REGULAR ARTICLE

Changes in gene expression of DOR and other thyroid hormone receptors in rat liver during acute-phase response

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Abstract Non-thyroidal illness is characterized by low tri-iodothyronine (T3) serum level under acute-phase conditions. We studied hepatic gene expression of the newly identified thyroid hormone receptor (TR) cofactor DOR/TP53INP2 together with TRs in a rat model of aseptic abscesses induced by injecting intramuscular turpentine-oil into each hind limb. A fast (4-6 h) decrease in the serum level of free thyroxine and free T3 was observed. By immunohistology, abundant DOR protein expression was detected in the nuclei of hepatocytes and ED-1$^+$ (mononuclear phagocytes), CK-19$^+$ (biliary cells), and SMA$^+$ (mesenchymal cells of the portal tract) cells. DOR signal was reduced with a minimum at 6-12 h after the acute-phase reaction (APR). Immunohistology also showed a similar pattern of protein expression in TRα1 but without a significant change during APR. Transcripts specific for DOR, nuclear receptor co-repressor 1 (NCoR-1), and TRβ1 were down-regulated with a minimum at 6-12 h, whereas expression for TRα1 and TRα2 was slightly and significantly up-regulated, respectively, with a maximum at 24 h after APR was initiated. In cultured hepatocytes, acute-phase cytokines interleukin-1β (IL-1β) and IL-6 down-regulated DOR and TRβ1 at the mRNA level. Moreover, gene expression of DOR and TRs (TRα1, TRα2, and TRβ1) was up-regulated in hepatocytes by adding T3 to the culture medium; this up-regulation was almost completely blocked by treating the cells with IL-6. Thus, TRβ1, NCoR-1, and the recently identified DOR/TP53INP2 are abundantly expressed and down-regulated in liver cells during APR. Their down-regulation is attributable to the decreased serum level of thyroid hormones and most probably also to the direct action of the main acute-phase cytokines.

Abbreviations

APP	Acute-phase protein
APR	Acute-phase reaction
cDNA	Complementary deoxyribonucleic acid
IL-1ß	Interleukin 1 beta
IL-6	Interleukin 6
LPS	Lipopolysaccharide
NCoR-1	Nuclear receptor co-repressor 1
NTI	Non-thyroidal illness
PBS	Phosphate-buffered saline
SMA	Smooth muscle actin
T3	3,3',5-triiodothyronine
T4	Thyroxine
THs	Thyroid hormones
TR	Thyroid hormone receptor
TO	Turpentine oil

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Introduction

Acute-phase reaction (APR) is the response of the body to tissue injury. It is clinically characterized by fever, weakness, adynamia, somnolence, and loss of appetite. Leucocytosis, an increased erythrocyte sedimentation rate, dramatic changes in serum levels of acute-phase proteins, and a change in serum levels of several hormones are observed in the blood. In addition to the up-regulation of the serum level of corticosteroids, the concentration of thyroid hormones (THs) decreases (Bartalena et al. 1994). Metabolic changes during APR are attributable to the effects of interleukin (IL)-1-like beta (IL-1β), tumor necrosis factor-α, and IL-6-like cytokines (IL-6, oncostatin M, and others), through the activation of transcription factors, i.e., nuclear factor kappa B, activator protein 1, signal transducer and activator of transcription 3/5, or CCAAT/enhancer-binding protein β. These signaling cascades result in increased plasma levels of a number of positive acute-phase proteins (APPs), including clotting proteins, transport proteins, antiproteases, and complement factors, with a parallel decrease in negative APPs, such as albumin or transferrin (Ramadori and Christ 1999). Synthesis of the major APPs can be increased up to 1000-fold over normal levels under acute-phase conditions. This group includes serum amyloid A and either C-reactive protein in humans or its homolog in mice (Ramadori et al. 1985a) and alpha-2 macroglobulin in rat hepatocytes (Pierzchalski et al. 1992).

The state of altered TH metabolism observed under acute-phase conditions is called non-thyroidal illness (NTI), which is characterized by decreased serum TH levels (De Groot 1999). Indeed, during the acute-phase response induced by tissue injury, significant changes take place in the regulation of TH serum levels and in the molecules involved in signaling pathways. The mechanisms inducing the changes at various levels of synthesis and of THs (NTI) action during APR are still poorly defined. Although the possibility of inducing normal thyroxine (T4) and 3,3',5-triiodothyronine (T3) plasma levels in intensive-care patients through hormonal stimulation of the anterior pituitary gland has been shown (Van den Berghe et al. 1999), the changes observed during the acute phase might be part of the defense strategy of the body. Indeed, therapeutic administration of THs to intensive-care patients might improve metabolic parameters (Van den Berghe et al. 1999, 2002) but not the patient outcome (Becker et al. 1982; Brent and Hershman 1986).

T3, the biologically active form of TH, plays a central role in metabolic homeostasis, development, cell differentiation, and growth under normal metabolic conditions (Fowden 1995; Yen 2001). THs act at the cellular level through diverse receptors. The gene expression of receptors is known to be dependent on the hormone level. T3 regulates gene expression by interacting with isoforms of the TH receptor (TR) derived from two different genes, viz., TRα and TRβ (Brent et al. 1989a, 1989b). TRs are differentially distributed in various tissues and during developmental stages, indicating distinct and specific functional roles. TRα is mainly present in the central nervous system and muscle but is also found in the liver (Amma et al. 2001; Ercan-Fang et al. 1996). In liver, two isoforms of TRα are present: the canonical TRα1 and the alternatively spliced TRα2, which lacks the T3-binding domain and therefore acts as the dominant negative inhibitor of TRα1 and TRβ1 at higher concentrations (Laudet et al. 1991). The predominant active form of TR in a murine liver cell line is TRβ1 (Ventura-Holman et al. 2007), but another report also underlines the important role of TRα in the liver (Macchia et al. 2001). In addition, TRα2 has been shown to have an inhibitory effect on the other TR isoforms (Koenig et al. 1989). Recent experiments with knockout mice have demonstrated that absence of both TRαs or TRα 2 alone results in a higher T3 sensitivity; this has been hypothesized to be attributable to the inhibitory effect of TRα2, which is missing in these knockout mice (Macchia et al. 2001). Another TH co-repressor protein, called nuclear receptor co-repressor 1 (NCoR-1; Horlein et al. 1995), is known to promote chromatin condensation and prevents access to the transcription machinery in the gene transcription regulated by the TR and retinoic X receptor (Grignani et al. 1998; Horlein et al. 1995).

Recently, a novel component of TH action, DOR/TP53INP2, has been identified, which is repressed in diabetic rat muscle. DOR is localized within the nucleus and has been shown to be crucial for the transcriptional regulation of T3-controlled genes by direct interaction with TRα1 (Baumgartner et al. 2007).

In the present study, we have demonstrated that the TRs are down-regulated in the liver and liver cells in a rat model of APR. This may be attributable not only to a reduced TH serum level, but also to the direct effect of acute-phase mediators on the regulation of the TR gene expression in liver cells, possibly explaining the lack of clinical effectiveness of T3 administration under acute-phase conditions.

Materials and methods

Animals

Male Wistar rats of about 170-200 g body weight were purchased from Harlan-Winkelmann (Brochen, Germany).

Table 1 Rat primer sequences used (*TR* thyroid hormone receptor, *NCoR* nuclear receptor co-repressor, *DOR* TR co-factor, *UBC* ubiquitin C)

Primer	5′——3′ Forward	5′——3′ Reverse
TRα1	TGCCCTTACTCACCCCTACA	AAGCCAAGCCAAGCTGTCCT
TRα2	TGAGCAGCAGTTTGGTGAAG	GAATGGAGAATTCCGCTTCG
TRβ1	AGCCAGCCACAGCACAGTGA	CGCCAGAAGACTGAAGCTTGC
NCoR-1	AGTCGCTACAGCCCAGAGTC	CTCCTCTCTGGGGATTTTCC
DOR	AACCACAGCCTGCTTCTAATACCTT	TCAGCCAGTCTCAACACAAAACAC
β-Actin	ACCACCATGTACCCAGGCATT	CCACACAGAGTACTTGCGCTCA
UBC	CACCAAGAAGGTCAAACAGGAA	AAGACACCTCCCCATCAAACC

The rats were kept under standard conditions with 12 h light/dark cycles and *ad libitum* access to fresh water and food pellets. All animals were cared for according to the University's guidelines, the German convention for the protection of animals, and NIH guidelines.

Antibodies

A mouse monoclonal antibody directed against CK-19 (marker for biliary cells) was purchased from Novocastra (Newcastle upon Tyne, UK), a mouse monoclonal antibody directed against smooth muscle actin (SMA) from Sigma (Munich, Germany), a rabbit polyclonal anti-TRα1 from Abcam (Cambridge, UK), and a mouse anti-rat ED-1 (marker for mononuclear phagocytes) monoclonal antibody from Serotec (Duesseldorf, Germany). A rabbit polyclonal antibody directed against human DOR/TP53INP2 (a TR co-factor) was a gift from Prof. Antonio Zorzano (University of Barcelona, Spain). Detection by immunofluorescence was carried out as described previously (Malik et al. 2010).

Induction of APR

APR was induced in ether-anesthetized rats by intramuscular injection of 5 mg/kg turpentine oil (TO) into both the right and left hind limbs of the animals. Control animals were treated in the same way for each time-point with saline injection into both limbs. Animals were killed 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 60, and 72 h after TO injection under pentobarbital anesthesia. Livers were excised, rinsed with physiological sodium saline, snap-frozen in liquid nitrogen, and stored at -80°C until further use.

Measurement of free thyroxine and free tri-iodothyronine levels

At time-points ranging from 1-24 h after TO injection, blood samples from the inferior vena cava were collected from controls and TO-injected rats and used for the detection of free thyroxine (FT4) and free tri-iodothyronine (FT3) levels in the serum of rats by using a standard protocol (University Medical Center, Göttingen).

Immunofluorescent staining

Double-immunofluorescence was performed according to a protocol described previously (Malik et al. 2010). Briefly, cryostat sections (~5 μm thick) were fixed in acetone for 10 min. Afterwards, the rabbit polyclonal primary antibody against DOR and a mouse monoclonal anti-CK-19, a rabbit monoclonal anti-α-SMA, and a mouse monoclonal anti-ED-1 primary antibody (1:50) were incubated with the sections overnight at 4°C.
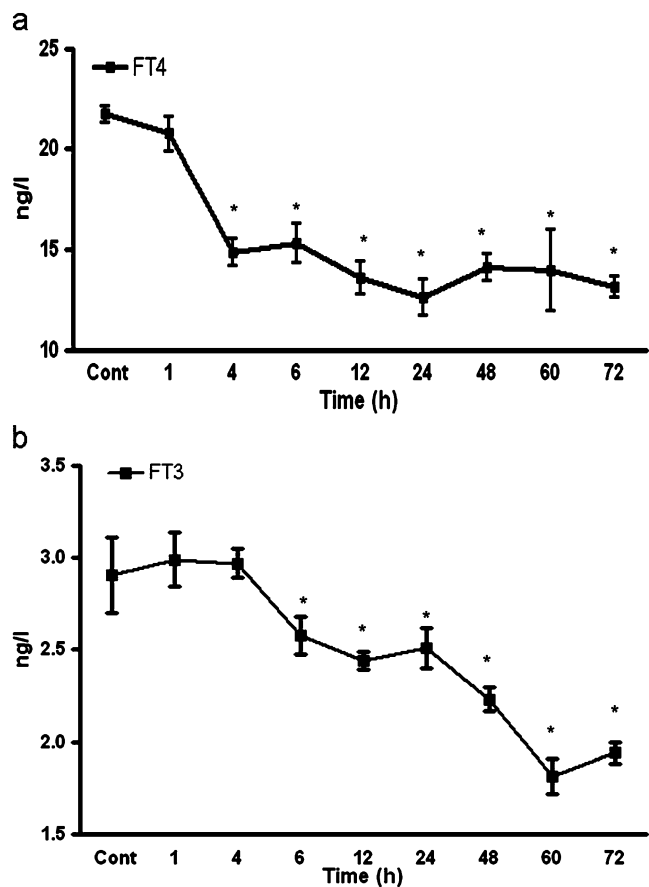
Fig. 1 Serum-free thyroxine (*FT4*; **a**) and free tri-iodothyronine (*FT3*; **b**) levels in rats during acute-phase reaction (APR). Values on the *y-axis* are serum concentration values of FT3 and FT4 measured compared with saline-treated controls. Results represent means±SEM of three animals; *P<0.05

Following a short washing step in phosphate-buffered saline (PBS), incubation was carried out with Alexa-Fluor-conjugated goat anti-rabbit and anti-mouse secondary antibody (1:200; Molecular Probes, Germany) at room temperature for 1 h. The sections were washed 3 times for 5 min in PBS. Finally, nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI), and sections were washed and mounted.

Protein isolation and Western blot analysis

Proteins were isolated as described previously (Tron et al. 2005). Livers at various time-points after TO treatment were lysed in hot Laemmli buffer (95°C) and processed with sodium dodecyl sulphate polyacrylamide gel electrophoresis under reducing conditions according to (Laemmli 1970). The protein content of the cellular lysates was calculated by the Coomassie Protein Assay (Pierce,

Germany) in which β-actin was used as the loading control. Proteins were transferred onto Hybond–ECL nitrocellulose hybridization transfer membranes according to Towbin et al. (1979). We performed immunodetection studies according to the ECL Western blotting protocol of GE Healthcare (Germany). The primary antibody to DOR was used at a 1:100 dilution, whereas the anti-rabbit immunoglobulins were each used at a 1:2000 dilution.

Isolation of hepatocytes and culture conditions

Hepatocytes were isolated from normal animals according to Seglen (1972) with the modification as described in Ramadori et al. (1990). The purity of the isolated cell populations was determined by phase-contrast microscopy and by immunocytochemistry with antibodies against laminin or glial fibrillary acidic protein in order to identify stellate cells (both by Sigma, Germany) or ED-1 and ED-2
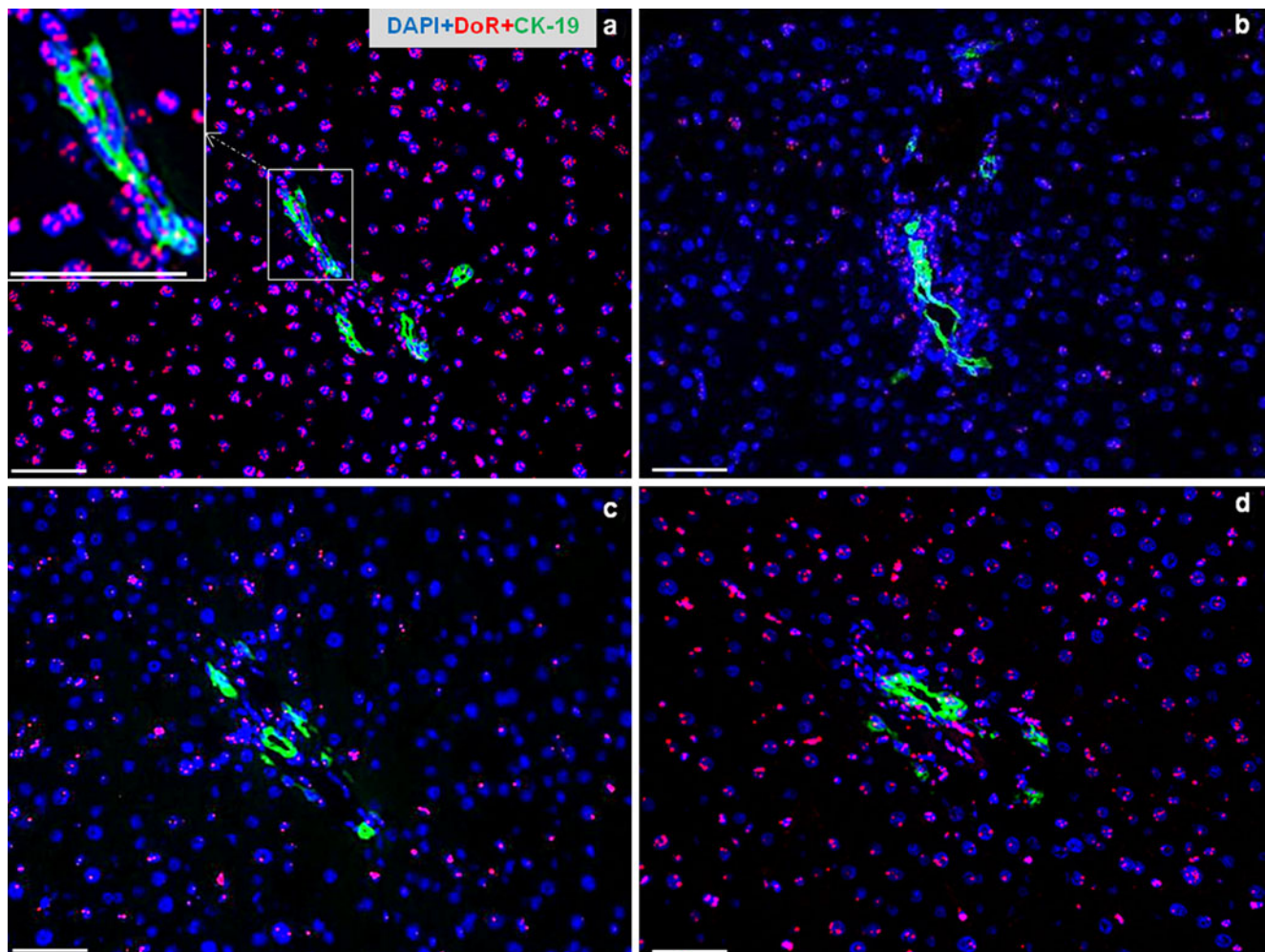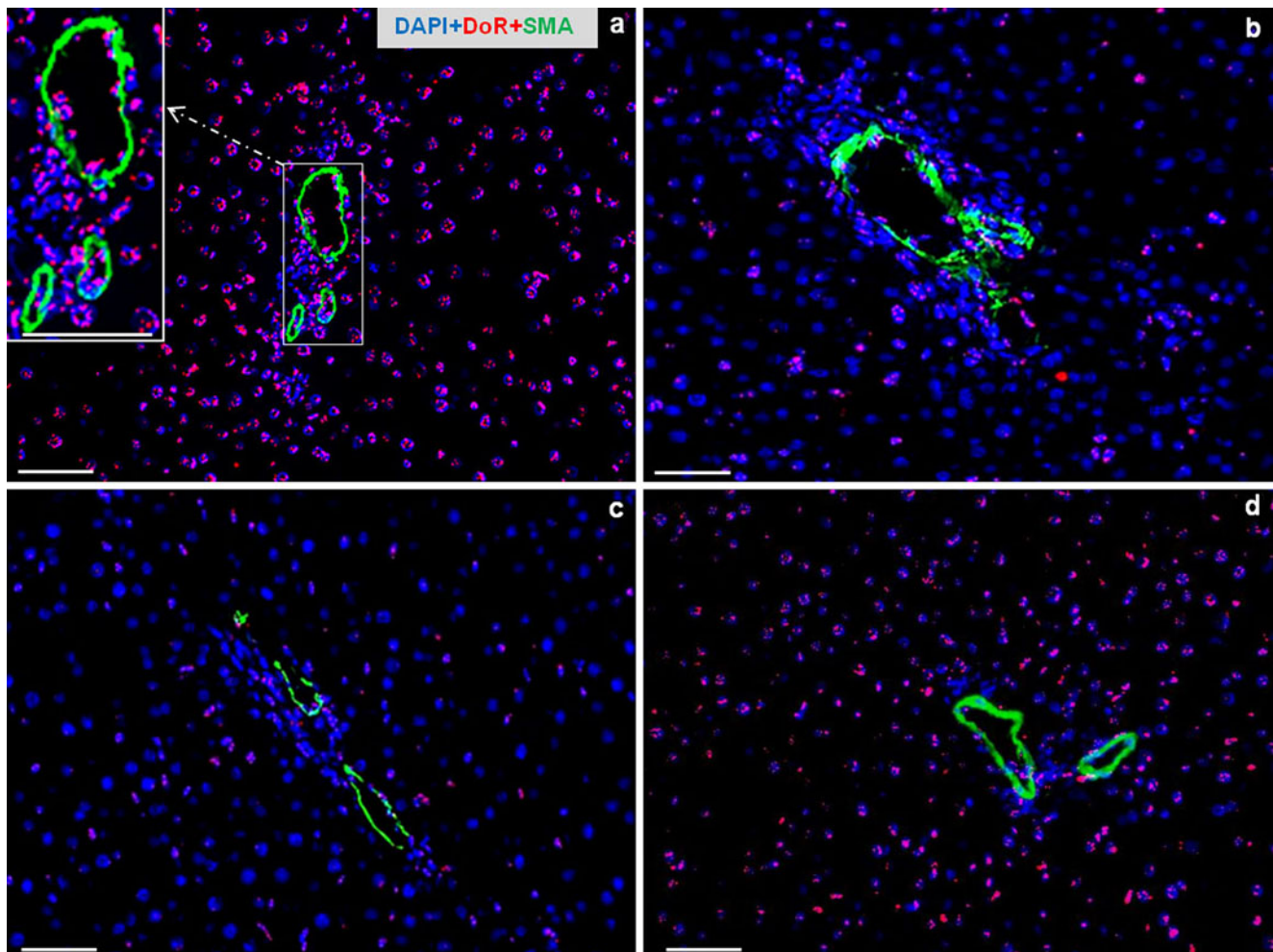

Fig. 2 Double-staining of liver sections with polyclonal antibody directed against DOR (red) and monoclonal antibody against human CK-19 (green) followed by fluorescence immunodetection in sections of rat liver during APR after TO treatment. a Control liver. Inset Higher magnification of boxed area in a. b At 6 h after TO treatment. c At 12 h after TO treatment. d At 24 hours after TO treatment. Representative results from three animals and six slides per time-point. Original magnification: ×200. Bars 100 μm

(gift from C. Dijkstra; Dijkstra et al. 1985) for macrophages. The cells were incubated at 37°C in a 95% air/5% CO_2 atmosphere. DMEM (Biochrom, Germany) was supplemented with 10% fetal calf serum (PAA, Germany), 0.05% insulin (Roche, Germany), and 10^{-7} dexamethasone (Sigma, Germany). Hepatocytes were exposed to a single treatment of 100 ng/ml recombinant IL-6 and IL-1β (PeproTech, Germany) after a dose-dependent (10, 100, and 1000 ng/ml) study at 6 and 12 h following stimulation, for both cytokines in 3 ml cell-culture medium. Cells were also stimulated with 100 nM T3 in 3 ml cell-culture medium in the presence or absence of IL-6 as described previously (Dace et al. 2000; Sheikh et al. 2006). Hepatocytes were harvested in all in vitro experiments at 1, 3, 6, 12, and 24 h after cytokine treatment. Non-treated cells at all studied time-points served as controls in all experiments.

RNA isolation and quantitative real-time polymerase chain reaction

Total RNA was isolated from liver samples and hepatocytes by means of guanidine isothiocyanate extraction, caesium chloride density-gradient ultracentrifugation, and ethanol precipitation according to a previously described method (Chirgwin et al. 1979), with some modifications as described elsewhere (Ramadori et al. 1985b). The RNA obtained was quantified by measuring the absorbance at 260 nm. cDNA was generated by reverse transcription of 1 mg total RNA with 100 nM dNTPs, 50 pM primer oligo(dT)$_{15}$, 200 U Moloney murine leukemia virus reverse transcriptase, 16 U protector RNase inhibitor, 1× reverse transcription buffer, and 2.5 ml 0.1 M dithiothreitole for 1 h at 40°C. Gene expression was analyzed by using Platinum SybrGreen qPCR mix UDG

Fig. 3 Double-staining of liver sections with polyclonal antibody directed against DOR (red) and monoclonal antibody against human SMA (green) followed by fluorescent immunodetection in sections of rat liver during APR after TO treatment. a Control liver. Inset Higher magnification of boxed area in a. b At 6 h after TO treatment. c At 12 h after TO treatment. d At 24 hours after TO treatment. DOR-positive cell numbers decreased in liver during APR. Representative results from three animals and six slides per time-point. Original magnification: ×200. Bars 100 μm

(Invitrogen, Germany). The housekeeping genes ubiquitin C (UBC) and β-actin were used as normalizers. The primer sequences used are shown in Table 1. Amplification was performed at 95–60°C for 45 cycles in an ABI prism 7000 sequence detection system. All samples were assayed in duplicate. The results were normalized to the housekeeping gene, and the fold-change in expression was calculated by using threshold cycle values.

Statistical analysis

The data were analyzed by using Prism Graph Pad 4 software (San Diego, USA). All experimental errors are shown as SEM. Statistical significance was calculated by Student's -test, one-way analysis of variance, and Dunnett's post hoc tests. Significance was accepted at $P < 0.05$.

Results

Changes in FT4 and FT3 serum levels in rats during APR

The FT3 serum level was significantly decreased below control values at 4 h after TO injection; this reduction lasted up to 72 h with a minimum of 60 h. A similar pattern was also observed for FT4 at the serum level (Fig. 1a, b).

### Subcellular DOR and TRα1 localisation in liver sections from untreated and APR animals

DOR protein was detected in the large nucleus of hepatocytes and was organized in well-defined spots, similar to that in HeLa cells (Baumgartner et al. 2007). The intensity of the DOR dots decreased 6–12 h after APR was initiated and began to increase thereafter (Fig. 2). Double-staining of biliary cells (CK-19$^+$), mesenchymal cells (SMA$^+$), and
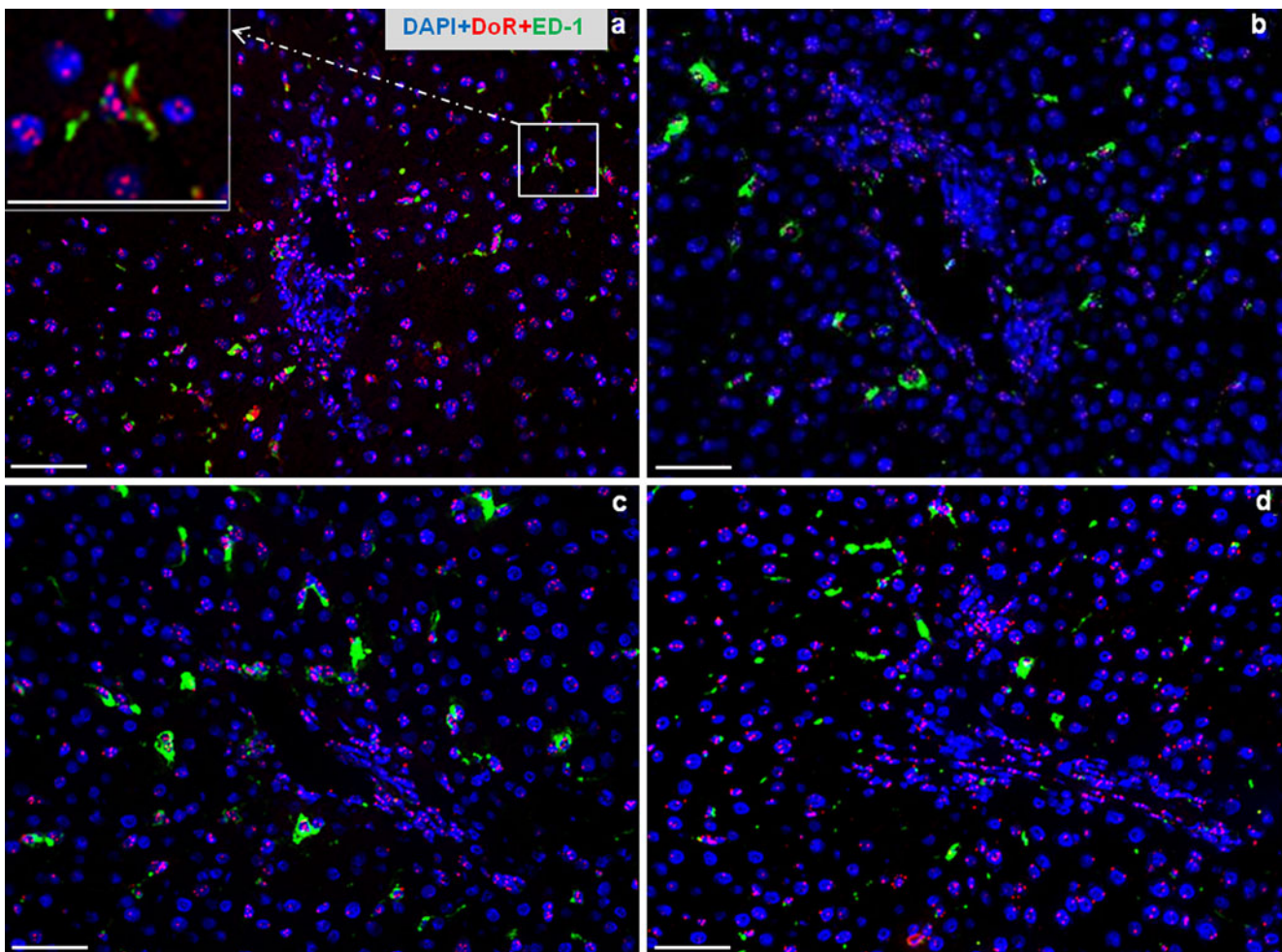
Fig. 4 Double-staining of liver sections with polyclonal antibody directed against DOR (*red*) and monoclonal antibody against human ED-1 (*green*) followed by fluorescent immunodetection in sections of rat liver during acute-phase response after TO treatment. **a** Control liver. *Inset* Higher magnification of *boxed area* in **a**. **b** At 6 h after TO treatment. **c** At 12 h after TO treatment. **d** At 24 hours after TO treatment. Representative results from three animals and six slides per time-point. Original magnification: ×200. *Bars* 100 μm

Fig. 5 Immunofluorescent staining of liver sections with polyclonal antibody directed against TRα1 (*green*) followed by fluorescence immunodetection in sections of rat liver during APR after TO treatment. **a**, **c**, **e** Control liver. **b**, **d**, **f** At 6 h after TO treatment. **a**, **b** Anti-TRα1 and 4,6-diamidino-2-phenylindole (DAPI) staining (*blue*). **c**, **d** Anti-TRα1 staining. **e**, **f** DAPI staining. *Insets* Higher magnification of respective *boxed areas*. *Inset* in **a** Anti-TRα1 and DAPI staining (*arrows* cells of portal area are also positive for TRα1). *Inset* in **c** Anti-TRα1 staining alone. Representative results from three animals and six slides per time-point (*PV* portal vein, *CV* central vein). Original magnification: ×200. *Bars* 100 μm
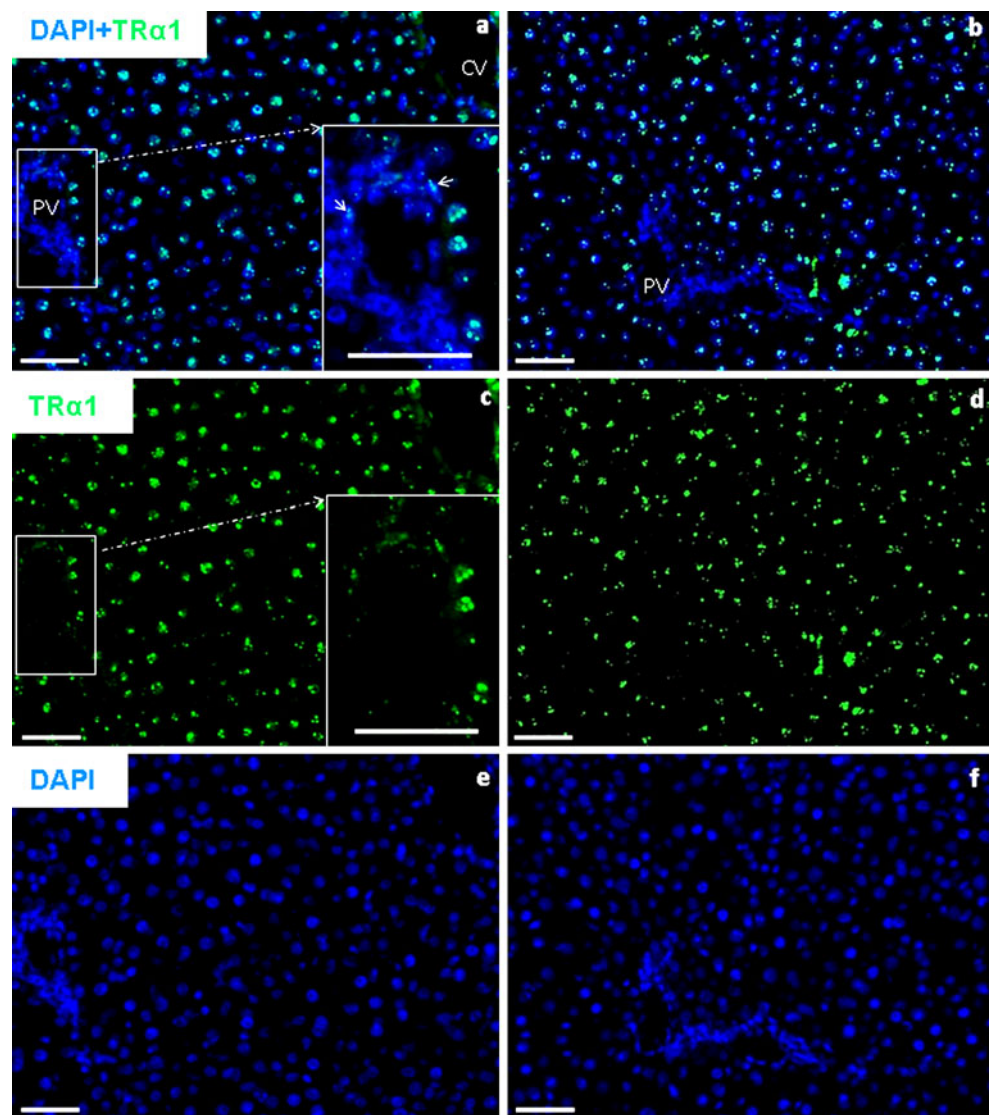
macrophages (ED-1$^+$) also revealed DOR-positive dots in these cells. The intensity of dots decreased in CK-19$^+$ cells, SMA$^+$, and in ED-1$^+$ cells during APR (Figs. 2, 3, 4).

Strong nuclear positivity for TRα1 was found in liver sections from untreated animals and was evenly distributed in periportal and pericentral areas. In addition, positive dots were also noted around the cells of vessel walls of portal and central areas. Immunohistology revealed no significant change in the protein expression of TRα1 at any time-point during APR (Fig. 5a–f).

DOR protein level in liver tissue during APR

In accordance with the immunohistological detection, DOR protein levels decreased in nuclei during APR as detected by Western blot analysis of nuclear extracts. At the same time, the concentration of HO-1 protein, a positive control for APR, peaked at 12 h during APR, as previously described, confirming successful APR induction (Tron et al. 2005; Fig. 6a, b).
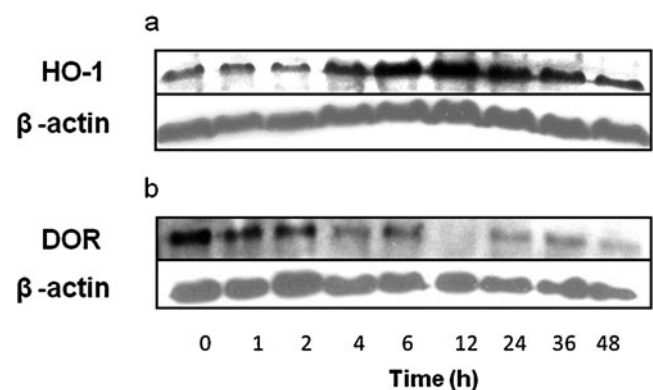
Fig. 6 Western blot analysis of protein from rat liver under APR conditions at various time-points with antibodies specific for (**a**) HO-1 and β-actin and for (**b**) DOR and β-actin using total protein and nuclear extract, respectively

APR-induced changes of amount of TR transcripts in liver tissue

A decrease of DOR gene expression from 30 min to 24 h, reaching a minimum at 6 h, could be observed after the onset of the acute phase. Accordingly, a significant down-regulation of NCoR-1-specific mRNA was found with a minimum at 6 h (Fig. 7a). TRβ1 was slightly up-regulated in the early phase followed by a significant down-regulation with a minimum at 12 h and normalization of mRNA levels by 48 h. A down-regulation was also observed in the later phase lasting up to the 72-h time-point. TRα1 steady state mRNA levels did not change during APR, whereas TRα2 showed significant up-regulation, which peaked at 4 h after treatment followed by down-regulation with minimum expression 60 h after treatment (Fig. 7b).

Modulation of TR gene expression in cultured rat hepatocytes

DOR mRNA level in hepatocytes significantly decreased compared with untreated controls upon exposure to IL-6 or IL-1β. The most pronounced decrease was observed at 6
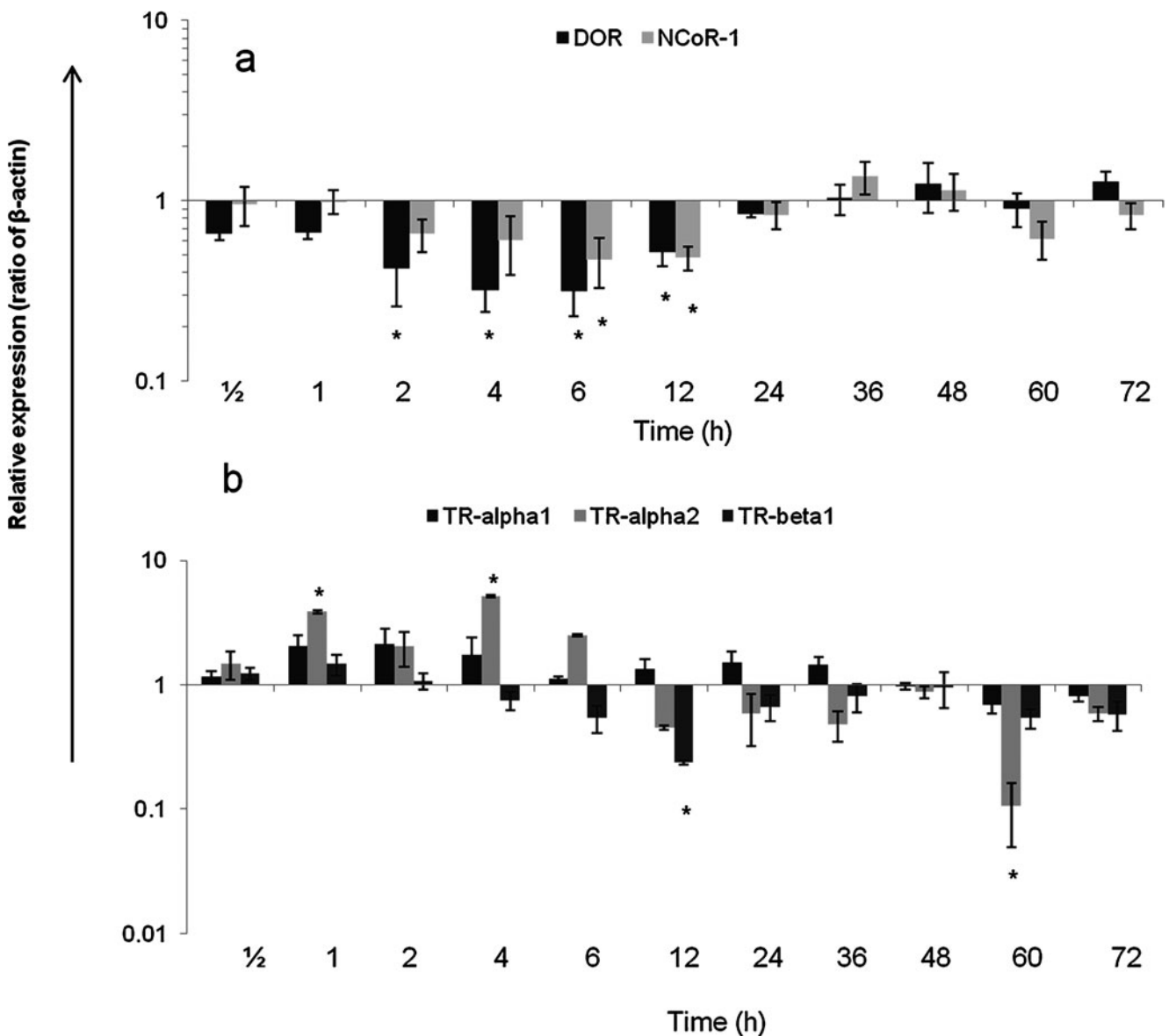
Fig. 7 Quantitative reverse transcription with polymerase chain reaction (qRT-PCR) analysis of total RNA from rat liver during APR. Fold-change in mRNA expression of DOR, NCoR1, TRα1, TRα2, and TRβ1 in the TO-treated liver at various time-points (1–72 h) relative to saline-treated controls for each time-point. qRT-PCR was normalized by using two housekeeping genes: β-actin and ubiquitin C. Results represent means±SEM of three animals; *$P<0.05$

and 12 h after the start of cytokine treatment. The effect of IL-6 on reducing DOR expression was stronger than that induced by IL-1β treatment. Similarly, an early (3-6 h) down-regulation of TRβ1 was also observed after treatment with IL-6 or IL-1β. The impact of IL-6 was also stronger than IL-1β in cultured hepatocytes. No significant change in the gene expression of NCoR-1 was observed after IL-6 exposure; however, a rapid significant reduction (1-6 h) was detected after IL-1β treatment. In contrast, an early (1 h) up-regulation of TRα1 and TRα2 was observed after treatment with IL-6 followed by a slight down-regulation thereafter. Surprisingly, a fast down-regulation (1 h) of both genes was detected followed by up-regulation at 3-6 h in hepatocytes when treated with IL-1β (Figs. 8, 9).

T3 treatment of hepatocytes significantly induced early gene expression of DOR, NCoR-1, and TRα1 and this steadily increased with a maximum at 24 h. An early significant up-regulation at 1 h was also detected for the gene expression of TRα2 and TRβ1 with a maximum of induction at 12 h. The up-regulating effect was completely cancelled by the addition of IL-6 to the culture medium (Fig. 8).

Discussion

In the present study, we investigated the expression levels of key receptors of the THs mainly responsible for hormone
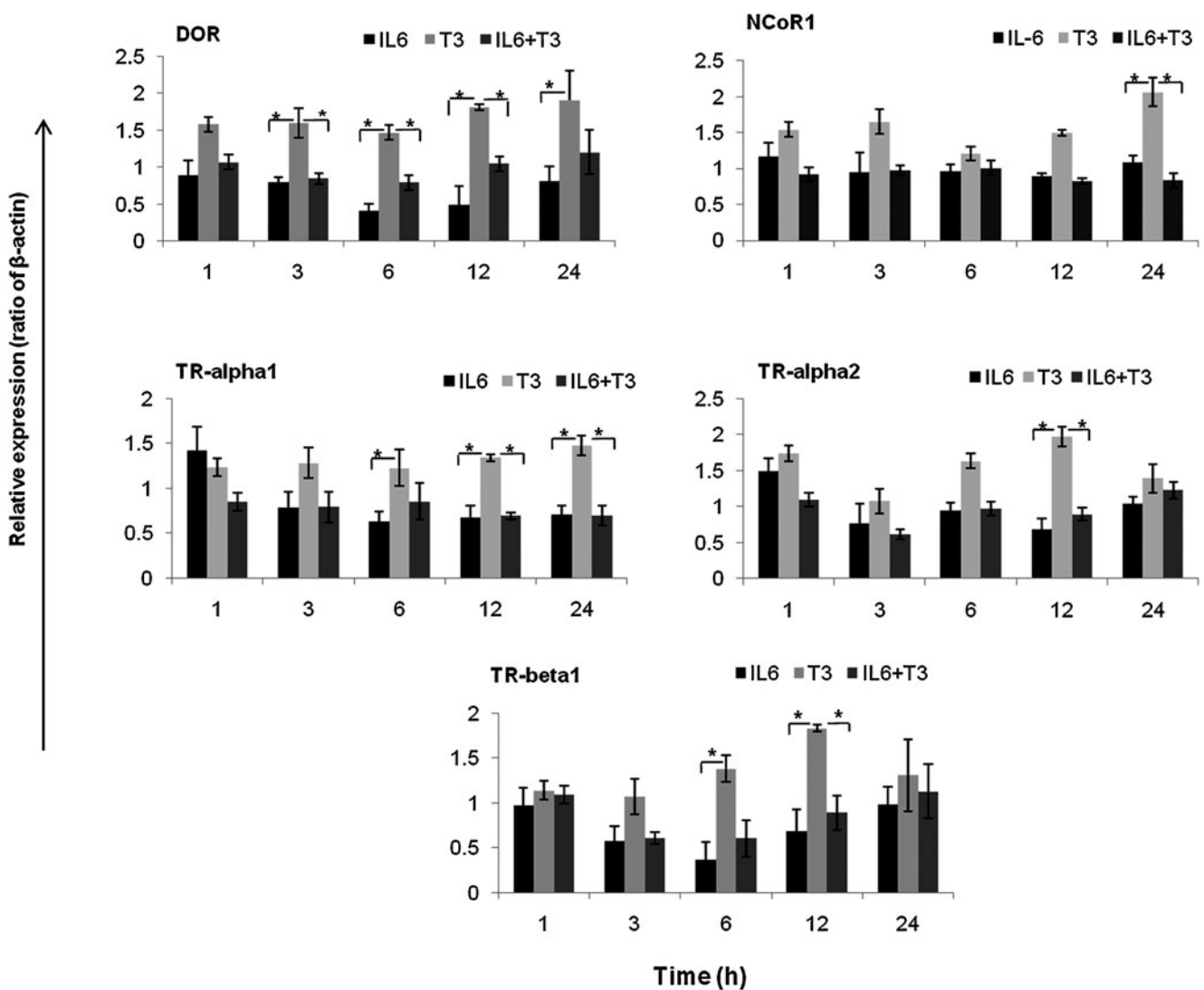
Fig. 8 qRT-PCR analysis of total RNA from isolated rat hepatocytes treated with IL-6, T3, or IL-6+T3. Data are shown as fold-changes in mRNA expression of DOR, NCoR-1, TRα1, TRα2, and TRβ1 at various time-points relative to untreated controls for each time-point. qRT-PCR was normalized by using two housekeeping genes: β-actin and ubiquitin C. Results represent means±SEM of three experiments; *P<0.05

Fig. 9 qRT-PCR analysis of total RNA from isolated rat hepatocytes treated with IL-1β. Data are shown as fold-changes in mRNA expression of DOR, NCoR-1, TRα1, TRα2, and TRβ1 at various time-points relative to untreated controls for each time-point. qRT-PCR was normalized by using two house-keeping genes: β-actin and ubiquitin C. Results represent means±SEM of three experiments; *P<0.05
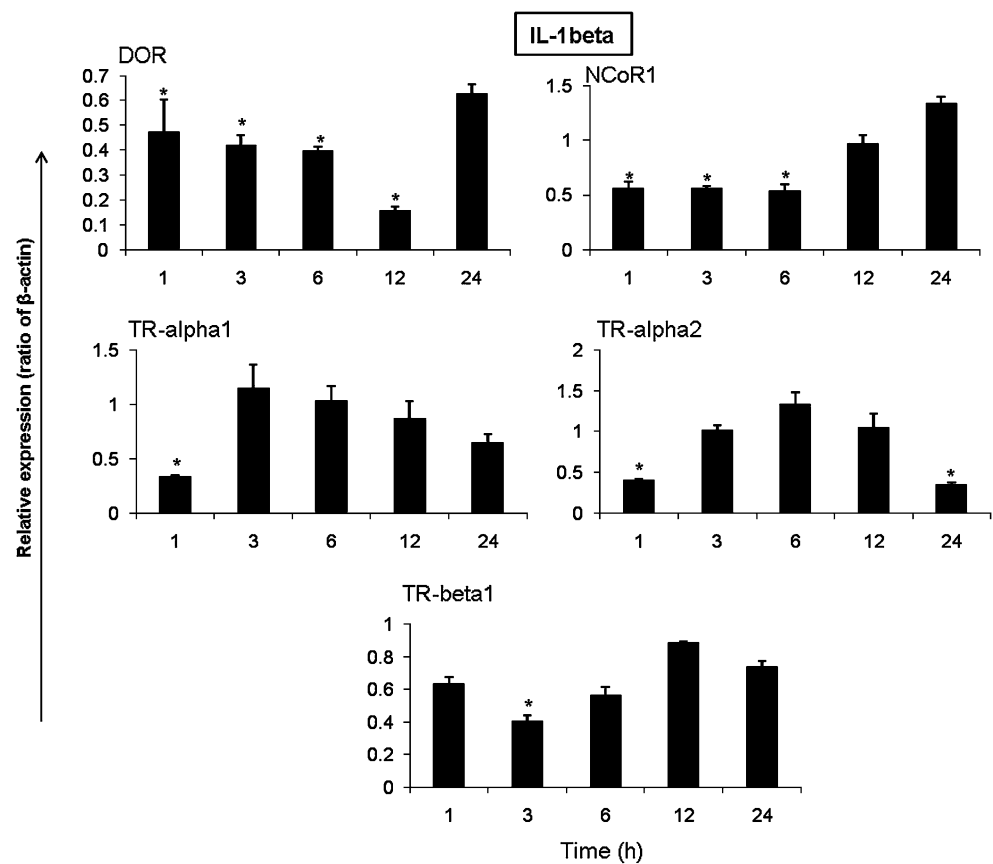
signaling, namely TRα1, TRα2, TRβ1, NCoR-1, and the newly identified TR cofactor DOR/TP53INP2, in the rat liver during APR in parallel with the serum level of the THs. In isolated hepatocytes treated with acute-phase mediators, with or without TH, we also studied changes of TR gene expression. The results of the current study demonstrate that hepatocytes, macrophages, mesenchymal cells, and bile duct cells express abundant amounts of nuclear DOR with a decrease during the first 6-24 h of APR. In contrast, TRα1 immunoreactivity shows a uniform, equally distributed, and constant nuclear positivity throughout the liver. In accordance with the immunohistology and Western blot analysis, DOR gene expression is also down-regulated at the mRNA level in the liver. Commensurate with the down-regulation observed for the DOR gene, similar patterns of expression have been detected for TRβ1 and NCoR-1 at the mRNA level, whereas TRα1 is only slightly up-regulated, and TRα2 is significantly up-regulated during APR.

To address the question of whether the reduction in DOR and TRβ1 gene expression during APR might have also been attributable to the direct effect of acute-phase mediators (IL-6 and IL-1β) on the genes studied, a major population of the liver cells (hepatocytes) was isolated and treated with two "major" acute-phase cytokines (IL-6 and IL-1β). Similar to the observations in the liver tissue during APR, a reduction in gene expression of DOR and TRβ1 was found after treatment

with acute-phase cytokines (IL-6 and IL-1β). Likewise, an up-regulation of TRα1 and the negative factor TRα2 was also observed. Whereas treatment of hepatocytes with T3 increased the gene expression of DOR and of the other TRs, such an increase was inhibited completely by the addition of IL-6 to the culture medium.

THs are known to regulate gene expression through receptors (TRs: TRα and TRβ), and changes in their gene expression are accompanied by progressive changes in TH level (Brent et al. 1989b).

Our current study has shown that transcription of T3-controlled TR genes is severely affected during APR; this might be more dependent on cytokines and the TH level than on the TH level alone. The association between acute-phase cytokines and TH might entail a causal link, as we have found that the T3 signaling molecules TRβ1, DOR, and NCoR1 are down-regulated in the liver during APR, whereas the negative signaling factor TRα2 is up-regulated. Tissue injury induces an acute-phase response, which is a major mechanism of the body to restore homeostasis; APR is heavily regulated by inflammatory cytokines such as IL-6 (Ramadori and Christ 1999; Sheikh et al. 2006) and is negatively related to serum T3 levels (Bartalena et al. 1994). Thus, the involvement of acute-phase cytokines in the pathogenesis of APR classifies NTI as part of the acute-phase response.

In other words, the decrease in TRβ1 and in the expression of the newly identified gene DOR is attributable not only to the decrease in TH serum concentration, but possibly also to the direct effect of acute-phase cytokines produced during APR on hepatocytes. Our study is moreover the first to show that the expression of the newly identified gene DOR is similar to that of the major TR, viz., TRβ1, in rat liver under APR.

The gene expression of TRs in NTI has been partially described in previous studies. However, the clinical impact, with a comprehensive study of major TRs, including the newly identified gene DOR, under acute-phase conditions has not been previously established. Furthermore, previous reports examining the changes in expression of TRs in models of NTI also show somewhat conflicting findings.

Boelen et al. (2006) have reported a rapid decrease in liver TRβ1 mRNA after lipopolysaccharide (LPS) administration in an animal model of APR. A lowering of TRβ1 mRNA in response to IL-1β also occurs in vitro in a hepatoma cell line (Kwakkel et al. 2006). In the rabbit model of prolonged (7 days) burn injury, TRs are unchanged in the hypothalamus-pituitary-thyroid axis (Mebis et al. 2009).

In contrast to treatments inducing an acute systemic response and also liver damage, such as the administration of the bacterial endotoxin LPS (Boelen et al. 2006), TO is known to induce aseptic local abscesses without detectable injury to other tissues (Ramadori and Meyer Zum Buschenfelde 1990; Tron et al. 2005; Wusteman et al. 1990). Therefore, the TO-induced APR model allows us to study the effect of cytokines produced at distant sites to the liver. It reproduces the changes observed in human disease states caused by localized tissue injury (Gabay and Kushner 1999; Halter et al. 2005; Kim et al. 2002; Stoeck et al. 2006) making it an ideal model for studying human APR. Use of this model in the current study is also of special interest as the down-regulation of TRβ1 and up-regulation of TRα2 have been observed, which might be attributable to the inhibitory effect of TRα2 on TRβ1, as previously reported (Koenig et al. 1989). This fact has not been reported in any other acute-phase animal model so far.

In conclusion, our data suggest that therapeutic replacement under acute-phase conditions might not be effective, as activating TRs are not available, and their inhibitor (TRα2) is up-regulated at the same time.

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