The acute-phase response of the liver in relation to thyroid hormone-induced redox signaling

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Abstract

Recently, we reported that 3,3′,5-triiodothyronine (T_3) induces the expression of redox-sensitive genes as a nongenomic mechanism of T_3 action. In this study, we show that T_3 administration to rats (daily doses of 0.1 mg/kg ip for 3 consecutive days) induced a calorigenic response and liver glutathione depletion as an indication of oxidative stress, with higher levels of interleukin (IL)-6 in serum (ELISA) and hepatic STAT3 DNA binding (EMSA), which were maximal at 48–72 h after treatment. Under these conditions, the protein expression of the acute-phase proteins haptoglobin and β-fibrinogen is significantly augmented, a change that is suppressed by pretreatment with α-tocopherol (100 mg/kg ip) or gadolinium chloride (10 mg/kg iv) before T_3. It is concluded that T_3 administration induces the acute-phase response in rat liver by a redox mechanism triggered at the Kupffer cell level, in association with IL-6 release and activation of the STAT3 cascade, a response that may contribute to reestablishing homeostasis in the liver and extrahepatic tissues exhibiting oxidative stress.

Keywords: Thyroid hormone; Oxidative stress; Liver; Kupffer cells; Interleukin-6; Signal transducer and activator of transcription 3; Acute phase response; Free radicals

Introduction

Significant progress has been made recently in the understanding of the processes underlying thyroid hormone (TH) action, pointing to the variety and complexity of the molecular mechanisms involved. Most effects of TH are mediated by a direct modulation of gene transcription through interaction of TH–nuclear receptor (TR) complexes with specific DNA sequences, the TH response elements [1]. This genomic mechanism requires the heterodimerization of TR with retinoic acid receptor, leading to (i) transcriptional activation in the presence of bound 3,3′,5-triiodothyronine (T_3) and coactivators or (ii) basal repression in the absence of bound T_3 and presence of corepressors [1]. In addition, T_3-responsive genes that do not interact with TR may involve an indirect induction mechanism via the activation of intermediate factors, as proposed for the nuclear respiratory factors 1 and 2 or the peroxisome proliferator-activator receptor-γ coactivator 1 [2].

Actions of TH that are independent of intranuclear liganded-TH complexes or T_3-induced activation of intermediate factors are called nongenomic [3]. The latter mechanisms mediate complex, usually rapid, cellular responses such as plasma membrane transport [4], enzyme activities related to mitochondrial respiration [5] or cytoplasmic signal-transducing processes [3], intracellular protein trafficking [6], or production of reactive oxygen species (ROS) [7,8]. Recently, work from our laboratory has established that T_3-induced calorigenesis in the rat increases the oxidative stress status of the liver [9], triggering the expression of redox-sensitive genes as a novel nongenomic mechanism of TH action [10]. The proposed mechanism underlies the phosphorylation of inhibitor of κB-α, suggesting the activation of the IκB kinase (IKK) complex [11], leading to a higher activating potential of nuclear factor-κB (NF-κB) and transcription of NF-κB-sensitive genes [10]. At the Kupffer cell level, up-regulation of cytokine-encoding genes by T_3 is

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observed, resulting in a significant tumor necrosis factor-α (TNF-α) response [12], a cytokine that exhibits autocrine and paracrine actions in the liver [13,14]. The effects of TNF-α are exerted through its interaction with surface receptors in target cells in order to mediate signaling from cell membrane to nucleus, TNF-α receptor 1 coupling being important in the homeostatic response of the liver under conditions of moderate pro-oxidant status and low levels of transient TNF-α expression [13,15]. This view is supported by the T_3-induced mRNA expression of the NF-κB-responsive genes for inducible nitric oxide synthase (iNOS) [11], manganese superoxide dismutase (MnSOD), and Bcl-2 [16], changes that are abrogated by the administration of α-tocopherol before T_3 [11,16].

The acute-phase response (APR) is a major pathophysiologic reaction, in which normal homeostatic mechanisms are replaced by new set points that may contribute to defensive or adaptive capabilities [17]. The APR is mainly focused on the liver, being commonly associated with inflammation and a number of states not regarded as inflammatory, including oxidative stress [18]. This reaction is mediated by both interleukin (IL)-1-like cytokines (IL-1, TNF-α) and IL-6-like cytokines (IL-6, oncostatin M, and others), through the activation of the transcription factors NF-κB, activator protein 1 (AP-1), signal transducer and activator of transcription 3/5 (STAT3/5), or CCAAT/enhancing-binding protein β (C/EBPβ) [17,18]. These signaling cascades result in an increase in the 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 [17,18]. In view of these considerations, the objective of this study was to evaluate the APR of the liver in relation to T_3-induced redox signaling. For this purpose, the hepatic expression of the APR proteins haptoglobin and β-fibrinogen was assessed in relation to the serum levels of IL-6 and liver STAT3 DNA binding, the main signaling pathway involved in hepatic APR [19]. Studies were performed both in control rats and animals given T_3 and in separate groups subjected to treatment with either α-tocopherol or the Kupffer cell inactivator gadolinium chloride ($GdCl_3$) [20] before hormone administration. The results obtained were correlated with the oxidative stress status achieved by the treatments, measured by the hepatic levels of total GSH equivalents.

Materials and methods

Animals and treatments

Female Sprague–Dawley rats (Bioterio Central, ICBM, Facultad de Medicina, Universidad de Chile) weighing 200–300 g were housed on a 12-h light/dark cycle and were provided with rat chow and water ad libitum. Animals received daily ip injections of 0.1 mg of T_3/kg body wt for 3 consecutive days or equivalent volumes of hormone vehicle (0.1 N NaOH, controls) and studies were performed at the indicated times after treatment. Thyroid hormone-induced calorigenesis was assessed by the rectal temperatures of the animals measured with a thermocouple (Cole-Parmer Instrument, Chicago, IL, USA) and blood samples were taken from the tail vein for the measurement of serum T_3 levels by GammaCoat(I^{125})T_3 Radioimmunoassay (assay sensitivity limit of 9 ng T_3/dl, between-assay variation of 4%, and intra-assay variation of 3.5% at 33 ng/dl and 2.7% at 290 ng/dl; Baxter Healthcare, Cambridge, MA, USA). Separate groups of rats were subjected to either (i) 100 mg of α-tocopherol/kg ip [21] 17 h before the first dose of T_3 or (ii) 10 mg of $GdCl_3$/kg iv [20] 24 h before T_3, and studies were carried out at the indicated times after hormone treatment. In the α-tocopherol-treated groups, the levels of α-tocopherol in plasma were measured by reverse-phase HPLC after extraction with hexane [22]. For this purpose, a Symmetry C-18 column (Waters Corp., Milford, MA, USA), 3.5 μm, with dimensions of 4.6 × 100 mm, was employed. Separation was done using a flow rate of 1 ml/min of the mobile phase (7% v/v dichloromethane in methanol) and detection was performed at 292 nm. All animals used were cared for according to the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals* by the National Academy of Sciences (National Institutes of Health Publication No. 86-23).

Enzyme-linked immunoabsorbent assay (ELISA) for IL-6

Serum from rat blood was obtained by cardiac puncture, separated, and stored at −80°C, and IL-6 levels were measured by ELISA (Immunoassay Kit KRC0061C; BioSource International, Inc., Camarillo, CA, USA) according to the manufacturer's specifications. Serum samples containing high IL-6 levels were repeated after dilution to ensure assay results within the standard curve.

Electromobility shift assay (EMSA)

Nuclear protein extracts from liver samples were prepared according to Deryckere and Gannon [23]. The samples were subjected to EMSA for assessment of STAT3 DNA binding using the STAT3 probe 5′-GTCGACATTTCCCGTAAATC-GTCGA-3′ (Invitrogen Life Technologies, Carlsbad, CA, USA), labeled with [α-^{32}P]dCTP using the Klenow DNA Polymerase Fragment I (Invitrogen), as previously described [24]. The specificity of the reaction was determined by a competition assay using 100-fold molar excess of unlabeled DNA probe. Samples were loaded on nondenaturing 6% polyacrylamide gels and run until the free probe reached the end of the gel; STAT3 bands were detected by autoradiography and quantified by densitometry using Scion Image (Scion Corp., Frederick, MD, USA).

Western blot analysis of haptoglobin and β-fibrinogen

At selected times after T_3 treatment, liver samples (100–500 mg) frozen in liquid nitrogen were homogenized and suspended in a buffer solution, pH 7.9, containing 10 mM Hepes, 1 mM EDTA, 0.6% Nonidet P-40, 150 mM NaCl, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 mM orthovanadate). Soluble protein fractions (50 μg) were separated on 12% polyacrylamide

gels using SDS–PAGE [25] and transferred to nitrocellulose membranes [26], which were blocked for 1 h at room temperature with TBS containing 5% nonfat dry milk. The blots were washed with TBS containing 0.1% Tween 20 and hybridized with rabbit polyclonal antibodies for either human haptoglobin or β-fibrinogen (Dako Corp., Carpinteria, CA, USA). In all determinations, mouse monoclonal antibody for rat β-actin (ICN Biomedicals, Inc., Aurora, OH, USA) was used as internal control. After extensive washing, the antigen–antibody complexes were detected using horseradish peroxidase-labeled goat anti-rabbit IgG or goat anti-mouse IgG and a SuperSignal West Pico chemiluminescence kit detection system (Pierce, Rockford, IL, USA).

Assay for liver GSH content

Animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the liver was perfused in situ with 150 ml of a cold solution containing 150 mM KCl and 5 mM Tris, pH 7.4, in order to remove blood. Total GSH equivalents were measured by the enzymatic assay of Tietze [27] in liver homogenates (1:100) prepared in 0.5 N $HClO_4$ and neutralized with 1.75 M K_3PO_4.

Statistics

Values shown correspond to the means ± SEM for the number of separate experiments indicated. One-way ANOVA and the Newman–Keuls test assessed the statistical significance of differences between mean values.

Results

T_3 treatment significantly increased the serum levels of T_3 compared with control values [controls, 53 ± 5 (n = 4) ng/dl; T_3-treated rats at 24, 48, and 72 h, 338 ± 27 (n = 4), 314 ± 47 (n = 4), and 274 ± 19 (n = 4), respectively ($p < 0.05$ versus controls)]. Under these conditions, T_3 administration led to (i) a calorigenic response evidenced by a significant increase in the rectal temperature of the animals in the 12- to 74-h time interval studied (Fig. 1A) and (ii) significantly higher IL-6 levels in serum at different times after treatment (Fig. 1B), resulting in daily net increases of 94, 110, and 356 pg h/ml assessed by the integration of the area under the average curves of IL-6 levels in serum between 0 and 24 h, 24 and 48 h, and 48 and 72 h, respectively.

T_3 administration to rats, involving enhanced serum levels of T_3 and the associated calorigenic and IL-6 responses (Fig. 1), elicited an enhancement in the DNA binding of STAT3 in liver, with significant transient 4.1- and 3.5-fold increases at 18 and 22 h after treatment that leveled off in the 24–46 h period, followed by peak increases of 6.2- and 6.5-fold ($p < 0.05$) at 48 and 54 h, respectively (Fig. 2A). Integration of the area under the average curves of liver STAT3 DNA binding between 0 and 24 h, 24 and 48 h, and 48 and 72 h (Fig. 2A) resulted in daily net increases of 20, 16, and 81 (arbitrary units) h, respectively. The 6.2-fold elevation in liver STAT3 DNA binding observed at 48 h after T_3 administration was diminished by 94 and 98% ($p < 0.05$) by $GdCl_3$ and α-tocopherol pretreatment, respectively, in the absence of significant changes induced by $GdCl_3$ or α-tocopherol alone (Fig. 2B). The effects elicited by α-tocopherol pretreatment were seen concomitant with significant increases in the serum levels of the antioxidant [(a) controls, 11.7 ± 0.5 (n = 4) μM; (b) T_3 (48 h), 13.7 ± 0.4 (3); (c) α-tocopherol, 20.8 ± 0.3 (4); (d) α-tocopherol + T_3, 20.4 ± 0.5 (3); (c) and (d), $p < 0.05$ versus (a) and (b)]. T_3 induced a significant 63% decrease in liver GSH content, an effect that is inhibited by 75 and 45% by α-tocopherol and $GdCl_3$ pretreatment, respectively [(a) controls, 6.97 ± 0.50 (n = 5) μmol/g liver; (b) T_3 (48 h), 2.55 ± 0.09 (n = 5); (c) α-tocopherol, 5.85 ± 0.27 (n = 8); (d) α-tocopherol + T_3, 4.75 ± 0.30 (n = 3); (e) $GdCl_3$, 7.22 ± 0.34 (n = 3); $GdCl_3$ + T_3, 4.75 ± 0.26; (b) $p < 0.05$ versus (a), (c), (d), (e), and (f)].

To examine the effects of T_3 treatment on the expression of APR proteins in rat liver, Western blot analysis of hepatic haptoglobin and β-fibrinogen was performed. Fig. 3A shows significant increases in haptoglobin expression at 62–72 h after

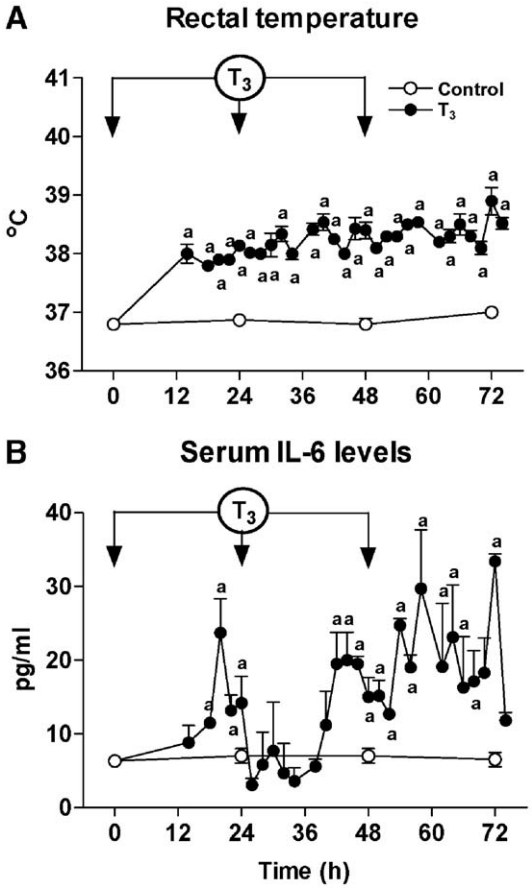

Fig. 1. Time course study of the effects of T_3 administration on (A) the rectal temperature of the animals and (B) the serum levels of interleukin-6 (IL-6). Fed rats received daily ip injections of 0.1 mg T_3/kg body wt for 3 consecutive days or hormone vehicle (arrows) and studies were performed at the indicated times. Data are means ± SEM from 3 to 12 different animals per group. Significance studies (one-way ANOVA followed by the Newman–Keuls test): $^a p < 0.05$ compared with either the respective control values at different times or the average of all control data indicated at time 0.

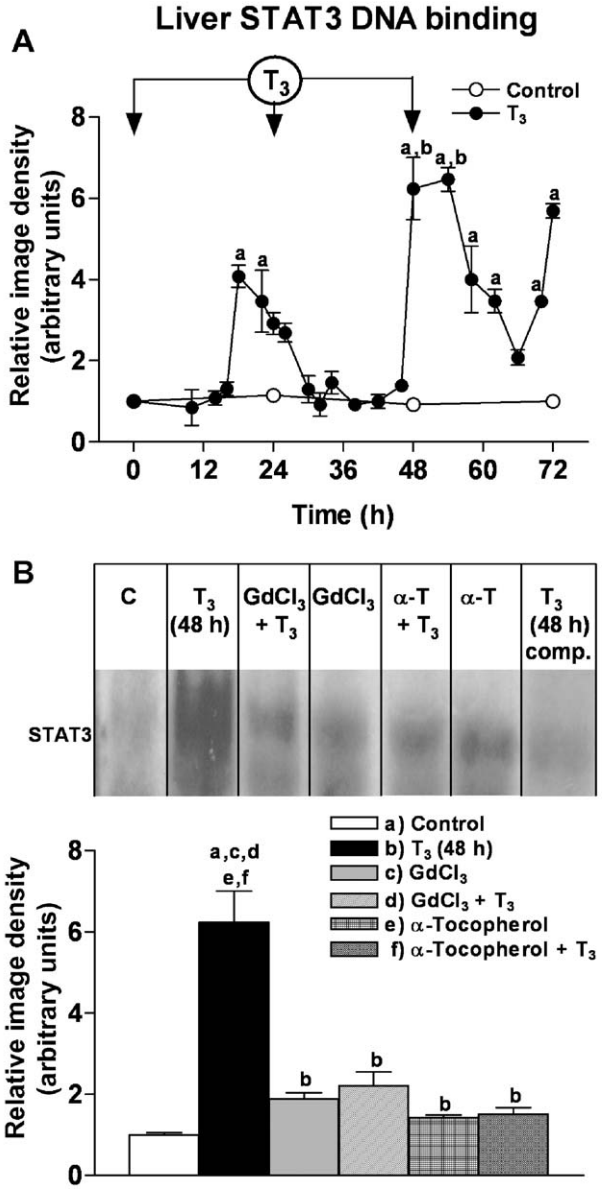

Fig. 2. Time course study of (A) the effects of T_3 administration on rat liver signal transducer and activator of transcription 3 (STAT3) DNA binding and (B) the effect of gadolinium chloride ($GdCl_3$) or α-tocopherol (α-T) pretreatment at 48 h after hormone treatment. Data are means ± SEM for three different animals per experimental group. Significance studies were done by one-way ANOVA and the Newman–Keuls test. (A) Densitometric quantification of relative STAT DNA binding evaluated by EMSA in control and T_3-treated rats at different times after treatment. Mean value at time 0 was arbitrarily set to unity, and values at other time points were normalized to this. $^{a}p < 0.05$ compared with control values; $^{b}p < 0.05$ compared with T_3-treated rats at the other experimental times. (B) Representative autoradiograph of STAT3 DNA binding in nuclear extracts of control or T_3-treated rats and in separate groups of animals pretreated with either $GdCl_3$ or α-tocopherol before hormone administration and in competition experiments with 100-fold excess of the unlabeled oligonucleotide [lane T_3 (48 h) comp.] (top), with the respective densitometric normalized quantification shown at the bottom. Significance studies: $p < 0.05$ as shown by the letters identifying each experimental group.

T_3 administration, with peak values found at 66–70 h. The threefold enhancement in haptoglobin expression observed at 66 h after T_3 treatment (Fig. 3A) is abolished by pretreatment with either $GdCl_3$ or α-tocopherol before hormone administration (Fig. 3B). Similarly, T_3 led to enhanced β-fibrinogen expression, which is significant at 66 h after hormone

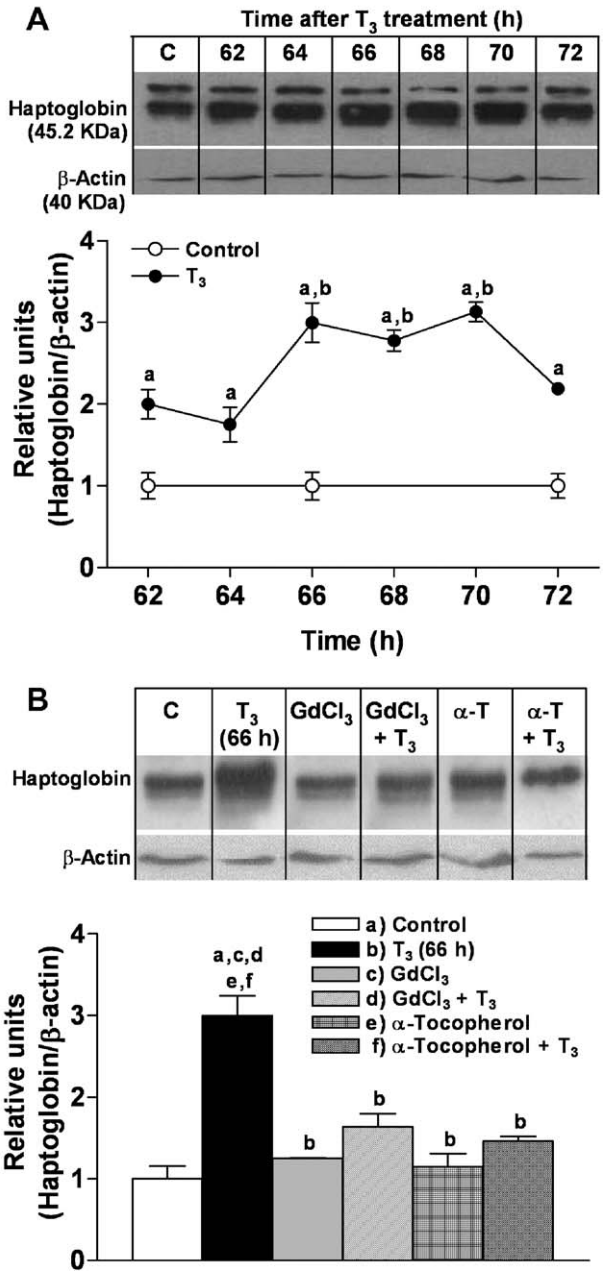

Fig. 3. Time course of (A) the effects of T_3 administration on liver haptoglobin protein expression and (B) the effect of gadolinium chloride ($GdCl_3$) or α-tocopherol pretreatment at 66 h after hormone treatment. Data are means ± SEM for three or four different animals per experimental group. Significance studies were done by one-way ANOVA and the Newman–Keuls test. The upper panels of A and B show representative SDS–PAGE of liver soluble protein fractions from control rats (C) and T_3-treated animals for detection of haptoglobin and β-actin. The lower panels of A and B show the respective densitometric quantification expressed as the haptoglobin/β-actin ratios. Average data from all control rats were set to unity, and values at other experimental time points or treatments were set against this. Significance studies: (A) $^{a}p < 0.05$ compared with control values or $^{b}p < 0.05$ compared with T_3-treated animals at other experimental times; (B) $p < 0.05$ as shown by the letters identifying each experimental group.

treatment, exhibiting maximal effects at 68–70 h (Fig. 4A), and is suppressed by $GdCl_3$ or α-tocopherol pretreatment (Fig. 4B). $GdCl_3$ or α-tocopherol administration did not modify liver haptoglobin and β-fibrinogen expression in euthyroid rats (Figs. 3 and 4).

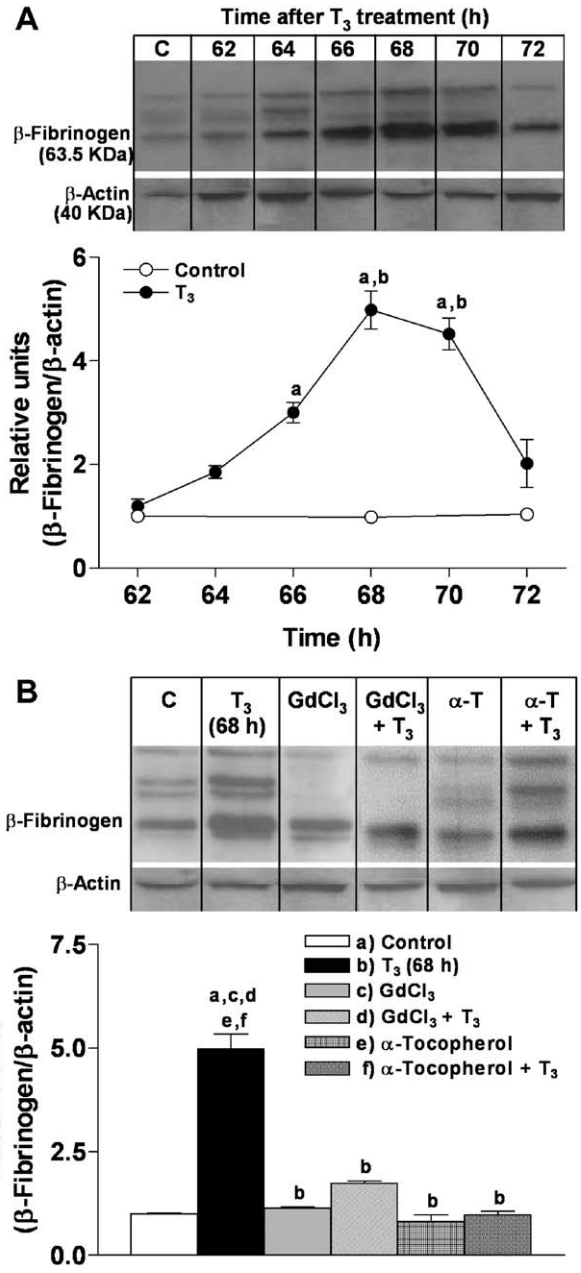

Fig. 4. Time course of (A) the effects of T_3 administration on liver β-fibrinogen protein expression and (B) the effect of gadolinium chloride ($GdCl_3$) or α-tocopherol pretreatment at 66 h after hormone treatment. Data are means ± SEM for three or four different animals per experimental group. Significance studies were done by one-way ANOVA and the Newman–Keuls test. The upper panels of A and B show representative SDS–PAGE of liver soluble protein fractions from control rats (C) and T_3-treated animals for detection of β-fibrinogen and β-actin. The lower panels of A and B show the respective densitometric quantification expressed as the β-fibrinogen/β-actin ratios. Average data from all control rats were set to unity, and values at other experimental time points or treatments were set against this. Significance studies: (A) $^{a}p < 0.05$ compared with control values or $^{b}p < 0.05$ compared with T_3-treated animals at other experimental times; (B) $p < 0.05$ as shown by the letters identifying each experimental group.

Discussion

The major function of the immune system is resistance to various pathologic processes, with the liver playing a central role in the regulation of both innate and adaptive immunity [28,29]. Hepatic involvement in innate or nonspecific immunity includes (i) production of APPs by hepatocytes and (ii) immunologic surveillance by nonparenchymal cells such as Kupffer cells, sinusoidal endothelial cells, stellate cells, dendritic cells, and liver-associated lymphocytes, thus promoting survival under stress conditions such as infection or severe injury [19,28,29]. Data presented in this work indicate that T_3 administration to rats induces the APR of the liver, which is produced under thermogenic conditions by a redox mechanism triggered at the Kupffer cell level due to its suppression by α-tocopherol and $GdCl_3$ pretreatment. This response is evidenced by the significant increase in the protein expression of two representative APPs, namely, the type I APP haptoglobin and the type II APP β-fibrinogen. T_3-induced liver APR is observed at 62 to 72 h after hormone treatment, similar to the induction of iNOS [11], MnSOD, and Bcl-2 [16], a time period in which achievement of significant oxidative stress is encountered, as shown by the maximal increases in lipid peroxidation and protein oxidation [11] that are preceded by substantial GSH depletion.

The hepatic APR induced by T_3 is associated with IL-6 signaling and activation of the Janus kinase (JAK)/STAT3 cascade, considering the maximal enhancement in IL-6 levels in serum and hepatic STAT3 DNA binding observed within the period of 48–72 h after T_3 administration. These observations suggest that a net signaling output is determined in terms of intensity and duration by T_3, reaching the signaling threshold requirement for activation of hepatic APR genes through IL-6 [30]. However, further studies in mutant mice carrying null mutations (knockout, KO) of either individual ligands ($IL\text{-}6^{KO}$) or their corresponding receptors ($gp130^{KO}$) [30] and transcription factors ($STAT3^{KO}$) [31] are needed to directly link T_3-induced IL-6 response, STAT3 activation, and APR. IL-6, the major stimulator of most APPs, is primarily produced by Kupffer cells, the resident macrophages of the liver representing the largest fixed population of macrophages in the body [18,28]. Up-regulation of IL-6 by T_3 may be related to the enhanced respiratory burst activity of Kupffer cells observed under conditions of liver macrophage hyperplasia [32], leading to the redox activation of NF-κB [10,11,16] controlling IL-6 expression [33].

As a member of the IL-6 family of cytokines, IL-6 stimulates the expression of the type II APPs such as β-fibrinogen through binding to the specific receptor α subunit [30]. This induces the homodimerization of the signal transducer glycoprotein 130 (gp130), leading to the activation of JAK, STAT3 phosphorylation and dimerization, and translocation of the dimers to the

nucleus where they bind to response elements present in the promoter regions of the type II APP genes (Fig. 5) [17,30,34]. STAT3-binding sites are also found in the promoter of type I APP genes [18], thus raising the possibility that T_3-induced activation of the IL-6/STAT3 cascade may stimulate also the expression of type I APP genes synergistically with the IL-1 family of cytokines (Fig. 5) [17,30]. The involvement of the latter pathway in T_3-induced liver APR is supported by the redox activation of NF-κB and the NF-κB-dependent expression of TNF-α observed at the Kupffer cell level after hormone administration [11,16]. TNF-α plays a crucial role in the up-regulation of hepatic type I APPs such as haptoglobin (Fig. 5), either through the NF-κB and/or AP-1 pathways or by stimulation of mitogen-activated protein kinases involving C/EBPβ [17,18,35], which may provide a point for cross-talk with the type II APP signaling pathway [17]. This proposal merits further investigation in the hyperthyroid state using either an antisense oligonucleotide against TNF-α, which abolishes the TNF-α response and markedly reduces liver oxidative stress induced by T_3 [12], or the TNF-α receptor-1 knockout mice lacking TNF-α signaling [14,35].

Up-regulation of the hepatic APR by T_3 may represent a defense mechanism against the detrimental effects of oxidative stress occurring both in the liver and in extrahepatic tissues with a calorigenic response [9], considering that the goal of the APR is to restore homeostasis [19]. This is achieved by redirecting the gene expression of the liver into a pattern fulfilling immune, transport, coagulation, and antioxidant functions that adapt tissues to the metabolic perturbations occurring under inflammatory and oxidative stress conditions [18,19]. In addition to the APR, up-regulation of iNOS [11], MnSOD, Bcl-2 [16], and uncoupling proteins (UCPs) [36] by T_3 may also contribute to cell defense from excessive cytokine-mediated lethality and ROS toxicity. (i) Induction of iNOS can deactivate NF-κB by scavenging superoxide radicals ($O_2^{\bullet -}$) and/or allowing NF-κB p50 nitrosylation [37]; (ii) MnSOD up-regulation will increase $O_2^{\bullet -}$ removal from mitochondria and cytosol, whose production is enhanced by T_3 [9]; (iii) Bcl-2 induction will diminish

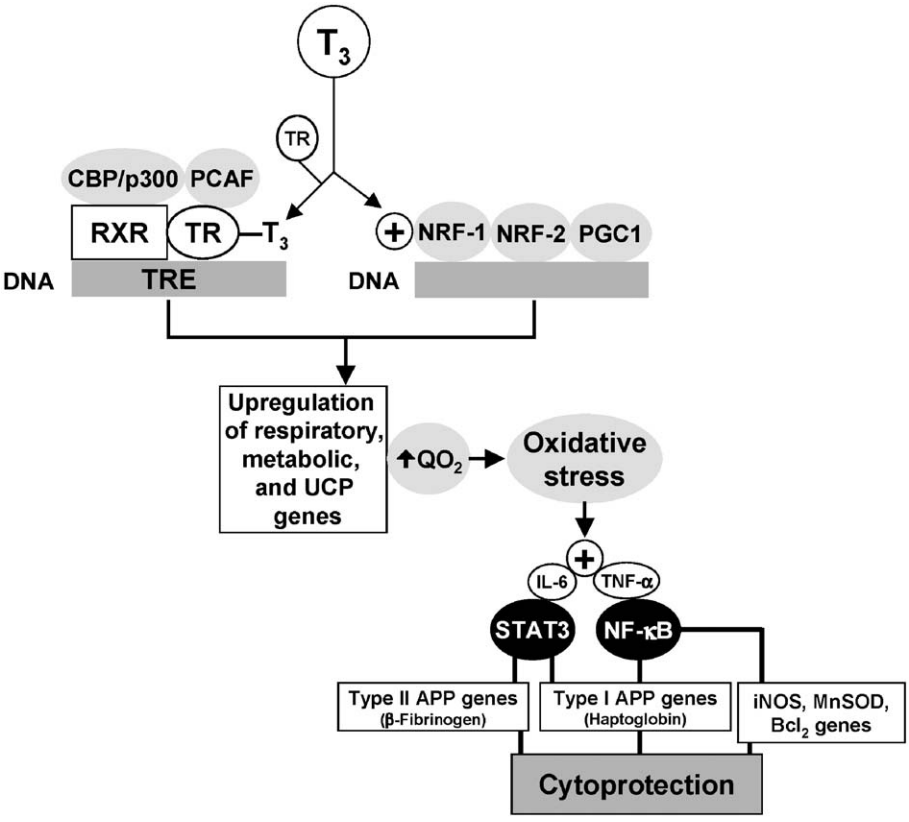

Fig. 5. Diagram summarizing T_3-induced transcriptional activation mechanisms and their relationships with O_2 consumption, oxidative stress, and redox up-regulation of gene expression. Genomic pathways triggered by T_3 involve (i) liganded-thyroid hormone receptors (TR), dimerized with retinoic acid receptor (RXR) and bound to coactivators such as the CREB binding protein (CBP)/CBP-related protein (p300) complex and p300/CBP-associated factor (PCAF), interacting with TH response elements (TRE) in DNA and/or (ii) indirect activation of intermediate factors such as nuclear respiratory factor-1 (NRF-1), NRF-2, and peroxisome proliferator-activated receptor-γ coactivator-1 (PGC1), interacting with specific binding sites in DNA, thus up-regulating the expression of respiratory genes. The consequent increase in the synthesis of enzymes involved in energy metabolism leads to higher rates of O_2 consumption (QO_2) and ATP production, the latter being partially balanced by intrinsic uncoupling afforded by induction of uncoupling proteins (UCP) by T_3. T_3-induced acceleration of energy metabolism enhances the generation of reactive oxygen species, which determines the consumption of cellular antioxidants and inactivation of antioxidant enzymes, thus inducing oxidative stress and up-regulation of gene expression. In the liver, this nongenomic mechanism is initiated by Kupffer cell-derived mediators such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), with the activation of the redox-sensitive transcription factors and signal transducer and activator of transcription 3 (STAT3) and nuclear factor-κB (NF-κB), respectively. Enhancement in the expression of genes controlled by STAT3 and NF-κB is achieved, namely, of type I and II acute phase proteins (APP), inducible nitric oxide synthase (iNOS), manganese superoxide dismutase (MnSOD), and Bcl-2 genes, thus conferring cytoprotection.

apoptosis commitment and increase the antioxidant potential by glutathione repletion [38]; (iv) up-regulation of UCPs may decrease mitochondrial ROS generation due to mild uncoupling [36] and/or allow the transport of peroxidized unsaturated fatty acid anions to the outer side of the inner mitochondrial membrane, thus decreasing oxidative damage to mitochondrial DNA and proteins [39].

In conclusion, T_3 administration achieves the redox up-regulation of the APR of the liver, as evidenced by the induction of the APPs haptoglobin and β-fibrinogen. The response is associated with the Kupffer cell-dependent release of IL-6 and activation of the JAK/STAT3 cascade, which may be contributed by the T_3-induced TNF-α release activating the IKK/NF-κB pathway [11,16]. The proposed mechanisms represent nongenomic processes triggered by ROS produced by the calorigenic action of T_3, which is secondary to redox-independent genomic pathways (Fig. 5). The latter contention is supported by the lack of effects of α-tocopherol or *N*-acetylcysteine pretreatment on T_3-induced liver mRNA expression of mitochondrial glycerol-3-phosphate dehydrogenase and the adenine nucleotide translocator 2, two target proteins of thyroid hormone action at the transcriptional level [40]. Data presented are in agreement with the increasing effect of thyroxine (T_4) administration on the hepatic synthesis of the APPs α_1-acid glycoprotein, α_2-(acute phase) globulin, haptoglobin [41], and ceruloplasmin [42], although the mechanisms underlying these effects of T_4 were not studied. In the case of the APP ferritin, T_3 was shown to up-regulate its hepatic expression posttranscriptionally, by increasing the iron-induced displacement of rat and human iron regulatory protein from the iron-responsive element present in ferritin mRNA [43]. Collectively, these findings point to a variety of nongenomic processes and signaling cascades influenced by thyroid hormones, which, in addition to those of genomic nature, may provide a better understanding of T_3 action and the outcome of thyroid gland dysfunction.

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