Regulation of Hepatocyte Thyroxine 5′-Deiodinase by T3 and Nuclear Receptor Coactivators as a Model of the Sick Euthyroid Syndrome*

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The syndrome of nonthyroidal illness, also known as the sick euthyroid syndrome, is characterized by a low plasma T3 and an “inappropriately normal” plasma thyrotropin in the absence of intrinsic disease of the hypothalamic-pituitary-thyroid axis. The syndrome is due in part to decreased activity of type I iodothyronine 5′-deiodinase (5′-D-I), the hepatic enzyme that converts thyroxine to T3 and that is induced at the transcriptional level by T3. The hypothesis tested is that cytokines decrease T3 induction of 5′-D-I, resulting in decreased T3 production and hence a further decrease in 5′-D-I. The proposed mechanism is competition for limiting amounts of nuclear receptor coactivators between the 5′-D-I promoter and the promoters of cytokine-induced genes. Using primary cultures of rat hepatocytes, we demonstrate that interleukins 1 and 6 inhibit the T3 induction of 5′-D-I RNA and enzyme activity. This effect is at the level of transcription and can be overcome by exogenous steroid receptor coactivator-1 (SRC-1). The physical mass of endogenous SRC-1 is not affected by cytokine exposure, and exogenous SRC-1 does not affect 5′-D-I in the absence of cytokines. The data support the hypothesis that cytokine-induced competition for limiting amounts of coactivators decreases hepatic 5′-D-I expression, contributing to the etiology of the sick euthyroid syndrome.

Under ordinary circumstances, plasma levels of thyroid hormone are tightly regulated by a homeostatic feedback loop in which hypothalamic thyrotropin-releasing hormone stimulates secretion of TSH1 by the anterior pituitary, which in turn stimulates secretion of thyroid hormones by the thyroid gland. 3,5,3′-triiodothyronine represses the synthesis and secretion of thyrotropin-releasing hormone and TSH to complete the negative feedback loop. However, this regulatory system is perturbed by virtually any medical illness or surgical stress, resulting in what is known as the syndrome of nonthyroidal illness or the sick euthyroid syndrome (for reviews, see Refs. 1 and 2). The characteristic features of this syndrome are low plasma T3 concentration with an “inappropriately normal” TSH. The syndrome is seen with both acute and chronic illnesses such as trauma, myocardial infarction, infection, malignancy, and renal failure. The more severe the nonthyroidal illness, the more depressed is the T3 level. Hospitalized patients will often have a frankly low TSH, and occasionally the T4 level also is low in very sick individuals. In fact, a correlation exists between the magnitude of the thyroid function test abnormalities and the mortality rate. The thyroid function test abnormalities resolve if the patient recovers from the nonthyroidal illness.

The mechanism that underlies the sick euthyroid syndrome is poorly understood but is clearly multifactorial. Both central (pituitary or hypothalamic) and peripheral defects are apparent. The central defect is manifest by abnormally low secretion of TSH in response to low circulating thyroid hormone levels. Multiple peripheral defects in the distribution and metabolism of thyroid hormone have been observed, but perhaps the most important is a decrease in the conversion of T4 to T3 (3, 4). It is estimated that direct thyroid secretion accounts for only approximately 20% of plasma T3. The majority of plasma T3 derives from thyroxine deiodination, primarily by the hepatocyte enzyme type I iodothyronine 5′-deiodinase. The activity of this enzyme is diminished in the sick euthyroid syndrome, thus accounting for the low plasma T3 despite a normal plasma T4.

Hepatocyte 5′-D-I is induced at the transcriptional level by T3 (5). Thus, if a signal leads to a decrease in hepatocyte 5′-D-I, the result will be a decrease in plasma T3, which will then lead to a further decrease in 5′-D-I production, thereby creating a self-reinforcing downward spiral of plasma T3 concentration. In the sick euthyroid syndrome, it is presumed that an illness or stress leads to such a signal, creating this downward spiral. Because the sick euthyroid syndrome occurs with essentially any medical illness or surgical stress, the initial signal must be something quite general. In this regard, attention has focused on the role of cytokines (6), which are known to be elevated under a wide array of pathological circumstances.

In this paper, the following hypothesis is tested using primary cultures of rat hepatocytes. Cytokines induce the expression of multiple genes, resulting in competition for limiting amounts of transcriptional coactivators between those genes and the T3-regulated 5′-D-I gene. The resulting coactivator deficiency limits the T3 induction of 5′-D-I, which results in decreased T3 generation, thus initiating the downward spiral. The return of availability of the coactivators results in resolution of the syndrome.
Experimental Procedures

Hepatocyte Cultures—Hepatocytes were isolated from male Harlan Sprague Dawley rats weighing 280–350 g, as described previously (7). Animal use was approved by the University of Michigan Committee on Use and Care of Animals. Hepatocytes in serum-free Williams E media were plated at 1.25 × 10^6 cells/cm² into Falcon Primaria 6-well clusters and maintained at 37 °C, 5% CO₂. After 24 h, the media were replenished, and T3 was added or not. In addition, at the same time, recombinant rat TNFα, IL-1β, IL-6, or vehicle was added. All cytokines were from PharMingen (San Diego, CA). Cytokine doses were selected based upon ED₅₀ data provided by PharMingen. A middle dose was chosen to be 2-fold higher than the stated ED₅₀, and concentrations 10-fold below and above that level were chosen as low and high doses. This resulted in TNFα concentrations of 5, 50, and 500 pg/ml, IL-1 concentrations of 1, 10, and 100 ng/ml, and IL-6 concentrations of 0.2, 2, and 20 ng/ml. Generally, the cells were harvested 24 h after the addition of T3 and/or cytokines. However, the media were replenished at that time, including T3 and cytokines, in the experiments that extended an additional 24 h.

Northern Blots—The influence of T3 and the above-listed cytokines on the expression of 5'-D-I, malic enzyme, and spot 14 RNAs as assessed by Northern blot. Total RNA was prepared using Trizol (Life Technologies, Inc.). Twenty micrograms of RNA were electrophoresed/ lane of a 1% agarose, 2.2 M formaldehyde gel and transferred to a nylon membrane. Membranes were hybridized with 32P-labeled cDNA probes derived from rat 5'-D-I, rat malic enzyme, rat spot 14, and rat GAPDH as a neutral control. Probes were labeled neutral using a Strip-EZ random priming kit (Ambion, Austin, TX) to facilitate stripping and reprobing. Blots were analyzed using a Molecular Dynamics PhosphorImager. The signals for 5'-D-I, malic enzyme, and spot 14 were normalized to GAPDH. For each experiment, the normalized value in the absence of T3 or cytokines was assigned a value of 1, and all other experimental conditions were expressed relative to that assigned value.

5'-D-I Enzyme Activity—Enzyme activity of cell lysates was measured as the release of 125I from 125I-reverse T3 as described (8). Results were calculated per microgram of cellular protein and were normalized for each experiment so that the value for cells without T3 or cytokines was 100.

Transient Transfections—Hepatocyte cultures were transfected using LipofectAMINE Plus (Life Technologies, Inc.) 24 h after plating. To assess the effects of IL-1 and SRC-1 on glucocorticoid induction of 5'-D-I gene expression, transfections similar to those above were performed. Priming kit (Ambion, Austin, TX) to facilitate stripping and reprobing. Blots were analyzed using a Molecular Dynamics PhosphorImager. The signals for 5'-D-I, malic enzyme, and spot 14 were normalized to GAPDH. For each experiment, the normalized value in the absence of T3 or cytokines was assigned a value of 1, and all other experimental conditions were expressed relative to that assigned value.

To assess the effects of IL-1 and SRC-1 on glucocorticoid induction of 5'-D-I gene expression, transfections similar to those above were performed. The human 5'-D-I gene (GenBank™ accession number AL031427) extending from base pair −3152 to +29 was inserted into the firefly luciferase vector, pGL3-Basic (Promega, Madison, WI). One microgram of this vector was transfected well along with 10 ng of the internal control vector, pRL-SV40 (which expresses Renilla luciferase; Promega) and 50 ng of rat TRβ1 in the vector pCDM (9). In some experiments, 10 ng of expression vector for SRC-1 (10), PCAF (11), CBP (12), or empty vector pcDNA3.1 (Invitrogen, Carlsbad, CA) cotransfected. Following transfection, the cells were cultured with or without T3 and cytokines as indicated. Cell lysates were prepared 48 h later for analysis of firefly and Renilla luciferases using the Promega Dual Luciferase Reporter Assay System. Renilla luciferase was used to normalize for transfection efficiency. The human 5'-D-I promoter was studied because the rat promoter has not been characterized.

RESULTS

Effect of Cytokines on Hepatocyte 5'-D-I Gene Expression—Primary cultures of rat hepatocytes were maintained ±T3 and were treated with TNFα, IL-1, IL-6, or vehicle. 5'-deiodinase gene expression was measured at the RNA level by Northern blot and was normalized to GAPDH. The results of a typical Northern blot are shown in Fig. 1, and the PhosphorImager quantification is shown in Fig. 2. These data are from 24 h of culture ±T3 ±cytokines, but identical results were achieved after 48 h of treatment. As expected, T3 induced 5'-D-I RNA 3-fold (Fig. 1A, lane 2 versus lane 1; Fig. 2, bar 2 versus bar 1). TNFα had no effect on 5'-D-I expression, either in the absence or the presence of T3 (Fig. 1A, lanes 4–7; Fig. 2, bars 3–8). However, both IL-1 and IL-6 inhibited the T3 induction of 5'-D-I in a dose-dependent manner. At the highest dose, IL-1 nearly eliminated the T3 effect (Fig. 1A, lane 11 versus lane 2; Fig. 2, bars 13–14 versus bars 1–2), and IL-6 decreased the T3 effect by about 50% (Fig. 1A, lane 15 versus lane 2; Fig. 2, bars 19–20 versus bars 1–2).

To confirm these results at the protein level, 5'-D-I enzyme activity was measured. After 24 h of culture, neither T3 nor any of the cytokines affected enzyme activity (data not shown). However, 48-h exposure to T3 resulted in a 1.6-fold increase in enzyme activity, which was blocked by treatment with IL-1 or IL-6 (Fig. 3). It is assumed that the lack of response at 24 h reflects the slower rate of change of protein expression than RNA expression. It was not possible to continue the cultures beyond 2 days of treatment, as the cells tended to detach from the plates.

Effect of Cytokines on Hepatocyte Malic Enzyme and Spot 14 Gene Expression—If the underlying hypothesis is correct that cytokines decrease the T3 induction of 5'-D-I by limiting the availability of transcriptional coactivators, then one would expect that other T3-responsive hepatocyte genes would be similarly affected, presuming they utilize the same coactivators. Therefore, to determine whether the effect of cytokines on the
T3 induction of 5′-D-I was specific to that gene, Northern blots were reprobed for two classically T3-inducible hepatocyte RNAs, malic enzyme (14) and spot 14 (15, 16). The results were similar to those achieved for 5′-D-I (Fig. 4). Thus, malic enzyme was induced about 3-fold by T3 and spot 14 about 2.5-fold. TNFa had no effect on either malic enzyme or spot 14 gene expression, but IL-1 and IL-6 inhibited the T3 induction of both RNAs. The major conclusion from the above studies is that IL-1 and IL-6 inhibit the expression of 5′-D-I and other T3-responsive hepatocyte genes and that this inhibitory effect is seen primarily in the presence of T3.

Effect of IL-1 and IL-6 on 5′-D-I Promoter Activity—Given that the induction of 5′-D-I by T3 is known to be at the transcriptional level (5), it seemed likely that the inhibitory effects of IL-1 and IL-6 also would be at that level. To assess this possibility, primary cultures of rat hepatocytes were transfected with a 3.2-kilobase pair 5′-D-I promoter-firefly luciferase reporter gene construct, along with a Renilla luciferase plasmid as an internal control. Luciferase activities were measured after a 48-h exposure to ±T3, ±cytokines. The low and high doses are from separate experiments. *, p < 0.05 versus ±T3, no cytokines.

Rescue of the IL-1 Effect by a Nuclear Receptor Coactivator—If the inhibitory effects of cytokines on 5′-D-I promoter activity are caused by limiting amounts of coactivators, then it should be possible to overcome the cytokine effect by supplying exogenous coactivators. Several different thyroid hormone receptor coactivator antibodies have been described (see Ref. 17 for a review), but the physiological roles of each of these coactivators are uncertain. We tested three coactivators for the ability to use as a base line for studying repression by cytokines. Therefore, the cells also were cotransfected with a TRβ1 expression plasmid. The results of these experiments are shown in Fig. 5. The data indicate that T3 induced 5′-D-I promoter activity ~4.5-fold. IL-1 virtually abolished this T3 induction and also modestly inhibited reporter gene activity in the absence of T3. IL-6 inhibited T3 induction by about 50% and did not affect activity in the absence of T3. Thus, the effects of T3, IL-1, and IL-6 in this reporter gene assay are similar to the effects seen on endogenous 5′-D-I gene expression. The data are consistent with the primary effect of cytokines on 5′-D-I being at the transcriptional level.

**Fig. 2.** PhosphorImager analysis of hepatocyte 5′-D-I RNA normalized to GAPDH from cells cultured ±T3, ±cytokines. Cytokine doses are low (L), medium (M), or high (H) as described under “Experimental Procedures.” The low and high doses are from separate experiments. *, p < 0.05 versus ±T3, no cytokines.

**Fig. 3.** 5′-D-I enzyme activity from hepatocytes cultured ±T3, ±100 ng/ml IL-1, or 20 ng/ml IL-6 for 48 h. For each experiment, enzyme activity was calculated per microgram of total protein, and the data were normalized to a value of 100 for the cultures without cytokines or T3. *, p < 0.05 versus ±T3, no cytokines.

**Fig. 4.** PhosphorImagery quantification of malic enzyme and spot 14 RNAs from hepatocytes cultured ±T3, ±cytokines. RNA expression was analyzed by Northern blot and normalized to GAPDH. Cytokine doses are medium (M) or high (H) as described under “Experimental Procedures.” *, p < 0.05 versus ±T3, no cytokines; **, p < 0.05 versus ±T3, no cytokines.
overcome the cytokine effect: CBP, SRC-1, and PCAF. These were chosen for study because they are structurally unrelated and ubiquitous, and data exist to support the importance of all three in thyroid hormone receptor action (18–21). We used cotransfection to examine the ability of each of these coactivators to overcome the IL-1 inhibition of 5’ D-I promoter activity, as the IL-1 effect was more profound than the IL-6 effect. As shown in Fig. 6, SRC-1 was able to partially overcome the IL-1 inhibition of 5’ D-I promoter activity (compare bar 12 versus 10), whereas PCAF and CBP were not (bars 14 and 16 versus 10). None of these coactivators affected luciferase activity in the absence of IL-1 (bars 3–8 versus 1–2), suggesting that endogenous coactivators are not rate-limiting under those circumstances.

Because SRC-1 is a coactivator for many nuclear receptors, we also examined the effects of IL-1 and SRC-1 on glucocorticoid-responsive gene expression. Luciferase driven by a cotransfected MMTV promoter was induced 11 ± 1.5-fold by dexamethasone, and this induction was reduced to 3.6 ± 0.72-fold in the presence of IL-1. However, in the presence of cotransfected SRC-1, dexamethasone induced luciferase 11 ± 3.3-fold in the absence of IL-1 and 8.9 ± 1.2-fold in the presence of IL-1 (p < 0.05 versus +IL-1, -SRC-1, n = 3). Thus, IL-1 and SRC-1 have effects on glucocorticoid induction of the MMTV promoter that parallel their effects on T3 induction of the 5’ D-I promoter. This finding suggests that, in the presence of cytokines, SRC-1 may be generally limiting for gene activation by nuclear receptors.

IL-1 Does Not Affect the Mass of Endogenous SRC-1—The data presented above suggest that IL-1 inhibits T3 induction of the 5’ D-I promoter at least in part by reducing the availability of SRC-1. Since IL-1 activates the transcription of a large number of genes (22), a simple explanation for this result is that these genes compete with the 5’ D-I promoter for limiting amounts of SRC-1. Alternatively, it is possible that IL-1 decreases the mass of this coactivator. To assess this possibility, the expression of endogenous SRC-1 in hepatocytes cultured ±IL-1 was assessed by Western blot. The results, shown in Fig. 7, indicate that IL-1 does not affect the mass of SRC-1.

**DISCUSSION**

The sick euthyroid syndrome is a response to virtually any acute or chronic illness characterized by a decreased plasma T3 and “inappropriately normal” TSH. Whether the body is actually euthyroid or hypothyroid is uncertain (23). The fact that the TSH rises upon resolution of the syndrome, often to levels above normal, until the plasma T3 normalizes (24) would argue that the body is actually hypothyroid, but that this is a physiological response to illness. Teleologically, it is argued that the sick euthyroid syndrome evolved to lower metabolic rate and conserve energy. Presumably, the syndrome is adaptive during evolution, but whether it may be maladaptive under certain circumstances is debated (23). The correlation between the magnitude of thyroid blood test abnormalities and mortality rate (25) suggests a causal relationship and has led to small trials of thyroid hormone therapy in extremely sick patients (26, 27). No clinical trial has demonstrated an improved outcome when such patients are treated with thyroid hormone. However, an interpretation of the studies to date is clouded by the small number of patients studied, as well as by uncertainties regarding the appropriate dose and form of thyroid hormone and the patient populations that should be treated. It remains possible that the sick euthyroid syndrome is maladaptive for certain subsets of very ill patients, but who those patients are and how they should be treated remain unknown. A better understanding of the pathophysiology that underlies the sick euthyroid syndrome would provide insight into these important questions.

The sick euthyroid syndrome is characterized by abnormalities in the secretion of TSH (inappropriately “normal” TSH despite low serum T3) and the production of T3 (decreased 5’ deiodination of T4). Interest in the role of cytokines in the pathophysiology of the sick euthyroid syndrome derives from...
the fact that cytokines are elevated in most medical illnesses and thus could explain the broad circumstances under which the sick euthyroid syndrome is known to occur. A single injection of lipopolysaccharide into cattle, which raises circulating cytokine levels, caused decreases in the plasma T3 concentration and hepatocyte 5′-D-I activity (28). One injection of IL-6 into healthy humans resulted in a transient decrease in serum T3 and TSH (29), changes that are reminiscent of the sick euthyroid syndrome. An analysis of 100 consecutive hospitalized patients revealed a weak inverse correlation between the serum levels of IL-6 and T3 (30). These data are consistent with studies in wild-type versus IL-6 null mice (31). For example, following injection of lipopolysaccharide, serum T3 fell in both wild-type and null mice, but the drop was smaller in the IL-6 null animals. Hepatic 5′- D-I RNA decreased in all mice, but again the decrease was smaller in the IL-6 null animals. Taken together, the data suggest that circulating IL-6 plays a role in the pathogenesis of the sick euthyroid syndrome but that it cannot, by itself, fully account for the fall in serum T3. On the other hand, a single injection of IL-6 into wild-type mice had no effect on hepatocyte 5′-D-I RNA, but a single injection of IL-1 caused a transient decrease (32). Thus, IL-1 also may be implicated in the pathogenesis of the sick euthyroid syndrome. Overall, a combination of various cytokines probably plays a role in the pathogenesis of the sick euthyroid syndrome, although other factors also are likely to be involved.

Cytokines ultimately exert their effects by regulating gene expression. The binding of IL-6 to its receptor results in activation of the JAK-STAT pathway (STATs 1 and 3), as well as activation of MAP kinase (for reviews see Refs. 33 and 34). Consequently, a large number of hepatocyte genes are induced, such as those encoding the classical acute phase proteins, and transcription factors such as JunB, c-Fos, and CEBPβ. IL-1 also activates a large number of genes, including the IL-6 gene. Although the signal transduction pathways are not as clear cut, activation of NF-κB is an important step in the action of IL-1 (for reviews, see Refs. 22 and 34). IL-1 also activates MAP kinase.

Nuclear receptors, including the thyroid hormone receptor, have served as an important paradigm for studying the mechanisms of gene induction by enhancer-binding proteins. The TR contains a transcriptional activation domain known as activator function 2 (AF-2) within its ligand binding domain. The binding of T3 to the TR results in a conformational change, allowing AF-2 to interact with coactivator proteins. The coactivator proteins, in turn, activate gene expression, probably by a variety of mechanisms including acetylation of histones (35) and physical interactions with basal transcription factors (36). Thus, although the TR is bound to DNA even in the absence of hormone, ligand binding is required for the AF-2 domain to activate transcription. A large number of coactivators have been identified that can interact with the AF-2 domain of the TR and other nuclear receptors. The first to be described, SRC-1 (37), is now known to be part of a family of structurally related 160-kDa coactivators. SRC-1 is able to form a complex with at least two other coactivators, CBP (12) and PCAF (38), each of which also can bind the TR directly (18, 39). Immunoneutralization studies in cell culture indicate that all three of these coactivators are important for the T3 induction of reporter genes (18–20). In addition, SRC-1 null mice are partially resistant to T3, demonstrating the importance of this coactivator in vivo (21).

Despite the name CREB-binding protein, CBP functions as a general transcriptional coactivator, stimulating gene expression by a large array of transcription factors including CREB (12), nuclear receptors (18), NF-κB (40), STAT proteins (41), p53 (42), Smads (43), and serum response factor (44). Because PCAF was first identified as a CBP-binding protein, it also likely serves as a coactivator with broad specificity. Although SRC-1 originally was thought to be a specific coactivator for nuclear receptors, it too now appears to have a broader role. Thus, SRC-1 has been shown to be a coactivator for numerous other transcription factors, such as NF-κB (45), AP-1 (46), and serum response factor (47).

It has been suggested that broad spectrum coactivators may become limiting for gene induction under circumstances in which a large number of genes are induced simultaneously (38). It is this idea, coupled with the knowledge that 5′-D-I is induced by T3, that has led to the following working model of inhibition of hepatocyte 5′-D-I in the sick euthyroid syndrome. Cytokines such as IL-1 and IL-6 are induced by nonthyroidal illness and lead to the induction of a broad array of hepatocyte genes. These genes compete with the 5′-D-I gene for limiting amounts of one or more coactivators, resulting in a decrease in the T3-supported level of 5′-D-I activity. This leads to a decrease in circulating T3, which serves to further decrease 5′-D-I gene transcription, thus creating a self-reinforcing downward spiral of T3 production and plasma T3 concentration. This situation would reverse itself if coactivators became increasingly available. This hypothesis does not address the mechanism of decreased TSH secretion, nor does it address other potential contributors to the sick euthyroid syndrome such as decreased T4 entry into cells (48), and thus it is meant to explain only one aspect of the syndrome.

The data presented herein largely support this proposed mechanism. We find that IL-1 and IL-6 inhibit the T3 induction of 5′-D-I in primary cultures of rat hepatocytes and that a similar inhibition is seen with other T3 responsive genes. Reporter gene experiments using the 5′-D-I promoter indicate that the cytokine effect is transcriptional, which is not surprising given that the T3 effect is transcriptional. Importantly, this cytokine effect can be overcome partially by cotransfection of the coactivator SRC-1. However, SRC-1 does not affect T3 induction in the absence of cytokines, indicating that this coactivator is not rate-limiting up to the cytokines are present. In theory, IL-1 and IL-6 could make SRC-1 rate-limiting in a number of ways. They could, for example, decrease the physical mass of SRC-1. However, this explanation has been excluded by Western blot analysis. We favor the concept that coactivators become limiting because they are being sequestered on the promoters of genes activated by the cytokines, as this seems to be a direct and simple explanation that is consistent with our data. However, other explanations would be possible in theory, such as changes in coactivator phosphorylation. In this regard, SRC-1 is a phosphoprotein, but the functional significance of this phosphorylation is not known (49).

Exogenous SRC-1 only partially overcame the inhibitory effect of IL-1 in our experimental system. This result could simply be an issue of experimental details; a stronger effect may require expression of this coactivator prior to the addition of T3 and IL-1, or for a longer period of time, or at a higher level. Also, it is possible that other coactivators play an important role, or that coactivator-independent mechanisms contribute to the cytokine effect. It should be noted that even a modest increase in 5′-D-I promoter activity due to increased coactivator availability would likely be important. This is because 5′-D-I enzyme activity produces T3, which will further induce the promoter.

If the working hypothesis is correct, it would have important implications for the treatment of the sick euthyroid syndrome. Treatment with T3 might be harmful, rather than helpful, because this would serve to draw coactivators away from IL-1.
and IL-6-induced genes that presumably serve important adaptive functions. A more logical approach might be to increase the amounts of SRC-1 or other key coactivators, which presumably would require a better understanding of the factors that regulate coactivator levels.

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