Positive and Negative Regulation of Retinoid X Receptor Gene Expression by Thyroid Hormone in the Rat

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CONTROLS BY THYROID HORMONE*

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The 9-cis-retinoic acid receptors (RXRs), belonging to the members of the steroid/thyroid hormone receptor superfamily, act as auxiliary proteins, heterodimerizing with other nuclear receptors such as retinoic acid receptors (RARs), vitamin D receptor, thyroid hormone receptors, and peroxisome-proliferator activated receptor, thereby transactivating target genes in a ligand-dependent manner. We have previously reported that in the rat, thyroid hormone (TH) positively and negatively regulates the hepatic mRNA levels of RXRβ and RXRγ, respectively. In the present study, we have tried to elucidate the level at which TH regulates the gene expression of RXRβ and RXRγ in the rat. A RNA synthesis inhibitor (actinomycin D), but not a protein synthesis inhibitor (cycloheximide), blocked the induction of RXRβ mRNA by TH. On the other hand, none of these drugs inhibited the decrease of RXRγ mRNA levels caused by TH. Nuclear run-on assays showed that the transcription rate of the RXRγ gene was positively regulated by TH, whereas the transcription of RXRγ gene was not controlled by TH. Taken together, these results indicate that the gene expression of RXRβ is positively regulated by TH at transcriptional level, while the negative regulation of the RXRγ gene expression by TH may occur at a post-transcriptional level in intact rat. Thus, the RXR-mediated signal transductions may be modulated in part through TH control of the levels of RXRβ and RXRγ.

The nuclear retinoid receptors belong to a superfamily of nuclear receptors, which are ligand-dependent transcription factors (1–4). The nuclear retinoid receptors consist of two classes. One class consists of the three retinoic acid receptors (RARα, β, and γ), which bind both all-trans-retinoic acid and 9-cis-retinoic acid with high affinity (5, 6). The other class consists of the three 9-cis-retinoic acid receptors (RXRα, β, and γ) (6–10). The RAR and RXR homodimers and RAR-RXR heterodimers recognize their cognate response elements, i.e., retinoic acid and retinoid X response elements, in the promoters of the target genes, resulting in ligand-dependent transcriptional activation (11). Moreover, it has recently been shown that vitamin D receptor, thyroid hormone receptor (TR), and peroxisome-proliferator activated receptor, to bind to their respective target DNA sequences efficiently, thereby activating transcription in a ligand-dependent manner (12, 13). Therefore, RXR is supposed to affect retinoid, vitamin D, thyroid hormone, and peroxisome-proliferator signaling.

Since changing levels of nuclear receptor protein can drastically affect the signaling of its cognate ligand, the study of the regulation of nuclear receptor genes is very important for understanding the signal transduction of that ligand. In our previous report, we had shown that TH (but not retinoid or vitamin D) regulates positively and negatively the mRNA levels of two RXRs (β and γ) out of the three RXRs in the rat liver (14). In the present study, we examined whether such regulation of RXR genes by TH might be due to the altered rates of transcription or to the control of the half-life of mRNA. Nuclear run-on assay with isolated hepatic nuclei and drug study using protein synthesis and RNA synthesis inhibitors indicated that TH may regulate the transcription of RXRβ gene, whereas the negative effect of TH on RXRγ gene expression may occur at a post-transcriptional level. These data imply that TH may control its own receptor signaling, as well as those of retinoid, vitamin D, and peroxisome-proliferator in part through the regulation of RXRβ and RXRγ gene expression.

MATERIALS AND METHODS

Animals—Wistar rats (3 weeks old) were fed on a 6-propyl-2-thiouracil (PTU)-containing diet for 30 days to induce goiters indicating hypothyroidism, and control rats were fed on the AIN-76 diet. Hypothyroidism was judged by the thyroid grand weight (more than 5-fold increase). The average body weights for hypothyroid rats was not significantly different from that for non-treated rats (15). For replenishment, these rats were intraperitoneally administered 100 or 500 μg of 3,5,3′-triiodothyronine (T3), and they were decapitated at the indicated times.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated from the various tissues of rats by the acid guanidinium thiocyanate-phenol chloroform method (16). Total RNA (40 or 80 μg) was fractionated on a 1.1% formaldehyde, 1% agarose gel. The RNA was transferred to a nitrocellulose filter (Schleicher & Schuell, BAS 85) by capillary blotting in 20 × SSC (1 × SSC: 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7). The filters were baked for 2 h at 80 °C and prehybridized at 42 °C in 50% formamide, 5 × SSPE (1 × SSPE: 0.1 M sodium chloride, 0.03 M NaH₂PO₄, 1 mM EDTA, pH 7), 5 × Denhardt’s solution (1 × Denhardt’s solution: 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.02% Ficoll 400), 1 mg/ml salmon sperm DNA, and 0.1% SDS for 4 h. Then the filters were hybridized at 42 °C for 18 h in 50% formamide, 5 × SSPE, 1 × Denhardt’s solution, 0.2 mg/ml salmon sperm DNA, and 1 × 10⁶ cpm/ml specific probe. The filters were washed at room temperature for 15 min in 2 × SSPE, 0.03% NaPP₃, 0.1% SDS, then at 65 °C for 15 min in 1 × SSPE, 0.03% NaPP₃, 0.1% SDS, then in 1 × 0.1×

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1 The abbreviations used are: RAR, retinoic acid receptor; RXR, retinoïd X receptor/9-cis-retinoic acid receptor; TR, thyroid hormone receptor; CHX, cycloheximide; Act D, actinomycin D; TH, thyroid hormone; T₃, 3,5,3′-triiodothyronine; PTU, 6-propyl-2-thiouracil.
SSPE, 0.03% NaPP₀, 1% SDS. The filters were exposed to x-ray film at –80 °C for 5 or 10 days with intensifying screens. The filters were dehydrated at 90 °C for 15 min in 0.1 x SSPE, 0.1% SDS, and rehydrated with another specific probe (5). The cDNA probes were labeled with [³²P]dCTP by the random priming method. The full-length cDNAs of rat RXRa (7) and mouse RXRβ (17) were cloned by the reverse transcription polymerase chain reaction method and verified by sequencing (10). The mouse RXRγ cDNA (12) was a gift from Prof. P. Chambon. Rat β-actin cDNA was used as an internal control to normalize RNA content (15).

All animal experiments using more than three rats per group were repeated at least two times. To obtain quantitative data on RXR transcripts, we used densitometric analysis of autoradiograms. The relative abundance of RXR mRNAs is shown as the mean ± S.D. for at least three samples from each different rat after normalizing with the β-actin mRNA levels.

Nuclei Isolation and Nuclear Run-on Assay—Rat liver nuclei were prepared from the rats as previously described (19). Briefly, 0.3 g of liver was minced and homogenized in 10 volumes (v/w) of ice-cold buffer A (15 mM Tris-HCl, pH 7.5, 15 mM KCl, 15 mM 2-mercaptoethanol, 2 mM EDTA, 0.5 mM EGTA, 0.15 mM spermine, 0.5 mM spermidine, 1.9 M sucrose, 0.1% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride) by a glass/Teflon homogenizer. The homogenates were filtered through nylon gauze to remove debris and then diluted by the addition of an equal volume of buffer B (buffer A without Triton X-100). The sample was centrifuged for 90 min at 90,000 × g. The supernatant was poured off, and the pellet was resuspended, homogenized in 200 µl of buffer C (15 mM Tris-HCl, pH 7.5, 15 mM NaCl, 60 mM KCl, 15 mM 2-mercaptoethanol, 0.15 mM spermine, 0.5 mM spermidine, 0.34 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride), and centrifuged for 5 min at 3,000 × g. The pellet was resuspended at concentration of 1 mg/ml DNA in buffer C and was used as liver nuclei for run-on assay (20). The 20 µl of liver nuclei was added in 40 µl of run-on buffer (80 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mg/ml heparin, 0.6% Sarkosyl, 350 mM (NH₄)₂SO₄, 400 µM ATP, 400 µM GTP, 400 µM UTP, 50 µCi of [³²P]CTP (800 µCi/ml, ICN), and incubated at 37 °C for 30 min. 1 µl of 10 µg/ml tRNA, 175 µl of TC buffer (0.02 mM Tris-HCl, 0.01 M CaCl₂), and 25 µl of protease-DNase buffer (1 mg/ml proteinase K, 1 mg/ml DNase I) were then added to reaction mixture. After incubation at 37 °C for 30 min, 25 µl of 10% SDS and 25 µl of 0.2 M EDTA were added to the each sample. After incubation of at 37 °C for 20 min, the labeled RNA was phenol/chloroform-extracted, precipitated in 10% trichloroacetic acid, and resuspended with SET buffer (10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 1% SDS). Equal amounts of radioactivity (1 × 10⁹ cpm) of the labeled RNA were hybridized to nylon filter (DuPont NEN) containing 20 µg of RXR cDNA and pSG5, and 5 µg of actin cDNA at 42 °C in 50% formamide, 0.7 M NaCl, 0.04 M NaPO₄, 0.2 µl Dextran’s, 0.2 µg EDTA, 0.1 mg/ml salmon sperm DNA, 0.1% SDS. After hybridization, filters were rinsed in 2 x SSC,
Thyroid Hormone Regulates the Expression of RXRβ and RXRγ Genes—In our previous study, we have shown that TH affects the transcript levels of RXRβ and RXRγ in the adult rat liver (14). To confirm these observations, we first examined the effect of TH on the expression of RXR genes in other tissues. As shown in Fig. 1 (A and B), the levels of RXRβ mRNAs clearly decreased in heart and brain of rats fed a PTU-containing diet, and the intraperitoneal administration of T3 (100 µg/rat) to the PTU-treated rats rapidly (within 6 h) restored the levels of RXRβ mRNAs, as observed in liver (14). Moreover such positive regulation was confirmed by the induction of the RXRβ gene with excess doses of T3 (500 µg/rat) to normal rats (Fig. 1, C and D). In contrast, the transcript levels of RXRγ increased in the tissues examined of rats fed on a PTU-containing diet, and remarkably, decreased upon replenishment of T3 in the PTU-treated rats and the normal rats given T3 (Fig. 1, A–D). These actions of TH on RXR gene expression were observed also in all tissues examined (data not shown). These results support our previous hypothesis that the gene expression of RXRβ and RXRγ are positively and negatively regulated by TH. Throughout these experiments, no alteration of the transcript levels of RXRα by TH was observed in any tissues examined.

We next examined by a time-course study to speculate whether the regulation of RXRβ and RXRγ genes by TH occurs in a direct or indirect manner. Normal rats were administrated 500 µg of T3 and killed at indicated times. Fig. 2 shows that the brain mRNA levels of RXRβ were clearly increased (2-3-fold) 3 h after TH administration, but this induction was reduced quickly. On the other hand, the transcript levels of RXRγ were gradually decreased 6-24 h after TH administration. Similar patterns were also observed in the other tissues of the PTU-treated rats given T3 (100 µg/rat) (data not shown). These results suggest that TH may directly modulate the expression of RXRβ at level of transcription, while TH may repress the transcription of the RXRγ gene and/or destabilize its mRNA.

Effect of Inhibitors on Thyroid Hormone-modulated RXR Gene Expression—To elucidate the level at which TH modulates the expression of RXRβ and RXRγ genes, the inhibitors of transcription (actinomycin D) and translation (cycloheximide) were used in rats (Fig. 3). Either actinomycin D (Act D; 0.15 mg/rat) or cycloheximide (CHX; 2 mg/rat) was injected, and after 1 h T3 (500 µg/rat) was administrated. After 4 h of T3 administration, the mRNA levels of RXRs were estimated. Act D abolished the TH-induced increase in the RXRβ mRNA levels, whereas the protein synthesis inhibitor CHX did not, indicating that the induction of the RXRβ gene by TH may result from an enhanced rate of transcription. In contrast, Act D and CHX did not affect the decrease in RXRγ mRNA levels by TH, implying that TH may control RXRγ mRNA stability (21-23).

DISCUSSION

We had previously shown that, in the liver of rats treated with PTU, administration of TH enhanced the mRNA levels of RXRβ but reduced those of RXRγ without affecting RXRα gene expression (14). In the present study, the positive and negative effects of TH on the RXR gene expression could be also confirmed in various tissues (Fig. 1). Furthermore, we investigated a possible mechanism of gene regulation of RXRβ and RXRγ by TH. Nuclear run-on assay using the isolated liver nuclei revealed that TH directly controls the transcription rate
of the RXRβ gene, strongly suggesting that the positive control of RXRα gene expression by TH may be under transcriptional control. This hypothesis was also supported by the drug study, where the induction of RXRα mRNA by TH was inhibited by actinomycin D, but not cycloheximide. In contrast, it is most likely that the negative regulation of RXRγ gene by TH occurs at a post-transcriptional level such as control of the half-life of mRNA, because transcription of the RXRγ gene was not affected by TH in the nuclear run-on analysis and drug study. As it is well described that TH regulates the expression of target genes at transcriptional and post-transcriptional levels (21–23), the molecular mechanism of the hormonal regulation of RXRα and RXRγ genes by TH may be classified into the well characterized mechanisms already described in the other target genes.

RXRs play critical roles in the signal transductions for retinoid, vitamin D, thyroid hormone, and peroxisome-proliferator, since they serve as auxiliary proteins heterodimerizing with the nuclear receptors of those ligands (12, 13). In addition to various combinations of RXRs with those receptors upon sequence-specific DNA binding, the binding of 9-cis-retinoic acid and the ligand on the receptor dimers is involved in the positive and negative controls of receptor-mediated transactivation. Recent studies have demonstrated that the ligand (9-cis-retinoic acid) for RXRα positively and negatively modulates ligand-induced transactivation mediated with the receptors for TH and vitamin D in a response element-specific manner (24–28). Although such ligand-induced regulations on receptor-mediated transactivation have been studied extensively in the transient expression assay, the effects of ligands on the gene expression of nuclear receptors themselves have not been fully investigated. The present study clearly shows that the gene expression of RXRβ and RXRγ is regulated by TH through distinct levels, indicating that TR-RXR-mediated signal transductions may be controlled in a thyroid hormone response element-specific manner not only by TH-induced transactivation through TR-RXR heterodimer but also by the altered levels of RXRs by TH. Moreover, these results imply a novel action of TH that the RXR-mediated signal transductions for retinoid, vitamin D, and peroxisome-proliferator may be modulated through the altered levels of RXRβ and RXRγ by TH.

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