

The role of thyroid hormone calorogenesis in the redox regulation of gene expression

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ABSTRACT

Thyroid hormone (TH; 3,3',5-triiodothyronine, T₃) is required for the normal function of most tissues, with major effects on O₂ consumption and metabolic rate. These are due to transcriptional activation of respiratory genes through the interaction of T₃-liganded TH receptors with TH response elements or the activation of intermediate factors, with the consequent higher production of reactive O₂ species (ROS) and antioxidant depletion. T₃-induced oxidative stress in the liver triggers the redox upregulation of the expression of cytokines (tumor necrosis factor- α [TNF- α], interleukin-10), enzymes (inducible nitric oxide synthase, manganese superoxide dismutase), and anti-apoptotic proteins (Bcl-2), via a cascade initiated by TNF- α produced by Kupffer cells, involving inhibitor of κ B phosphorylation and nuclear factor- κ B activation. Thus, TH calorogenesis triggers an expression pattern that may represent an adaptive mechanism to re-establish redox homeostasis and promote cell survival under conditions of ROS toxicity secondary to TH-induced oxidative stress. Mechanisms of expression of respiratory and redox-sensitive genes may be functionally integrated, which could be of importance to understand the complexities of TH action and the outcome of thyroid gland dysfunction.

Key terms: Thyroid hormone; Calorogenesis; Oxidative stress; Gene expression

INTRODUCTION

Thyroid hormones (TH) are required for the normal function of most tissues of the body, playing essential roles in growth, development, differentiation, and metabolism, with major effects on O₂ consumption (QO₂) and metabolic rate (Videla, 2000). Current available data indicate that TH calorogenesis is achieved by both (i) a short-term nongenomic signaling mechanism mediated by 3,5-diiodothyronine and 3,3',5-triiodothyronine (T₃) leading to the allosteric activation of cytochrome-c oxidase (Moreno et al., 2002), and (ii) a long-term pathway upregulating nuclear and mitochondrial gene transcription through T₃ signaling (Fig. 1) (Yen, 2001; Weitzel et al., 2001;

Lanni et al., 2003). In several target organs such as liver, a higher pro-oxidant activity is developed as result of the functional interdependence established between TH calorogenesis, cellular QO₂, and generation of reactive oxygen (ROS) (Fernández et al., 1985; 2003; Fernández & Videla, 1993a) and nitrogen (RNS) species (Fernández et al., 1997). This respiratory component accounts for 16-25% of the net increase in total QO₂ (Fernández & Videla, 1993b). T₃-induced free radical activity decreases the cellular antioxidant defenses, leading to oxidative stress (Fernández & Videla, 1996) in liver and in extrahepatic tissues of experimental animals exhibiting a calorogenic response (Fig. 1), a phenomenon also observed in human hyperthyroidism (Videla, 2000). Recent

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studies by Fernández et al. (2005a; 2005b) highlighted a novel nongenomic mechanism by which TH achieve the redox regulation of gene expression through activation of redox-sensitive transcription factors, as an adaptive response to re-establish redox homeostasis (Fig. 1).

REGULATION OF GENE TRANSCRIPTION BY THYROID HORMONE

Activation of gene transcription by T_3 involves its binding to different thyroid hormone (TH) receptor (TR) isoforms that are ligand-regulatable transcription factors

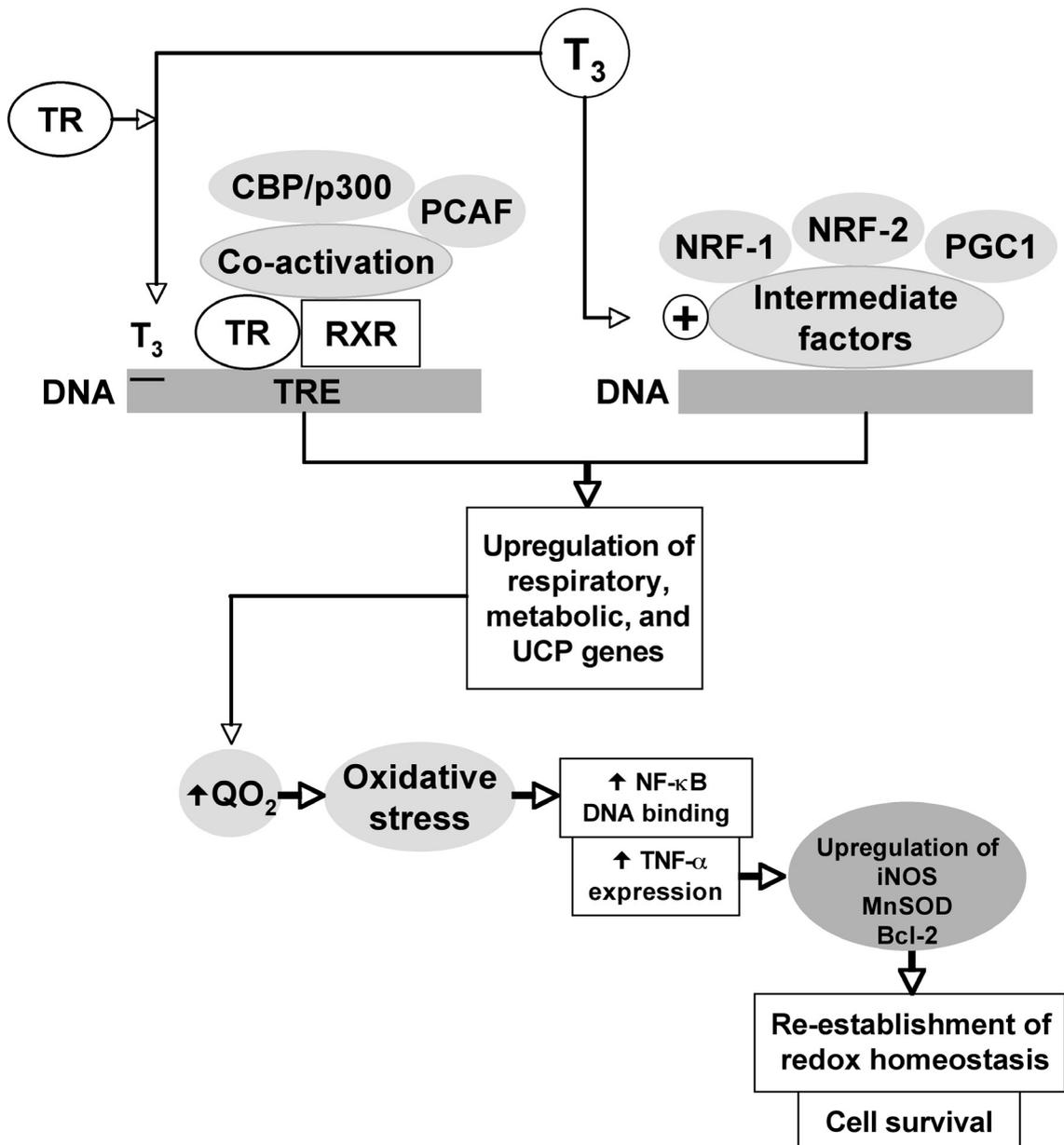


Figure 1: Relationship between transcriptional activation by T_3 , O_2 metabolism and oxidative stress, and the redox regulation of gene expression. Abbreviations: CBP, CREB binding protein; iNOS, inducible nitric oxide synthase; MnSOD, manganese superoxide dismutase; NF- κ B, nuclear factor- κ B; p300, CBP related protein; PCAF, p300/CBP-associated factor; T_3 , L-3,3',5'-triiodothyronine; TNF- α , tumor necrosis factor- α .

having a similar domain organization. Liganded TR isoforms can bind to TH response elements (TRE) in DNA, specially in the form of TR/retinoic acid receptor (RXR) heterodimers, which can form complexes with specific co-activators, thus regulating histone acetylation and determining gene transcription (Fig. 1). Alternatively, T₃-responsive genes that do not interact with TR may involve an indirect induction mechanism via the activation of intermediate factors, such as nuclear respiratory factor-1 (NRF-1) and NRF-2 or the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1)(Yen, 2001; Weitzel et al., 2001). These T₃-dependent signaling mechanisms induce the synthesis of the enzymes involved in energy metabolism leading to higher rates of O₂ consumption (QO₂), oxidative phosphorylation, and ATP production. The latter process being partially balanced by intrinsic uncoupling afforded through induction of uncoupling proteins (UCP) by T₃ (Lanni et al., 2003)(Fig.1). T₃-induced QO₂ may be also contributed by energy expenditure due to (i) higher active cation transport or from futile cycles coupled to increased catabolism and anabolism, (ii) higher activity of membrane-bound respiratory enzymes due to altered lipid composition of mitochondrial membranes, and (iii) O₂ utilization in oxidative stress induced by thyroid hormone calorogenesis (Soboll, 1993; Videla, 2000).

OXIDATIVE STRESS INDUCED BY THYROID HORMONE

In the liver, T₃-induced acceleration of QO₂ leads to elevation in superoxide radical and hydrogen peroxide generation at mitochondrial, microsomal, or cytosolic subcellular sites, as well as nitric oxide by nitric oxide synthase. T₃ also leads to hyperplasia and hypertrophy of Kupffer cells, with the resulting enhancement in the respiratory burst activity (Videla, 2000). This T₃-induced free radical activity diminishes the antioxidant

defenses leading to oxidative stress (Fernández & Videla, 1996), a condition that may lead to a variety of responses depending on the cell type, the level of pro-oxidants achieved, and the duration of the exposure. ROS and RNS species occur at low levels under normal conditions, however, persistent production of large amounts of them may induce significant oxidation of biomolecules, persistent changes in gene expression and signal transduction, thus leading to cell death (Dröge, 2002; Martindale & Holbrook, 2002). Contrarily, transient fluctuations in ROS and RNS levels may elicit regulation of protein function through reversible oxidation and/or nitrosation of protein sulfhydryls (Klatt & Lamas, 2000). In addition, regulation of gene expression is also achieved, through modulation of the activity of kinases, phosphatases, and redox-sensitive transcription factors (Thannickal & Fanburg, 2000; Poli et al., 2004).

REDOX REGULATION OF GENE EXPRESSION BY THYROID HORMONE

T₃-induced calorogenic effect, which becomes significant at 10-12 h after hormone administration, coincides with increases in liver QO₂ and in the serum levels of tumor necrosis factor- α (TNF- α) (Fernández et al., 2002). The latter response is maintained up to 22 h and is determined by actions exerted at the Kupffer cell level and these are related to the oxidative stress status achieved, as it is abolished by pretreatment with (i) the Kupffer-cell inactivator gadolinium chloride (GdCl₃), (ii) the antioxidants α -tocopherol and N-acetylcysteine (NAC), and (iii) an antisense oligonucleotide targeting the primary RNA transcript of TNF- α , prior to hormone administration (Fernández et al., 2002). These pretreatments also markedly reduced liver glutathione (GSH) depletion and the enhancement in biliary glutathione disulfide efflux by T₃, supporting the involvement of oxidative stress in the effects elicited by TH (Fernández et al., 2002). T₃-induced TNF- α response is paralleled by activation of

hepatic nuclear factor- κ B (NF- κ B), as assessed by electrophoretic mobility shift assay, which is suppressed by α -tocopherol, N-acetylcysteine, and GdCl₃ (Tapia et al., 2003). In agreement with the crucial role of NF- κ B in controlling the transcriptional activation of cytokine-encoding genes (Tsukamoto, 2002; Dröge 2002), livers from hyperthyroid animals with enhanced NF- κ B DNA binding show induced mRNA expression of TNF- α and interleukin (IL)-10, which correlates with increases in the serum levels of the cytokines (Tapia et al., 2003). Upregulation of the IL-1 α gene also occurred after T₃ administration, a response that may be due to TNF- α induction (Tapia et al., 2003). The latter changes could play a role in the onset of TH calorogenesis, as TNF- α and IL-1 α are considered endogenous pyrogens due to their direct effects on the hypothalamus, leading to activation of responses that decrease heat loss and increase heat production (Dinarello, 1999).

TNF- α released from Kupffer cells exhibits autocrine and paracrine actions in the liver through interactions with two surface receptors in target cells, TNF- α receptor 1 (TNFR-1) and TNFR-2, in order to mediate TNF- α -dependent signals from cell membrane to nucleus (Garg & Aggarwal, 2002; Tsukamoto, 2002). Signal cascades operating after TNF- α -TNFR-1 coupling are important in the homeostatic response of the liver to oxidative stress. These cascades trigger defense and reparative processes against injury under conditions of moderate pro-oxidant status and low levels of transient TNF- α expression (Garg & Aggarwal, 2002). Daily T₃ administration for three consecutive days resulted in a progressive increase in the oxidative stress status of the liver, with maximal increases in lipid peroxidation and protein carbonylation at 72 h after hormone treatment (Fernández et al., 2005a). Under these conditions, the serum levels of TNF- α are also enhanced, concomitantly with higher liver inhibitor of κ B- α (I κ B- α) serine 32 phosphorylation, NF- κ B DNA binding, and the mRNA expression of the NF- κ B-responsive genes encoding inducible NOS

(iNOS) (Fernández et al., 2005a), manganese superoxide dismutase (MnSOD), and Bcl-2 (Fernández et al., 2005b). The latter changes and the increase in the hepatic activity of NOS and MnSOD induced by T₃ are abrogated by the administration of α -tocopherol prior to T₃ (Fernández et al., 2005a; 2005b). This is in agreement with the normalization or diminution of oxidative stress-related parameters induced by hyperthyroid state in response to α -tocopherol (Asayama et al., 1989; Fernández et al., 2002), NAC (Fernández et al., 2002), or ascorbic acid (Seven et al., 1998), as well as antithyroid therapy alone (Videla et al., 1988; Adali et al., 1999) or combined with α -tocopherol (Adali et al., 1999). Suppression of T₃-induced gene expression by α -tocopherol and NAC, antioxidants having different mechanisms of action, strengthens the contention that the underlying mechanisms are oxidant dependent (Macdonald et al., 2003). In this context, non-antioxidant ligand-induced effects on specific proteins have been proposed to mediate cell signaling and regulation of gene expression by α -tocopherol (Azzi et al., 2004). However, the latter non-antioxidant mechanism is not imitated by structurally related (γ -tocopherol) or unrelated (NAC) antioxidants (Azzi et al., 2004) and its relationship with the redox activation of signaling cascades has not been established. Finally, the finding that the mRNA expression of two target proteins of TH action, namely, mitochondrial glycerol-3-phosphate dehydrogenase and the adenine nucleotide translocator 2, is not modified by α -tocopherol and NAC (Videla et al., 2007), suggests that redox regulation of gene transcription by T₃ is a secondary mechanism to those triggered by genomic pathways (Fig. 1).

CONCLUDING REMARKS

Recent data suggest that T₃ elicits the redox upregulation of gene expression as a secondary mechanism of ROS produced by TH calorogenesis, which is triggered by redox-independent (i) direct T₃-liganded

TR interactions with TRE in DNA and/or (ii) indirect induction processes. Upregulation of cytokine-encoding genes in Kupffer cells leads to a T_3 -induced TNF- α response, with concomitant I κ B- α phosphorylation suggesting the activation of the I κ B kinase complex, although the participation of other signaling kinases cannot be discarded.

Upregulation of hepatic iNOS, MnSOD, and Bcl-2 expression by T_3 may represent a defense mechanism by protecting the liver from cytokine-mediated lethality and ROS/RNS toxicity (Dröge, 2002; Martindale & Holbrook, 2002), which can be accomplished through different actions. First, high levels of NO that are expected upon iNOS expression may limit the redox activation of NF- κ B through $O_2^{\bullet-}$ scavenging, NF- κ B p50 nitrosylation, and/or I κ B induction and stabilization (Laroux et al., 2000). Second, upregulation of MnSOD will increase $O_2^{\bullet-}$ removal, whose production is increased by T_3 in mitochondria (Fernández & Videla 1993a; Venditti et al., 2003), microsomes (Fernández et al., 1985; 2003), and cytosol (Huh et al., 1998). Third, upregulation of hepatic Bcl-2 would diminish apoptosis commitment and increase the antioxidant potential of the liver due to a higher intracellular availability of GSH (Voehringer & Meyn, 2000). In addition, antioxidant defense mechanisms may involve the upregulation of the T_3 -responsive nuclear genes for UCP, considering that (i) mild uncoupling by UCP decreases the mitochondrial membrane potential below a critical level, thus increasing QO_2 and reducing $O_2^{\bullet-}/H_2O_2$ generation (Lanni et al., 2003) and (ii) UCP induction may transport peroxidized unsaturated fatty acid anions, in addition to native fatty acid anions, from the inner to outer side of the inner mitochondrial membrane (Goglia & Skulachev, 2003), with the consequent diminution in the oxidative damage to mitochondrial proteins and DNA (Lanni et al., 2003; Goglia & Skulachev, 2003). These observations, and the finding that the CREB binding protein and the related protein p300 activating liganded TR can

function as coactivator for various transcription factors including NF- κ B (Yen, 2001), suggest the integration of different T_3 -signaling inputs to achieve metabolic and redox balance under TH calorogenic conditions. Recent evidence has suggested alternate nongenomic mechanisms of TH action involving cell surface G protein-coupled TH membrane-bound binding sites and the activation of signal transducing kinases (Davis et al., 2002). This nongenomic pathway was recently shown to enhance $O_2^{\bullet-}$ generation and myeloperoxidase activity in rat and human neutrophils, either after addition of 3,5-diiodothyronine, T_3 , and T_4 in vitro (Mezosi et al., 2005) or following T_3 administration *in vivo* (Videla et al., 1993; Fernández & Videla, 1995), which may be of importance as a cellular defense mechanism (Mezosi et al., 2005). Considering that oxidative stress activates numerous important signaling cascades by regulating the function of a variety of enzymes and transcription factors determining transcriptional activation, the influence of TH on these regulatory processes merits further investigation to understand the complexities of TH action and the outcome of thyroid gland dysfunction. Of particular importance is the role that T_3 administration may have in liver preconditioning, based on the redox upregulation of hepatic proteins involved in cell survival achieved (Fig. 1) (Fernández et al., 2005a; 2005b) and the actions of T_3 as a primary hepatic mitogen (Malik et al., 2003), mechanisms that may increase the tolerance of the liver to a subsequent injury (Romanque et al., 2005). This novel hepatic preconditioning strategy is currently under study in our laboratory using the ischemia-reperfusion liver injury, a model that is relevant to temporary clamping of the hepatoduodenal ligament during liver surgery and graft failure after liver transplantation in man (Romanque et al., 2005).

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