Intraperitoneal insulin is more potent than subcutaneous insulin at restoring hepatic insulin-like growth factor-I mRNA levels in the diabetic rat: a functional role for the portal vascular link


Department of Endocrinology and Chemical Pathology and *Division of Biochemistry, United Medical and Dental Schools of Guys and St Thomas’, St Thomas’ Hospital, London SE1 7EH, U.K.

(Requests for offprints should be addressed to C. R. Thomas)

RECEIVED 13 May 1992

ABSTRACT

There is evidence that the hormonal control of hepatic IGF-I production is mediated by GH and insulin. To elucidate the role of these hormones further we administered s.c. or i.p. insulin (at 2.5 and 5.0 IU/day) and/or GH (0.8 IU/day) to rats made diabetic with streptozotocin 16 days previously. Hepatic IGF-I production was then assessed by quantifying hepatic IGF-I mRNA levels by autoradiography of Northern blots. Diabetes resulted in a fivefold reduction in hepatic IGF-I mRNA levels (optical density (OD) of the 0.7–1.1 kb band; controls, 1.3±0.09; diabetics, 0.28±0.08; P<0.01), which was not significantly changed by treatment with s.c. insulin (OD: low dose, 0.55±0.05; high dose, 0.58±0.05) or low dose i.p. insulin (OD: 0.40±0.03). High dose i.p. insulin enhanced hepatic IGF-I mRNA levels (OD: 0.93±0.23) compared with diabetic rats (P<0.01) and those given high dose s.c. insulin (P<0.04), despite the blood glucose values being similar in the treated groups (i.p., 4.72±0.29 mmol/l; s.c., 3.32±0.03 mmol/l). Administration of GH alone partially restored the hepatic IGF-I mRNA level (OD: GH-treated, 1.00±0.05; diabetic, 0.28±0.08; P<0.01), whilst having no effect on blood glucose values (diabetic, 36.35±0.45 mmol/l; GH-treated, 38.65±2.39 mmol/l). Additional administration of s.c. insulin completely restored IGF-I mRNA levels to those of controls (OD: low dose, 1.35±0.14; high dose, 1.27±0.18). These observations indicate that insulin and GH are required for full expression of hepatic IGF-I mRNA and that insulin given i.p. is more potent than that given s.c. at stimulating hepatic synthesis of IGF-I.

Journal of Molecular Endocrinology (1992) 9, 257–263

INTRODUCTION

Insulin-dependent diabetes is associated with a reduction in somatic growth (Tattersall & Pike, 1973) which is often accompanied by low serum levels of insulin-like growth factor-I (IGF-I) (Amiel et al. 1984). Insulin therapy has been shown to restore growth in human patients (Rudolf et al. 1981) and other animals (Scott & Baxter, 1986) and to increase serum IGF-I levels towards normal values. An infusion of purified human IGF-I into diabetic rats has been reported to enhance growth significantly (Scheiwiller et al. 1986). IGF-I is a circulating polypeptide with a chemical structure similar to proinsulin (Rinderknecht & Humbel, 1978), with both mitogenic (Sara & Hall, 1990) and metabolic (Rossetti et al. 1991) actions. These may be mediated by IGF-I acting in an autocrine, paracrine and endocrine manner (Holly & Wass, 1989). IGF-I can mimic the growth-promoting effect of growth hormone (GH), and is thought to mediate many of the actions of GH on target tissues (Froesch et al. 1985). Whilst IGF-I and IGF-I mRNA have been found in many tissues (D’Ercole et al. 1984; Murphy et al. 1987b; Sara & Hall, 1990), the predominant site of synthesis of circulating IGF-I is the liver...
(Schwander et al. 1983). The primary regulator of IGF-I levels is GH (Mathews et al. 1986) but there is some evidence to suggest that nutritional state (Bornfeldt et al. 1989; Goldstein et al. 1991; Vandehaar et al. 1991) and insulin (Böni-Schnetzler et al. 1991) may exert independent influences. The role of the direct portal vascular link between the pancreas and the liver with respect to mobilization of hepatic glycogen stores by glucagon is established (Baron et al. 1987), whilst the importance of this route for the actions of insulin is controversial and poorly understood (Starzl et al. 1962; Botz et al. 1976). With respect to the effect of insulin on hepatic IGF-I synthesis, Griffen et al. (1987) have shown that insulin delivered via the portal circulation may be more effective than systemic insulin at restoring circulating IGF-I levels in diabetic rats, but they did not show whether this is due to increased production or decreased clearance of IGF-I. This may explain why, despite intensive s.c. insulin therapy, circulating IGF-I levels in diabetic patients remain either low or, at best, low normal (Rudolf et al. 1981).

The aim of this study was thus to investigate the effects of GH, insulin and the route of insulin delivery on liver IGF-I gene expression. We therefore injected insulin (s.c. or i.p., to mimic hepatic portal delivery) and GH into streptozotocin diabetic rats for 16 days and assessed the levels of hepatic IGF-I mRNA using Northern blot analysis.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats were obtained from commercial breeders (Banting and Kingman, Hull, Humberside, U.K.) and acclimatized in holding cages for approximately 1–2 weeks prior to the study. Water and chow were available ad libitum throughout.

Experimental design

Twenty-four rats were rendered diabetic by a single i.p. dose of 50 mg streptozotocin in sodium citrate/kg body weight. Diabetes was confirmed 3 days after injection by 24-h urinary glucose excretion. Animals were randomly divided into eight groups of three animals plus four controls. The groups were: controls, untreated diabetic (D), diabetic treated with low dose s.c. Ultratard human insulin (Novo, Copenhagen, Denmark; 2.5 IU/24 h; D + ↓I s.c.), diabetic treated with high dose s.c. insulin (5.0 IU/24 h; D + ↑I s.c.), diabetic treated with low dose i.p. insulin (D + ↓I i.p.), diabetic treated with high dose i.p. insulin (D + ↑I i.p.), diabetic treated with recombinant human GH (rhGH; Genotropin; Kabivitrum, Stockholm, Sweden; 0.8 IU/24 h; D + GH), diabetic treated with rhGH plus low dose s.c. insulin (D + GH + ↓I s.c.) and diabetic treated with rhGH plus high dose s.c. insulin (D + GH + ↑I s.c.). Subcutaneous injections were given into the right rear leg. All injections were performed between 09.00 and 10.00 h for 16 days. A blood sample was obtained from the tails of all rats on day 8 of treatment. Between 15.00 and 17.00 h on day 16 of treatment the animals were stunned, a cardiac blood sample was obtained, and the animals were killed by cervical dislocation. Livers were rapidly excised, weighed and snap-frozen in liquid nitrogen. The hearts and kidneys were removed and weighed, and the gastrocnemius muscle from the left leg was dissected out of all rats by the same person to ensure uniformity of sampling. To determine the glycaemic control of our injection regime a parallel study was performed in six streptozotocin-induced diabetic rats. The rats were randomly assigned to either s.c. or i.p. high dose insulin injections, and on the final day of dosing four blood samples were taken from the tail vein over a 24-h period.

RNA extraction and hybridization

Total RNA was extracted from duplicate liver samples (approximately 400 mg) by homogenization, using a Polytron (PTA 10–35; Kinematica, Lucerne, Switzerland), in 5 M guanidium isothiocyanate, 1% (v/v) β-mercaptoethanol, followed by ultracentrifugation (Kontron TST 55·5 rotor; 165 000 g at 20 °C for 18 h) through 5·7 M caesium chloride (Chirgwin et al. 1979). The RNA pellet was solubilized in TES buffer (10 mM Tris, 5 mM EDTA, 0·1% (w/v) sarkosyl, pH 7·0) and stored until use under ethanol at −70 °C. RNA was pelleted by centrifugation, dried and resuspended in TES buffer and the concentration determined spectrophotometrically at 260 nm. The integrity of all RNA samples was confirmed by illumination with u.v. light and visual inspection after electrophoresis through a 1% agarose gel containing formaldehyde and staining with ethidium bromide. Samples of RNA (50 μg) were separated by electrophoresis in a 1·2% agarose–formaldehyde gel, transferred to nylon membranes (Hybond N+; Amersham International plc, Amersham, Bucks, U.K.) using a vacuum blower (Hybaid, Teddington, Middlesex, U.K.) and cross-linked with u.v. radiation (UVP transilluminator; UVP Ltd, Cambridge, Cambs, U.K.) (Sambrook et al. 1989).
Membranes were hybridized at 42 °C in a solution containing 50% formamide, 50 mM sodium dihydrogen phosphate, pH 7.0, 5 × SSC (1 × SSC is 0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0), 1 mM EDTA, 2 × Denhardt's solution, 1% sodium dodecyl sulphate (SDS) and 100 μg sonicated denatured salmon sperm DNA/ml. The IGF-I probe was rat IGF-I cDNA (Murphy et al. 1987; a gift from G. I. Bell, Howard Hughes Medical Institute, University of Chicago, IL, U.S.A.) labelled with deoxycytidine 5'([α-32P]triphosphate (Amersham International plc) to a specific activity of approximately 10⁶ c.p.m./μg (Feinberg & Vogelstein, 1984). After hybridization, membranes were washed initially at room temperature with 2 × SSC, 0.1% (w/v) SDS and then with 0.1 × SSC, 0.1% (w/v) SDS for 1 h at 55 °C. This probe detected a number of transcript sizes, the smallest of which was the most abundant, as found by others using this cDNA probe (Murphy et al. 1987a,b; Murphy, 1988), and was used for quantitation. Membranes were also hybridized with human hypoxanthine phosphoribosyltransferase cDNA (Jolly et al. 1983), as a control for differences in the amount of RNA loaded on the gel. Autoradiographs were analysed by laser densitometry (UltroScan XL laser densitometer using 2400 GelScan XL; LKB, Turku, Finland).

### Glucose and GH determinations

Serum glucose was measured by the glucose oxidase method using a glucose analyser (Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.). Serum GH was measured by an immunometric assay as previously described (Salomon et al. 1989).

#### Statistical analyses

Data were analysed using the Number Cruncher Statistical System (Hintze, 1987) and are expressed as means±S.E.M. Differences between groups were determined by analysis of variance and multiple comparisons were performed with Fisher's least significant difference test using appropriately corrected probability values (Armitage, 1980).

#### RESULTS

Diabetic rats and diabetic rats treated with rhGH or rhGH plus low dose insulin had higher mean blood glucose values than control animals (P<0.04; Table 1). There was a tendency for the groups treated with high dose insulin to have lower blood glucose levels than those treated with low dose insulin (Table 1). However, in those rats in which we investigated the 24-h glucose profile, the mean blood glucose values were: i.p., 9.37±1.96 mmol/l; s.c., 9.47±2.37 mmol/l. There were no differences between the blood glucose values at any of the sampling times.

In all groups treated with rhGH, the mean blood GH level was 30.7±8.8 mU/l; there was no difference between groups.

At the start of the experiment there were no significant differences between body weights of the animals in all groups (P=0.7, not significant (NS); mean weight, 223.5±18 g). Following treatment, the diabetic rats weighed less than control rats (P<0.04; Table 1). Although treatment of diabetic rats with rhGH alone did not increase their body

### TABLE 1. Blood glucose and weights of body, gastrocnemius and liver of control rats and diabetic rats with or without i.p. or s.c. hormone treatment. Values are means±S.E.M. of four controls and three animals in all treatment groups. D+I s.c. = diabetic treated with low dose (2.5 IU/24 h) s.c. insulin, D+I s.c. = diabetic treated with high dose (5.0 IU/24 h) s.c. insulin, D+I i.p. = diabetic treated with low dose i.p. insulin, D+I i.p. = diabetic treated with high dose i.p. insulin, D+GH = diabetic treated with s.c. recombinant human GH (rhGH, 0.8 IU/24 h), D+GH+I s.c. = diabetic treated with rhGH plus low dose s.c. insulin, D+GH+I s.c. = diabetic treated with rhGH plus high dose s.c. insulin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose (mmol/l)</th>
<th>Body weight (g)</th>
<th>Gastrocnemius weight (g)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.43±0.38</td>
<td>241.8±2.8</td>
<td>1.55±0.04</td>
<td>9.59±0.32</td>
</tr>
<tr>
<td>Diabetic</td>
<td>36.35±0.45</td>
<td>219.3±8.2</td>
<td>1.15±0.09</td>
<td>10.5±0.69</td>
</tr>
<tr>
<td>D+I s.c.</td>
<td>9.87±3.3</td>
<td>239.3±11.4</td>
<td>1.37±0.15</td>
<td>9.13±0.54</td>
</tr>
<tr>
<td>D+I s.c.</td>
<td>3.32±0.03</td>
<td>256.6±6.6</td>
<td>1.51±0.05</td>
<td>9.27±0.15</td>
</tr>
<tr>
<td>D+I i.p.</td>
<td>10.73±2.40</td>
<td>230.0±1.5</td>
<td>1.46±0.06</td>
<td>10.6±0.6</td>
</tr>
<tr>
<td>D+I i.p.</td>
<td>4.72±0.09</td>
<td>243.0±7.4</td>
<td>1.49±0.02</td>
<td>10.5±1.27</td>
</tr>
<tr>
<td>D+GH</td>
<td>38.65±2.39</td>
<td>249.6±10.5</td>
<td>1.39±0.13</td>
<td>11.7±0.64</td>
</tr>
<tr>
<td>D+GH+I s.c.</td>
<td>13.9±4.26</td>
<td>276.3±2.3</td>
<td>1.67±0.11</td>
<td>13.1±1.5</td>
</tr>
<tr>
<td>D+GH+I s.c.</td>
<td>5.36±0.49</td>
<td>298.0±4.0</td>
<td>1.76±0.05</td>
<td>13.2±0.3</td>
</tr>
</tbody>
</table>

*P<0.04, **P<0.02, ***P<0.01 compared with control rats.

*P<0.01, **P<0.02, ***P<0.01 compared with diabetic rats (analysis of variance).
weight above that of controls, the administration of rhGH plus low or high dose insulin resulted in an increase in body weight compared with control animals (P<0.04; Table 1). Treatment of diabetic rats with either s.c. or i.p. low dose insulin did not result in a body weight greater than that of the diabetic animals, whilst treatment with high dose insulin (s.c. or i.p.) or rhGH resulted in a significant increase (P<0.04, Table 1). The weight of the left gastrocnemius muscle in diabetic rats was reduced when compared with controls (P<0.01), and was restored in all groups except in diabetic rats treated with s.c. low dose insulin or rhGH alone. Liver weights were greater than that in controls in the diabetic rats treated with rhGH alone (P<0.04) and in rats given rhGH and insulin (P<0.01). There was no significant difference in the weights of the kidneys (P=1.02, NS; mean for all groups, 2.06±0.06 g) or the hearts (P=0.84, NS; mean for all groups, 0.92±0.04 g) between the groups.

The apparent sizes of the hepatic IGF-I mRNA transcripts were 7.0, 1.8 and 0.7-1.1 kb. The 7.0 kb transcript was not always seen because of the vacuum-blotting method used, in which the high molecular weight RNA transfer was sometimes blocked by the apparatus.

Streptozotocin-induced diabetes resulted in a substantial reduction in hepatic IGF-I mRNA expression compared with control rats. Representative Northern blots of RNA from all treatment groups are shown in Fig. 1. Laser densitometric analyses of multiple autoradiographs indicated differences (F=13.77, P<0.001) between treatment groups. For comparison, results for the amount of IGF-I mRNA are expressed as percentages of that in control animals (Fig. 2). Hepatic IGF-I mRNA was less in the diabetic animals (optical density (OD) of the 0.7-1.1 kb band: 0.28±0.08) than in controls (OD: 1.32±0.09; P<0.01), the rhGH-treated groups (OD: D + GH, 1.00±0.05; D + GH + I s.c., 1.35±0.14; D + GH + I s.c., 1.27±0.18; all P<0.01) and diabetic animals treated with high dose i.p. insulin (OD: 0.92±0.23; P<0.01). Administration of high dose i.p. insulin to diabetic rats was more effective at restoring IGF-I mRNA towards control levels than high dose s.c. insulin (P<0.04). There was no difference between low dose i.p. insulin (OD: 0.40±0.03) and both low (OD: 0.55±0.05) and high dose (OD: 0.58±0.05) s.c. insulin administration on IGF-I mRNA levels. Diabetic animals injected with rhGH alone had less hepatic IGF-I mRNA than controls (P<0.05); in contrast, diabetic animals treated with rhGH and high or low dose insulin had IGF-I mRNA levels which were not significantly different from controls (Fig. 2).

**FIGURE 1.** (a) Northern blot analysis of RNA extracted from livers of all treatment groups. Approximately 50 µg of each sample was hybridized to radiolabelled rat IGF-I cDNA. Groups are: C = control, D = untreated diabetic, D + I s.c. = diabetic treated with low dose (2.5 IU/24 h) s.c. insulin, D + I s.c. = diabetic treated with high dose (5.0 IU/24 h) s.c. insulin, D + I p. = diabetic treated with low dose i.p. insulin, D + I i.p. = diabetic treated with high dose i.p. insulin, D + GH = diabetic treated with recombinant human GH (rhGH, 0.8 IU/24 h), D + GH + I s.c. = diabetic treated with rhGH plus low dose s.c. insulin, D + GH + I s.c. = diabetic treated with rhGH plus high dose s.c. insulin. (b) Hybridization of the same membrane with cDNA for hypoxanthine phosphoribosyltransferase (HPRT) as a control for gel loading.

**DISCUSSION**

The primary finding in these studies was that the reduced levels of hepatic IGF-I mRNA, characteristic of diabetes, were more effectively restored towards normal values by i.p. than by s.c. insulin at 5-0 IU/24 h. Insulin is almost entirely absorbed from the peritoneum by the hepatic portal circulation (Selam et al. 1990) and thus insulin injected i.p. more closely reflects the normal delivery of insulin than insulin injected s.c. Administration of GH increased hepatic IGF-I mRNA levels in the diabetic rats but additional insulin at high or low dose was required to reach levels similar to those in control animals.

Streptozotocin-induced diabetes caused grossly elevated blood glucose levels, which were reduced more by high dose insulin given either i.p. or s.c. than by low dose insulin. There were no differences in blood glucose values between diabetic rats receiving the same insulin dose either i.p. or s.c., similar
FIGURE 2. Amount of hepatic IGF-I mRNA in control rats (C), diabetic rats (D) and diabetic rats treated with low dose (2·5 IU/24 h) s.c. insulin (D + ▼ s.c.), high dose (5·0 IU/24 h) s.c. insulin (D + ▼ s.c.), low dose i.p. insulin (D + ▼ i.p.), high dose i.p. insulin (D + ▼ i.p.), recombinant human GH (rhGH; 0·8 IU/24 h; D + GH), rhGH plus low dose s.c. insulin (D + GH + ▼ s.c.) or rhGH plus high dose s.c. insulin (D + GH + ▼ s.c.). Results are expressed as percentages of the control ± S.E.M.

to findings in anaesthetized pancreatectomized dogs (Botz et al. 1976). This is in contrast to the results of Griffen et al. (1987), who reported that, at low dose, insulin delivered via the hepatic portal vein in diabetic rats was more effective at lowering blood glucose than a similar dose given via the jugular vein. However, at high dose no such differences were found. Administration of GH alone did not change blood glucose levels in the diabetic rats. Additional administration of low dose insulin reduced the hyperglycaemia to levels which were higher than, but not significantly different from, those in rats receiving low dose insulin alone, suggesting that these supraphysiological doses of GH may have induced insulin resistance. This is in accord with results from diabetic rats bearing a GH tumour, which required considerably higher insulin doses to reduce glycaemia to an equivalent extent (Fagin et al. 1989).

Diabetes resulted in a slight loss in body weight over the time of the experiment, as found in other studies (Scheiwiller et al. 1986; Bornfeldt et al. 1989), but this was ameliorated by treatment with insulin or GH. However, GH alone was less effective than GH plus insulin at increasing body weight or muscle weight above values in diabetic rats, and confirms the findings that GH and insulin are required for effective growth and weight gain in hypophysectomized (Murphy et al. 1987a) and diabetic (Fagin et al. 1989) rats.

The approximately fivefold reduction in hepatic IGF-I mRNA levels in diabetic rats compared with controls reported here is similar to the findings of other workers (Murphy, 1988; Böni-Schnetzler et al. 1989; Bornfeldt et al. 1989; Fagin et al. 1989). Despite similar blood glucose values, the IGF-I mRNA level in GH-treated diabetic rats was higher than that in untreated diabetic rats. Thus, expression of hepatic IGF-I mRNA may be independent of blood glucose concentration in these animals. GH is required for insulin to induce hepatic IGF-I mRNA levels; in hypophysectomized rats, GH partially restored hepatic IGF-I mRNA levels (Roberts et al. 1986), whilst insulin had no consistent effects (Salamon et al. 1989). However, in the present study, we have shown that GH will increase levels of hepatic IGF-I mRNA in the presence of the severely reduced endogenous insulin levels in rats made diabetic with streptozotocin. It is worth noting that in streptozotocin-induced diabetic rats the levels of circulating GH are reduced, in contrast to diabetic humans in whom levels are elevated. Diabetic rats given GH and diabetic rats given GH plus insulin showed 3·6- and 4·7-fold increases in hepatic IGF-I mRNA respectively; this compares with the respective 3·8- and 6·7-fold increases found in isolated hepatocytes (Böni-Schnetzler et al. 1989). However, Johnson et al. (1989) did not find a further increase in hepatocyte IGF-I mRNA levels when insulin was added to GH. Whilst Böni-Schnetzler et al. (1991) found an additive effect of different insulin and GH concentrations on isolated cultured hepatocytes, the results of the present in-vivo study indicate that increasing insulin from low to high dose in the
presence of GH did not increase hepatic IGF-I mRNA levels further.

Griffen et al. (1987) reported that diabetic rats receiving 2 IU insulin/kg per day delivered via the hepatic portal vein had higher plasma IGF-I levels than those receiving the same insulin dose delivered via the systemic circulation. It was not known, however, whether this was due to increased production or reduced clearance of the IGF-I. In the present study, the increased mRNA for IGF-I in the group given i.p. high dose insulin demonstrates for the first time a similar effect at the level of mRNA expression. It seems likely, therefore, that the differences found by Griffen et al. (1987) were due to increased production. Anaesthesia and operative procedures, like other catabolic states, are associated with reduced circulating IGF-I levels (Wilmore, 1991) and we thus avoided operative procedures in our animals by giving i.p. injections instead of surgically placed catheter vessels.

In conclusion, the delivery of insulin to the liver via the portal route is associated with enhanced expression of hepatic IGF-I mRNA when compared with peripherally administered insulin. This may explain why patients with type-I diabetes who are treated with s.c. insulin have low IGF-I levels, despite good blood glucose control (Rudolf et al. 1981). The lower IGF-I levels in these patients are thought to lead to deleterious GH hypersecretion, due to reduced negative feedback. Additionally, patients with type-I diabetes with residual β-cell function show a significantly greater hepatic IGF-I response to the administration of rhGH than patients with type-I diabetes with no residual function (Wurzburger et al. 1991). The contribution of insulin to the regulation of body growth (Hill & Milner, 1985) may thus be potentiated by the direct link between the pancreatic β cell and the liver.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Special Trustees of St Thomas' Hospital. We should like to thank Dr H. Jenkins, Vision Research Unit, The Rayne Institute, UMDS, St Thomas' Hospital, for the use of his laser densitometer and Dr M. Wheeler for measuring the GH.

REFERENCES


---

*Journal of Molecular Endocrinology* (1992) 9, 257-263