***In Vivo* Regulation of Hepatic Insulin-Like Growth Factor-1 Messenger Ribonucleic Acids with Thyroid Hormone**

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Abstract

Insulin-like growth factor-1 (IGF-1) is synthesized primarily by the liver in response to growth hormone (GH). Thyroid hormone plays a major role in mediating pituitary GH secretion. In order to clarify the effect of thyroid hormone on IGF-1 gene expression, we measured hepatic IGF-1 mRNA levels in rats with thyroid dysfunction. Female Wistar rats were rendered hypothyroid by surgical thyroidectomy or hyperthyroid by daily injections of thyroxine (12 µg/day) for 2 weeks. Northern gel analysis of hepatic poly (A) RNA revealed the multiple sizes of the RNA transcripts ranging from 1.6 to 9.0 kb. After 4 weeks, hepatic IGF-1 mRNA levels were suppressed in hypothyroid rats, to <20% of control euthyroid animals. These suppressed mRNA levels were restored to euthyroid levels by thyroid hormone replacement for 2 weeks. Hyperthyroid rats, however, did not contain altered levels of hepatic IGF-1 mRNA as compared to euthyroid rats. The γ -actin mRNA hybridization signal was not altered in hypothyroid or hyperthyroid rats.

These results suggest that thyroid hormone regulates the *in vivo* expression of hepatic IGF-1 mRNA, probably through the mechanism of GH regulation.

It is well known that insulin-like growth factor-1 (IGF-1) mediates many of the growth-promoting actions of GH (Froesch *et al.*, 1985). The source of circulating IGF-1 has been traditionally believed to be primarily hepatic (Van Wyk., 1985). In the rat, concentrations of immunore-

active IGF-1 in a number of tissues are reduced by hypophysectomy and restored to normal by GH replacement (D'Ercole *et al.*, 1984). GH itself stimulates IGF-1 biosynthesis in the perfused rat liver (Schwander *et al.*, 1983). In contrast, serum levels of GH and IGF-1 are very low in thyroidectomized rats (Yaffe and Samuels., 1984) and thyroid hormone itself stimulates GH gene expression in rat pituitary cells (Dobner *et al.*, 1981). Furthermore, thyroid hormone directly regulates IGF-1 gene

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expression in GH3 rat pituitary cells (Fagin *et al.*, 1987; Yamashita *et al.*, 1989). These observations suggest the presence of an interaction between thyroid hormone and IGF-1 at the gene level.

In the present study, therefore, we have investigated the effects of thyroidectomy and thyroid hormone replacement on IGF-1 mRNA levels in rat liver to establish whether previously observed effects of thyroid hormone status on serum and tissue IGF-1 concentrations are mediated at the level of hepatic IGF-1 mRNA abundance.

Materials and Methods

Animals

Female Wistar rats (Shizuoka Animal Center, Shizuoka, Japan), aged 3 months and weighing 220 g, were surgically thyroidectomized or sham operated under pentobarbital anesthesia (0.05 mg/gBW) and maintained for 4 weeks. During this period, failure to gain weight confirmed the hypothyroid state of the thyroidectomized group. Thyroidectomized rats (group 1) were sacrificed by decapitation and their livers aseptically removed, quickly frozen and stored at -70°C for RNA extraction. Trunk blood was simultaneously collected for hormone RIA. Thyroidectomized rats (group 2) were injected daily with thyroxine (12 $\mu\text{g}/\text{day}$) for five days and sacrificed by decapitation. A third group of rats (normal control rats) was injected daily with T_4 (12 $\mu\text{g}/\text{day}$) for two weeks and then sacrificed. Sham-operated control rats were similarly subjected to hepatic RNA extraction and blood sampling at identical times of sacrifice. Serum T_3 and T_4 were measured by radioimmunoassay (Eiken Chemical Co., Ltd. Tokyo, Japan).

RNA extraction

Hepatic RNA was extracted by acid-guanidinium thiocyanate and phenol-chloroform (Chomczynski and Sacchi, 1987) and poly (A) RNA was isolated with poly (U) messenger affinity paper (Werner *et al.*, 1984). The UV absorbance was measured at 260 nm. The ratio of A 260/280 was usually 2.0. RNA was stored at -70°C until hybridization assays.

Preparation of cDNA probe

The mIGF-1 cDNA insert (721 bp) was excised from the plasmid kindly provided by G. I. Bell (Bell *et al.*, 1986) by restriction enzyme digestion with EcoRI. Radioactive labelled mIGF-1 cDNA probe was prepared by multi-prime DNA labelling system (Amersham) with ^{32}P -CTP (11.1×10^4 GBq, Amersham) to obtain a specific activity of $5-10 \times 10^7$ dpm/ μg DNA template. The γ -actin cDNA (Ponte *et al.*, 1983) was similarly labelled.

Dot blot hybridization

Denatured poly (A) RNA (5 μg) was immobilized on prewetted Hybond N nylon membrane paper (Amersham, Japan) mounted in a micro-sample filtration vacuum minifold (Schleicher and Schuell, Keene, NH) and supported on NO. 470 paper (White and Bancroft, 1982). The paper was prehybridized, hybridized, washed, and autoradiographed with the modifications described previously (Thomas, 1980). Briefly, after prehybridization in 50% formamide, $1 \times$ Denhardt's solution 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA, $5 \times$ SSPE and 1 $\mu\text{g}/\text{l}$ SDS at 42°C overnight, hybridization was performed with ^{32}P -labelled mIGF-1 cDNA probe for 48 h. The hybridization signal was quantified by integrated densitometry of the autoradiographic images (Melmed *et al.*, 1985). Filters were subsequently washed twice with 0.1% sodium dodecyl sulfate (SDS) and $0.2 \times$ sodium chloride-sodium citrate (SSC) at 95°C for 30 min, and rehybridized with [^{32}P] γ -actin cDNA.

RNA gel hybridization (Northern) analysis

Fifteen micrograms of hepatic poly (A) RNA was denatured by incubation at 60°C and electrophoresed in a 1% agarose gel, as described previously (Yamashita and Melmed, 1986). After RNA transfer from the gel to nylon membrane paper, the paper was prehybridized and hybridized with 10^7 cpm [^{32}P] mIGF-1 cDNA probe before exposure to X-ray film for autoradiography (Murphy *et al.*, 1987).

Statistical analysis

All data are presented as the mean $\pm$ SEM. Significance was assessed by nonpaired Student's *t*-test.

Table 1. Circulating Thyroid Hormone Levels in Rats with Thyroid Dysfunction

	Shams (n=10)	Group 1 (thyroidectomized) (n=10)	Group 2 Thyroidectomized (T ₄ supplemented) (n=5)	Group 3 (hyperthyroid) (n=10)
T ₃ (ng/ml)	98±6	31±4*	100±2	199±29**
T ₄ (μg/ml)	4±0.2	1.4±0.2*	4.2±1	18±2**

Each value represents mean±SEM ; *, ** p<0.001 vs shams

Results

Circulating thyroid hormone levels

As depicted in Table 1, serum thyroid hormone levels in thyroidectomized rats were significantly lower than in euthyroid control animals. Five days after T₄ re-

placement, serum T₃ and T₄ were restored to control levels. Two weeks after T₄ injection to normal rats, serum levels of T₃ and T₄ were significantly higher than those of control rats.

Expression of hepatic IGF-1 mRNA

Northern gel analysis of 15 μg poly (A) RNA extracted from rat liver is shown in Fig. 1. Discrete bands of IGF-1 mRNA transcripts were apparent in normal rat liver. After thyroidectomy, the level of hepatic IGF-1 mRNA transcripts was decreased in a time dependent manner. All the IGF-1 mRNA species (1.6 Kb, 4.4 Kb and 7-9.0 Kb, respectively) were reduced

Fig. 1. Northern gel analysis. A: Poly (A) RNA (15 μg) extracted from normal and thyroidectomized liver was electrophoresed in each lane for 3.5 h (100 V, 30 mA) on 1.0% agarose in 6% formaldehyde. The gel was blotted on nylon membrane paper (Hybond N, Amersham), hybridized with (³²P)-labelled mIGF-I cDNA, (s.a. 1×10⁸ cpm/μg DNA), and autoradiographed. Standard size markers (Hind III digested λDNA) were electrophoresed in a parallel lane and the size of the hybridizing bands is indicated on the left. B: The same paper was reprobed with (³²P)-labelled γ-actin cDNA. The hybridization signal (ca. 2.0 Kb) was not altered by these treatments. N: normal rat liver. TX1: hypothyroid rat liver (2 weeks after thyroidectomy). TX2: hypothyroid rat liver (4 weeks after thyroidectomy).

Fig. 2. Representative autoradiograph of dot blot hybridization of hepatic poly (A) RNA. Triplicate blots (5 μ g poly (A) RNA each) from each liver were immobilized and hybridized with (32 P)-labelled mIGF-I cDNA as described in the text. N: Normal rat liver, TX: Hypothyroid rat liver, TX+T₄: TX with T₄ replacement, T₄: Normal rat liver following T₄ administration.

to a similar degree in the liver after thyroidectomy. The γ -actin mRNA levels were not changed by this treatments.

Regulation of hepatic IGF-1 mRNA

When hepatic poly (A) RNA derived from hypothyroid rats was hybridized with the 32 P-labelled IGF-1 cDNA probe, the intensity of the IGF-1 mRNA transcripts was altered. Triplicate dots in a representative autoradiography are shown in Fig. 2. These suppressed mRNA levels were restored to euthyroid levels by thyroid hormone replacement. In contrast, hyperthyroid rats did not contain altered levels of hepatic IGF-1 mRNA as compared to euthyroid rats. The intensity of each dot was measured by optical densitometry (Fig. 3). The level of hepatic IGF-1 mRNA in Tx rats was significantly decreased compared to that of normal rats ($p < 0.001$). However, T₄ administration in normal rats did not affect the level of hepatic IGF-1 mRNA. In contrast, the γ -actin mRNA hybridization signal was not altered in hypothyroid or hyperthyroid rats.

Fig. 3. Densitometric analysis of dot blot hybridization. Each bar represents the mean $\pm$ SEM of triplicate dots.

Discussion

The present findings demonstrate the effect of thyroidectomy and thyroid hormone replacement on the abundance of hepatic IGF-1 mRNA. Rat liver contains several mRNA transcripts (1.6, 4.4, 7.0–9.0 Kb) which hybridize to a mouse IGF-1 cDNA probe. Signal intensities of all hybridizing IGF-1 mRNAs were lower in hepatic poly (A) RNA derived from thyroidectomized animals than in normal rats.

Previous studies have shown the distribution of hepatic IGF-1 mRNA (Murphy *et al.*, 1987; Daughaday and Potwein, 1989) and the GH dependence of IGF-1 mRNA levels in rat liver (Hynes *et al.*, 1987). Since serum GH levels were dramatically suppressed in hypothyroid rats (Matsuo *et al.*, in press), our data demonstrate the same effect of thyroidectomy on hepatic IGF-1 mRNA. Furthermore, thyroid hormone replacement in thyroidectomized rats (GH: 49 ± 17 ng/ml) restored circulating GH to normal euthyroid levels (GH: 190 ± 31 ng/ml), and also reversed the attenuation of hepatic IGF-1 mRNA in these animals. However, hyperthyroid rats did not demonstrate changes in hepatic IGF-1 mRNA in our study. This remains to be further investigated. A direct effect of T_4 on the expression of hepatic IGF-1 mRNA transcription or stability cannot presently be excluded. Nevertheless, these observed changes are probably secondary to the induction of GH secretion by the exogenous T_4 in thyroidectomized rats, although the tissue origins of circulating IGF-1 and intracellular mechanisms regulating IGF-1 synthesis are not clearly established. The strong correlations between hepatic IGF-1 content and circulating IGF-1 levels indicate the liver as a major source of IGF-1 in the circulation (Schwander *et al.*, 1983). Furthermore, recent evidence indicates hepatic IGF-1 regulation at the mRNA

level (Hynes *et al.*, 1987; Lowe *et al.*, 1988). The fluctuation in mRNA levels could reflect changes in the transcriptional rate or in mRNA stability. IGF-1 gene expression in both man (Ullrich *et al.*, 1984) and rat (Shimatsu and Rotwein, 1987) appears to include the possibility of alternative RNA processing, although alternative processing seems unlikely based on the co-ordinate fall in different mRNA transcripts with hypothyroid liver. The induction of IGF-1 mRNA by estrogen was observed in the rat uterus (Murphy *et al.*, 1987) and a relative increase in IGF-1 mRNA in compensatory renal hypertrophy was also reported (Fagin and Melmed, 1987). Furthermore, in streptozotocin-diabetes rats, hepatic IGF-1 mRNA was decreased (Goldstein *et al.*, 1988). If a thyroid hormone responsive element resides within the IGF-1 gene, a direct action of thyroid hormone on IGF-1 gene expression may be speculated. However, the promoter regions of the IGF-1 gene have not been sufficiently delineated to permit speculation as to the likely site of distorted regulation in hypothyroid rats. Recently we have demonstrated the *in vivo* regulation of IGF-1 receptor by T_3 in thyroidectomized rats (Matsuo *et al.*, 1990). Taken together, thyroid hormone may play an important role in regulating IGF-1 action at the IGF-1 production site and/or at the IGF-1 targeting site..

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