Effects of cholinergic modulation on serum insulin-like growth factor-I and its binding proteins in normal and diabetic subjects

I. S. Ismail, J. P. Miell, M. F. Scanlon and J. R. Peters
Section of Endocrinology, Metabolism and Diabetes, Department of Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, and tDepartment of Medicine, King’s College School of Medicine, Denmark Hill, London SE5 9RS, UK
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Summary

OBJECTIVE We wished to study alterations in serum insulin-like growth factor-I (IGF-I) and its binding proteins in subjects with insulin dependent diabetes mellitus (IDDM) and possible relations with metabolic and GH secretory status, before and after cholinergic modulation. In addition, we have investigated whether cholinergic modulation exerts any effects on IGF-I secretion, independently of any actions on GH secretory status.

DESIGN All subjects received GH releasing hormone (GHRH) 1–44; 80 µg i.v.) alone and 60 minutes following 120 mg of pyridostigmine orally or 200 mg of pirenzepine orally. The three tests were carried out in random order at least one week apart. Blood was sampled at 15-minute intervals over 120 minutes.

PATIENTS Twelve male subjects with IDDM and no clinical evidence of complications were selected on the basis of HbA1c levels to provide a wide range of metabolic control. Six normal male subjects were also studied.

MEASUREMENTS Serum IGF-I, IGF-binding protein 1 (IGFBP-1) and IGFBP-3 were measured at regular intervals throughout the study. Fasting plasma glucose and HbA1c were measured before each study to provide measures of metabolic control.

RESULTS Serum IGF-I and IGFBP-3 levels were significantly lower while serum IGFBP-1 levels were significantly higher in the diabetic subjects. Pirenzepine had no effect on serum IGF-I, IGFBP-1 or IGFBP-3 in diabetic subjects but caused a significant increase in serum IGF-I and IGFBP-3 levels in normal subjects.

Pyridostigmine had no effect on IGF-I, IGFBP-1 or IGFBP-3 in either diabetic or normal subjects. IGFBP-1 levels were significantly correlated with fasting plasma glucose but no correlation was demonstrated between measures of diabetic control and serum IGF-I or IGFBP-3 levels in diabetic subjects, nor was there any correlation between GH responses to GHRH alone or after pirenzepine or pyridostigmine pretreatment and serum levels of IGF-I, IGFBP-1 or IGFBP-3.

CONCLUSION These data confirm that subjects with IDDM have reduced serum IGF-I and IGFBP-3 and increased IGFBP-1 levels, the latter being directly related to the fasting plasma glucose concentrations. The absence of any relation between changes in the IGF-I system and altered GH neuroregulation after cholinergic modulation suggests that changes in IGF-I are not the sole contributors to the altered GH neuroregulation which occurs in IDDM. We have also shown an acute stimulatory effect of pirenzepine on serum IGF-I and IGFBP-3 in normal subjects which is not present in IDDM although the underlying mechanism is unknown.

In patients with insulin dependent diabetes mellitus (IDDM), increased 24-hour growth hormone (GH) secretion and elevated GH responses to provocative stimuli occur commonly (Hansen, 1970; Hayford et al., 1980; Holly et al., 1988a) but the underlying mechanisms are not fully understood. Pituitary secretion is under hypothalamic control via the stimulatory effects of GH-releasing hormone (GHRH) and the inhibitory actions of somatostatin (SS) (Dieguez et al., 1988). GH stimulates insulin-like growth factor-I (IGF-I) production which exerts a negative feedback on GH release in animal studies (Berelowitz et al., 1981; Yamashita & Melmed, 1986). Conflicting data have been reported concerning IGF-I in patients with IDDM, both normal (Horner et al., 1981; Lamberton et al., 1984) and low (Amiel et al., 1984; Tan & Baxter, 1986; Dills et al., 1990) levels having been described. It is possible that altered negative feedback by IGF-I contributes to the changes in GH secretory status in IDDM so that increased GH secretion compensates for a partial block in IGF-I production.

It is also possible that alterations in central control mechanisms contribute to this phenomenon and we have recently provided evidence consistent with the presence of...
increased hypothalamic cholinergic tone in patients with IDDM, which could reduce hypothalamic SS release leading to GH hypersecretion (Ismail et al., 1993). Briefly, cholinergic augmentation with pyridostigmine (PD) did not increase GH responses to GHRH in diabetic subjects in contrast to normals while cholinergic blockade with pirenzepine (PIR) reduced the GH responses to GHRH in diabetic subjects but not to the same extent as in normals. There was no correlation between metabolic control as measured by fasting plasma glucose and HbA1c and the altered GH responses. We now report data on alterations in serum IGF-I and its binding proteins in this same group of normal and diabetic subjects and discuss possible relations with metabolic and GH secretory status.

Methods

The study design and clinical characteristics of the subjects have been reported previously and are shown in Table 1 (Ismail et al., 1993). The study was granted ethical approval by the Ethics Subcommittee of the University Hospital of Wales. Briefly, 6 normal males and 12 males with IDDM and no clinical evidence of diabetic complications were studied. The studies were carried out on 3 separate days, at least one week apart, and in random sequence. On the day before each test, diabetic patients received their last dose of long-acting insulin in the evening and no further insulin was administered until the end of the test on the following day. After an overnight fast, subjects were admitted to the Investigation Unit and all subjects received GHRH (80 μl) i.v. alone (control study) and after pretreatment with pirenzepine (PIR 200 mg orally) or pyridostigmine (PD 120 mg orally) one hour prior to GHRH which was given at the same clock time on each occasion. Blood was sampled for serum GH, IGF-I, IGF binding protein-1 (IGFBP-1) and IGFBP-3. Blood was sampled at 15-min intervals over 120 minutes for measurement of GH levels and the other parameters were measured on selected samples as indicated below.

IGF-I was measured, after acid–ethanol extraction of its binding proteins, by radioimmunoassay, using a polyclonal rabbit antiserum (R557A) raised against purified human IGF-I. Results are expressed in μg/l. The limit of detection of the assay was 5 μg/l; interassay coefficients of variation were 9-0, 4-5 and 6-2% at analyte levels of 654, 231 and 78-4 μg/l, respectively, with an intra-assay CV of 4% at 231 μg/l. Serum concentrations of IGFBP-1 were measured by a specific radioimmunoassay (Mielik et al., 1993). Purified antigen was obtained from Dr S. Drop (Rotterdam, The Netherlands). Tracer was prepared by iodination of antigen using the chloramine T method, followed by separation on a short Sephadex G-75 column. Antiserum (Taylor et al., 1990) was used at a final dilution of 1:10 000 which bound approximately 60% of iodinated tracer. Bound and free antigens were separated using a solid-phase second antibody, donkey anti-rabbit coated cellulose (Sac-Cel, Welcome, Beckenham, UK). The minimum detection limit of the assay was 6 μg/l. The interassay CV at 55 μg/l was 6-2%, and the intra-assay CV at 35 μg/l was 4%. IGFBP-3 was measured by a specific RIA as previously described (Blum et al., 1990). This assay has a sensitivity of 0-03 mg/l and the intra and inter-assay coefficients of variation at 50% specifically bound tracer were 3-5 and 7-3% respectively.

Plasma glucose was measured by the glucose oxidase method (Yellow Springs Instruments Model 23AM glucose analyser). HbA1c was measured by the agar gel electrophoresis method (GLYTRAC, Corning Medical, Pala Alto, California, USA). The normal range was 5–8%.

To better characterize the effect of cholinergic modulation, percentage suppression or augmentation of GH responses was calculated by dividing GH responses after cholinergic modulation by GH responses during the control study. The changes in serum IGF-I, IGFBP-1 and IGFBP-3 were measured by subtracting the baseline value from the value at the end of the study. Statistical analysis used paired and unpaired Student's t-test. Changes in IGF-I and

<table>
<thead>
<tr>
<th>Table 1 Clinical characteristics of subjects</th>
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<tr>
<td><strong>Subject</strong></td>
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<tr>
<td>IDDM (12 males)</td>
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<tr>
<td>Mean ± SEM</td>
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<tr>
<td>Range</td>
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<tr>
<td>Normal (6 males)</td>
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<tr>
<td>Mean ± SEM</td>
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<tr>
<td>Range</td>
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Table 2 The effect of GHRH alone, PIR 200 mg and GHRH, and PD 120 mg and GHRH, on IGF-I, IGFBP-1 and IGFBP-3 levels in patients with IDDM and normal subjects

<table>
<thead>
<tr>
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<th>Normal subjects</th>
<th>IDDM</th>
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<tbody>
<tr>
<td>Basal IGF-I (µg/l)</td>
<td>183.3 ± 8.2*</td>
<td>132.6 ± 8.2</td>
</tr>
<tr>
<td>ΔIGF-I after GHRH</td>
<td>−5.7 ± 9.4</td>
<td>−7.5 ± 7.6</td>
</tr>
<tr>
<td>ΔIGF-I after PIR</td>
<td>72.7 ± 16.0†</td>
<td>4.8 ± 11.1</td>
</tr>
<tr>
<td>ΔIGF-I after PD</td>
<td>−6.7 ± 7.2</td>
<td>−12.7 ± 8.6</td>
</tr>
<tr>
<td>Basal IGFBP-1 (µg/l)</td>
<td>26.6 ± 5.3*</td>
<td>218.9 ± 26.7</td>
</tr>
<tr>
<td>ΔIGFBP-1 after GHRH</td>
<td>−12.8 ± 4.3‡</td>
<td>−43.0 ± 26.0</td>
</tr>
<tr>
<td>ΔIGFBP-1 after PIR</td>
<td>−0.7 ± 2.4</td>
<td>−13.4 ± 15.0</td>
</tr>
<tr>
<td>ΔIGFBP-1 after PD</td>
<td>−0.5 ± 2.0</td>
<td>−40.3 ± 24.7</td>
</tr>
<tr>
<td>Basal IGFBP-3 (mg/l)</td>
<td>3.7 ± 0.2*</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>ΔIGFBP-3 after GHRH</td>
<td>0.1 ± 0.7</td>
<td>−0.01 ± 0.2</td>
</tr>
<tr>
<td>ΔIGFBP-3 after PIR</td>
<td>0.5 ± 0.1†</td>
<td>0.10 ± 0.2</td>
</tr>
</tbody>
</table>

*P < 0.01 vs IDDM; †P < 0.01 vs basal; ‡P < 0.05 vs basal.

IGFBP-3 were analysed using the paired t-test. Correlation analysis was used where appropriate with the basal data from all 3 study days in subjects with IDDM. Fasting plasma glucose levels from individual subjects on each of the three study days were compared using ANOVA. P < 0.05 was considered statistically significant. All values are expressed as mean ± SEM.

Results

The GH data have been reported (Ismail et al., 1993) and fasting plasma glucose did not differ significantly on the three study days.

Effect on IGF-I concentrations (Table 2, Figs 1 and 2)

Basal IGF-I concentrations were significantly lower in the diabetic subjects compared with normals on all three study days. IGF-I levels did not change after GHRH alone in either diabetic or normal subjects. PIR did not change IGF-I concentrations in diabetic subjects but stimulated a significant increase in serum IGF-I concentrations in normals. PD pretreatment did not affect the IGF-I concentrations in either diabetic or normal subjects.

There was no significant correlation between IGF-I concentrations and either of the measures of diabetic control, namely HbA1c and fasting plasma glucose, during the control study with GHRH alone. Similarly, there was no correlation between IGF-I concentrations and other measured variables including age, BMI and duration of disease. During the control study, there was no correlation between IGF-I concentrations and the GH responses to GHRH as measured by peak or mean GH. Similarly, there was no correlation between serum IGF-I concentrations and percentage change of peak or mean GH after PIR or PD pretreatment.

Effect on IGFBP-1 concentrations (Table 2)

Basal IGFBP-1 concentrations were significantly higher in the diabetics on all study days. There was no change in IGFBP-1 levels after GHRH alone in diabetics whereas normals showed a significant decrease in IGFBP-1 during the control study associated with an increase in GH concentrations. Neither PIR nor PD altered IGFBP-1 concentrations in either diabetic or normal subjects. In diabetics there was a significant correlation between IGFBP-1 and fasting plasma glucose (r = 0.79, P < 0.01). There was no correlation between IGFBP-1 and other variables including age, BMI or duration of disease.

During the control study, there was no correlation between IGFBP-1 and the GH response to GHRH as measured by peak or mean GH. There were no significant correlations between IGFBP-1 concentrations and either percentage suppression of GH response with PIR or percentage augmentation of GH responses with PD.
markedly reduced in keeping with previous data (Tan compared
Consequently, it is possible that increased secretion of GH
caused a significant increase in normals.

Discussion
Serum IGF-I concentrations in diabetic subjects were
markedly reduced in keeping with previous data (Tan &
Baxter, 1986; Taylor et al., 1988; Schapter et al., 1990).
Consequently, it is possible that increased secretion of GH
in IDDM may compensate for a partial block in IGF-I
production. However, there was no correlation between
serum IGF-I concentrations and GH responses to GHRH
which could indicate that reduced negative feedback by
IGF-I at the pituitary level may not be the sole contributor
to GH hypersecretion in IDDM.

The cause of the reduced IGF-I concentration is unclear.
Besides GH, nutrition and insulin are important regulators
of the serum IGF-I concentration (Furlanetto, 1990). However,
the comparable BMI of the diabetic and normal subjects in
this study argues against malnutrition as a likely cause of the
low IGF-I concentrations in the diabetic subjects and we
found no correlation between IGF-I concentrations and BMI.
Another possible explanation for the reduced IGF-I levels in
IDDM is the failure of low functional insulin concentrations
to stimulate the expression of hepatic GH receptors or to
stimulate IGF-I production at an intracellular, post-GH
receptor site (Maes et al., 1986). In accord with this view, it is
known that intensive insulin treatment and improved
metabolic control in IDDM is associated with increased
IGF-I levels and responsiveness to GH administration (Lanes
et al., 1985; Tamborlane et al., 1981; Amiel et al., 1984). On
the other hand, we and others (Salardi et al., 1986; Taylor et
al., 1988; Schaper et al., 1990; Massa et al., 1993) have been
unable to demonstrate any correlation between serum IGF-I
concentrations and HbA1c or fasting plasma glucose levels,
although this lack of correlation by no means excludes insulin
deficiency as a possible cause of the low IGF-I levels. It should
be emphasized that this lack of correlation may relate to the
small numbers of subjects studied in this cross-sectional
analysis.

Serum IGFBP-1 concentrations in IDDM were signifi-
cantly higher than in normal subjects and correlated with
fasting plasma glucose as previously reported by others
(Brismat et al., 1988; Suikkari et al., 1988; Hall et al., 1989;
Holly et al., 1990; Batch et al., 1991). These data are
consistent with the view that insulin is the main regulator of
hepatic IGFBP-1 production (Holly et al., 1988b; Suikkari
et al., 1988; Conover et al., 1992). We did not find any
relation between IGFBP-1 and long-term diabetic control as
measured by HbA1c, in contrast to the findings of others
(Holly et al., 1990; Batch et al., 1991). However, the absence
of such a correlation was not surprising because IGFBP-1
relates to insulin status over the preceding few hours whereas
HbA1c reflects status over the preceding weeks. In
addition, the marked acute circadian changes in IGFBP-1
(Holly et al., 1988b) further complicate the comparison with
chronic measures of glycaemic control. The decline in
IGFBP-1 levels in normal subjects in this study probably
reflects the normal circadian rhythm (Baxter & Cowell,
1987; Holly et al., 1988b).

**Fig. 2** The mean (±SEM) of serum a, IGF-I and b, IGFBP-3
congentations during a control study with i.v. GHRH alone and
c after PIR 200 mg pretreatment followed by i.v. GHRH in
6 normal subjects.

**Effect on IGFBP-3 concentrations (Table 2, Figs 1 and 2)**
IGFBP-3 concentrations were measured only during the
date and PIR studies in view of the significant effect of
PIR on IGF-I in normal subjects. Basal IGFBP-3 con-
centrations were significantly lower in the diabetic subjects
compared with normals on all three study days. Serum
IGFBP-3 levels did not change after GHRH alone in either
the diabetic or normal subjects. PIR did not change the
serum IGFBP-3 concentration in the diabetic subjects but
caused a significant increase in normals.

There was no significant correlation between IGFBP-3
concentrations and either of the measures of diabetic
control, namely HbA1c and fasting plasma glucose, during
the control study. Similarly, there was no correlation
between IGFBP-3 concentrations and other measured
variables including age, BMI and duration of disease.
During the control study day, there was no correlation
between IGFBP-3 concentrations and the peak or mean GH
responses to GHRH.
There was no correlation between IGF-I or IGFBP-1 and either the percentage suppression or augmentation of GH responses by PIR or PD respectively, actions which are probably effected at the hypothalamic level (Locatelli et al., 1986; Wehrenberg et al., 1992). These data suggest that IGF-I and IGFBP-1 do not contribute in a major way to the altered hypothalamic control mechanisms in IDDM.

Serum IGFBP-3 concentrations in diabetic subjects were significantly reduced compared with normal subjects, in keeping with published data (Baxter & Martin, 1986; Batch et al., 1991). The association of reduced IGFBP-3 with IGF-I in diabetic subjects despite elevated serum GH concentrations is consistent with the view that IGF-I, rather than GH, is the primary physiological regulator of circulating IGFBP-3. This view is supported by both in-vivo studies involving GH or IGF-I administration to hypophysectomized rats (Zapf et al., 1989) and expression of IGF-I in transgenic GH deficient mice which restores IGFBP-3 levels (Camacho-Hubner et al., 1991) as well as in-vitro studies where IGF-I, but not GH, stimulates IGFBP-3 production by cultured bovine fibroblasts (Conover, 1990).

In diabetic subjects, cholinergic modulation had no effect on IGF-I or IGFBP-1 concentrations but cholinergic muscarinic blockade with PIR significantly increased serum IGF-I and IGFBP-3 concentrations in normal subjects over a period of 3 hours. The increase in IGF-I was not a result of the GHRH induced increase in GH levels because a similar effect was not seen during the control study with GHRH alone and infusions of GH produce increases in IGF-I that begin only after 6 hours and peak at 16-28 hours (Copeland et al., 1980). Furthermore, PIR pretreatment caused marked suppression of GH responses to GHRH. The observation of a parallel increase in IGF-I and IGFBP-3 after PIR is in keeping with the hypothesis that IGF-I is the primary regulator of circulating IGFBP-3. However, not all studies support this and it was demonstrated recently that mean 24-hour IGFBP-3 levels in healthy adults were reduced after daily treatment with subcutaneous recombinant human IGF-I (Baxter et al., 1993). In view of this, it is possible that the primary effect of PIR in this study would be to increase IGFBP-3 with a secondary increase in IGF-I. The mechanism underlying the stimulatory effects of PIR on IGF-I and IGFBP-3 is uncertain at present but the absence of this effect in IDDM indicates a further disturbance of the GH–IGF-I axis in these patients. Further studies are now required to determine whether this action of PIR is due to a specific cholinergic muscarinic mechanism.

In conclusion, we have confirmed that subjects with IDDM have reduced serum IGF-I and IGFBP-3 and increased IGFBP-1 compared with normal subjects. There were no correlations between these alterations and measures of metabolic control. In addition, there was no correlation between changes in the IGF-I system and altered GH responses after cholinergic modulation, suggesting that the changes in IGF-I may not be the only contributors to the altered GH neuroregulation in IDDM. We have also shown an acute stimulatory action of pirenzepine on serum IGF-I and IGFBP-3 in normal subjects which is not present in IDDM, although the underlying mechanism is not known.

References


