Growth hormone regulation of insulin-like growth factor-I mRNA in rat adipose tissue and isolated rat adipocytes

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ABSTRACT

The effects of hypophysectomy and hormonal replacement therapy on insulin-like growth factor-I (IGF-I) mRNA in rat adipose tissue and adipocytes were studied. The effects of GH and IGF-I in vitro on IGF-I mRNA and IGF-I production were also studied in cultured rat adipocytes. Male rats were hypophysectomized at about 50 days of age and given replacement therapy with cortisol (400 µg/kg per day) and thyroxine (10 µg/kg per day). GH was given as a single i.v. or s.c. injection and also as a continuous s.c. infusion for 6 days. Epididymal fat pads were excised and used either for isolation of adipocytes or for determination of IGF-I mRNA in adipose tissue. A solution hybridization assay was used. The IGF-I mRNA content of adipocytes was analysed either immediately after isolation or after short-term (2–3 days) culture with or without GH or IGF-I. Hypophysectomy resulted in a marked decrease in IGF-I mRNA in both tissue and cells. Replacement therapy (in vivo) with cortisol and thyroxine alone had no effect, whereas additional treatment with GH caused a dose-dependent increase in IGF-I mRNA. IGF-I mRNA was also increased after a continuous s.c. infusion of GH. A single i.v. injection of GH (100 µg) resulted in an increase in IGF-I mRNA after approximately 2 h, with maximal levels around 6 h after the injection. In cultured adipocytes, addition of GH to the culture medium increased IGF-I mRNA in a dose-dependent manner and a marked increase was observed with a concentration of GH of 1 ng/ml. Addition of IGF-I (100 ng/ml) had no effect. The increase in IGF-I mRNA after addition of GH (100 ng/ml) was detectable after 3 h. The concentration of IGF-I in the culture medium was increased 24 h after the addition of GH. These results demonstrate that GH induces IGF-I mRNA in both adipose tissue and isolated fully differentiated adipocytes and that this increase in IGF-I mRNA results in increased IGF-I production.

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INTRODUCTION

Apart from its effects on somatic growth, growth hormone (GH) is also an important regulator of intermediary metabolism (Goodman & Schwartz, 1974). Lack of GH results in an increase in body fat, whereas GH excess, as in acromegaly, results in a decrease in body fat (Edén, Bengtsson & Oscarsson, 1990). In paradox to the latter effects of GH was the finding that the number of fat cells was low in patients with pituitary insufficiency (Brook, 1972) and that it was normalized after treatment with GH (Bonnet, Vanderschueren-Lodeweyckx, Eekels & Malvaux, 1974; Ginsberg-Felner, 1981). Over the last few years, increasing evidence suggests that GH is important for differentiation of preadipocytes to adipocytes (Morikawa, Nixon & Green, 1982; Nixon & Green, 1984; Green, Morikawa & Nixon, 1985).

Insulin-like growth factor-I (IGF-I) or somatomedin C is a GH-dependent peptide that is structurally related to insulin and that stimulates cell proliferation in a wide range of cell types. IGF-I has been shown to be synthesized in a number of different tissues and cell types (Humbel, 1990). The effects of GH on growth and differentiation have been suggested to be an initial reaction with progenitor cells or stem cells and to involve a subsequent local production of IGF-I in the target tissue (Green et al. 1985). Gaskins, Kim, Wright et al. (1990) presented immunocytochemical data indicating IGF-I synthesis by mature adipocytes in primary cell cultures derived from porcine adipose tissue.

The present study was initiated to investigate the possible effects of GH on the regulation of IGF-I production by fully differentiated adipocytes using well-characterized systems in which metabolic effects
of GH have been demonstrated previously (Edén, Schwartz & Kostyo, 1982; Gause, Edén, Isaksson, DiGirolamo & Smith, 1985).

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats, 45–55 days old and purchased from Alab Laboratory Services (Stockholm, Sweden), were used. They were kept at constant temperature with a 14-h light:10-h darkness cycle. Tap water and pelleted food (Type R36, Ewos, Södertälje, Sweden) were freely available. Hypophysectomy was performed by the standard parapharyngeal approach. Body weight was measured daily and rats gaining more than 0.5 g/day in body weight were excluded.

Hormonal treatment

L-Thyroxine (T4) (Nynomed Ltd, Oslo, Norway; 10 µg/kg per day) and cortisol (Solu-Cortef; Upjohn, Puurs, Belgium; 400 µg/kg per day) were diluted in saline and given as a daily s.c. injection to hypophysectomized rats. Recombinant human GH (Genotropin, 2-7 IU/mg) and IGF-I (lot 1005; 4100 IU/mg) were generously provided by Kabi AB (Stockholm, Sweden), and bovine GH was generously provided by American Cyanamide Co (Princeton, NJ, U.S.A.). Human GH was used when effects of single injections of GH were investigated and was given either as an i.v. or s.c. injection depending upon the design of the experiment. Human GH was also used when the effects of GH in vitro were investigated. Bovine GH (1 mg/kg per day) was diluted in 0.05 mol phosphate buffer/l (pH 8.6) containing 1-6% (v/v) glycerol and 0.02% (w/v) sodium azide and given via Alzet osmotic mini-pumps (Alza Corporation, Palo Alto, CA, U.S.A.) implanted on the back of the animal. In these experiments, the rats were killed after 6 days of treatment.

Tissue preparation and cell culture

Rats were killed and epididymal fat pads excised. The fat pads were either immediately frozen in liquid nitrogen or used for preparation of adipocytes. The method of cell preparation was modified from that of Rodbell (1964) and Gause et al. (1985). The fat pads were incubated in 20 ml Dulbecco's modified Eagle's medium (DMEM) (Gibco, Paisley, Scotland, U.K.), 25 mmol Hepes/l, 20 mmol HCO3/l, 4% (w/v) bovine serum albumin (BSA, fraction V, Wilfred Smith Ltd, Edgware, Middx, U.K.), 0.5 µg amphotericin B/ml (Fungizone, Gibco), 50 µg gentamicin sulphate/ml (Gentamycin; Sigma, St Louis, MO, U.S.A.) and 0.1% (w/v) collagenase (125 U/mg; Worthington Biochemical Co., Freehold, NJ, U.S.A.) at 37 °C for 90–120 min in a reciprocal shaking water bath. After digestion the cell suspension was filtered through double-layered gauze and separated by floating. The cells were washed three times with DMEM supplemented as described above but without collagenase and with a lower BSA concentration (0.1%). The adipocytes were either immediately processed for preparation of total nucleic acids, as described below, or seeded at a cell density of 1·75–3·0 × 106 cells/Falcon culture flask (75 mm²).

IGF-I mRNA probe

The radioactive probe was prepared as described by Melton, Krieg, Rebagliati et al. (1984). In brief, the DNA clone used was a 153 bp genomic subclone (in pSP64 in both orientations) of mouse IGF-I corresponding to exon 3 (by analogy with human IGF-I) (Mathews, Norstedt & Palmiter, 1986). Analyses of cDNA clones for IGF-I indicate that two forms of IGF-I mRNA could exist (Rotwein, 1986). The structure of the probe used in this study would allow detection of both forms of IGF-I mRNA.

Northern blot analysis

Total RNA was prepared according to Chomczynski & Sacchi (1987). RNA (20 µg) was electrophoresed in an agarose (1%, w/v)/formaldehyde (2·2 mol/l) gel. The RNA was transferred to a membrane (Hybond-N, Amersham, Bucks, U.K.) with a vacuum transfer system (LKB, Stockholm, Sweden), and baked at 80 °C for 3 h. The membranes were prehybridized for 12–24 h at 57 °C in a buffer consisting of 50% (v/v) formamide, 25 mmol NaHPO4/l, 25 mmol Na2HPO4/l, 5 × standard saline citrate solution (SSC), 0·1% (w/v) sodium dodecyl sulphate (SDS), 1 mmol EDTA/l, 0·05% (w/v) BSA, 0·05% (w/v) Ficoll, 0·05% (w/v) PVP, 200 µg calf liver RNA/ml and 200 µg salmon sperm DNA/ml and hybridized for 24 h in the prehybridization buffer with the addition of [32P]UTP-labelled IGF-I RNA probe (Carlsson, Nilsson & Billig, 1991). The membranes were washed in 0·1 × SSC, 0·1% (w/v) SDS at 60 °C and further treated with RNase A (1 µg/ml) at 37 °C, and washed in 0·1 × SSC, 0·1% (w/v) SDS. Autoradiography was performed at −70 °C using Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY, U.S.A.). The hybridization signal was related to ribosomal RNA (Leeuw, Slagboom & Vrij, 1989).

Solution hybridization assay

Total nucleic acids (TNA) were prepared essentially according to Durman & Palmiter (1983). Briefly, the frozen tissue or the washed cells were transferred to a sterile test tube containing 1% (w/v) SDS, 20 mmol
Tris–HCl/l (pH 7.5) and 4 mmol EDTA/l and homogenized by a polytron (Ultra-turrax T25; Janke & Kunkel GMBH & Co. KG, Staufen IBR, Germany) for 15 s with maximum speed. TNA were prepared by overnight digestion with proteinase K added to the homogenate. A subsequent extraction with phenol–chloroform was then performed. The mouse IGF cRNA probe, radiolabelled with [³²P]UTP, was hybridized at 70 °C to TNA samples with a total incubation volume of 40 μl and formamide concentration was 25% (v/v) (Isgaard, Möller, Isaksson et al. 1988). After overnight incubation, the TNA samples were treated with 40 μg RNase A and 2 μg RNase T₁ (Boehringer, Mannheim, Germany) in the presence of 100 μg herring sperm DNA for 45 min at 37 °C in a volume of 1 ml. Protected probe was precipitated with 100 μl trichloroacetic acid (6 mol/l). The precipitates were then collected on glass-fibre filters (GF/C Whatman; Whatman International Ltd, Maidstone, Kent, U.K.) and counted in a scintillation counter. The hybridization signal was compared with that of a tissue standard curve originally compared with known amounts of the synthetic 153 nucleotide mRNA strand, as described previously (Mathews et al. 1986; Isgaard et al. 1988). Results are expressed as the amount of IGF-I mRNA/DNA (amol/μg). Each sample was analysed in duplicate. The within-assay coefficient of variation (C.V.) was less than 20% in the range of 40–400 amol of the RNA standard. The between-assay C.V. was estimated by repeated analysis of the same TNA preparation and was 23%.

DNA analysis

DNA analysis was performed essentially according to the method of Labarca & Paigen (1980). Salmon testis DNA (Sigma) served as standard and each TNA preparation was analysed in duplicate.

IGF-I assay

IGF-I concentrations in the culture media were determined by radioimmunoassay after acid ethanol extraction according to the manufacturer’s protocol (Nicols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.) in one single assay in duplicate without dilution. The intra-assay C.V. was 3·4% and kit controls were well within the range given by the manufacturer.

Statistics

Values are expressed as means ± s.e.m. Comparisons between groups were performed with analysis of variance followed by Student–Newman–Keul’s multiple-range test (Woolf, 1968). When appropriate, values were transferred to logarithms. Values of P less than 0·05 were considered significant.

RESULTS

Northern blot analysis of RNA from adipose tissue with the IGF-I probe revealed a major transcript with an estimated size of 7·0 kb. Two minor expressed transcripts at 1·7 and 1·2–0·9 kb could also be demonstrated. Hypophysectomy resulted in a decrease of all transcripts. Treatment with GH (bGH; 1 mg/kg per day for 1 week by continuous infusion) resulted in an increase of all transcripts towards normal (Fig. 1). In isolated adipocytes transcripts of the same sizes as in adipose tissue were detected (Fig. 1).

In adipose tissue of hypophysectomized rats given replacement therapy with T₄ and cortisol, levels of IGF-I mRNA were markedly reduced compared with sham-operated animals (Fig. 2). Single i.v. injection of human GH (100 μg) resulted in increased mRNA levels detectable after 2 h and maximal levels were observed after 6 h. Thereafter levels gradually declined (Fig. 2). However, levels of IGF-I mRNA were below those found in normal rats also after injection of GH. In another experiment, two doses of human GH (100 and 1000 μg) were given as a single s.c. injection. After 10 h, the animals were killed and IGF-I mRNA levels determined in epididymal fat pads. In that experiment IGF-I mRNA levels increased to those of normal rats in hypophysectomized rats given the higher dose of GH (9·6 ± 0·6 amol/μg DNA in controls, 1·2 ± 0·1 in hypophysectomized rats, 3·4 ± 0·4 in hypophysectomized animals given 100 μg hGH and 8·6 ± 1·49 amol/μg DNA in animals given 1000 μg hGH; mean ± s.e.m.; five observations in each group).

There was no effect of replacement therapy with cortisol and T₄ on IGF-I mRNA levels in adipose tissue of hypophysectomized rats (0·7 ± 0·2 amol/μg DNA in untreated hypophysectomized animals compared with 0·6 ± 0·1 amol/μg DNA in hypophysectomized animals given replacement therapy; five observations in each group). Additional treatment for 1 week with bovine GH (1 mg/kg per day) by continuous s.c. infusion resulted in a significant increase in IGF-I mRNA levels (3·7 ± 0·5 amol/μg DNA; P < 0·01 compared with hypophysectomized animals).

Hypophysectomy resulted in a marked reduction in IGF-I mRNA levels also in isolated adipocytes (16·6 ± 1·4 amol/μg DNA in cells from control animals compared with 0·47 ± 0·1 amol/μg DNA in cells from hypophysectomized animals; five independent observations in each group). Treatment of hypophysectomized rats with bovine GH (1 mg/kg per day for 6
Materials. GH-treated rats were hypophysectomized at 50 days of age and then given replacement therapy with L-thyroxine and cortisol. One week after hypophysectomy, 100 μg hGH was injected into the tail vein under light ether anaesthesia. The rats were killed at different times after the injection. Sham-operated, age-matched rats were given an i.v. injection of saline ( ). Epididymal fat pads were excised and immediately frozen. IGF-I mRNA levels were determined by a solution hybridization assay. There were five or six rats in each group with one observation from each rat. Values represent means ± S.E.M. of the five to six observations. *P < 0.01 compared with time 0 (one-way analysis of variance followed by Student–Newman–Keul's test between individual groups).

When adipocytes from normal animals were cultured for 24 h, IGF-I mRNA levels were lower than those in cells immediately after isolation (4.0 ± 1.4 amol/μg DNA and 16.6 ± 0.9 amol/μg DNA respectively; three observations from each cell culture). Addition of GH (100 ng/ml) to the culture medium 24 h after cell isolation resulted in a time-dependent increase in IGF-I mRNA levels in the cultured adipocytes reaching maximal levels after about 24–72 h (Fig. 3). The effect of GH was already apparent at a concentration of 1 ng/ml, and a further increase was observed at 10–100 ng/ml. However, culture of the cells in the presence of IGF-I (100 ng/ml) had no effect on IGF-I mRNA levels (data not shown).

Concentrations of IGF-I were measured in the culture media from adipocytes cultured in the presence or absence of GH (100 ng/ml) (Table 1). In the absence of GH, IGF-I concentrations were low throughout the culture period. In the presence of
FIGURE 3. Time-course of the effect of GH on IGF-I mRNA in cultured rat adipocytes. Isolated adipocytes from epididymal fat pad were seeded out in Dulbecco's modified Eagle's medium. After an overnight incubation, 100 ng human GH/ml was added to the culture flasks. At each time point, indicated three cultures were processed for IGF-I mRNA determination using a solution hybridization assay. Values represent the mean of each triplicate. This experiment was repeated twice with similar results. Control (■), plus GH (□).

GH, IGF-I concentrations had increased after 24 h of culture.

DISCUSSION

The results of the present study demonstrate that GH regulates the levels of IGF-I mRNA in rat epididymal adipose tissue and in cultured adipocytes. Since GH in vitro increased IGF-I mRNA levels and IGF-I production in cultured adipocytes, this effect of GH must be the result of a direct interaction with fully differentiated cells.

A solution hybridization assay was used to quantify IGF-I mRNA. This assay has previously been employed to measure IGF-I mRNA levels in many tissues including liver, rib growth plate and skeletal muscle (Mathews et al. 1986; Isgaard et al. 1988; Isgaard, Nilsson, Vikman & Isaksson, 1989). Northern blots of mRNA encoding for IGF-I have revealed multiple-sized mRNA species in mouse and rat ranging from 0.7 kb to more than 7 kb (Lund, Moats-Straats, Hynes et al. 1986; Mathews et al. 1986; Roberts, Brown, Graham et al. 1986). The biological significance of the existence of multiple-sized IGF-I mRNA species is not clear. At present it is not clear whether the different mRNAs encode unknown precursor forms of IGF-I or whether some of these different mRNAs are not translated. A probe identical with the one used in the present study has hybridized to IGF-I mRNA species of 0.8–1.5 and 7.5 kb in rat adipose cells (Doglio, Dani, Fredrikson et al. 1987) in line with the present results. Studies by Bell, Stempień, Fong & Rall (1986) and Shimatsu & Rotwein (1987) suggest that two forms of IGF-I mRNA exist in mouse and rat: IGF-IA mRNA and IGF-IB mRNA. The rat IGF-IB mRNA is predominant in the liver and is barely detectable or undetectable in other tissues (Hoyt, Van Wyk & Lund, 1988; Lowe, Lasky, LeRoith & Roberts, 1988). Both IGF-IA and IGF-IB mRNAs can be induced by GH administration to hypophysectomized rats (Lowe et al. 1988). The structure of the probe used in the present study corresponds to a part of the C peptide, the whole A and D peptide and that part of the E peptide which is identical in the IA and IB pre-pro IGF-I molecules. The probe therefore permitted detection of both the IA and IB forms of IGF-I mRNA, but did not discriminate between the two forms. Thus potential differences in the regulation of various transcripts cannot be excluded from the present results.

Both human and bovine GH were used. Since it has been demonstrated that human GH induces antibody formation and has a lower biopotency in long-term experiments (Groesbeck & Parlow, 1987), bovine GH was used in our long-term experiments. Human GH has a lactogenic effect in rodents and binds to the rat liver prolactin receptor (Postel-Vinay, 1976). Since bovine GH, which only binds to the somatogenic GH receptor, had similar effects on IGF-I mRNA to those of human GH, the observed effects of GH were most probably mediated through the somatogenic action of GH. Furthermore, prolactin does not seem to bind to rat adipocytes (Gause & Edén, 1986).

Studies of GH regulation of IGF-I mRNA in different tissues have revealed that the effects are comparatively rapid, with peak levels of IGF-I mRNA detected within 3–12 h after a single GH injection

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<tr>
<th>Culture time (h)</th>
<th>GH</th>
<th>IGF-I concentration (µg/l)</th>
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<tr>
<td>0</td>
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<td>1.89 ± 0.21 (3)</td>
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<tr>
<td>24</td>
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<td>0.93 ± 0.05 (3)</td>
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<td>24</td>
<td>+</td>
<td>5.13 ± 0.66 (5)*</td>
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*P < 0.01 compared with all other groups (one-way analysis of variance followed by Student–Newman–Keul's test between individual groups).

Adipocytes from epididymal fat pad of normal rats were isolated and cultured overnight. GH (100 ng/ml) was then added and culture continued for another 24 h. IGF-I concentrations were measured by radioimmunoassay in media obtained from three to five different experiments.
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