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Thyroid Hormone Controls the Expression of Insulin-Like Growth Factor I Receptor Gene at Different Levels in Lung and Heart of Developing and Adult Rats*

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ABSTRACT

Thyroid hormone exerts profound effects on the insulin-like growth factors (IGFs)/IGF factor I receptor (IGF-IR) system through its action on the production of IGF-I peptide and IGF-binding proteins. Most of these actions are mediated by the direct control of pituitary GH gene by thyroid hormone. In this work, we have analyzed the possible effect of hypothyroidism on the expression of IGF-IR gene, both in adult and developing animals. Our results show that in the lung and heart, thyroid hormone exerts a negative effect on IGF-IR gene expression in the adult animals and during perinatal life (from day 15 onwards). This negative effect is exerted at different levels. In the heart, this regulation occurs at a pretranslational level, indicated by the fact that parallel changes in the number of membrane IGF-I receptors and IGF-IR transcripts were observed, whereas in lung, no effect of thyroid hormone was noted in the amount of IGF-IR transcripts, suggesting a translational or posttranslational control. GH does not seem to mediate T_3 effects on this gene. In contrast, retinoic acid increases the expression of IGF-IR gene at a transcriptional or posttranscriptional level in adult lung and heart. Because the IGFs/IGF-IR system is depressed in hypothyroid animals, the specific increase in the number of IGF-IRs in the lung and heart of these animals could represent a mechanism to ameliorate the negative effects of hypothyroidism on these important organs. (*Endocrinology* **138:** 1194–1203, 1997)

T_3 PLAYS an important role in growth and development of most vertebrate tissues (1). The majority of the actions of T_3 are mediated by nuclear receptors present in those tissues. These receptors are members of the nuclear receptor superfamily, which includes the receptors for steroid hormones, vitamin D_3, retinoids, and the so-called orphan receptors, without known ligand (2). Several isoforms of thyroid hormone receptors are produced from two different genes (3). These proteins are ligand-dependent transcription factors that control directly the expression of a limited number of genes, those containing a thyroid response element (TRE) (4, 5) in their promoter. Thyroid hormone receptors also can exert direct actions on gene expression through protein-protein interaction with other transcription factors (6). T_3 control of these specific genes most probably initiates a cascade of events in which many other genes are indirectly influenced by thyroid hormone. A clear example of this indirect control is the transcriptional regulation by T_3 of pituitary GH gene and the subsequent effects of this hormone. Therefore, the knowledge of which genes are directly or indirectly regulated by T_3 in the different tissues is necessary for a complete understanding of thyroid hormone action.

Insulin-like growth factor I (IGF-I) is involved in tissue growth and development during fetal and neonatal life and acts as a mitogen and a differentiation factor (7), mediating many of the effects of pituitary GH (8). The plasma membrane receptor for IGF-I (IGF-IR) is coded by a single gene and shows considerable sequence homology and functional similarity with the insulin receptor. Both receptors have heterotetrameric structures formed by two α and two β subunits with a tyrosine kinase domain in the cytoplasmic portion of the molecules (9). IGF-IR also binds IGF-II, although with lower affinity (10), and it is thought to mediate many of the effects of this growth factor (11). IGF-binding proteins (IGFBPs) bind circulating IGF-I and IGF-II with high affinity modulating their receptor-binding properties and their biological actions (12). IGFBPs are coded by at least six different genes that are expressed in a tissue- and developmental-specific manner (13). All these molecules (IGF-I, IGF-II, IGF-IR, and IGFBPs) constitute the IGFs/IGF-IR system, which acts in an endocrine and auto/paracrine manner and plays a major role in vertebrates' growth. This system is a target of thyroid hormone action, because it is known that T_3 regulates the expression of IGF-I and several IGFBP genes in diverse tissues (14, 15), and it is thought to mediate many of the thyroid hormone effects on somatic growth (14). The effects of thyroid hormone on the IGFs/IGF-IR system are mainly exerted through its control of GH gene expression (16), although GH-independent mechanisms also have been described (17).

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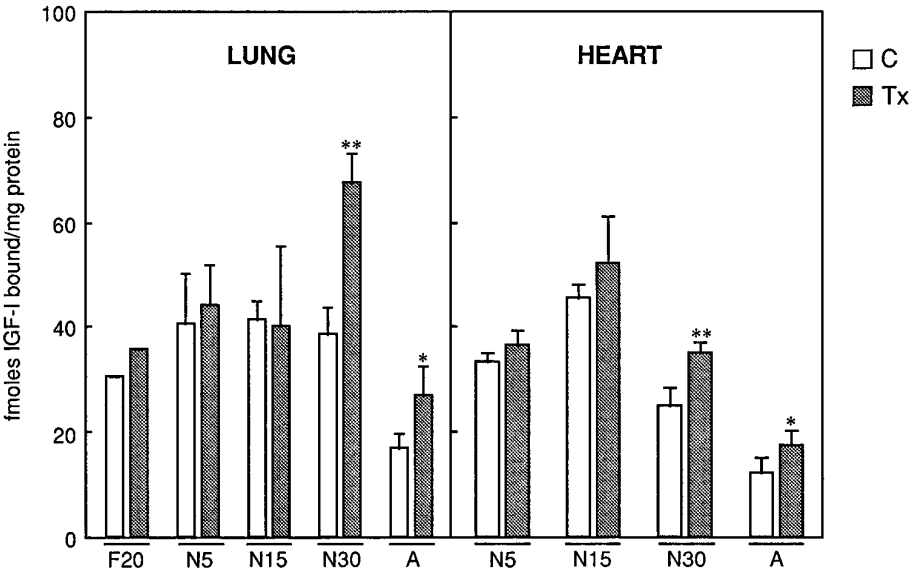

FIG. 1. Effect of hypothyroidism on IGF-I binding to lung and heart membranes during development. Lung and heart membranes from control (C) and hypothyroid animals (Tx) were prepared, as indicated in *Materials and Methods*. F, Fetuses; N, neonates; A, adults. [^{125}I]-IGF-I binding (1.1×10^{-10} M) was determined, and the values given in the text are the average of specific binding of at least three different samples, or two for 20-day-old fetuses. The *bars* represent SD. *, $P < 0.05$; **, $P < 0.01$ *vs.* C.

In this work, we have studied whether in addition to IGF-I and IGFBP genes, the IGF-IR gene could be under thyroid hormone control throughout development and in adult life. We have focused our study on the lung and heart, two tissues that are important targets of both thyroid hormone (18, 19) and the IGFs/IGF-IR system (20, 21).

Our results showed that in the rat, IGF-IR gene expression is repressed by thyroid hormone in the two tissues studied, lung and heart, from day 15 of postnatal life. Consequently, IGF-I binding was increased in hypothyroid animals and diminished after T_3 administration to these animals. The changes in IGF-IR messenger RNA (mRNA) levels in the heart paralleled those of IGF-I binding. However, this was not the case in the lung, where T_3 had no effect on the content of IGF-IR transcripts, suggesting that this hormone acts at different levels in both tissues. On the other hand, retinoic acid (RA) increased the expression of IGF-IR gene at a pretranslational level in both tissues in adult animals. Finally, GH does not seem to mediate T_3 effects on this gene.

Materials and Methods

Animals

Female Wistar rats were mated and the day of appearance of the vaginal plug was considered as day zero of fetal age. To induce fetal and neonatal hypothyroidism, dams were given 0.02% methyl mercaptoimidazole (MMI) in their drinking water at day 9 of pregnancy. MMI administration was continued throughout gestation and the lactating period. At postnatal day 5, pups were injected with ^{131}I (150 μCi/100 g of BW) to destroy all thyroidal tissue. Adult hypothyroidism was induced with MMI treatment for 3 weeks. With these treatments, cytosolic T_3 fell below detectable levels. Treatment with T_3 or RA was performed by ip injection of 200 μg T_3 or 100 μg RA/100 g of BW. Control rats received an injection of the vehicle. The rats were decapitated at the indicated ages and the tissues rapidly removed and frozen at −80 C until use, except for membrane preparation, for which fresh tissues were used.

Membrane isolation

Lung and heart membranes were prepared, as previously described (22, 23). Briefly, the lungs were homogenated with a polytron in 10 vol buffer L (10 mM HEPES pH 7.5, 30 mM NaCl, 1 mM DTT, and 5 μM PMSF as protease inhibitor), the homogenates were filtered through a double gauze, then layered over a 41% (wt/vol) sucrose solution in buffer L, and centrifuged at 95,000 × *g* for 1 h. The membrane fraction was recovered from the interface, washed twice with L buffer, resuspended in 10 mM HEPES pH 7.4, and kept at −80 C until use. Hearts were homogenized with a polytron in 3.5 vol buffer H (10 mM Imidazole/HCl pH 7.0, 0.6 M sucrose, and 5 μM PMSF as protease inhibitor), centrifuged 30 min at 12,000 × *g*, and the supernatants were diluted 2.5-fold with MOPS/KCl buffer (20 mM MOPS pH 7.4 and 160 mM KCl) and centrifuged again 1 h at 96,000 × *g*. Pellets were resuspended in MOPS/KCl buffer (4 ml/g), layered on a 30% (wt/vol) sucrose solution containing 0.1 M Tris-HCl pH 8.3, 50 mM Na-pyrophosphate and 0.3 M KCl, and centrifuged 1.5 h at 95,000 × *g*. The membrane fraction was recovered from the interface, washed with MOPS/KCl buffer, resuspended in the same buffer, and kept at −80 C until use. All the steps described for membrane preparation were carried out at 4 C. Protein content was determined by the method of Bradford (24). The activity of the enzyme 5′-nucleotidase (EC 3.1.3.5.) (25) was used as an index of membrane purification.

Binding assay

Membrane fractions (100 μg protein) were incubated with 1.1–1.5 × 10^{-10} M [^{125}I]-IGF-I (2000 Ci/mmol; Amersham Corp., Aylesbury, UK) at 0–2 C during 16–20 h in Krebs HEPES solution (NaCl 118 mM, KCl 5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 2.5 mM, and HEPES 25 mM, pH 7.4), containing 1% BSA and 0.5 mg/ml bacitracin. Bound and free polypeptides were separated by centrifugation, and the membrane protein recovered was determined to correct binding values. The recovery was approximately 60% of the initial amount of protein and no differences were found among the different samples. Nonspecific binding was considered as the radioactivity bound in the presence of 10^{-7} M unlabeled IGF-I. For saturation analysis, membranes were incubated, as described, with [^{125}I]-IGF-I (1.1–1.6 × 10^{-10} M) and increasing concentrations of unlabeled IGF-I (10^{-10} M-5 × 10^{-7} M). The data were analyzed with the computer program Ligand and the affinity and number of binding sites calculated. Degradation of [^{125}I]-IGF-I, as measured by 10% trichloroacetic acid precipitation, was negligible (2.5%).

Affinity labeling and SDS-PAGE

For IGF-I receptor labeling, membrane-bound [^{125}I]-IGF-I was chemically cross-linked to the receptor with the homobifunctional reagent, disuccinimidyl suberate (Pierce Chemical, Rockford, IL), at a final concentration of 0.5 mM. Affinity-labeled membranes were solubilized and analyzed by SDS-PAGE in 7.5% acrylamide gels under reducing conditions (2.5% β-mercaptoethanol) (26). The gels were dried, exposed to x-ray films, and the signal in the film quantified by computer-assisted densitometry.

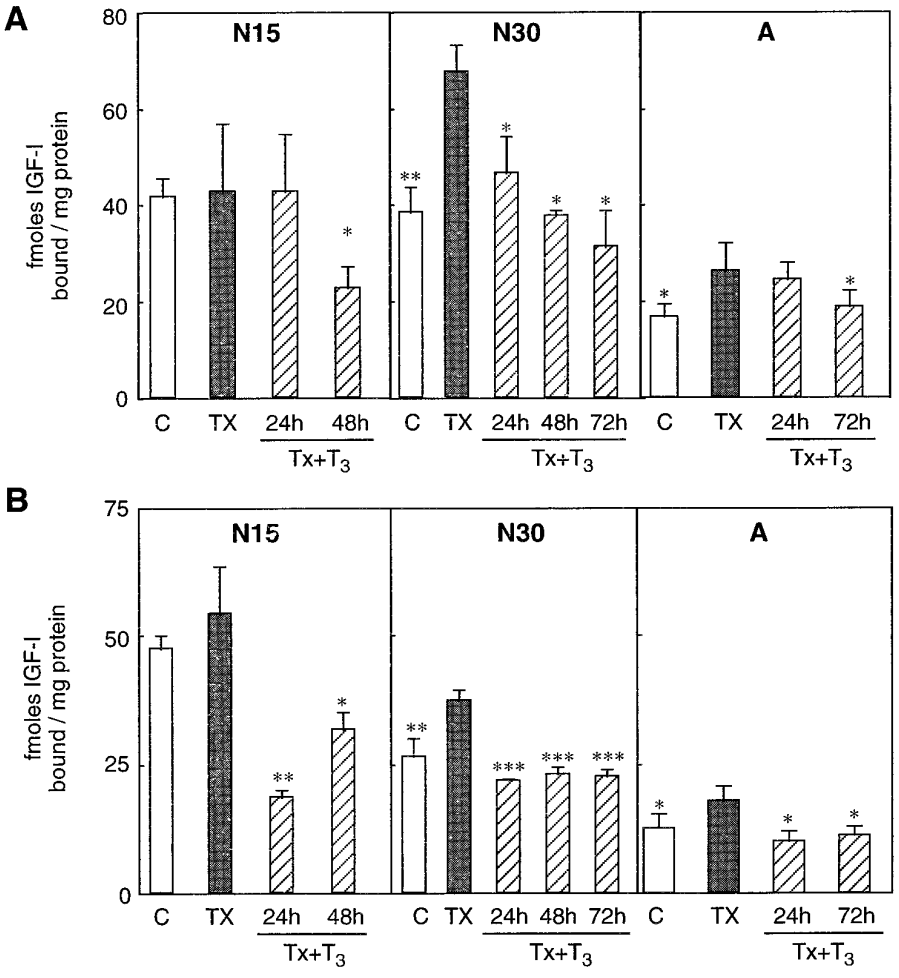

FIG. 2. Effect of T_3 administration on IGF-I binding to lung (A) and heart (B) membranes. Lung and heart membranes from control (C), hypothyroid (Tx), and hypothyroid animals injected daily with T_3 (200 μg/100 g BW) (Tx+T_3) were obtained, as indicated in *Materials and Methods*. Animals were killed at the indicated times after the first injection. N, Neonates; A, adults. [^{125}I]-IGF-I binding (1.1×10^{-10} M) was determined, and the values given are the average of specific binding of at least three different samples. The *bars* represent SD. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* Tx.

Ribonuclease (RNase) protection assay

Total RNA from the different tissues was obtained according to the method of Chomczynski and Sacchi (27) and checked for integrity by 1% agarose gel electrophoresis. For RNase protection assay, a rat homologous IGF-I receptor complementary DNA (cDNA) probe of 510 bp was generated by PCR amplification of rat brain cDNA. (Forward primer: 5′-AAA AGG AAT GAA GTC TGG CTC C-3′, reverse primer: 5′-GTA GTT ATT GGA CAC CGC ATC C-3′). The amplified fragment was subcloned in the pCR vector (Invitrogen Corporation, San Diego, CA) and sequenced in an Applied Biosystems 373A DNA Sequencer, using the dideoxynucleotide chain-terminator method. A [^{32}P]-labeled cRNA probe of 643 nucleotides was generated by SP6 RNA polymerase and [α-^{32}P]UTP (400 Ci/mmol; Amersham Corp., Aylesbury, UK), and 5×10^5 cpm were allowed to hybridize overnight at 45 C with 20 μg of total RNA. A 248-bp cyclophilin cRNA (from 1–248) (28), labeled with [α-^{32}P]UTP (400 Ci/mmol), was used as control. The RNA was then digested and the remaining fragments separated in a sequencing gel, as described by Sambrook *et al.* (29). The gels were exposed and the autoradiograms quantified by computer-assisted densitometry. For quantitation corrections, the values of cyclophilin signal were used. We have observed previously that cyclophilin values are not modified in lung and heart as a consequence of hypothyroidism or T_3 treatment (data not shown). Development caused a small decrease in the ratio cyclophilin/28S rRNA signal; this was taken into account for the final quantitation analysis (see Figs. 5 and 6), where mRNA IGF-IR signal was compared at different ages.

Results

Effect of thyroid hormone on IGF-I binding

Membranes from lung and heart of control and hypothyroid animals were isolated at the following ages: 20-day-old fetuses (F-20) and 5- (N-5), 15- (N-15), and 30-day-old (N-30) neonates and adult (A) animals. The binding of IGF-I was determined, as indicated in *Materials and Methods*, and the results are shown in Fig. 1. In lung, IGF-I binding increases after birth (30 fmol/mg protein in F-20 and 41 fmol/mg protein in N-5) and decreases in the adult animal (17 fmol/mg protein). Congenital hypothyroidism did not cause any significant change to lung membrane IGF-I binding in fetuses and N-5 and N-15 animals. However, a marked increase in binding was observed in the membranes of hypothyroid N-30 and adult animals compared with age-matched controls (1.8- and 1.6-fold increases, respectively). The IGF-I binding profile in heart membranes differed from that observed in the lung. The highest binding activity was observed in N-15 (47.5 fmol/mg protein), and a more pronounced reduction than that in the lung was observed in N-30 (25.8 fmol/mg protein) and adult (12.79 fmol/mg protein) heart membranes. Hypothyroidism, as seen in lung, caused a significant increase of IGF-I binding in N-30 and adults (1.4-fold

increase in both cases compared with age-matched controls) but not in the other groups studied (Fig. 1).

In the hypothyroid animals, the changes observed in IGF-I binding to lung and heart membranes were not caused by differences in IGF-I degradation or an artifact of membrane purification. The rate of degradation was the same in control and hypothyroid membranes (2.5%), and 5′-nucleotidase activity was similarly enriched in both groups (9- and 11-fold in lung and heart, respectively), indicating no differences in the membrane fraction selected.

To determine whether the observed effect of hypothyroidism on IGF-I binding to lung and heart membranes could be corrected by thyroid hormone treatment, hypothyroid N-30 and adult rats were injected daily with a saturating dose of T_3, killed at the indicated times, and IGF-I binding determined. The results for lung membranes are shown in Fig. 2A. Twenty-four hours after T_3 injection, a significant reduction in IGF-I binding was observed in N-30 (from 67.5 to 46.7 fmol/mg protein), and after 48 h, the binding was indistinguishable from control animals. In the adults, T_3 reduced IGF-I binding only after 72 h of T_3 administration, and no significant effect was observed within 24 h, suggesting a delayed response to T_3 in these animals when compared with the neonates. Also, N-5 and N-15 hypothyroid rats were injected with T_3 and, as shown in Fig. 2A, a decrease in binding was detected in N-15 after 48 h of T_3 administration, suggesting that, at this age, the animals are sensitive to T_3 action, although no differences could be observed on steady-state values. On the contrary, N-5 hypothyroid neonates did not respond to T_3 treatment (data not shown). In heart membranes, as shown in Fig. 2B, T_3 administration to hypothyroid N-30 and adult rats decreased IGF-I binding to reach control values within 24 h. In hypothyroid N-15, T_3 also reduced IGF-I binding far below control levels after 24 h (18.4 fmol/mg protein compared with 47.48 fmol/mg protein in controls), although, as observed in the lung, no differences were seen in steady-state values.

It is known that IGF-I also binds to IGF-II and insulin receptors, although with lower affinity. To eliminate the possibility of IGF-I cross-binding with these receptors, cross-linking experiments were performed using lung and heart membranes. As shown in Fig 3, a labeled band of approximately 130 kDa was observed in lung membranes of N-30 and adults in all the experimental groups studied. This band was displaced by the simultaneous incubation with unlabeled IGF-I (10^{-7} M), but only slightly by insulin (10^{-6} M), indicating that it corresponds to the α subunit of the IGF-I receptor. Similar results were observed using heart membranes (data not shown). A labeled band of approximately 260 kDa was observed in all samples analyzed and probably represents IGF-I receptor α subunit dimmers, because its levels are proportional to the 130 kDa-band.

Scatchard analysis of IGF-I binding was performed to determine whether the increased binding observed in hypothyroid animals was caused by a change in the number of binding sites and/or changes in affinity. As shown in Fig. 4, curvilinear plots, probably reflecting a negative cooperativity in IGF-I binding (30), were obtained for all the groups studied. The number of total IGF-I binding sites in hypothyroid lung membranes compared with the control were

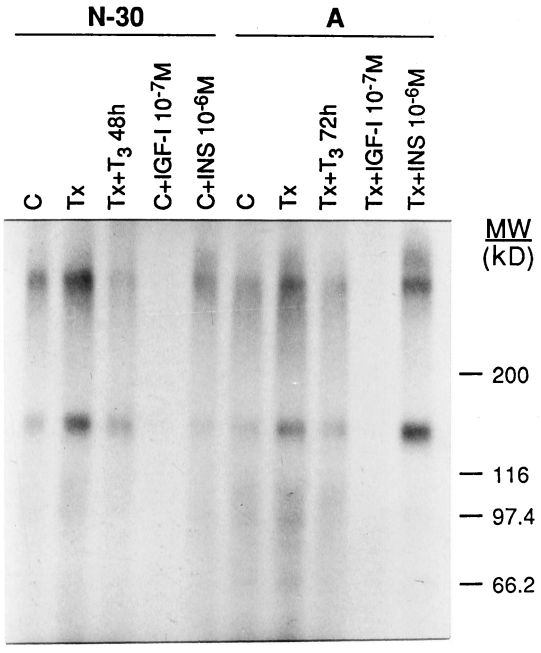

FIG. 3. [^{125}I]-IGF-I cross-linking to lung membranes. Bound [^{125}I]-IGF-I (1.5×10^{-10} M) in the absence or presence of unlabeled IGF-I (10^{-7} M) or insulin (10^{-6} M) was chemically cross-linked to its receptor with 0.5 mM disuccinimidyl suberate. The samples (50 μg protein/lane) were solubilized under reducing conditions and subjected to SDS-PAGE in 7.5% acrylamide slab gels. N-30, 30 days-old neonates; A, adults; C, control; Tx, hypothyroid.

2.7-fold higher in N-30 and 2.4-fold higher in adults (Bmax 470 and 160 fmol/mg protein in control N-30 and adults, respectively) (Fig. 4A). The administration of T_3 to hypothyroid animals decreased the number of IGF-I receptors. In the heart, a 3.2-fold increase was observed in N-30 hypothyroid animals (Bmax 620 fmol/mg protein in controls) (Fig. 4B). The high affinity-binding constant was similar in all the groups studied (kDa 1.06 nM). These results clearly indicate that the increases in IGF-I binding (10^{-10} M) in lung and heart membranes of hypothyroid animals were caused by an increase in the number of binding sites.

Effect of thyroid hormone on IGF-I receptor mRNA content

To analyze the mechanism by which thyroid hormone affected IGF-I binding, we determined the amount of IGF-IR transcripts in total RNA isolated from lung and heart of control and hypothyroid animals at the indicated ages. The amount of IGF-I mRNA was determined by RNase protection assay, as indicated in *Materials and Methods*, and in the same assay, the amount of cyclophilin was determined and used as a control. The results are shown in Figs. 5 and 6. The developmental profiles of heart and lung IGF-IR mRNA levels were similar to those observed for IGF-I binding. A moderate increase of IGF-IR mRNA after birth and a decrease in the adults suggests a developmental control of this gene at a pretranslational level.

Hypothyroidism did not significantly alter the levels of IGF-IR mRNA in lung during the period studied, although the average tends to be higher in the hypothyroid group in N-30, in accordance with IGF-I binding data, and lower in the

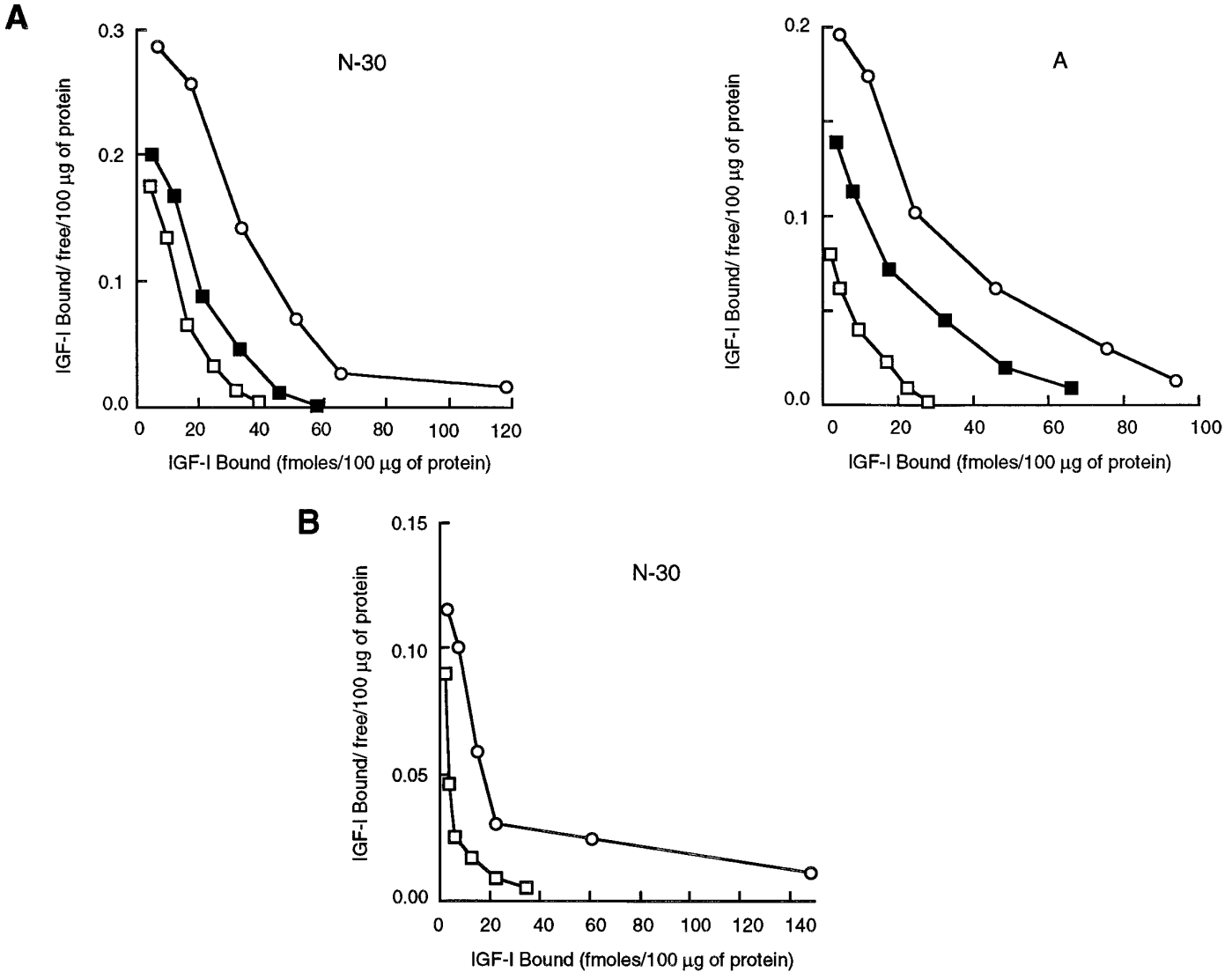

FIG. 4. Scatchard analysis of IGF-I binding to lung (A) and heart (B) membranes. IGF-I binding was analyzed at different polypeptide concentrations (10^{-10} M-5 $\times$ 10^{-7} M) and the values represented according to Scatchard. N-30, 30 day-old neonates; A, adults; □, control; ○, hypothyroid; ■, hypothyroid injected with T_3 and killed 48 h (N-30) and 72 h (A) later.

adults, in spite of the higher binding activity (Fig. 5B). In contrast to the observations in lung and in agreement with the binding values, a significant increase in IGF-IR mRNA content in the heart was observed in hypothyroid N-30 and adult rats (2.8- and 1.8-fold increase, respectively) (Fig. 6B).

When the amount of IGF-IR mRNA was determined in T_3-injected hypothyroid N-15, N-30, and adult animals, no changes were observed in the lung (Fig. 7A), even after 72 h of treatment, a period during which a decrease in IGF-I binding was observed in all groups. These results confirm the lack of effect of T_3 on the content of IGF-IR transcripts in lung and suggest that IGF-IR gene is controlled by thyroid hormone at the translational and/or posttranslational level in this tissue. In keeping with thyroid hormone repression of IGF-IR binding, T_3 injection also decreased IGF-IR mRNA content in the heart of hypothyroid animals at all ages studied (Fig. 7B). In N-15 animals, a marked 2.6-fold decrease in the content of IGF-IR transcripts was already observed 12 h after T_3 injection. These results indicate that in heart, in contrast with lung, thyroid hormone exerts its effects on IGF-IR gene at a pretranslational level, and changes in IGF-IR mRNA are a probable cause for the observed decrease in binding sites.

Effect of GH on IGF-I binding and IGF-I receptor mRNA content

It is well established that GH production is under T_3 control in rat pituitary of both adult and developing rats (16), and it has been shown that GH alters IGF-I binding in some systems (31, 32). Given these evidences, we investigated whether the effect of thyroid hormone on IGF-IR could be mediated by GH. We studied the effect of GH administration on IGF-I binding and IGF-IR mRNA content in lung and heart. For this purpose, N-30 hypothyroid rats were sc injected once daily with human GH (50 μg/100 g of BW), and

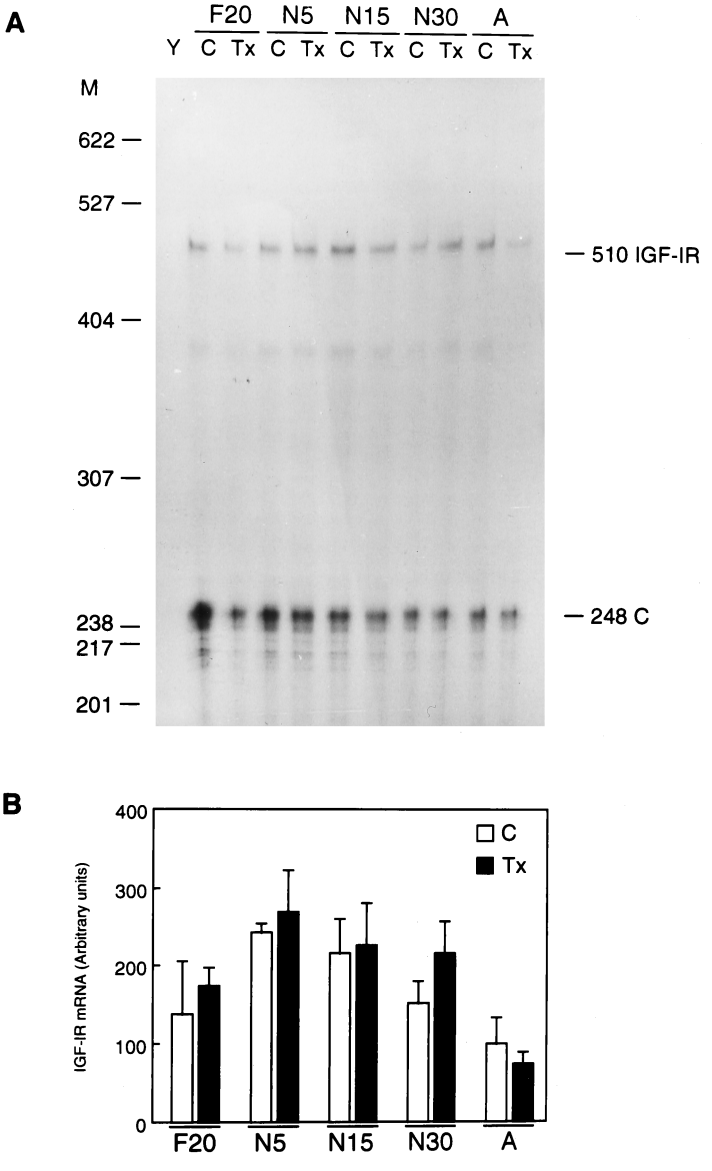

FIG. 5. Effect of hypothyroidism on IGF-IR mRNA levels in lung during development; Representative RNase protection experiment (A) and densitometric quantification of IGF-IR mRNA content (B). Total RNA was isolated from lung of control (C) and hypothyroid (Tx) animals at the indicated ages and IGF-IR mRNA content determined, as described in *Materials and Methods*. F, Fetuses; N, neonates; A, adults; M, DNA ladder; Y, yeast tRNA (10 μg) was used for hybridization. Values refer to the intensity of the band in the adult control and represent the average of three (F-20), four (N-5 and N-15), and eight (N-30 and A) different samples. The *bars* represent SD.

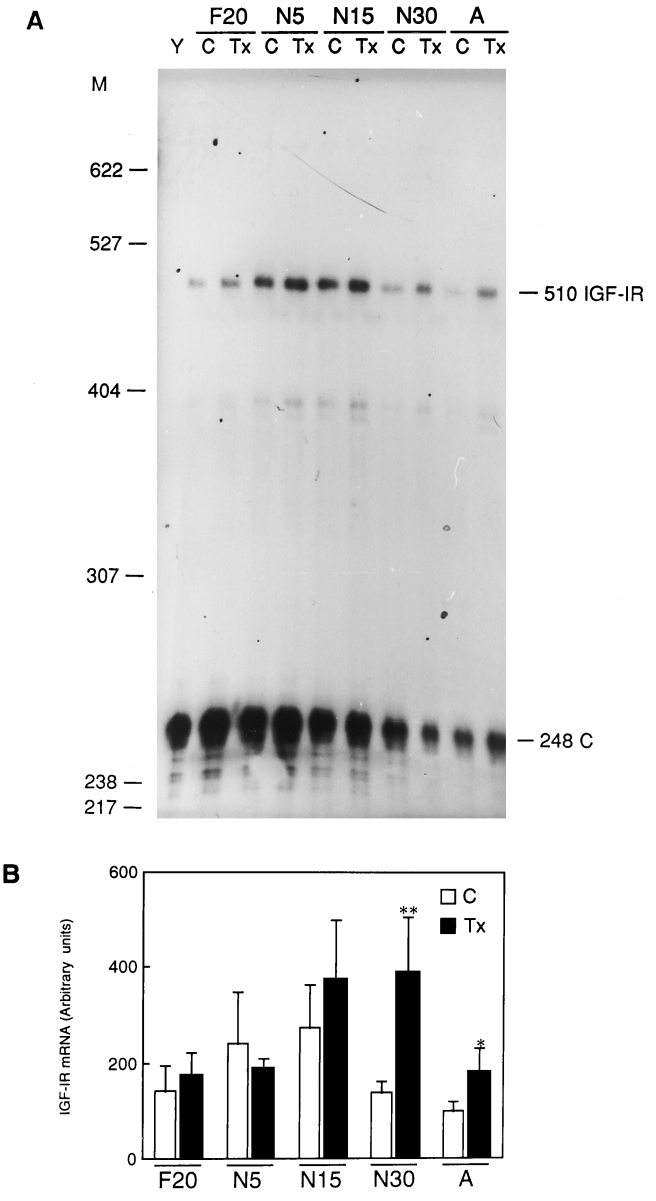

FIG. 6. Effect of hypothyroidism on IGF-IR mRNA content in heart during development; representative RNase protection experiment (A) and densitometric quantification of IGF-IR mRNA levels (B). Total RNA was isolated from heart of control (C) and hypothyroid (Tx) animals at the indicated ages and IGF-IR mRNA content determined, as described in *Materials and Methods*. F, Fetuses; N, neonates; A, adults. M, DNA ladder. Y, yeast tRNA (10 μg) was used for hybridization. Values refer to the intensity of the band in the adult control and represent the average of 3 (F-20) and 4 (neonates and adult) different samples. The *bars* represent SD. *, $P < 0.05$; **, $P < 0.01$ *vs.* C.

different groups of animals were killed 24 and 72 h later to determine IGF-I binding and IGF-IR mRNA content. As shown in Fig. 8A, GH had no effect on IGF-I binding in either tissue. No alterations in IGF-I degradation or in 5′nucleotidase activity during membrane purification was observed in GH-treated animals. Moreover, no alterations in IGF-IR mRNA content were observed in the heart and only a moderate increase in the lung was detected after 72 h (Fig. 8B), in spite of the fact that no changes were observed on IGF-I binding to lung membranes. All these results suggest that GH does not mediate the observed alterations on IGF-IR expression caused by thyroid hormone.

Effect of RA on IGF-I binding and IGF-IR mRNA content in lung and heart

RA receptor is a close relative of thyroid hormone receptor; they bind to similar hormone regulatory elements, can heterodimerize, and frequently can regulate the same set of

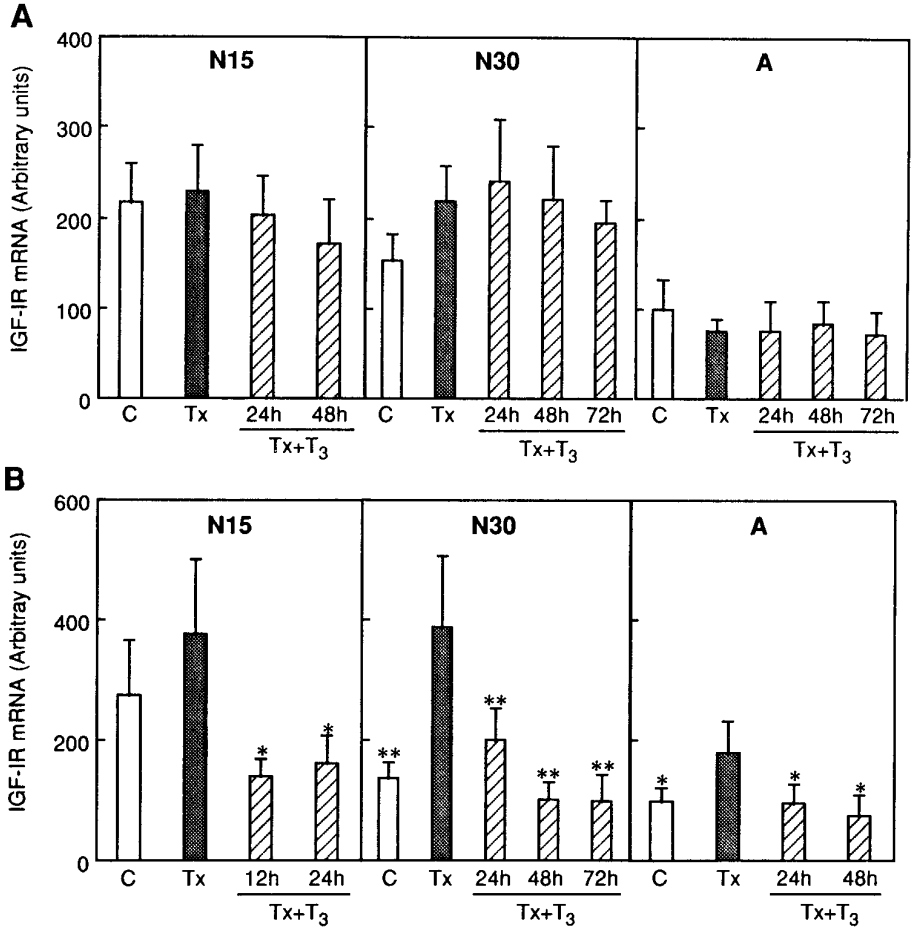

FIG. 7. Effect of T_3 administration on IGF-IR mRNA content in lung (A) and heart (B). Total RNA was isolated from lung and heart of control (C), hypothyroid (Tx) and hypothyroid animals injected daily with T_3 (200 μg/100 g BW) (Tx+T_3), and killed at the indicated times. IGF-IR mRNA content was determined, as indicated in *Materials and Methods*. N, Neonates; A, adults. IGF-IR mRNA content was quantified by computer-assisted densitometry. Values refer to the intensity of the band in the adult control and represent the average of at least four different samples. The *bars* represent SD. *, $P < 0.05$;* **, $P < 0.01$ *vs.* Tx.

genes in a similar way (33). For this reason, RA (100 μg/100 g BW was injected ip into hypothyroid adult rats and then IGF-I binding and the amount of IGF-IR mRNA determined. As shown in Fig. 9A, RA increased IGF-IR mRNA levels after 24 h of treatment, both in heart and lung of hypothyroid animals, with a higher effect in the heart (3.7-fold compared with 2.4-fold in lung). In agreement with the increase in IGF-IR mRNA content, IGF-I binding was augmented in lung membranes after RA treatment (Fig. 9B). Scatchard analysis indicated that this increase in IGF-I binding was the consequence of a higher number of IGF-IR (data not shown). These results suggest that RA regulates IGF-IR gene at a pretranslational level in both tissues.

Discussion

In this work, we have demonstrated that the expression of IGF-I receptor is under thyroid hormone control in the lung and heart of neonatal and adult rats. These results, in addition to other known effects of thyroid hormone on the expression of IGF-I and IGFBPs genes in humans and experimental animals, imply a tremendous dependence of the IGFs/IGF-IR system on the thyroidal state.

The parallelism between the developmental profile of IGF-I binding and IGF-IR mRNA content in lung and heart suggests a control of the IGF-IR gene at a pretranslational level, as previously indicated (34). Our results are in agreement with reported data of IGF-I binding in developing rat lung (35) and heart membranes (36), although changes in affinity also have been described (36). An effect of thyroid hormone on IGF-I binding, in lung and heart during development, was not observed until day 15 of postnatal life, although in this group of animals, the administration of a saturating dose of T_3 was required to detect a decrease in binding, in contrast to the N-30 animals, where an increase in IGF-I binding was observed in hypothyroid animals compared with controls. This suggests that IGF-I receptor gene responsiveness to thyroid hormone is established between days 5 and 15 of postnatal life, and it is maintained throughout adult life. The negative effect exerted by thyroid hormone on IGF-IR gene expression in lung and heart is in contrast to the reported effects of T_3 on IGF-I binding in other systems. In adult rats, it has been shown that T_3 increases IGF-I binding in the pituitary (37), and also, T_3 increases IGF-I binding in smooth muscle (38) and pituitary tumor cells in culture (39). This differential response indicates that the effect of thyroid hormone is tissue specific, which may be very important in relation to IGF-I regulation of specific functions. Another result that supports this conclusion is that we have not observed any effect of thyroidal state on IGF-I binding and IGF-IR mRNA in the cerebral cortex of developing rats and in the skeletal muscle of adult animals (data not shown).

Interestingly, T_3 acts by different mechanisms in lung and

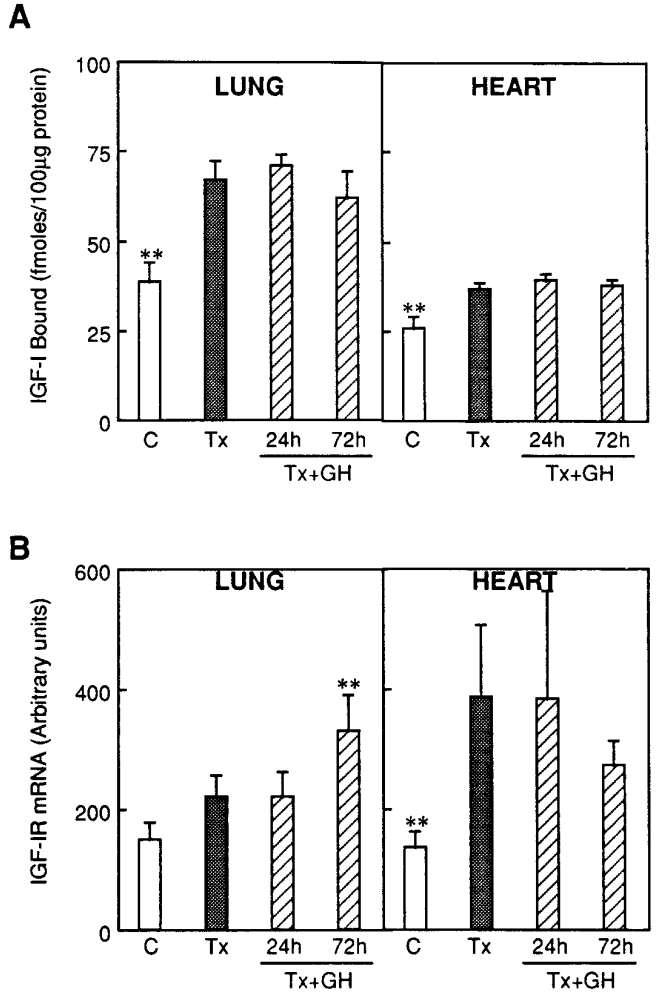

FIG. 8. Effect of GH administration on IGF-I binding (A) and IGF-IR mRNA content (B) in lung and heart of N-30 hypothyroid rats. Hypothyroid 30 day-old neonatal rats were injected daily sc with human GH (50 μg/100 g BW) and groups of animals were killed 24 and 72 h later. Membranes and total RNA were isolated from lung and heart and IGF-I binding and IGF-IR mRNA content determined, as indicated in *Materials and Methods*. C, Control; Tx, hypothyroid. Given values represent the average of four (C and Tx) or three (GH injected animals) different animals in (A) and four in (B). The *bars* represent SD. **, $P < 0.01$ *vs.* Tx.

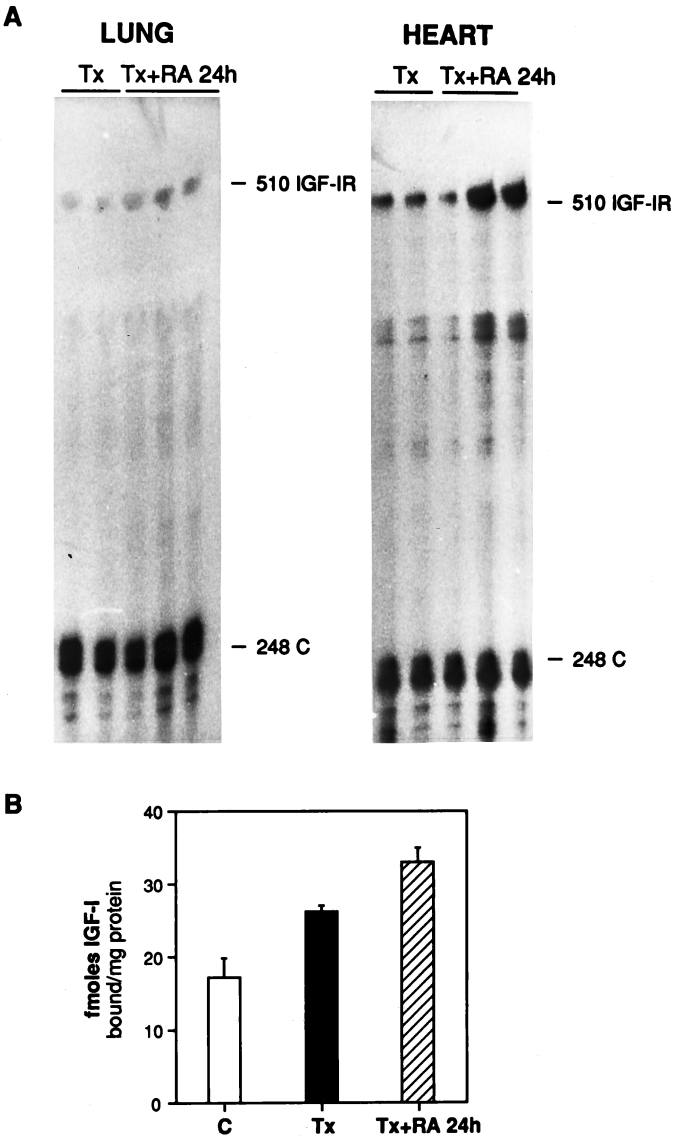

FIG. 9. Effect of RA administration to hypothyroid adult rats on IGF-IR mRNA content (A) and IGF-I binding (B). A, A representative RNase protection assay; B, binding values for at least three different lung membrane samples. Adult hypothyroid animals were intraperitoneally injected with RA (100 μg/100 g BW) and killed 24 h later. Total RNA was isolated from lung and heart and IGF-IR mRNA content determined. Lung membranes were obtained and IGF-I binding measured, as indicated in *Materials and Methods*. Tx, hypothyroid. The *bars* represent SD.

heart. In heart, IGF-IR mRNA and IGF-I binding change in parallel, both in steady-state and after hormone administration, suggesting a control of this gene at a pretranslational level. In contrast, in the lung, no significant differences were observed in IGF-IR mRNA content, in spite of the differences observed in IGF-I binding, suggesting an effect of thyroid hormone on translational efficiency or half-life of the protein. In the literature, both levels of control have been reported for IGF-IR gene. Proportional changes in mRNA levels and binding have been shown in diverse rat tissues during fasting (40) and in cells in culture after the administration of progestins (41). Regulation at a translational or posttranlational level has been shown also in mesangial cells from normal and diabetic mice (42). The structure of IGF-I receptor mRNA could provide the molecular basis for the control of this gene at the translational level. Rat IGF-IR mRNA has a long 5′-untranslated region, a G/C rich sequence with an open reading frame of 84 nucleotides (43), that has been implicated in the regulation of translation (44). Unusually long 5′-untranslated regions have been observed in other genes regulated at the level of translation (45). In fact, thyroid hormone also has been shown to control gene expression at a translational and/or posttranslational level (46). Although the mechanisms for T_3 control at these two levels are not well established, the mechanism is probably indirect and implies the existence of previous control by T_3 of other genes at a transcriptional level.

Although it has been shown previously that IGF-IR could

be down-regulated by GH in cells in culture (31) and in female rats (32), our results indicate that GH does not mediate the effect of T_3 on IGF-IR gene expression in lung and heart, because its administration to hypothyroid N-30 did not modify IGF-I binding. Another mediator of T_3 effect could be the IGF-I peptide, because the circulating levels and tissue concentrations of IGF-I are low in hypothyroid animals (14). Also, IGF-I has been shown to down-regulate its own receptors (47). Consequently, the low levels of IGF-I could cause the increased IGF-I binding and IGF-IR mRNA observed in hypothyroid animals. This possibility, however, seems unlikely because after hormone administration, the normalization of the number of receptors and mRNA is very rapid, especially in the heart. Also, no increase in IGF-I receptors has been reported in the lung and heart of hypophysectomized rats in spite of the marked reduction in serum and tissue levels of IGF-I (48). In other tissues, like spleen, hypophysectomy has been reported not to alter IGF-IR mRNA levels (49), and contradictory results have been reported in kidney (50, 51). On the other hand, because thyroid hormone receptors are present in the heart and lungs of adults and developing rats (52), an effect of T_3 at the cellular level in these tissues seems to be the most probable explanation.

RA receptor is a close relative of thyroid hormone receptor, and both share the regulation of multiple genes (33). We also have shown in this work that in adult animals, RA increases IGF-I binding and IGF-IR mRNA, both effects being quantitatively larger in the heart compared with the lung. The effect of RA is not dependent on the thyroidal state of the animal, because this effect was observed also in control rats, and it is exerted at a pretranslational level in both tissues studied. This action of RA has not been shown previously, although there were some indications that vitamin A and its derivatives could exert some effects on IGFs and IGFBPs (53, 54).

In summary, we have showed a tissue-specific increase in IGF-IR gene expression in the lung and heart in animals deprived of T_3, animals in which the IGFs/IGF-IR system is profoundly depressed. These results suggest a mechanism by which the negative consequences of hypothyroidism can be mitigated in organs as important as the heart and lung.

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