Insulin-like growth factor I reduces thyroid hormone receptors in the rat liver. Evidence for a feed-back loop regulating the peripheral thyroid hormone action

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Abstract

Tri-iodothyronine (T_3) is known to be involved in the regulation of the growth hormone (GH)–insulin-like growth factor I (IGF-I) axis. In previous studies we demonstrated that IGF-I and GH reduced the metabolic response to T_3 measured as the activity of two T_3-dependent enzymes, mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and cytosolic malic enzyme (ME) in cultured rat liver cells. In this study we analysed *in vivo* the effect of IGF-I administered to rats on the activity of α-GPD and ME. IGF-I (240 μg/100 g body weight (BW) every 12 h for 48 h) significantly diminished α-GPD ($P<0·01$) and ME ($P<0·05$) activities. Serum basal glucose concentration was not significantly modified 12 h after the administration of recombinant human IGF-I (240 and 480 μg/100 g BW every 12 h for 48 h). Under similar conditions, no significant change in serum total thyroxine (TT_4) concentration was observed, although free thyroxine (FT_4) was diminished ($P<0·02$) and total T_3 (TT_3) was increased ($P<0·03$). To explore the participation of the nuclear thyroid hormone receptor (THR) in the mechanism of IGF-I action we measured the maximal binding capacity and the affinity constant (K_a) of THR by Scatchard analysis, and concentrations of messenger RNAs (mRNAs) that code for the isoforms of THR present in the liver (β_1, α_1 and α_2) by Northern blot. IGF-I (240 μg/100 g BW every 12 h for 48 h) significantly reduced maximal binding capacity to 37% of the control value ($P<0·01$) without changes in the K_a. β_1, α_1 and α_2 THR mRNAs were significantly reduced ($P<0·01$) by 120–480 μg/100 g BW IGF-I administration every 12 h for 48 h. Time-course studies indicated that this effect was obtained 12 h after the administration of 240 μg/100 g BW IGF-I ($P<0·05$). These results indicate that IGF-I administration to rats diminishes the metabolic thyroid hormone action in the liver by a mechanism that involves, at least in part, a reduction in the number of THRs and in their level of expression.

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Introduction

The participation of 3,5,3′-tri-iodothyronine (T_3) in the regulation of the growth hormone (GH)–insulin-like growth factor I (IGF-I) axis is well established (Rodriguez-Arnao *et al.* 1993). T_3 is required for normal GH gene expression and thyroid hormone responsive elements have been identified in the GH gene promoter (Koenig *et al.* 1987, DeGroot *et al.* 1988, Williams & Brent 1995). Pituitary GH secretion and GH mRNA level are reduced in hypothyroid rats and restored to normal after treatment with T_3 (Hervas *et al.* 1975, Nyborg *et al.* 1985). In primary culture of pituitary cells, IGF-I, the major mediator of growth promoting effects of GH, inhibits T_3 induction of GH mRNA and GH release (Melmed & Yamashita 1986). In contrast, in the perfused rat liver, physiological doses of T_3 stimulate the synthesis and release of IGF-I (Ikeda *et al.* 1991). In hypothyroid rats, serum IGF-I concentration and IGF-I gene expression in liver are reduced (Harakawa *et al.* 1990).

The introduction of recombinant GH and IGF-I for the treatment of growth disorders has renewed interest in the study of the effect of GH and IGF-I on thyroid function. The impact of administration of GH to normal and GH-deficient patients on thyroid function has yielded conflicting results. Diverse reports have described a decrease in thyroid uptake of iodine-125, serum thyrotrophin (TSH) and serum thyroxine (T_4) and an increase in serum T_3 after administration of GH (Grunfeld *et al.* 1988, Jorgensen *et al.* 1989, 1994), whereas IGF-I administered to Laron-type dwarfs and healthy individuals induces a decrease in TSH and free T_4, with no change in the T_3 serum concentration (Klinger *et al.* 1992).

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However, the impact of GH and IGF-I on specific metabolic responses to T_3 in target tissues has received scant attention. In previous studies (Pellizas *et al.* 1996), we demonstrated that IGF-I and GH incorporated to cultured rat liver cells induced a time- and dose-dependent reduction in the activity of mitochondrial α-glycerophosphate dehydrogenase (α-GPD) and cytosolic malic enzyme (ME), two specific tissue markers of thyroid hormone action (Oppenheimer *et al.* 1995). Evidence that the GH effect was mediated by IGF-I was also obtained (Pellizas *et al.* 1996).

Although T_3 may exert its effects via different cellular loci, its major effect is the transcriptional regulation of target genes mediated by nuclear thyroid hormone receptors (THR) (Yen & Chin 1994). The liver represents an important target tissue for thyroid hormone action, with ligand binding studies suggesting abundant expression of high affinity receptors for T_3 (Brent 1994). The cloning of specific THR complementary DNA (cDNA) led to evidence of the expression of α_1, α_2 and β_1 THR messenger ribonucleic acids (mRNAs) in rat liver, although concentrations of these mRNAs were low compared with those present in other tissues (Schwartz *et al.* 1992). More recently, the development of specific antibodies has provided evidence for abundant expression of α_1, α_2 and β_1 THR proteins in the rat liver (Chamba *et al.* 1996).

Because THR are decisive in the initial steps of thyroid hormone action, we investigated putative modifications at the THR level that could explain the diminished metabolic response to T_3 induced by IGF-I. Results from our previous work provided evidence that the effect of IGF-I could be mediated by a reduction in the number of THRs, as the maximal α-GPD and ME activities attained after addition of T_3 to the liver cell culture in a concentration high enough to saturate the THRs fully, were significantly lower in the group pre-exposed to IGF-I (Pellizas *et al.* 1996).

In the present study using an *in vivo* model, we were able to induce a reduction of liver α-GPD and ME activities by the administration of IGF-I to normal rats. We also explored the mechanism of IGF-I action on the specific metabolic response to T_3 by measuring the effect of IGF-I on the maximal binding capacity and the affinity constant (K_a) of THR, in addition to the levels of the mRNAs that code for the THR isoforms in the liver.

Materials and Methods

Materials

Recombinant human IGF-I (rhIGF-I) was from Genentech Inc, San Francisco, CA, USA, [^{125}I]T_3 and [α^{32}P]ATP from Du Pont NEN (Boston, MA, USA). Nylon membranes (charge modified, 0·45 μm pore size) were from Sigma Chemical Co., St Louis, MO, USA, hybridization bags and EcoRI from Gibco BRL, Gaithersburg, MD, USA. The DNA labelling kit (Prime-a-gene Labeling System) was from Promega Corp., Madison, WI, USA. The radiographic films were from Eastman Kodak, Rochester, NY, USA and the intensifying screens from Amersham International plc, Amersham, Buckinghamshire, UK. The plasmid Bluescript SK+ TRβ1 (pBSK+β1) was generously donated by R. Koenig, Department of Internal Medicine, Endocrinology and Metabolism Division, The University of Michigan Medical Center, Ann Arbor, MI, USA. All chemicals were of reagent grade.

Animals and rhIGF-I treatment

Adult male Wistar rats (250 ± 50 g) were given free access to a balanced diet and tap water, and maintained under a 12 h light : 12 h darkness cycle. Rats were injected s.c. with vehicle (0·15 M NaCl) or rhIGF-I every 12 h for different periods of time. Rats were lightly anaesthetized, killed by cervical dislocation, and the liver removed and processed for different purposes. Animal procedures were in compliance with the Guidelines on the Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare, and the local Institutional Animal Care Committee.

The doses of rhIGF-I (60–480 μg/100 g BW every 12 h) were selected on the basis of previous reports indicating that a dose of 320 μg/100 g BW per day was able to induce IGF-I-dependent effects (Hinton *et al.* 1995, Lo *et al.* 1995).

Isolation of mitochondrial, cytosolic and nuclear fractions

The procedure was similar to that described previously, with slight modifications (Bernal *et al.* 1978*a*). In brief, liver tissue (1 g) homogenized in 0·32 M sucrose–1 mM $MgCl_2$ with a Teflon–glass motor-driven tissue grinder was centrifuged at 700 ***g*** to obtain the crude nuclear pellet. The supernatant was centrifuged at 8500 ***g*** for 10 min for the preparation of the mitochondrial fraction. The 8500 ***g*** pellet was washed once with 0·125 M potassium phosphate buffer (KBP), pH 7·5. The pellet containing the mitochondrial fraction was resuspended in 3 ml KBP. The initial 8500 ***g*** supernatant was centrifuged at 105 000 ***g*** to obtain the cytosolic fraction.

The crude nuclear pellet was resuspended in 2·3 M sucrose–1 mM $MgCl_2$–2 mM dithiothreitol (DTT)–0·1 mM phenylmethylsulphonyl fluoride and centrifuged at 114 000 ***g*** for 30 min in a Beckman 50·1 SW rotor. The pellet was resuspended in 0·32 M sucrose–1 mM $MgCl_2$–1 mM $CaCl_2$–20 mM Tris–2 mM DTT, pH 7·85 (SMCT) and 0·5% Triton X-100. After 1 min at 0 °C, the tube was centrifuged at 250 ***g*** for 3 min. The pellet was washed once with SMCT. The final pellet containing the purified nuclear fraction was resuspended in 10 ml 0·32 M sucrose–1 mM $MgCl_2$–20 mM Tris, pH 7·85 (SMT).

Enzyme activity assays

α-Glycerophosphate dehydrogenase [EC 1·1·99·5; sn-glycerol-3-phosphate: (acceptor) oxidoreductase] was assayed in the mitochondrial fraction by the method of Lee & Lardy (1965) and expressed as Δ A/min per mg DNA. Malic enzyme [EC 1·1.1·40; L-malate-$NADP^+$ oxidoreductase (decarboxylating)] was assayed in the cytosolic fraction by the method of Hsu & Lardy (1969) and expressed as U/mg DNA. One unit (U) was defined as the amount of the enzyme needed to catalyse the reduction of 1 nmol NADP/min using an extinction coefficient for NADPH of $E_{340}^{mmol/l}=6·3$ (Mariash *et al.* 1980).

The DNA content was evaluated in the total cellular homogenate and in the purified nuclear fraction by the method of Burton (1956).

[^{125}I]T_3-binding studies

The maximal binding capacity and the K_a of THR were measured in isolated nuclei as described previously (Bernal *et al.* 1978*b*). Briefly, nuclei (200–300 μg DNA/tube) were incubated in SMT with $7·8 \times 10^{-11}$–$2·5 \times 10^{-9}$ M [^{125}I]T_3 (2200 Ci/mmol). To assess non-specific binding, tubes containing $2·9 \times 10^{-7}$ M T_3 were run in parallel. After 2 h incubation at 20 ± 2 °C, the nuclei were ice-cooled and centrifuged at 1000 ***g*** for 10 min. The nuclear pellets were washed twice in SMCT plus 0·5% Triton X-100 and the radioactivity in the pellet measured. The amount of receptors released to the medium during the incubation period (Bernal & DeGroot 1977) was measured in the 1000 ***g*** supernatants by the resin test. Total T_3 binding was calculated by Scatchard analysis (Scatchard 1949) from the [^{125}I]T_3 bound to the nuclear pellet and to the supernatants (resin test).

Total RNA extraction

Total RNA was prepared by the one-step acid-guanidinium method described by Chomczynski & Sacchi (1987). In brief, 0·5 g liver was homogenized in 5 ml denaturing solution (4 M guanidinium isothiocyanate, 25 mM sodium citrate pH 7·0, 0·5% *N*-lauroyl sarcosine and 0·1% β-mercaptoethanol). After phenol–chloroform–isoamyl alcohol (50 : 49 : 1) extraction, RNA was precipitated in isopropanol, recovered by centrifugation and washed in 75% ethanol. After a further extraction, precipitation and wash, the RNA was dissolved in diethyl pyrocarbonate-treated water and quantified and checked for purity by spectrophotometry at 260 and 280 nm.

Northern blot

Forty micrograms total RNA were electrophoresed in 1% agarose gel containing 0·66 M formaldehyde (Fourney *et al.* 1988). This agarose concentration allowed a good separation of the three THR mRNA species: β_1, 6·0 kb; α_1, 5·0 kb; α_2, 2·6 kb (Strait *et al.* 1990). The gel was stained with ethidium bromide to visualize ribosomal RNA (rRNA). After electrophoresis, RNAs were transferred to a nylon membrane.

The membranes were incubated in prehybridization solution containing 30% deionized formamide–5 × Denhart's solution (0·1% Ficoll type 400–0·1% albumin–0·1% polyvinylpirrolidone (PVP))–5 × SSPE (0·75 M NaCl–0·05 M NaH_2PO_4–5 mM EDTA)–1% SDS–200 μg/ml DNA from herring testes, for 5 h at 42 °C in hybridization bags. Hybridizations with the probe were performed for 48 h at the same temperature. The entire $pBSK^+$ β_1 THR cDNA (Koenig *et al.* 1988) linearized with EcoRI was used as hybridization probe for Northern blots. The entire cDNA is approximately 1·4 kb, and includes the full coding sequence. As the DNA and ligand binding domain are very similar in THRα and THRβ, this cDNA has been used as a common probe for all THR isoforms (Koenig *et al.* 1988). To ensure an even loading, the same blots were hybridized using the entire pBR 322 with an 18S rRNA genomic probe. The probes were labelled by the random primer technique with [α-^{32}P]deoxy-ATP (3000 Ci/mmol). The specific activity of the labelled probes ranged from $2·6 \times 10^9$–$3·9 \times 10^9$ d.p.m./μg DNA. After hybridization, blots were washed in 2 × SSC (0·3 M NaCl–0·015 M sodium citrate)–1% SDS for 20 min at room temperature, followed by 2 × SSC–1% SDS for 20 min at 55 °C, 1 × SSC–1% SDS for 20 min at 55 °C and 0·2 × SSC–1% SDS for 20 min at 55 °C. The membranes were exposed to Kodak X-Omat film at −70 °C with intensifying screens, for 20 days in the case of the THR probe, and for 6 h in the case of the 18S rRNA probe. The bands were quantified densitometrically (Shimadzu Dual-Wavelength Chromato Scanner CS-930) at 500 nm and the levels of THR mRNA expressed as the absorbance of the THR signals normalized with that of the 18S rRNA in the same lane.

Glucose determination

The serum glucose concentration was evaluated in samples collected 12 h after the last rhIGF-I dose (48 h treatment), using a commercial kit (Enzymatic glycaemia, Wiener lab, Rosario, Argentina).

Serum thyroid hormone concentrations

Serum concentration of total T_4 (TT_4), free T_4 (FT_4) and total T_3 (TT_3) were measured in samples collected 12 h after the last rhIGF-I dose (48 h treatment) by radioimmunoassay using commercial kits (Diagnostic Products Corporation, Los Angeles, CA, USA). Each sample was assayed in duplicate. Intra- and interassay coefficients of variation were 3·3% and 8·1% for TT_4, 5% and 8% for FT_4, and 6·1% and 9·5% for TT_3.

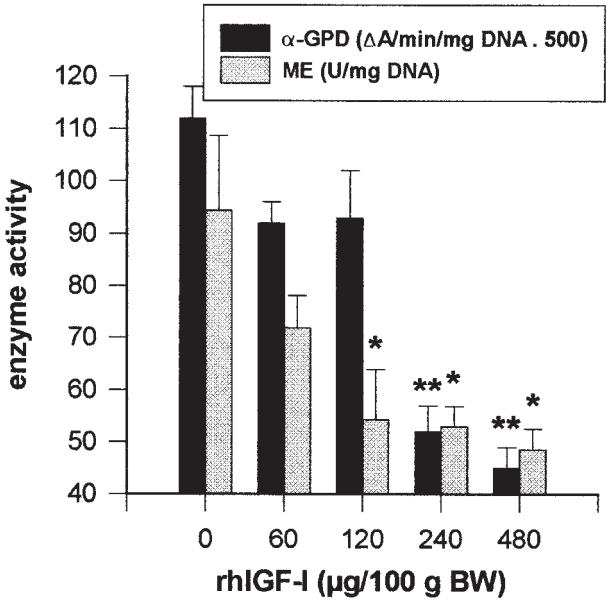

Figure 1 Effect of rhIGF-I administration to rats on liver α-GPD and ME activities. rhIGF-I was administered s.c. every 12 h for 48 h. Results are expressed as means ± S.E.M. Eight samples were analysed from each individual animal in each group, in two separate experiments; each analysis was performed at least in duplicate. *P<0·05, **P<0·01 compared with the control group by one-way ANOVA followed by Student–Newman–Keuls test.

Statistical analysis

Analysis of multiple intergroup differences in each experiment was conducted by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. Comparisons between two groups were made using the Student's t-test. P<0·05 was considered statistically significant.

Results

α-GPD and ME activities

Figure 1 shows the effect of increasing doses of rhIGF-I, administered to rats during 48 h, on the activity of α-GPD and ME. A reduction in ME activity was observed after 120 μg/100 g BW rhIGF-I (P<0·05). A similar effect on α-GPD activity was obtained by the administration of 240 μg/100 g BW (P<0·01).

Serum glucose concentration

Recombinant human IGF-I treatment every 12 h for 48 h did not induce significant changes in basal serum glucose concentration 12 h after the last IGF-I injection (mean ± S.D. values (mM): control 6·78 ± 1·56; rhIGF-I (240 μg/100 g BW) 6·72 ± 0·61; rhIGF-I (480 μg/100 g BW) 7·17 ± 0·72; n=6 in each group, Student's t-test).

Table 1 Effect of administration of rhIGF-I on serum thyroid hormone concentrations in rats. rhIGF-I (240 μg/100 g BW) was administered s.c. every 12 h for 48 h. Results are expressed as means ± S.D. for duplicate determinations. Data are from a representative experiment from a total of three with similar results

	TT_4 (μg/dl)	FT_4 (ng/dl)	TT_3 (ng/dl)
Control	5·6 ± 1·3 (n=9)	1·59 ± 0·32 (n=8)	59 ± 8 (n=7)
rhIGF-I	4·6 ± 1·1 (n=11)	1·12 ± 0·35* (n=8)	72 ± 9** (n=5)

*P<0·02, **P<0·03 compared with control group (Student's t-test).

Serum thyroid hormone concentrations

The changes in circulating iodothyronines after rhIGF-I administration every 12 h for 48 h are shown in Table 1. TT_4 concentrations tended to decrease with rhIGF-I treatment, but this failed to reach statistical significance (P=0·09). In contrast, rhIGF-I treatment induced a reduction in FT_4 to 70% of the control value (P<0·02), whereas there was a 22% increase in TT_3 compared with the control value (P<0·03).

$[^{125}I]T_3$ binding

The effect of rhIGF-I on the THR is shown in Fig. 2 and Table 2. The administration of rhIGF-I (240 μg/100 g BW) every 12 h for 48 h induced a significant reduction in the maximal binding capacity of THR (37% of control), whereas no significant changes were detected in the K_a.

As previously reported (Bernal *et al.* 1978*b*), in both groups (control and rhIGF-I treated), the K_a of THR was greater in the supernatant from the 'resin test' than in the nuclear pellet (supernatant/nuclear pellet ratio, control: 1·4, rhIGF-I treated: 1·6). However the maximal binding capacity of THR was greater in the nuclear pellet than in the supernatant (nuclear pellet/supernatant ratio, control: 1·4, rhIGF-I treated: 1·5).

The shape of the Scatchard plot from the control group did not indicate the presence of two different sets of sites for T_3 binding (high and low affinity), as the Hill coefficient (De Lean & Rodbard 1979) was 1·4 for this group.

THR mRNAs

The levels of liver THR mRNA isoforms (β_1, α_1 and α_2) after administration of rhIGF-I (120–480 μg/100 g BW for 48 h) are shown in Fig. 3. A significant reduction in β_1, α_1 and α_2 THR mRNAs was observed after the injection of 120 μg/100 g BW rhIGF-I. THR mRNAs were at the lowest level of detection after 240 and 480 μg/100 g BW rhIGF-I.

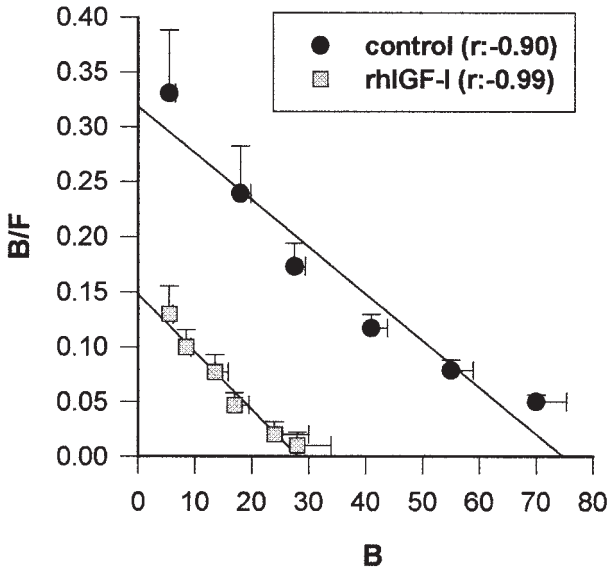

Figure 2 Scatchard plot of specific liver nuclear [^{125}I]T_3 binding in control rats or rats treated with rhIGF-I (240 μg/100 g BW every 12 h for 48 h). Plots were drawn by least squares computation. B, bound T_3 (fmol/100 μg DNA); B/F, ratio of bound T_3 to free T_3. Data are from a representative experiment from a total of two with similar results. Results are expressed as means ± S.D. Seven samples were analysed from each individual animal in each group. Maximal binding capacity and K_a values are given in Table 2.

Results of time-course studies of the effect of rhIGF-I (240 μg/100 g BW) on THR mRNAs are indicated in Fig. 4. A significant reduction in β_1, α_1 and α_2 THR mRNAs concentrations was registered after 12 h of rhIGF-I treatment and the values were at the lowest level of detection after 24 h.

The difference between the absorbance ratio for α_1 THR mRNA from control animals in Fig. 3 (zero dose of rhIGF-I) and Fig. 4 (zero time) is not of significance, because a wide range of β_1 THR mRNA/α_1 THR mRNA ratios has been reported previously (Strait *et al.* 1990).

Discussion

The present study demonstrated that the administration of rhIGF-I to normal rats resulted in a diminished metabolic response to T_3 in the liver, evaluated as the activity of α-GPD and ME, two T_3-responsive enzymes (Oppenheimer *et al.* 1995). This finding is in accordance with our previous results obtained in an *in vitro* system of cultured rat liver cells (Pellizas *et al.* 1996).

The doses of rhIGF-I that we used in this study were substantially greater than the sustitutive doses used in growth disorders in children (Klinger *et al.* 1992). However, serum IGF-I concentration in adult normal rats is about eightfold greater than that in human beings

Table 2 Effect of administration of rhIGF-I on the maximal binding capacity (MBC) and affinity constant (K_a) of liver nuclear thyroid hormone receptors in rats. rhIGF-I (240 μg/100 g BW) was administered s.c. every 12 h for 48 h. Values were obtained from Fig. 2 and expressed as means ± S.D. Seven samples were analysed from individual animals in each group

	MBC (fmol T_3/100 μg DNA)	K_a (nM^{-1})
Control	73·12 ± 14·07	2·50 ± 0·77
rhIGF-I	27·30 ± 2·50*	2·19 ± 0·74

*P<0·01 compared with the control group (Student's t-test).

(Yamamoto *et al.* 1991, Nanto-Salonen *et al.* 1993). Moreover, the lack of effect of IGF-I on the animals' behaviour or on glucose concentrations, suggests that the doses administered to rats may be considered to be in a physiological rather than a pharmacological range (Jones & Clemmons 1995).

The mechanism by which IGF-I reduced α-GPD and ME activities seems to be mediated by the type I IGF receptor. Although a lack of functional type I IGF receptors has been reported in rat hepatocytes (Massague & Czech 1982, Jaeggi-Groisman *et al.* 1990), other authors have reported their presence in male (Gruppuso *et al.* 1991) and female (Venkatesan & Davidson 1990) adult rat liver. Moreover, mRNA coding for IGF-I receptors has been reported recently (Lemmey *et al.* 1997). Other IGF-I-mediated effects have also been reported in the rat liver (Gosteli-Peter *et al.* 1994, Raper *et al.* 1995). In contrast, a possible crossreaction of infused IGF-I with the structurally similar liver insulin receptor would be expected to enhance, rather than suppress, the activity of α-GPD and ME (Wilson & McMurray 1981). In addition, although IGF-I is able to reduce insulin serum concentrations (Kolaczynski & Caro 1994), it seems not to be an explanation for the diminished liver T_3 response, as this effect was also observed in rat liver cells cultured in a medium containing a fixed concentration of insulin (Pellizas *et al.* 1996). The involvement of IGF-II/mannose-6-phosphate receptors in the effect of IGF-I is highly unlikely, as the affinity of these receptors for IGF-I is very low (Jones & Clemmons 1995).

The changes in serum thyroid hormone concentrations induced by IGF-I administration are in accordance with previous results obtained after administration of GH to normal and GH-deficient human beings (Grunfeld *et al.* 1988, Jorgensen *et al.* 1994). The reduction in FT_4 concentrations with a concomitant increase in TT_3 induced by IGF-I treatment, suggests an enhanced conversion of T_4 to T_3. However, short-term administration of IGF-I to normal adults and Laron-type dwarfs resulted in a reduction in FT_4 but failed to augment TT_3 concentrations (Klinger *et al.* 1992). Nevertheless, the changes in serum thyroid hormone concentrations induced by IGF-I

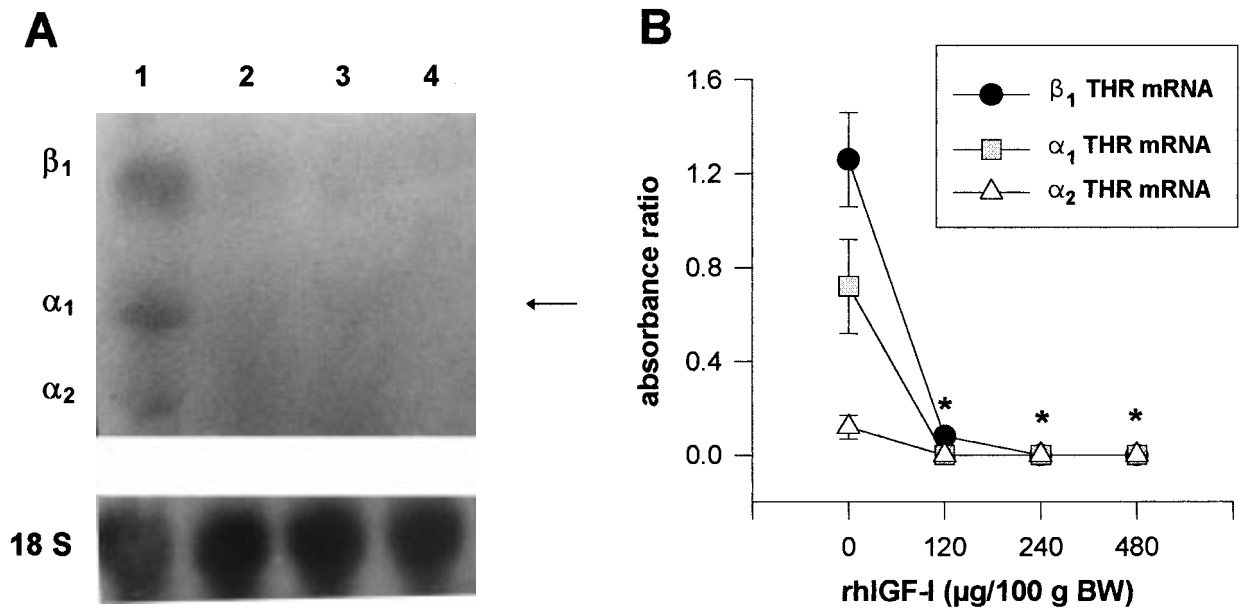

Figure 3 (A) Northern blot of liver β_1, α_1, and α_2 THR mRNAs after administration of rhIGF-I: lane 1, control; lane 2, 120 µg; lane 3, 240 µg; lane 4, 480 µg/100 g BW every 12 h for 48 h. Forty micrograms total RNA were applied to each lane. Blots were hybridized with THR (upper panel) and 18S rRNA probes (lower panel). Arrow indicates the 28S rRNA marker. (B) Densitometric analysis of Northern blots. Data are given as the ratio between the absorbance of each THR-specific signal and the absorbance of the 18S rRNA signal in the same lane. Data are from a representative experiment from a total of three with similar results. Results are expressed as means ± S.D. Three samples were analysed from each individual animal in each group. *P<0·01 compared with the control group by one-way ANOVA followed by the Student–Newman–Keuls test.

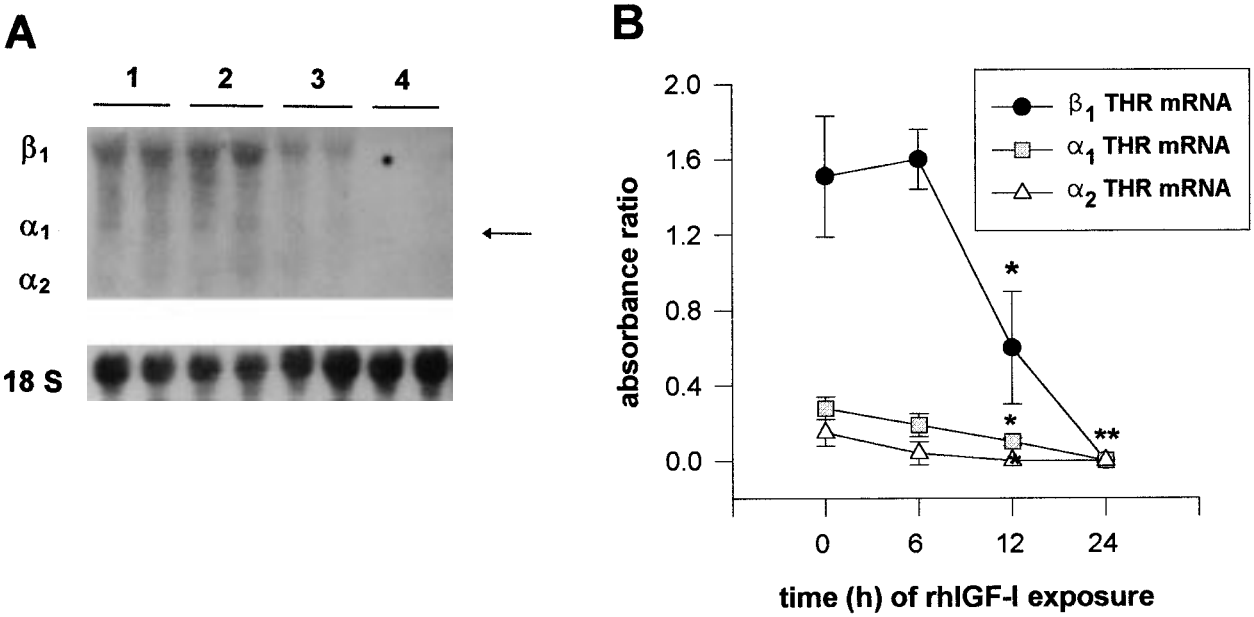

Figure 4 (A) Northern blot of rat liver β_1, α_1, and α_2 THR mRNAs after treatment with rhIGF-I (240 µg/100 g BW) for 6, 12 and 24 h. Lanes 1 and 2: control; lanes 3 and 4: 6 h; lanes 5 and 6: 12 h; lanes 7 and 8: 24 h. Forty micrograms of total RNA were applied to each lane. Blots were hybridized with THR (upper panel) and 18S rRNA probes (lower panel). Arrow indicates the 28S rRNA marker. (B) Densitometric analysis of Northern blots. Data are given as the ratio between the absorbance of each specific THR signal and the absorbance of the 18S rRNA signal in the same lane. Data are from a representative experiment from a total of three with similar results. Results are expressed as means ± S.D. Three samples were analysed from each individual animal in each group. *P<0·05, **P<0·01 compared with the control group by one-way ANOVA followed by the Student–Newman–Keuls test.

could not explain the inhibitory effect on the enzyme activities, because this effect was also seen *in vitro* in rat liver cells cultured with a fixed concentration of T_3 (1·0 nM) (Pellizas *et al.* 1996). Moreover, when cells were incubated with 1 μM T_3, a concentration large enough to saturate THRs fully (Mariash & Oppenheimer 1983), the failure to reach the maximal α-GPD and ME activities in the group pre-exposed to IGF-I (Pellizas *et al.* 1996) suggested that the effect of IGF-I might not be related to alterations in nuclear occupancy by T_3. Instead, such a response could involve a diminished number of functionally active nuclear T_3 binding sites (Sato & Robins 1981, Pellizas *et al.* 1996).

The magnitude of the cellular response to thyroid hormone depends on the abundance of nuclear receptors for T_3. It is therefore possible that physiologically or pharmacologically induced alterations in receptor numbers may be able to modify the tissue response to T_3 (Hodin *et al.* 1990). Previous reports indicate that, under several experimental conditions (DeGroot *et al.* 1977, Dillmann *et al.* 1978, Dillmann & Oppenheimer 1979, Recúpero *et al.* 1983, 1986, Kaji & Hinkle 1987), modifications in the number of THRs paralleled changes in the tissue response to thyroid hormone. Consequently, the diminished number of THRs induced by IGF-I treatment would explain, at least in part, the reduction in the metabolic response to T_3. However, in spite of the lack of known direct effects of IGF-I on α-GPD or ME, this possibility cannot be ruled out.

Although the amount of a specific protein cannot always be predicted on the basis of its mRNA level (Oppenheimer *et al.* 1995), the reduction in the concentrations of THR mRNAs after the administration of rhIGF-I (240 μg/100 g BW for 12 h) may account for the reduction in the expression of THR proteins. Regarding the failure to detect THR mRNAs after the administration of 240 and 480 μg rhIGF-I/100 g BW for 48 h, and 240 μg/100 g BW for 24 h, it may be that the use of intermediate doses and times of exposure would produce progressive detectable reductions in THR mRNAs. The diminished amounts of liver mRNA coding for the β_1, α_1 and α_2 THRs suggest that the mechanism of IGF-I action could involve either a reduction in the rate of transcription of genes coding for the THR isoforms, or an increase in the rate of disappearance of the THR mRNAs. Moreover, an effect of IGF-I that is mediated by a reduction in stimulatory transcriptional factors or by enhanced inhibitory transcriptional factors of THR-coding genes should also be considered.

The possibility that the mechanism of action of IGF-I involves an increase in the α_2 THR mRNA coding for a THR protein that lacks the ability to bind T_3 and is known to inhibit the active THRs (Koenig *et al.* 1989) was disregarded, as α_2 THR mRNA was concomitantly reduced.

The present results indicate that, in the rat liver, IGF-I modulates thyroid hormone action by a mechanism that involves, at least in part, an effect at the level of THR expression. Therefore, it is possible that IGF-I feeds back to limit the stimulatory action of T_3 on IGF-I synthesis in liver cells (Ikeda *et al.* 1991), through THR downregulation.

Even though these effects of IGF-I were observed in experimental animals, our findings may have relevance to a possible impact on thyroid function in patients receiving GH or IGF-I treatment.

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