big-GH represent an oligomeric series composed of 22K (major), 20K and one or more acidic GH monomers. The majority of these monomers are non-covalently associated, with a smaller fraction consisting of monomers linked by disulphide bridges (Stolar et al. 1984a,b). The physiological significance of the big-GH forms is unclear. In general, circulating big forms exhibit diminished receptor-binding activity and/or biological potency relative to those of monomeric GH (Gorden et al. 1973b, 1976).

There is growing evidence that the excessive GH secretion in poorly controlled diabetic patients may play a role in diabetic microvascular complications (Gerich 1984). Circulating levels of GH and of GH-dependent insulin-like growth factor or somatomedin are higher in Type 1.
diabetics with retinopathy than in non-diabetics or Type 1 patients without retinopathy (Merimee et al. 1983). Also, diabetic subjects with GH deficiency tend to be spared microvascular complications and hypophysectomy can prevent the progression of retinopathy (Merimee 1978; Kohner et al. 1976). We found no relationship between the proportions of GH forms and the presence of microvascular complications or the degree of metabolic control (HBA1 concentrations) in our diabetic patients. However, the molecular composition of the monomeric and oligomeric GH forms in diabetics in relation to microvascular complications remains to be assessed. Also, our study has only detected immunoreactive GH forms. It is possible that some diabetic subjects release GH forms of high bioactivity and little immunoreactivity which may have a major role in the development and progression of retinopathy.

References


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