Thyroid hormone-induced oxidative stress in rodents and humans: A comparative view and relation to redox regulation of gene expression ☆

Virginia Fernández [a], Gladys Tapia [a], Patricia Varela [b], Pamela Romanque [a], Denise Cartier-Ugarte [a], Luis A. Videla [a,*]

[a] *Programa de Farmacología Molecular y Clínica, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70000, Santiago-7, Chile*
[b] *Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70000, Santiago-7, Chile*

Abstract

Thyroid hormone (3,3′,5-triiodothyronine, T_3) exerts significant actions on energy metabolism, with mitochondria being the major target for its calorigenic effects. Acceleration of O_2 consumption by T_3 leads to an enhanced generation of reactive oxygen and nitrogen species in target tissues, with a higher consumption of cellular antioxidants and inactivation of antioxidant enzymes, thus inducing oxidative stress. This redox imbalance occurring in rodent liver and extrahepatic tissues with a calorigenic response, as well as in hyperthyroid patients, is further enhanced by an increased respiratory burst activity in Kupffer cells, which may activate redox-sensitive transcription factors such as NF-κB thus up-regulating gene expression. T_3 elicits an 80-fold increase in the serum levels of tumor necrosis factor-α (TNF-α), which is abolished by pretreatment with the antioxidants α-tocopherol and *N*-acetylcysteine, the Kupffer-cell inactivator $GdCl_3$, or an antisense oligonucleotide against TNF-α. In addition, T_3 treatment activates hepatic NF-κB, a response that is (i) inhibited by antioxidants and $GdCl_3$ and (ii) accompanied by induced mRNA expression of the NF-κB-responsive genes for TNF-α and interleukin (IL)-10. T_3 also increases the hepatic levels of mRNA for IL-1α and those of IL-1α in serum. Up-regulation of liver iNOS expression is also achieved by T_3, through a cascade initiated by TNF-α and involving IκB-α phosphorylation and NF-κB activation. In conclusion, T_3-induced oxidative stress in the liver enhances the DNA-binding of NF-κB and the NF-κB-dependent expression of cytokines and iNOS by actions primarily exerted at the Kupffer cell level.

Keywords: Thyroid hormone; Calorigenesis; Oxidative stress; Liver; Kupffer cell; Gene expression; Nuclear factor-κB; Cytokines

Contents

☆ This paper is part of a special issue of CBP dedicated to "The Face of Latin American Comparative Biochemistry and Physiology" organized by Marcelo Hermes-Lima (Brazil) and co-edited by Carlos Navas (Brazil), Tania Zenteno-Savín (Mexico) and the editors of CBP. This issue is in honour of Cicero Lima and the late Peter W. Hochachka, teacher, friend and devoted supporter of Latin American science.

* Corresponding author. Tel.: +56 2 9786256; fax: +56 2 7372783.
E-mail address: lvidela@med.uchile.cl (L.A. Videla).

1. Mechanisms regulating cellular respiration by iodothyronines

Thyroid hormones are key regulators of growth, development, and metabolism. The development of a hyperthyroid state in vertebrates leads to an enhancement in their basal metabolic rate (BMR) due to an increase in the rate of O_2 consumption in most tissues (Fig. 1), excluding the spleen, testis, and adult brain (Baker and Klitgaard, 1952). Current available data indicate that thyroid calorigenesis is achieved by both (i) a short-term signaling mechanism mediated by 3,5-diiodothyronine (3,5-T_2) and 3,3′,5-triiodothyronine (T_3) with the allosteric activation of cytochrome *c* oxidase (Arnold et al., 1998; Moreno et al., 2002), and (ii) a long-term pathway inducing nuclear and mitochondrial gene transcription through T_3 signaling (Oppenheimer et al., 1996; Goglia et al., 1999), thus stimulating basal thermogenesis (Fig. 1). The T_3-dependent long-term signaling mechanism induces the synthesis of the enzymes involved in energy metabolism and the components of the respiratory chain apparatus, leading to a higher capacity of oxidative phosphorylation (Soboll, 1993; Videla, 2000). The consequent increase in ATP production is partially balanced by the concomitant diminution in the efficiency of ATP synthesis due to intrinsic uncoupling, afforded by induction of uncoupling proteins (UCPs) by T_3 (Lanni et al., 2003), with the consequent enhancement in mitochondrial O_2 uptake (Fig. 1). Although these short- and long-term pathways are mainly responsible for the increased cellular respiration induced by hyperthyroid state, other processes may also play a role, namely, (i) energy expenditure due to higher active cation transport, (ii) loss of energy from futile cycles due to increases in catabolic and anabolic pathways of intermediary metabolism, (iii) higher activity of membrane-bound enzymes associated with electron transfer and metabolite carriers due to changes in the lipid composition of mitochondrial membranes (Soboll, 1993), and/or (iv) O_2 equivalents related to oxidative stress (Fig. 1) (Sies, 1986; Videla, 2000), a redox imbalance that leads to various pathophysiological events in the liver (Videla et al., 1995; Jaeschke et al., 2002).

2. Thyroid calorigenesis and liver oxidative stress

2.1. T_3-induced hepatic free-radical activity and depletion of antioxidant defenses

The relation between thyroid calorigenesis and oxidative stress has been studied extensively (Videla, 2000) in line with the significant correlation established for BMR and the lipid peroxidative potential of tissues from several mammalian species (Cutler, 1985).

Experimental animals made hyperthyroid by T_3 administration exhibit a thermogenic response that coincides with

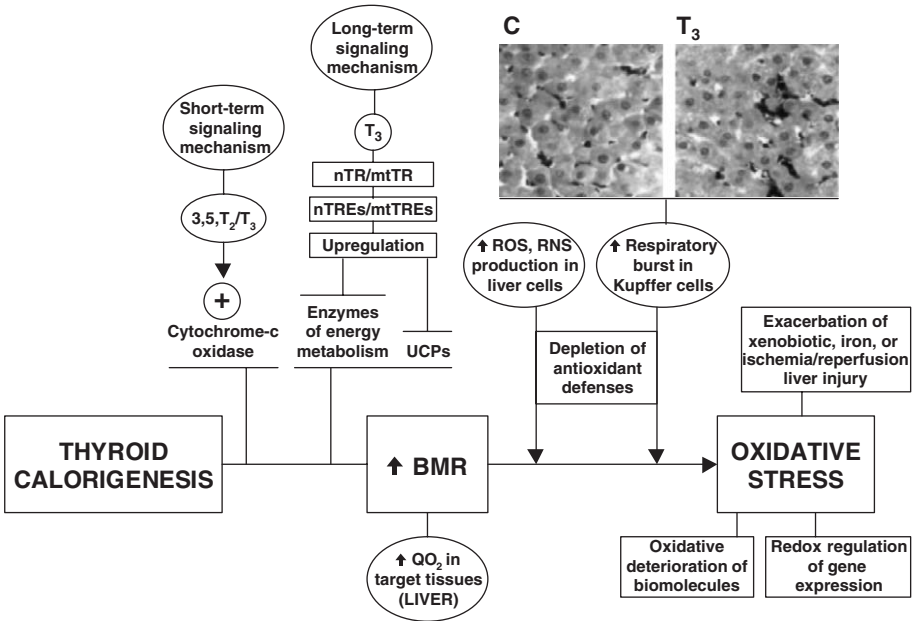

Fig. 1. Mechanisms involved in thyroid calorigenesis and its relationship to oxidative stress. Oxidative phosphorylation is mainly controlled by the ATP/ADP ratio at the cytochrome *c* oxidase level which is modulated by thyroid hormones, as 3,5-diiodothyronine (3,5-T_2) and 3,3′,5-triiodothyronine (T_3) eliminate the allosteric inhibition of the oxidase exerted by ATP [short-term mechanism]. Activation of nuclear (n) and mitochondrial (mt) gene transcription involve (i) a direct interaction of T_3 with nuclear T_3 receptors (nTR) and mtTR, which act as active transcription factors, and (ii) binding of the T_3–nTR and T_3–mtTR complexes to specific sites in DNA, the respective thyroid hormone responsive elements, nTREs and mtTREs, thus triggering the expression of most respiratory genes including those for uncoupling proteins (UCPs) [long-term mechanism]. Up-regulation of gene transcription by T_3 may also involve the stimulation of intermediate activators (such as nuclear respiratory factor 1 and 2) and/or coactivators (such as peroxisome proliferator-activated receptor γ coactivator-1). As a result, the basal metabolic rate (BMR) of the individual is enhanced, due to increases in the rate of O_2 consumption (QO_2) of target tissues. In the liver, thyroid calorigenesis induces the generation of reactive oxygen (ROS) and nitrogen (RNS) species and the respiratory burst activity in Kupffer cells, reducing the antioxidant potential of the liver, thus determining a higher oxidative stress status in the tissue and associated functional consequences. Structural characteristics of liver parenchyma in a control rat (C) and in a T_3-treated animal (T_3), perfused in vitro with colloidal carbon (0.5 mg/ml) at comparable flow rates for 15 min (Tapia et al., 1997), show T_3-induced hyperplasia and hypertrophy of Kupffer cells as visualized by carbon phagocytosis (hematoxylin–eosin; magnification=600×).

increases in the rate of O_2 consumption by the liver (Fig. 2) (Fernández et al., 1985). Acceleration of hepatic respiration during thyroid calorigenesis leads to a marked elevation in the rate of superoxide ($O_2^{\bullet -}$) production by liver submitochondrial particles in the presence of NADH (142%) or succinate (152%), with higher rates of hydrogen peroxide (H_2O_2) generation, either under basal conditions or in the succinate-supported process, both in the absence and presence of antimycin-A (Fernández and Videla, 1993a). Enhancement in liver mitochondrial H_2O_2 production also occurs in the transition from hypothyroid to hyperthyroid state as a function of the content of autoxidizable electron carriers (Venditti et al., 2003), an effect that is mimicked by cold-induced hyperthyroidism (Venditti et al., 2004). Development of a hyperthyroid state in rats results in the proliferation of the smooth endoplasmic reticulum, with higher activities of NADPH–cytochrome *P*450 reductase (Tata et al., 1962; Ram and Waxman, 1992) and NADPH oxidase (Fernández et al., 1985). The latter enzymatic activity represents the oxidase activity of cytochrome *P*450 responsible for $O_2^{\bullet -}$ and H_2O_2 production (Goeptar et al., 1995), which has been recently related to the induction of the highly pro-oxidant cytochrome *P*4502E1 isoform by T_3 (Fernández et al., 2003). These changes, and the increase in the activity of the NADPH-generating system glucose-6-phosphate dehydrogenase (Simon-Giavarotti et al., 1998), are likely to determine high rates of cytochrome *P*450 reduction in hyperthyroid state, thus explaining the increase in (i) NADPH-dependent antioxidant-sensitive rates of O_2 uptake (Fernández et al., 1988), (ii) NADPH-supported $O_2^{\bullet -}$ generation (Fernández et al., 1985), and (iii) the biotransformation of a variety of xenobiotics (Videla, 2000). In addition to the T_3-induced liver mitochondrial and microsomal capacity of reactive oxygen species (ROS) generation, cytosolic enzymatic mechanisms are also increased, namely, the well-known ROS generator xanthine oxidase (Huh et al., 1998) and the reactive nitrogen species (RNS)-producing system nitric oxide synthase (NOS) (Fernández et al., 1997). These changes are presumed to occur primarily at the parenchymal cell level, with Kupffer cells playing a secondary role, as evidenced by the enhancement in the respiratory burst activity after T_3 administration (Tapia et al., 1997). The latter process is mainly due to the activity of NADPH oxidase, with a smaller contribution by NOS (Wang et al., 1993), being the T_3-induced increase in liver NOS activity partially inhibited by the Kupffer cell inactivator gadolinium chloride ($GdCl_3$) (Fernández et al., 1997). T_3-induced liver free-radical generation occurs in concomitance with enhanced respiratory burst activity and chemiluminescent response in polymorphonuclear leukocytes, both in experimental and human hyperthyroidism (Videla et al., 1993; Fernández and Videla, 1995).

Thyroid hormone-induced liver free-radical activity is associated with a diminution in antioxidant defenses (Fig. 1), namely, (i) reduction in the activity of superoxide dismutase (SOD) and catalase (Asayama et al., 1987; Fernández et al., 1988), probably due to enzyme inactivation by the ROS/RNS produced, and (ii) depletion of reduced glutathione (GSH) (Fernández et al., 1988, 1991; Huh et al., 1998), α-tocopherol, β-carotene, and lycopene (Simon-Giavarotti et al., 1998), associated with increased consumption. GSH depletion, a major hepatic alteration induced by hyperthyroidism in experimental animals (Fernández et al., 1988, 1991; Huh et al., 1998) and man (Sir et al., 1987), is determined by both loss of the tripeptide into the blood and higher intracellular catabolism, despite the enhancement in the rate of GSH synthesis and in the GSH turnover rate triggered in the liver (Fernández et al., 1991; Fernández and Videla, 1996a).

In conclusion, a higher pro-oxidant activity is developed in the liver as result of the functional interdependence established between thyroid calorigenesis, hepatic respiration, and ROS/RNS generation (Fig. 1), which accounts for 16–25% of the net increase in the total rate of O_2 consumption by T_3 (Fernández and Videla, 1993b), including O_2 equivalents used in the oxidation of hepatic biomolecules. This T_3-induced liver free-radical activity is paralleled by a decrease in antioxidant defenses, leading to oxidative stress (Sies, 1986) in liver, as

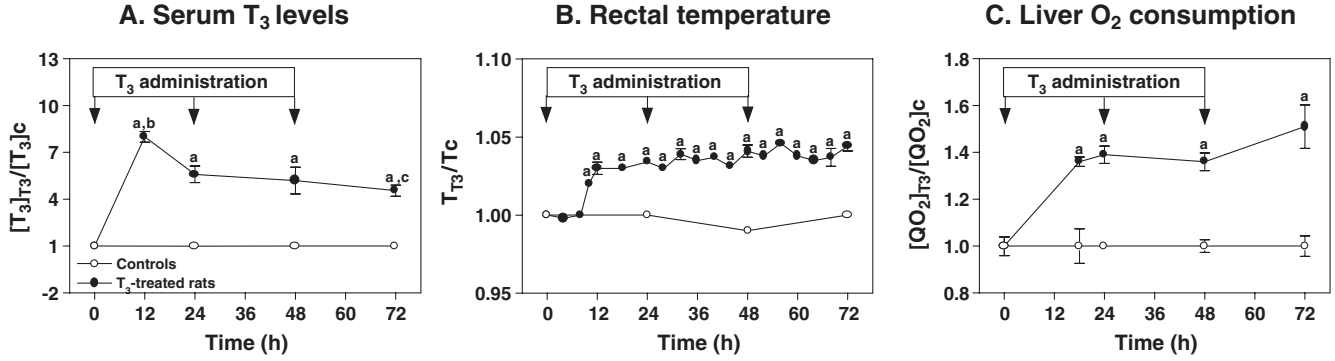

Fig. 2. Time course effects of T_3 administration on (A) the serum T_3 levels, (B) the rectal temperature of the animals, and (C) the rate of O_2 consumption of rat liver. Female Sprague-Dawley rats weighing 200–300 g fed at libitum received either daily doses of 0.1 mg T_3/kg (i.p.) for 3 consecutive days (arrows) or equivalent volumes of vehicle (0.1 N NaOH, controls) and studies were performed at the indicated times. Serum T_3 levels were determined with a radioimmunoassay, the rectal temperatures of the rats were measured with a thermocouple, and the basal rate of O_2 consumption by the liver was determined polarographically in perfusion studies. Data from control rats at time zero were set to unity, and values at other time points were normalized to this; each data point represents the mean ± S.E.M. for three to fifteen different animals; significance studies (one-way ANOVA and the Newman–Keuls test): $^{a}p<0.05$ vs. controls; $^{b}p<0.05$ vs. T_3-treated rats at 72 h; $^{c}p<0.05$ vs. T_3-treated rats at 12 h (modified from Tapia et al., 2003; Fernández et al., 2005).

well as in extrahepatic tissues exhibiting a calorigenic response (Videla, 2000).

2.2. Functional consequences of T_3-induced liver oxidative stress

At the cellular level, oxidative stress leads to a wide spectrum of responses, depending on the cell type, the level of ROS achieved, and the duration of the exposure (Martindale and Holbrook, 2002; Dröge, 2002). Under conditions of thyrotoxicosis, T_3-induced liver oxidative stress triggers different molecular changes associated with either cell dysfunction or adaptive responses to injury (Fig. 1).

As a consequence of the enhanced oxidative stress imposed on the liver by thyroid calorigenesis, damage to polyunsaturated fatty acids, proteins, and DNA have been evidenced by the increases in biochemical indicators of (i) lipid peroxidation [thiobarbituric acid reactants (TBARs) (Fernández et al., 1985; Fernández and Videla, 1996b; Venditti et al., 1997; Huh et al., 1998), hydroperoxide formation (Landriscina et al., 1988), and chemiluminescence (Marzoev et al., 1982; Fernández et al., 1985, 1988)], (ii) protein oxidation [content of protein hydrazone derivatives (Tapia et al., 1999)], and (iii) DNA oxidation [8-oxo-deoxyguanosine levels (Andican et al., 2004)], in experimental animals. In man, hyperthyroidism is characterized by significant changes in circulating parameters related to oxidative stress, including (i) increased levels of TBARs (Videla et al., 1988; Ademoglu et al., 1998; Seven et al., 1998; Adali et al., 1999; Bianchi et al., 1999; Sewerynek et al., 2000; Komosinska-Vessev et al., 2000; Guerra et al., 2001; Yavuz et al., 2004; Bednarek et al., 2004) and conjugated dienes (Komosinska-Vessev et al., 2000; Sewerynek et al., 2000), (ii) elevated levels of H_2O_2 and lipid hydroperoxides (Bednarek et al., 2004), and (iii) reduced levels of thiols (Wilson et al., 1989; Adali et al., 1999; Komosinska-Vessev et al., 2000), ascorbic acid (Ademoglu et al., 1998; Seven et al., 1998), α-tocopherol (Ademoglu et al., 1998; Bianchi et al., 1999), and coenzyme-Q (Bianchi et al., 1999). The above changes are either reduced or normalized by thyrostatic therapy or antioxidant supplementation, correlate with the elevation in urinary TBARs levels (Videla et al., 1988) and chemiluminescent response (Lissi et al., 1992), and occur concomitantly with a higher susceptibility of erythrocytes to an oxidant challenge (Videla et al., 1988). In the liver, T_3-induced oxidative stress exacerbates hepatic injury caused by other harmful stimuli, probably due to potentiation of the pro-oxidant state and increased Kupffer cell functioning due to macrophage hyperplasia and hypertrophy (Fig. 1) (Tapia et al., 1997; Videla, 2000).

In addition to the oxidative damage to hepatic biomolecules that may compromise their biological functions and cell viability, ROS/RNS may represent important signals regulating either protein function, through reversible oxidation and/or nitrosation of protein sulfhydryls (Klatt and Lamas, 2000), or gene expression (Fig. 1), in the different cells of the hepatic sinusoid (Tsukamoto and Lin, 1997; Martindale and Holbrook, 2002). The latter mechanism appears to be mediated by ROS and lipid oxidation products of ROS-dependent reactivity, modulating kinases, phosphatases, and/or redox-sensitive transcription factors (Thannickal and Fanburg, 2000; Poli et al., 2004).

3. T_3-induced redox regulation of gene expression in rat liver: role of Kupffer cells

3.1. Nuclear factor-κB (NF-κB) DNA binding

T_3 administration to rats, as depicted in Fig. 2, resulted in significantly higher serum T_3 levels over control values, reaching close to steady-state conditions between 24 h and 72 h after treatment (Fig. 2A), with the development of a concomitant calorigenic response shown by the increase in the

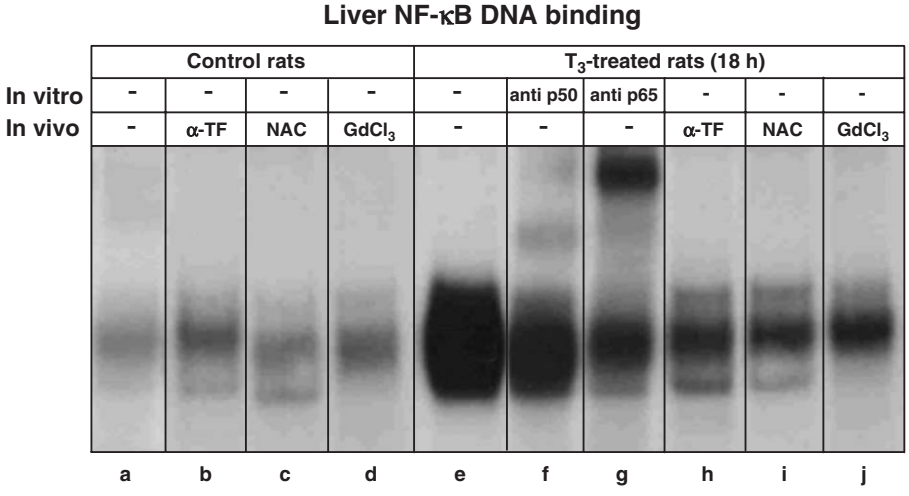

Fig. 3. Influence of T_3 administration on rat liver NF-κB DNA binding and effect of pretreatment with α-tocopherol (α-TF), *N*-acetylcysteine (NAC), and gadolinium chloride ($GdCl_3$) at 18 h after hormone administration. Representative autoradiographs of liver NF-κB DNA binding in control rats and T_3-treated animals and in separate groups of animals subjected to either (i) 100 mg α-tocopherol/kg (i.p.) 17 h prior to the first dose of T_3, (ii) 1 g *N*-acetylcysteine (NAC)/kg (i.p.) 30 min before T_3, or (iii) 10 mg gadolinium chloride ($GdCl_3$)/kg (i.v.) 24 h prior to T_3, assessed by electromobility shift assay (modified from Tapia et al., 2003).

rectal temperature of the animals, starting at 10 h and sustained up to 72 h after T_3 treatment (Fig. 2B). In addition, the rate of O_2 consumption of the liver was enhanced by 36%–51% ($p<0.05$) in the 18-h to 72-h time period studied (Fig. 2C), a response known to involve O_2 equivalents consumed in oxidative stress-related processes (Fig. 1) (Fernández and Videla, 1993b). Under these conditions, liver NF-κB DNA binding enhanced transiently by T_3 administration, an effect that was significant at 10 h and 14 h, maximal at 18 h (Fig. 3, e vs. a), and that returned towards control values at 20 h to 22 h (Tapia et al., 2003). The maximal increase in NF-κB DNA binding at 18 h after T_3 treatment involves the nuclear accumulation of NF-κB p50 and p65 subunits, as shown by supershift analysis (Fig. 3, f and g), and is significantly diminished by the in vivo pretreatment with α-tocopherol and *N*-acetylcysteine (Fig. 3, h vs. e and i vs. e, respectively). These data support a role for T_3-induced free-radical activity in the regulation of rat liver NF-κB activation, considering that pretreatment with α-tocopherol and *N*-acetylcysteine in euthyroid animals led to NF-κB DNA binding comparable to that in control rats (Fig. 3, b vs. a and c vs. a, respectively), thus discarding nonspecific actions of the antioxidants other than their scavenging of ROS (Thannickal and Fanburg, 2000). In agreement with data presented in Fig. 3, hepatic NF-κB DNA binding, and that of the redox-sensitive transcription factors AP-1 and STAT3, remain unaltered 0.5 h to 4 h after the administration of 0.2 mg T_3/kg (Pibiri et al., 2001), time period at which thyroid calorigenesis involving oxidative stress is not yet developed. Furthermore, the redox activation of hepatic NF-κB induced by T_3 is primarily due to Kupffer cell activity, considering its abolishment by $GdCl_3$ pretreatment (Fig. 3, j vs. e). This contention is supported by (i) the selective elimination of large Kupffer cells in periportal regions of the liver elicited by $GdCl_3$ (Hardonk et al., 1992), (ii) the lack of nonspecific effects of $GdCl_3$ in the experimental setup studied, revealed by the similar NF-κB DNA binding found in controls rats and euthyroid animals given $GdCl_3$ (Fig. 3, d vs. a), and (iii) the $GdCl_3$-dependent inhibition of particle phagocytosis (Tapia et al., 1997; Camandola et al., 1999), the associated respiratory burst activity (Tapia et al., 1997), and the T_3-induced oxidative stress-related parameters (Tapia et al., 1997; Simon-Giavarotti et al., 2002).

Direct evidence for the redox activation of NF-κB has been questioned by the rather large range of ROS concentrations required in vitro, which do not simulate their cellular generation in response to physiological stimuli (Thannickal

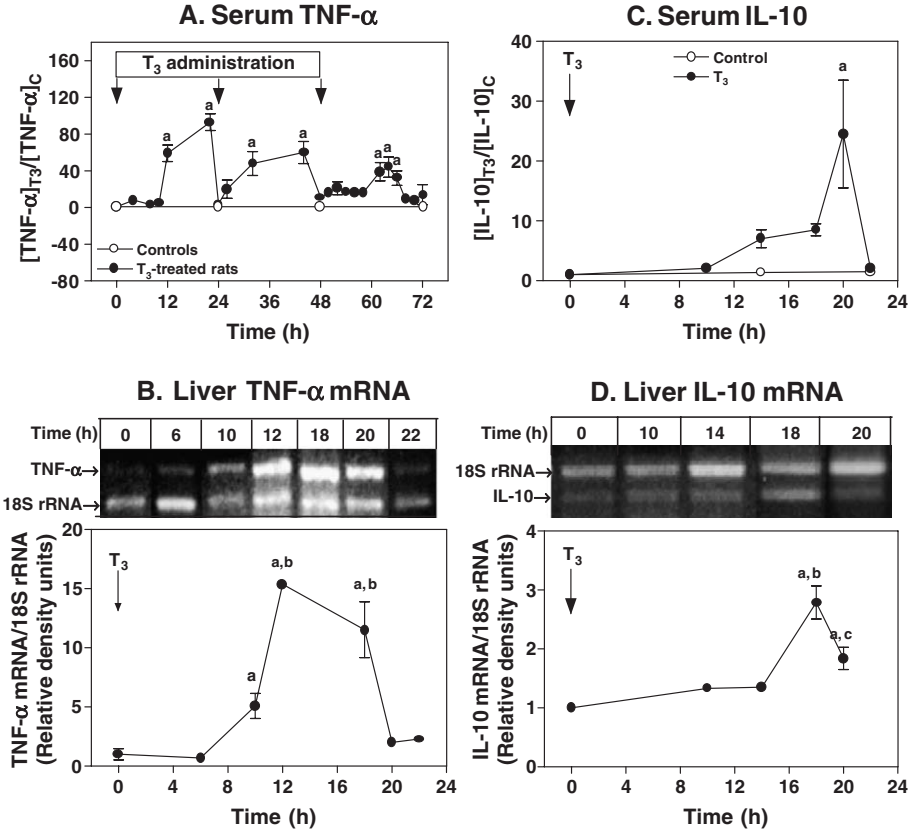

Fig. 4. Time course effects of T_3 administration on (A) tumor necrosis factor-α (TNF-α) levels in serum, (B) liver TNF-α mRNA content, (C) interleukin (IL)-10 levels in serum, and (D) liver IL-10 mRNA content. Animals were treated as described in the legend to Fig. 2. The serum levels of TNF-α and IL-10 were measured by enzyme-linked immunosorbent assays. Determination of TNF-α mRNA and IL-10 mRNA from isolated hepatic RNA were performed by reverse transcription–polymerase chain reaction assay. Cytokine mRNA data were expressed in terms of an invariant 18S rRNA to compare lane-lane equivalency in total RNA content. All data from control rats at time zero were set to unity, and values at other time points were normalized to this. Each data point represents the mean ± S.E.M. for three to twelve different animals. Significance studies (one-way ANOVA and the Newman–Keuls test): $^{a}p<0.05$ vs. control values; $^{b}p<0.05$ vs. T_3-treated rats at 6, 10, 20, and 22 h (in B) and at 10, 14, and 20 h (in D); $^{c}p<0.05$ vs. T_3-treated rats at 18 h (in D) (modified from Fernández et al., 2002; Tapia et al., 2003).

and Fanburg, 2000). Data presented indicate that thyroid calorigenesis represents a hormonal stimulus for the redox activation of hepatic NF-κB, a response that is triggered in Kupffer cells exhibiting higher respiratory burst activities. This is in agreement with studies showing that NF-κB activation can be achieved by physiological levels of ROS, which are produced during the respiratory burst after stimulation of isolated or cultured macrophages (Kaul and Forman, 1996) and in carbon-stimulated Kupffer cells in the isolated perfused rat liver (Romanque et al., 2003).

3.2. Cytokine and inducible nitric oxide synthase (iNOS) expression

NF-κB has prominent transcriptional control over the expression of several peptide mediators that are synthesized

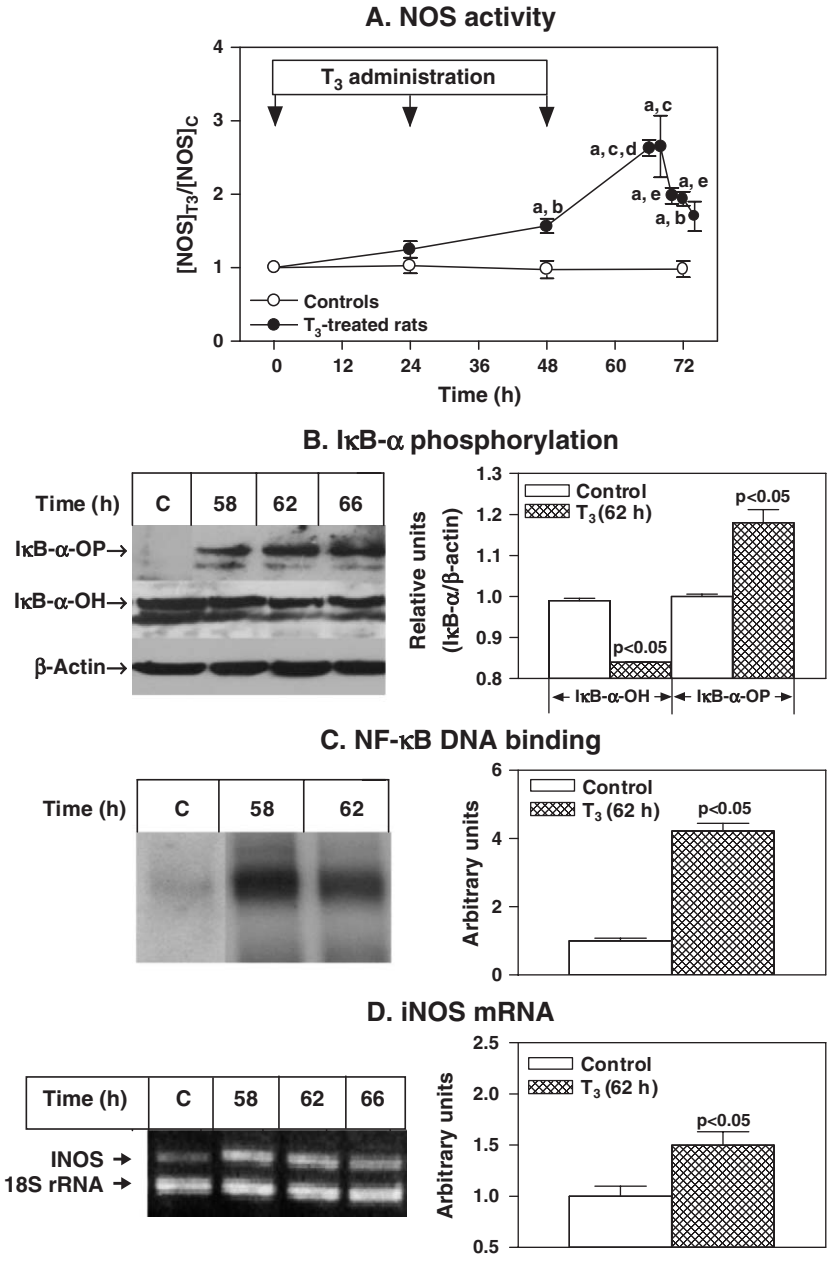

Fig. 5. Time course effects of T_3 administration on liver (A) nitric oxide synthase (NOS) activity, (B) inhibitor of κB-α (IκB-α) phosphorylation, (C) NF-κB DNA binding, and (D) inducible NOS (iNOS) mRNA levels. Animals were treated as described in the legend to Fig. 2. Total liver NOS activity was determined in cytosolic fractions by the oxidation of oxyhemoglobin to methemoglobin by NO monitored spectrophotometrically at 401 nm vs. 411 nm. Measurement of hepatic IκB-α phosphorylation was carried out by Western blot analysis and expressed in terms of β-actin to compare lane–lane equivalency in total protein content; NF-κB DNA binding was assessed by electromobility shift assay; iNOS mRNA content was measured by reverse transcription–polymerase chain reaction assay and expressed in terms of an invariant 18S rRNA to compare lane–lane equivalency in total RNA content. All data from control rats were set to unity, and values at 62 h after T_3 treatment were normalized to this. Each data point represents the mean±S.E.M. for three to thirteen different animals. Significance studies (one-way ANOVA and the Newman–Keuls test): $^{a}p<0.05$ vs. controls; $^{b}p<0.05$ vs. T_3-treated rats at 66 h and 68 h; $^{c}p<0.05$ vs. T_3-treated rats at 24 h and 48 h; $^{d}p<0.05$ vs. T_3-treated rats at 74 h; $^{e}p<0.05$ vs. T_3-treated rats at 24 h (modified from Fernández et al., 2005).

and released by Kupffer cells and other sinusoidal cells, including cytokines, growth factors, and chemokines (Tsukamoto and Lin, 1997). Under conditions of close to steady-state T_3 levels in serum (Fig. 2A) with sustained calorigenic (Fig. 2B) and liver respiratory (Fig. 2C) responses, T_3 up-regulated the expression of the NF-κB-responsive gene for tumor necrosis factor-α (TNF-α), leading to substantial but fluctuating increments in the serum levels of the cytokine (Fig. 4A). Within the period of 0 h to 24 h, TNF-α mRNA expression in the liver of T_3-treated rats was observed after 10 h to 18 h (Fig. 4B), with the parallel increase in the serum levels of TNF-α (Fig. 4A), which returned towards control values at 20 h to 24 h. Suppression of the T_3-induced TNF-α response may be due to the late expression of the NF-κB-responsive gene for interleukin (IL)-10, characterized by higher hepatic IL-10 mRNA levels at 18 h (Fig. 4D) and peak serum values of IL-10 at 20 h (Fig. 4C). This contention is supported by the NF-κB deactivating action exerted by IL-10, which seems to be due to preserved expression of the inhibitor of κB (IκB) (Lentsch et al., 1997). In agreement with data presented, T_3-induced TNF-α response is suppressed by α-tocopherol and NAC, $GdCl_3$, or the antisense oligonucleotide TJU-2755 targeting the primary RNA transcript for TNF-α (Fernández et al., 2002). T_3 also up-regulated the expression of IL-1α with a time profile closely related to that of TNF-α, pro-inflammatory responses that are abrogated by delayed IL-10 expression (Fig. 4C and D), thus limiting inflammatory injury (Tapia et al., 2003).

In addition to cytokines, NF-κB regulates the expression of various liver enzymes including inducible NOS (iNOS), both in Kupffer cells, endothelial cells, and hepatocytes (Tsukamoto and Lin, 1997). Under the pro-oxidant conditions imposed by thyroid calorigenesis (Fig. 2), ROS production is an early and sustained event (Fernández et al., 1985, 1988), whereas that of RNS by hepatic NOS is a slowly developing response, reaching peak values at 66 h to 68 h after hormone treatment (Fig. 5A). The latter effect of T_3 involves up-regulation of hepatic iNOS expression through a redox mechanism induced at the Kupffer cell level, with production and release of TNF-α, as shown by the increased serum levels of the cytokine at 62 h to 66 h after T_3 (Fig. 4A). Following interaction of TNF-α with surface TNF-α receptor 1 (TNFR-1) in target cells, intracellular signals may lead to the activation of receptor kinases, in line with the significant increase in liver IκB-α serine 32 phosphorylation (Fig. 5B) suggesting the activation of the IκB kinase complex, IKK (Janssen-Heininger et al., 2000). Ultimately, this intracellular signaling pathway and the proposed TNF-α-dependent phosphorylation of p65 at serine 529 (Wang and Baldwin, 1998) trigger NF-κB DNA binding (Fig. 5C) and iNOS mRNA expression (Fig. 5D), changes that are abolished by α-tocopherol and $GdCl_3$ treatment prior to T_3 (Fernández et al., 2005).

4. Concluding remarks

Thyroid calorigenesis resulting from acceleration of energy metabolism and secondary electron transfer processes leads to a higher generation of ROS in target tissues including the liver, both at the hepatocyte and Kupffer cell levels. This pro-oxidant condition enhances the oxidative stress status of the liver when the diminution in the antioxidant potential is not adequately compensated, leading to: (i) substantial oxidative deterioration of biomolecules, with loss of their functions that may compromise cell viability; (ii) a higher susceptibility of the liver to toxic stimuli that exacerbate liver injury; and (iii) up-regulation of gene expression.

Thyroid hormone-induced gene expression includes the antioxidant-sensitive expression of the cytokines TNF-α, IL-1α, and IL-10. Cell signaling is primarily exerted at the Kupffer cell level, which is the most important source of TNF-α, produced and released in response to several stimuli including ROS derived from activated NADPH oxidase (Forman and Torres, 2002). Subsequent TNF-α-TNFR-1 coupling may result in liver iNOS expression, through a cascade involving IκB-α phosphorylation and NF-κB activation. Up-regulation of iNOS can be interpreted as a defense response against T_3-induced oxidative stress by protecting the liver from pro-inflammatory cytokine-mediated lethality and ROS toxicity. This can be achieved by the rather large NO production that is expected in conditions of iNOS expression, which may (i) scavenge ROS to reduce the oxidation potential that oxidizes biomolecules and activates NF-κB, (ii) nitrosylate NF-κB p50 to diminish NF-κB DNA binding, and/or (iii) lead to the induction and/or stabilization of IκB (Janssen-Heininger et al., 2000; Laroux et al., 2000). In addition, induction of liver UCPs by T_3 (Fig. 1) may contribute to cell antioxidant defense, considering that (i) mild uncoupling by UCPs reduces the mitochondrial membrane potential, thus increasing O_2 uptake and blunting ROS production (Lanni et al., 2003), and that (ii) UCPs may transport peroxidized unsaturated fatty acid anions from the inner to the outer side of the inner mitochondrial membrane, with the consequent decrease in the oxidative damage to mitochondrial proteins and DNA (Goglia and Skulachev, 2003). Although the expression of cytokines and iNOS by T_3 seem to be a secondary mechanism of ROS induced by thyroid calorigenesis in the liver, its relation with the expression of other redox-regulated genes or with nuclear and mitochondrial transcription systems triggered by T_3 remains to be established.

Acknowledgments

Supported by grant 1030499 from FONDECYT (Chile).

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