Interleukin-1 β modulates endogenous thyroid hormone receptor α gene transcription in liver cells

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Abstract

One of the main characteristics of nonthyroidal illness (NTI) is a decrease in serum tri-iodothyronine, partly caused by a decrease in liver deiodinase type 1 (D1) mRNA and activity. Proinflammatory cytokines have been associated with NTI in view of their capability to decrease D1 and thyroid hormone receptor (TR) β 1 mRNA expression in hepatoma cells. Proinflammatory cytokine induction leads to activation of the inflammatory pathways nuclear factor (NF) κ B and activator protein (AP)-1. The proinflammatory cytokine interleukin (IL)-1 β decreases thyroid hormone receptor (TR) β 1 mRNA in an NF κ B-dependent way. The aim of this study was to unravel the effects of IL-1 β on endogenous *TR α* gene expression in an animal model and in a liver cell line. The *TR α* gene product is alternatively spliced in TR α 1 and TR α 2, TR α 2 is capable of inhibiting TR α 1-induced gene transcription. We showed that both TR α 1 and TR α 2

mRNA decreased not only after lipopolysaccharide administration in liver of mice, but also after IL-1 β stimulation of hepatoma cells (HepG2). Using the NF κ B inhibitor sulfasalazine and the AP-1 inhibitor SP600125, it became clear that the IL-1 β -induced decrease in TR α mRNA expression in HepG2 cells can only be abolished by simultaneous inhibition of NF κ B and AP-1. The IL-1 β -induced TR α 1 and TR α 2 mRNA decrease in HepG2 cells is the result of decreased *TR α* gene promoter activity, as evident from actinomycin D experiments. Cycloheximide experiments showed that the decreased promoter activity is independent of *de novo* protein synthesis and therefore most likely due to posttranslational modifications such as phosphorylation or subcellular relocalization.

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Introduction

Increased serum levels of proinflammatory cytokines have been associated with nonthyroidal illness (NTI; Boelen *et al.* 1993), which is a state of altered thyroid hormone regulation and metabolism during illness. NTI, among others, is characterized by low serum T₃ levels and decreased liver deiodinase type 1 (D1) activity (Peeters *et al.* 2003, Wiersinga 2005). It has been shown that proinflammatory cytokines are capable of decreasing liver D1 mRNA expression and activity (Yu & Koenig 2000, Jakobs *et al.* 2002), via the activation of nuclear factor (NF) κ B and activator protein (AP)-1 (Kwakkel *et al.* 2006). Liver D1 is involved in the peripheral conversion of thyroxine into T₃ and decreased activity of liver D1 therefore contributes to low serum T₃ levels. TR β 1, one of the thyroid hormone receptors through which T₃ exerts its actions in the liver, is also downregulated in an animal model of NTI. This suggests that impaired T₃ action is not only due to the low serum T₃ levels, but also because of lower TR expression in tissues (Boelen *et al.* 2004b), which could result in the downregulation of TR β 1 target genes as was described by Beigneux *et al.* (2003). Furthermore, it has been shown

that T₃-binding capacity in hepatoma cells is decreased by proinflammatory cytokines, interleukin (IL)-1 β being the most potent (Wolf *et al.* 1994). Recently we have demonstrated that adding IL-1 β to the human hepatoma cell line (HepG2) also results in a decrease of TR β 1 mRNA. Using specific inhibitors of the IL-1 β signal transduction pathways NF- κ B and AP-1, the IL-1 β -induced TR β 1 mRNA decrease in HepG2 cells appeared to be mediated via NF κ B (Kwakkel *et al.* 2006). Another important TR through which T₃ exerts its actions is the TR α 1. Although it is commonly thought that thyroid hormone action in the liver is mainly dependent on TR β 1, recent papers showed that TR α 1 also plays an important role in T₃ action in the liver. Amma *et al.* (2001) have shown that 30% of liver D1 mRNA expression is dependent on TR α 1. Furthermore, using a cDNA microarray, it has been shown that 40% of the T₃-regulated genes in the liver was independent of TR β 1, indicating a more prominent role for TR α 1 (Flores-Morales *et al.* 2002). This is supported by the observation that the zonal expression patterns of TR α 1 and TR β 1 in liver are different (Zandieh-Doulabi *et al.* 2003). The *TR α* gene gives rise to two major isoforms which share the same promoter

due to alternative splicing: TR α 1 and TR α 2. The TR α 1 isoform is a bona fide TR, which has a ligand-binding domain and a DNA-binding domain and modulates gene transcription. The TR α 2 isoform, however, does not have a ligand-binding domain and is not able to activate gene transcription. It has been shown that TR α 2 has a weak-inhibitory effect on the other TR isoforms (Koenig *et al.* 1989, Liu *et al.* 1995, Yen 2001). Recent experiments with knockout mice have shown that absence of both TR α 's or TR α 2 alone results in a higher T₃ sensitivity and it has been hypothesized that this is due to the inhibitory effect of TR α 2 that is missing in these knockout mice. In the case of the TR α 2^{-/-}, upregulation of TR α 1 could be an alternative explanation (Macchia *et al.* 2001, Salto *et al.* 2001). Nevertheless, changed tissue responsiveness to T₃ due to a difference in TR α 1 and TR α 2 expression might be an important regulating mechanism during NTI.

The aim of the present study is first to evaluate TR α 1 and TR α 2 mRNA expression in the liver of mice which received a sublethal dose of bacterial endotoxin lipopolysaccharide (LPS). LPS activates NF κ B and AP-1 and results in a rapid IL-1 β , IL-6, IL-12, and interferon (IFN) γ response in the liver (Boelen *et al.* 2004a, Palsson-McDermott & O'Neill 2004). Secondly, we studied the effects of one specific proinflammatory cytokine (IL-1 β) on the endogenous expression of TR α 1 and TR α 2 mRNA in a liver cell line and investigated the pathways and mechanisms involved. To this end, we used specific inhibitors of NF κ B and AP-1 and inhibitors of promoter activity and protein synthesis.

Materials and Methods

Animal experiment

Female Balb/c mice (Sprague-Dawley; Harlan, Horst, The Netherlands) were used at 6–12 weeks of age. The mice were kept in 12 h light:12 h darkness cycle in a temperature-controlled room.

Acute illness was induced by an i.p. injection of 150 μ g LPS (endotoxin, *Escherichia coli* 127:B8; Sigma) diluted in 0.5 ml saline. Control mice received 0.5 ml saline. Due to diurnal variations of TR α mRNA (Zandieh-Doulabi *et al.* 2004), each time point needs its own control and the experiment started at 0900 h. At time points 0, 1, 2, 3, 4, 6, and 24 h after LPS or saline administration injection, five mice per group (three mice per group at 24 h after LPS) were anesthetized with isoflurane and killed by cervical dislocation. The liver was obtained and immediately stored in liquid nitrogen. The study was approved by the local animal welfare committee.

Cell cultures

The human HepG2 (ATCC, Rockville, MD, USA) was cultured in Eagle's minimal essential medium (EMEM), supplemented with 10 U/ml penicillin, streptomycin,

fungizone, and 5% fetal calf serum (all from Cambrex, East Rutherford, NJ, USA). For the actinomycin D, sulfasalazine, SP600125, and cycloheximide experiments, 10% fetal calf serum was used. For the RNA-expression time-course experiments, 5 $\times$ 10⁴ cells per well were grown for 24 h in 24-well plates. For the actinomycin D, sulfasalazine, SP600125, and cycloheximide experiments, 1 $\times$ 10⁵ cells per well were grown for 24 h in a 24-well plates. Cells were stimulated with 10 ng/ml IL-1 β (Sigma) dissolved in PBS with 0.5% (w/v) BSA (PBS/BSA). PBS/BSA was added in the same amount in the negative control. For the inhibition of AP-1, 50 μ M SP600125 (Calbiochem, Darmstadt, Germany) were used. SP600125 is a specific inhibitor of the c-jun N-terminal kinase, which is an activator of the AP-1 pathway (Bennett *et al.* 2001). Sulfasalazine (Sigma) was used as an NF κ B inhibitor, which inhibits the NF κ B-inhibitory protein kinase (IKK), needed for NF κ B activation (Weber *et al.* 2000) and added in the concentration of 3 mM. Cycloheximide was added to inhibit protein synthesis at a concentration of 10 μ g/ml. Actinomycin D was added to inhibit promoter activity at a concentration of 5 μ g/ml. All inhibitors were dissolved in DMSO (Sigma). Final DMSO vehicle concentrations were used as controls, 1.2% (sulfasalazine control), 0.5% (SP600125 and actinomycin D controls), and 0.01% (cycloheximide control). We have previously shown that 3 mM sulfasalazine inhibits phosphorylation of I κ B α and 50 μ M SP600125 inhibits c-jun N-terminal kinase phosphorylation and therefore these concentrations were used in our experiments (Kwakkel *et al.* 2006). Cells were pre-incubated for 30 min with actinomycin D, cycloheximide, SP600125, sulfasalazine, or with both SP600125 and sulfasalazine. At reincubation, IL-1 β was added without washing the cells.

RNA isolation and RT-PCR

Liver mRNA was isolated using the Magna Pure LC mRNA Tissue kit, using 10 mg liver tissue. HepG2 cells were washed with PBS and subsequently lysed in 200 μ l lysis buffer with the Magna Pure LC RNA Isolation kit – High Performance (Roche Molecular Biochemicals). Liver mRNA and HepG2 RNA were isolated with the Magna Pure kit (Roche Molecular Biochemicals) using the protocol and buffers supplied with the corresponding kit. RNA amounts were measured using the Nanodrop (Wilmington, DE, USA) to be able to perform cDNA synthesis with equal RNA input for the HepG2 experiments. cDNA synthesis was performed using the First-Strand cDNA Synthesis kit for RT-PCR with oligo d(T) primers (Roche Molecular Biochemicals). Real-time PCR was performed using the Lightcycler (Roche Molecular Biochemicals). For all PCRs, Lightcycler DNA Master SYBR Green I kit (Roche Molecular Biochemicals) was used, adding 3 mM MgCl₂ (2 mM for TR α 2) and 50 ng (100 ng for TR α 2) primers (Biolegio, Nijmegen, The Netherlands) each. Primer pairs for TR α 1, TR α 2,

mouse hypoxanthine phosphoribosyl transferase (HPRT), and human HPRT were previously described (Bakker 2001, Sweet *et al.* 2001, Liu *et al.* 2003). PCR programs were as follows: denaturation 30 s at 95 °C, 40–45 cycles of 0–5 s at 95 °C, 10-s annealing temperature, 15–20 s at 72 °C. Annealing temperatures were 54 °C for mHPRT, 60 °C for hHPRT, 64 °C for TR α 1, and 66 °C for TR α 2. For quantification, a standard curve was generated of a sequence-specific PCR product ranging from 0.01 to 100 fg/ μ l. Samples were corrected for their mRNA content using HPRT as a housekeeping gene. Samples were individually checked for their PCR efficiency (Ramakers *et al.* 2003). The median of the efficiency was calculated for each assay, samples that had a greater difference than 0.05 of the efficiency median value, were not taken into account (0–5%).

Preparation of liver nuclear extracts and western blotting

Liver tissue was homogenized using a glass–teflon homogenizer in 4 vol cytosolic lysis buffer (CLB: 10 mM HEPES (pH 7.5), 1.5 mM MgCl₂, 10 mM KCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM Na₃VO₄, 2 mM NaF, 1 mM dithiothreitol, and protease inhibitor cocktail (Roche Molecular Biochemicals)). After a centrifugation step (5 min, 1500 g, 4 °C), the nuclear pellet was washed with CLB and lysed with 0.8 ml nuclear lysis buffer (50 mM Tris–HCl (pH 7.2), 140 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 1 mM PMSF, 2 mM Na₃VO₄, 2 mM NaF, protease inhibitor cocktail (Roche Molecular Biochemicals)). Following incubation for 15–30 min on ice, nuclear extracts were centrifuged (10 min, 10 000 g, 4 °C), SN was used as nuclear extract. Protein content was measured and 50 μ g was loaded on a 10% SDS–PAGE gel. Gels were blotted on Immobilon-P transfer membrane (Millipore, Bedford, MA, USA). Blots were blocked with 5% casein in PBS/0.01% Tween, for 1 h at room temperature (RT). Primary antibodies (PA1-211 (TR α 1) and PA1-216 (TR α 2; Affinity Bioreagents, Golden, CO, USA) diluted in blocking buffer 1:200 and 1:50 respectively) were incubated for 1 h at RT followed by an overnight incubation at 4 °C. Blots were washed three times for 5 min with PBS/T (Tween). Following 1-h incubation at RT with secondary antibody goat-anti-rabbit-HRP (1:10 000 diluted in blocking buffer; DAKO Cytomation, Glostrup, Denmark), blots were washed again and detected with Lumi-Light^{plus} chemiluminescent substrate (Roche Molecular Biochemicals). The emitted light was visualized and quantified on the Lumi-Imager (Roche Molecular Biochemicals).

Statistical analysis

Nonparametric Mann–Whitney *U*-tests (SPSS, Chicago, IL, USA) were performed to test statistical significance between groups. For time-course experiments, statistical significance was tested by two-way ANOVA (Excel Microsoft).

Results

Liver TR α mRNA and protein expression decreases after cytokine induction

In vivo LPS administration in mice resulted in decreased TR α 1 mRNA levels within 2 h which remained significantly lower than controls during the time course of the experiment (ANOVA, $P < 0.01$). TR α 2 mRNA levels, however, decreased slower and only resulted in a significant decrease at 6 h after LPS administration ($P < 0.05$). The observed mRNA decrease resulted in decreased TR α 1 and TR α 2 protein levels 24 h after LPS administration (Fig. 1A).

In vitro The effect of IL-1 β on endogenously expressed TR α 1 and TR α 2 mRNA in HepG2 cells resembles the effect of LPS on TR α mRNA expression in mice. TR α 1 mRNA was significantly decreased after 4 and 6 h IL-1 β (ANOVA, $P < 0.01$). TR α 2 mRNA was only significantly lower when compared with controls after 6-h IL-1 β stimulation ($P < 0.05$; Fig. 1B).

Involvement of NF κ B and AP-1 in IL-1 β -induced TR α mRNA decrease in HepG2 cells

NF κ B Inhibition of the NF κ B pathway by sulfasalazine had no effect on the IL-1 β -induced mRNA decrease of TR α 1 and TR α 2. Basal levels of TR α 1 mRNA were decreased by sulfasalazine alone, but adding IL-1 β to sulfasalazine-treated cells still resulted in a significant decrease of TR α 1 mRNA expression ($P < 0.05$; Fig. 2A).

AP-1 Inhibition of the AP-1 pathway by SP600125 did not have an effect on the IL-1 β -induced TR α 1 and TR α 2 mRNA decrease. SP600125 treatment significantly increased the basal levels of the TR α 2 mRNA. Adding IL-1 β to SP600125-treated cells, however, still resulted in a significant decrease of TR α 2 mRNA levels ($P < 0.01$; Fig. 2B).

NF κ B and AP-1 Inhibition of both NF κ B and AP-1 pathways simultaneously abolished the IL-1 β -induced TR α 1 and TR α 2 mRNA decrease (Fig. 2C).

For all experiments, DMSO alone was added as a vehicle control; this did not result in significant changes in TR α 1 or TR α 2 mRNA (data not shown).

Mechanism of IL-1 β -induced TR α mRNA decrease in HepG2 cells

To evaluate whether the TR α 1 and TR α 2 mRNA decrease was the result of increased mRNA degradation or decreased TR α promoter activity, preincubations with actinomycin D, an inhibitor of promoter activation, were performed. The IL-1 β -induced TR α 1 and TR α 2 mRNA decrease was abolished by pretreating cells with 5 μ g/ml actinomycin D (Fig. 3A), suggesting that the TR α 1 and TR α 2 mRNA decrease is the

Figure 1 (A) Relative expression of liver TR α 1 and TR α 2 mRNA after 0, 1, 2, 3, 4, and 6 h in saline (○) and LPS-treated mice (●). Mean values $\pm$ s.e.m. ($n=5$) are shown. Protein expression of liver TR α 1 (46 kDa) and TR α 2 (58 kDa) in nuclear extracts of control mice and mice 24 h after LPS treatment ($n=3$) was given. (B) Relative expression of TR α 1 and TR α 2 mRNA after 0, 2, 4, and 6 h in controls (○) and IL-1 β -treated HepG2 cells (●). Mean values $\pm$ s.e.m. ($n=6$) are shown. P values indicate differences between groups by ANOVA. Significances of separate time points were evaluated by Mann–Whitney U -tests, * $P \leq 0.05$, ** $P \leq 0.01$.

result of decreased promoter activity. After incubation with actinomycin D alone, TR α 1 mRNA decrease was more severe than the TR α 2 mRNA decrease when compared with the control. This indicates that TR α 1 mRNA degrades faster than TR α 2 mRNA.

To see whether the TR α 1 and TR α 2 mRNA decrease was a direct or indirect effect of IL-1 β , control and IL-1 β -treated cells were pretreated with 10 μ g/ml cycloheximide, an inhibitor of protein synthesis. After pretreatment with cycloheximide, IL-1 β stimulation still induced a decrease of TR α 1 and TR α 2 mRNA when compared with controls (Fig. 3B), indicating that the TR α 1 and TR α 2 mRNA decrease is induced by IL-1 β independently of *de novo* protein synthesis.

Discussion

Proinflammatory cytokines have differential effects on thyroid hormone metabolism (Wiersinga 2005) and are negatively related to serum T $_3$ levels in NTI patients (Boelen *et al.* 1993, 1995). In an animal model of NTI, decreased serum thyroid hormone levels were preceded by decreased TR β 1 mRNA levels (Boelen *et al.* 2004b), indicating altered T $_3$ responsiveness in the liver. In this study, we showed that besides TR β 1, liver TR α mRNA also decreased, TR α 1 faster when compared with TR α 2 mRNA, resulting in lower TR α 1 and TR α 2 protein levels 24 h after LPS administration. The difference in time course of the mRNA decrease might be of

Figure 2 Relative expression of TR α 1 and TR α 2 mRNA in HepG2 cells after 6-h incubation with medium and 10 ng/ml IL-1 β with or without 30-min pretreatment with (A) 3 mM sulfasalazine (Sulfa), (B) 50 μ M SP600125 (SP), and (C) 3 mM sulfasalazine and 50 μ M SP600125 simultaneous (Sulfa + SP). Mean values $\pm$ S.E.M. ($n=6$) are shown. P values indicate differences between groups evaluated by Mann–Whitney U -tests, * $P\leq 0.05$, ** $P\leq 0.01$.

Figure 3 Relative expression of TR α 1 and TR α 2 mRNA in HepG2 cells after 4-h incubation with medium and 10 ng/ml IL-1 β with or without 30-min preincubation with (A) 5 μ g/ml actinomycin D (ActD) and (B) 10 μ g/ml cycloheximide (Chx). Mean values $\pm$ S.E.M. ($n=6$) are shown. P values indicate differences between groups evaluated by Mann-Whitney U -tests, ** $P \leq 0.01$.

physiological relevance. NTI is thought to be an adaptive mechanism to downregulate thyroid hormone action during illness. Besides decreasing serum T₃ levels, this is also accomplished by decreasing the expression of the functional receptor TR α 1. The fact that TR α 2 is slightly later downregulated might result in a further downregulation of TR α 1 signaling due to the inhibitory properties of TR α 2 on TR-mediated transcription. Our results are in agreement with a study by Beigneux *et al.* (2003).

Our study is the first that tries to get insight into the pathways and mechanisms involved in the LPS-induced TR α mRNA decrease in mouse livers. To this end, we have used a human HepG2 which has endogenous TR α mRNA expression and stimulated these cells with IL-1 β . We preferred to use a cell-line expressing endogenous TR α 1

and TR α 2 mRNA, because we wanted to compare the *in vivo* and *in vitro* effects of cytokines on TR α gene expression. Transfection of the TR α gene does not give an adequate impression of the cellular situation because of the overexpression of one specific gene. Comparable with the *in vivo* situation after LPS administration, IL-1 β stimulation of HepG2 cells resulted in a rapid decrease of TR α 1 mRNA expression, whereas TR α 2 mRNA expression decreased more slowly. The IL-1 β -induced decrease of TR α 1 and TR α 2 in HepG2 cells was attenuated when compared with the LPS-induced changes *in vivo*. This is probably because LPS administration induces a number of cytokines (Boelen *et al.* 2004a) and therefore has more effect than IL-1 β alone. Studies from Jakobs *et al.* (2002) have shown that IL-1 β is the most potent cytokine to induce decreased D1 mRNA

expression in HepG2 cells *in vitro*; therefore, we used this cytokine to perform our *in vitro* studies. Actinomycin D experiments showed that the observed difference in time course of the TR α 1 and TR α 2 mRNA decrease is probably due to a difference in mRNA stability rather than a change in splicing direction. The difference in mRNA stability has been previously published (Lazar 1990).

In contrast to the IL-1 β -induced TR β 1 mRNA decrease, the IL-1 β -induced TR α mRNA decrease is not solely mediated via the NF κ B pathway. Only simultaneous inhibition of NF κ B and AP-1 abolished the TR α 1 and TR α 2 mRNA decrease in HepG2 cells, which is comparable with the IL-1 β -induced D1 mRNA decrease in HepG2 cells (Kwakkel *et al.* 2006).

An interesting observation, however, was that basal TR α 1 mRNA levels were decreased by the NF κ B inhibitor sulfasalazine, whereas the TR α 2 mRNA levels were increased by the AP-1 inhibitor SP600125. This suggests that NF κ B and AP-1 might play a role in basal TR α 1 and TR α 2 mRNA expression respectively, possibly via influencing TR α splicing. Besides mediating the inflammatory response, both the AP-1 and NF κ B pathways are known to be involved in the development, cell proliferation, and apoptosis (Baldwin 2001, Nishina *et al.* 2004, McDonald *et al.* 2006, Raivich & Behrens 2006). Thyroid hormone is also involved in these physiological processes (Su *et al.* 1999, O'Shea & Williams 2002).

Inhibiting promoter activity with actinomycin D abolished the IL-1 β -induced TR α 1 and TR α 2 mRNA decrease. From this experiment, it can be concluded that the TR α mRNA decrease is not due to an increase in mRNA degradation, but to decreased TR α gene promoter activity. Incubation with cycloheximide, a protein synthesis inhibitor, showed that the IL-1 β effect on the TR α promoter is a direct effect, independent of *de novo* protein synthesis. Posttranslational modifications such as phosphorylation or subcellular relocalization of proteins, thereby interfering with the TR α promoter, might play a role in the inhibitory effect of IL-1 β on TR α mRNA expression.

The TR α promoter is very GC-rich and has several binding sites for specificity protein (Sp)1, a ubiquitous transcriptional activator which binds a GC-rich sequence (Ishida *et al.* 1993). During activation of NF κ B and AP-1 by cytokines, multiple phosphorylation events take place (O'Neill 2000, Palsson-McDermott & O'Neill 2004). Although no direct relationship between NF κ B and AP-1 activation and Sp1 activity has been shown, it is known that posttranslational modifications such as phosphorylation are important regulatory mechanisms of Sp1 transcriptional activity (Chu & Ferro 2005) and thus possibly influence TR α promoter activity.

Other binding sites for transcription factors on the TR α promoter have been described, such as early growth-response (Egr)-1 site (also known as Krox-24; Laudet *et al.* 1993). Interestingly, Egr-1 is, like Sp1, a transcription factor that also binds a GC-rich sequence and is known to translocate to the nucleus within 1 h upon LPS stimulation in Kupffer cells

(Kishore *et al.* 2002) and osteoblasts after IL-1 β stimulation (Granet & Miossec 2004) and thereby possibly interfering with transcriptional activity of GC-rich promoters like TR α . Sp1-mediated gene activation can be downregulated by Egr-1 by displacing Sp1 from the promoter, as shown by transfection studies in HepG2 cells (Thottassery *et al.* 1999). Thus, besides the possible direct inhibitory effect on Sp1 by phosphorylation resulting in decreased TR α promoter activity, the Egr-1 translocation and interference with Sp1 transcriptional activation might be another potential mechanism for the IL-1 β -induced decrease of TR α promoter activity.

In contrast to the IL-1 β -mediated TR α decrease, the IL-1 β -induced decrease of TR β 1 mRNA is regulated via NF κ B alone. Transcriptional Element Search System analysis showed that the TR β 1 promoter, unlike the TR α promoter, does not have an Egr-1-binding site, suggesting different regulatory mechanisms of both TR promoters during inflammation (Schug & Overton 1998).

TR α mRNA-decreased promoter activity might also be due to competition for limiting amounts of coactivators-like steroid receptor coactivator (SRC)-1 and CREB-binding protein. These coactivators are also used by NF κ B and AP-1. This mechanism might be operative in the IL-1-induced decrease of D1 hepatocytes. Adding exogenous SRC-1 partially abolished the cytokine-induced D1 mRNA decrease *in vitro* (Yu & Koenig 2000). Recently this has been confirmed *in vivo* (Yu & Koenig 2006). Our study showed that the IL-1 β -induced effects on TR α mRNA expression were mediated via the same pathways as the IL-1 β -induced D1 mRNA decrease, namely via NF κ B and AP-1, indicating that this nonspecific mechanism might be involved in both the IL-1 β -induced D1 and TR α mRNA decrease.

In conclusion, our experiments indicate that IL-1 β decreases TR α 1 and TR α 2 mRNA both *in vivo* and *in vitro*. *In vitro*, the IL-1 β -induced TR α 1 and TR α 2 mRNA decrease in HepG2 cells is the result of decreased TR α promoter activity. This decreased promoter activity is independent of *de novo* protein synthesis and therefore most likely due to posttranslational modifications such as phosphorylation or translocation of proteins within the cell, which can only be abolished by simultaneous inhibition of the inflammatory pathways NF κ B and AP-1.

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