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Steroid Receptor Coactivator-1 Deficiency Causes Variable Alterations in the Modulation of T_3-Regulated Transcription of Genes *in Vivo*

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Thyroid hormone exerts its biological effect by binding to a TR. Both liganded and unliganded TRs regulate the transcription of T_3-responsive genes. Cofactors with activating or repressing function modulate the transcriptional regulation by TRs. We showed that steroid receptor coactivator 1 (SRC-1)-deficient mice (SRC-$1^{-/-}$) exhibit partial resistance to thyroid hormone at the level of the pituitary thyrotrophs. To determine whether SRC-1 deficiency affects globally T_3-dependent transcriptional regulation, we studied the effects of thyroid hormone deprivation and replacement on the expression of several genes in different tissues of SRC-$1^{-/-}$ and wild-type mice (SRC-$1^{+/+}$). Thyroid hormone deficiency was induced by a low iodine diet (LoI) supplemented with propylthiouracil (PTU) for 2 wk. L-T_3 was injected ip for the last 4 d in one group (PTU+T_3 group), and another group (PTU group) received only vehicle. Levels of mRNAs for T_3-responsive genes were determined by Northern blotting: GH and TSHβ in pituitary; type 1 iodothyronine 5′-deiodinase, spot 14 (S14), and malic enzyme in liver; and sarcoplasmic reticulum calcium adenosine triphosphatase 2 and myosin heavy chain α and β in heart. Serum parameters, TSH, total cholesterol, creatine kinase, and alkaline phosphatase (AP), were also measured.

Hypothyroidism produced a comparable increase in TSHβ mRNA in both genotypes, but its suppression by L-T_3 was attenuated in SRC-$1^{-/-}$ mice. In contrast, hypothyroidism failed to reduce S14 mRNA levels in SRC-$1^{-/-}$ mice. As a consequence, the response to L-T_3 was not observed in these mice. SRC-1 deficiency had no effect on the expression of the rest of the T_3-responsive genes examined. Of the four serum parameters, the T_3-mediated decrease in TSH and changes in AP were attenuated in SRC-$1^{-/-}$ mice. We conclude that SRC-1 deficiency altered the expression of only some of the T_3-responsive genes. SRC-1 appears to be involved not only in transcriptional activation by liganded TRs, but also in the suppression by liganded or unliganded TRs. Some of the effects of SRC-1 may be TR isoform specific. (*Endocrinology* 143: 1346–1352, 2002)

MOST METABOLIC EFFECTS of thyroid hormone require the binding of T_3 to a TR. Both liganded and unliganded TRs bind to T_3 response elements (TRE) on target genes to regulate their expression either positively or negatively (reviewed in Refs. 1 and 2). In the case of genes positively regulated by T_3, expression is usually repressed by the unliganded TR through association with corepressors. The conformational change in TR produced by T_3 binding results in corepressor release and recruitment of coactivators. Many of the coactivators possess intrinsic histone acetyltransferase activity that is essential to chromatin remodeling and promotes accessibility of transcription factors (3). Thus, this series of events causes transcriptional activation of genes targeted by T_3. In contrast, in genes negatively regulated by T_3, such as TSHα and -β, expression is activated in the absence of T_3, and T_3 binding to the TR causes repression of the transcription. However, the mechanism of T_3-dependent transcriptional repression is not fully understood.

The first coactivator of nuclear receptors that was identified is the steroid receptor coactivator 1 (SRC-1) (4, 5). It belongs to a family of 160-kDa nuclear coactivators that includes TIF/SRC-2/GRIP1 (6) and AIB1 (7)/ACTR (8)/RAC3 (9)/pCIP (10)/TRAM-1 (11)/SRC-3 (12). Other coactivator proteins have also been identified (reviewed in Ref. 13). Because tissue distribution of coactivators overlaps (11, 12, 14), it remained unclear how each coactivator contributes in the mediation of hormone action.

SRC-1-deficient mice are viable and fertile, but are partially resistant to steroid hormones, exhibiting reduced growth and development of the uterus, prostate, testis, and mammary glands after hormone treatment (14). These same mice also exhibit partial resistance to thyroid hormone at the level of the pituitary thyrotrophs. We previously reported that mice deficient in SRC-1 displayed elevated serum TSH levels despite high serum free T_4 and T_3 concentrations, and suppression of their serum TSH level by T_3 was attenuated (15). Therefore, it is suggested that SRC-1 is required to mediate full expression not only of steroid hormone-dependent genes but also of those genes regulated by thyroid hormone. However, it remains to be determined whether SRC-1 deficiency affects T_3-dependent transcriptional regulation globally or if the effect is restricted to a limited number of T_3-responsive genes.

Abbreviations: AP, Alkaline phosphatase; CK, creatine kinase; 5′DI, type 1 iodothyronine 5′-deiodinase; LoI, low iodine diet; ME, malic enzyme; MHC, myosin heavy chain; PTU, propylthiouracil; S14, spot 14; SERCA2, sarcoplasmic reticulum calcium adenosine triphosphatase 2; SRC-1, steroid receptor coactivator 1; TRE, T_3 response element.

To answer this question we studied how SRC-1-deficient mice (SRC-$1^{-/-}$) (14, 15) responded to thyroid hormone deprivation and replacement compared with wild-type mice (SRC-$1^{+/+}$). For this study we examined the expression of two or three T_3-responsive genes in each of three target organs: GH (16) and TSH β-subunit (TSHβ) (17) in pituitary; type 1 iodothyronine 5′-deiodinase (5′DI) (18), spot 14 (S14) (19) and malic enzyme (ME) (20) in liver; and sarcoplasmic reticulum calcium adenosine triphosphatase 2 (SERCA2) (21), myosin heavy chain α (MHCα), and -β (MHCβ) (22) in heart.

Our studies using Northern blot analysis show that SRC-1 deficiency does not affect the expression of GH, 5′DI, ME, and all three T_3-responsive genes in the heart. On the other hand, expression levels of TSHβ in the pituitary and of S14 in the liver were modified in the absence of SRC-1, but in different ways. These results suggest that for some T_3-responsive genes, the loss of SRC-1 either has little effect or can be compensated for by other coactivators, whereas for other genes SRC-1 is essential for normal T_3-mediated regulation.

Materials and Methods

Animal origin and handling

SRC-$1^{-/-}$ mice were created and maintained as reported previously (14, 15). Heterozygous (SRC-$1^{-/+}$) mice were interbred to generate litters containing homozygous (SRC-$1^{-/-}$) and wild-type (SRC-$1^{+/+}$) mice. The genotypes were confirmed by analysis of tail DNA as described previously (14). Mice were weaned during the fourth week after birth and were fed Purina rodent chow containing 0.8 parts/million iodine (no. 5053, Ziegler, Gardnerf, PA) *ad libitum* and given tap water. They were housed, three to five mice per cage, in an environment with a controlled temperature of 19 C and 12-h alternating dark and artificial light cycles. All animal procedures followed protocols approved by the animal care and use committee of the University of Chicago, where the experiments were performed.

Induction of hypothyroidism and treatment with L-T_3

All mice were male and 50–70 d old at the beginning of each experiment. As it has been shown that SRC-$1^{-/-}$ mice have elevated T_4 and T_3 levels (15), we induced hypothyroidism and provided L-T_3 supplementation to study the effect of thyroid hormone under the same degree of hormonal deficiency and supplementation in SRC-$1^{-/-}$ and SRC-$1^{+/+}$. In brief, thyroid hormone deficiency was induced by feeding a LoI supplemented with 0.15% propylthiouracil (PTU) purchased from Harlan Teklad Co. (Madison, WI). On d 11 of the PTU treatment, animals of each genotype were split into two groups. One group received daily ip injections of 0.8 μg L-T_3/100 g BW for 4 d (PTU+T_3 group), and the other group was given the vehicle only (PTU group). L-T_3 was purchased from Sigma (St. Louis, MO), and the solution for the injection was prepared as previously reported (15). Twelve to 16 h after the last injection, the experiment was terminated by exsanguination. There were 11–13 mice in each of the 4 experimental groups. However, not all tissues analyzed were available from all animals. Numbers for each determination are indicated in *Results*.

Measurements in serum samples

Serum TSH was measured in 50 μl serum using a sensitive, heterologous, disequilibrium, double antibody precipitation RIA, as previously described (23), with a sensitivity of 5 mU/liter expressed in bioassayable TSH units. Serum T_4 concentrations were measured by RIA (Diagnostic Products, Los Angeles, CA) using 25 μl serum with a sensitivity of 0.2 μg/dl (2.6 nmol/liter).

Cholesterol, CK, and AP were measured using a clinical chemistry autoanalyzer on serum samples obtained at the termination of the experiment. In addition to 10 μl serum for each determination, the autoanalyzer required 40 μg serum to prime the pump.

Northern blot analysis

Total RNA was extracted from pituitary, liver, and heart by the method of Chomczynski and Sacchi (24) and subjected to Northern blot analysis. Aliquots of 5, 15, and 20 μg total RNA from pituitary, liver, and heart, respectively, were subjected to Northern blot analysis. After denaturation, samples were separated by 0.8% agarose gel electrophoresis and transferred onto GeneScreen Plus (NEN Life Science Products, Boston, MA) as described previously (25).

cDNAs used as probes were those for mouse TSHβ (17), rat GH (26), heart SERCA2 [prepared by RT-PCR using 5′-acgatctgtgctctgtgtaatgactct-3′ (sense) and 5′-ggcgcgtcgttcacaccatcaccagtca-3 (antisense) primers and mouse heart RNA as templates], 5′DI (18), S14 (27), and ME (28). They were labeled with [α-^{32}P]dCTP (SA, 111 TBq/mmol; NEN Life Science Products) using a random primed DNA labeling kit (Roche Molecular Biochemicals, Mannheim, Germany). Conditions of hybridization and subsequent washing were previously reported (29), except for S14 mRNA (washing with 1% SDS was performed at 45 C instead of 65 C).

Probes to detect MHCα and -β were oligonucleotides, 5′-ttagagctgggtagcacaagatctactcctcattcaggcc-3′ for MHCα and 5′-tatgtttattgtggattggccacagcgagggtctgctgga-3′ for MHCβ. They were labeled with [γ-^{32}P]ATP (SA, 222 Tbq/mmol; NEN Life Science Products) using T4 polynucleotide kinase (TOYOBO Co. Ltd., Osaka, Japan) according to a protocol provided by the manufacturer.

Membranes were hybridized with the labeled probe in a buffer [1.0 M NaCl, 50 mM Tris-HCl (pH 7.5), 10% dextran sulfate, 1% SDS, and 100 μg/ml denatured herring sperm DNA] at 65 C for 20 h. Membranes were then washed twice with 2× SSC [300 mM NaCl, 30 mM sodium citrate (pH 7.0), and 0.1% SDS] under the following conditions: 5 min at 20 C, 10 min at 65 C, and 5 min at 20 C. The radioactivity of bands was measured using the Molecular Imager System (GS-363, Bio-Rad Laboratories, Inc., Hercules, CA). The accuracy of RNA delivery in Northern blots was monitored by rehybridization with ^{32}P-labeled cDNA for 18S ribosomal RNA.

Data presentation and statistics

Values are expressed as the mean ± SEM. Statistical analysis was performed using StatView 5.0 software (Abacus Concepts, Inc., Berkley, CA). For each variable, differences between the two genotypes and the response to thyroid hormone deprivation and supplement were analyzed using one-way ANOVA and Bonferroni/Dunn test. $P < 0.0083$ was considered significant.

Results

T_3-dependent transcriptional regulation of pituitary TSHβ and GH mRNAs

As shown in a previous report (15), treatment with LoI/PTU lowered serum T_4 levels below the limit of assay sensitivity (<2.6 nmol/liter) and elevated serum TSH concentrations to the same extent in both SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice (6800 ± 700 and 7800 ± 850 mU/liter, respectively). Although the dose of 0.8 μg L-T_3/100 g BW/d (on the average, 0.2 μg/mouse/d) reduced the serum TSH concentration in both genotypes, the decline was blunted significantly in SRC-$1^{-/-}$ mice (Fig. 1).

It is well known that the transcription of TSHα and -β genes is markedly activated by thyroid hormone deficiency and suppressed by L-T_3 (30). Thus, the changes in serum TSH levels are thought to be due mainly to T_3-induced down-regulation of TSH subunit mRNAs. In the present study we examined whether SRC-1 is involved in T_3-dependent transcriptional regulation of the TSHβ gene. As shown in Fig. 1, the levels of TSHβ mRNA in SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice

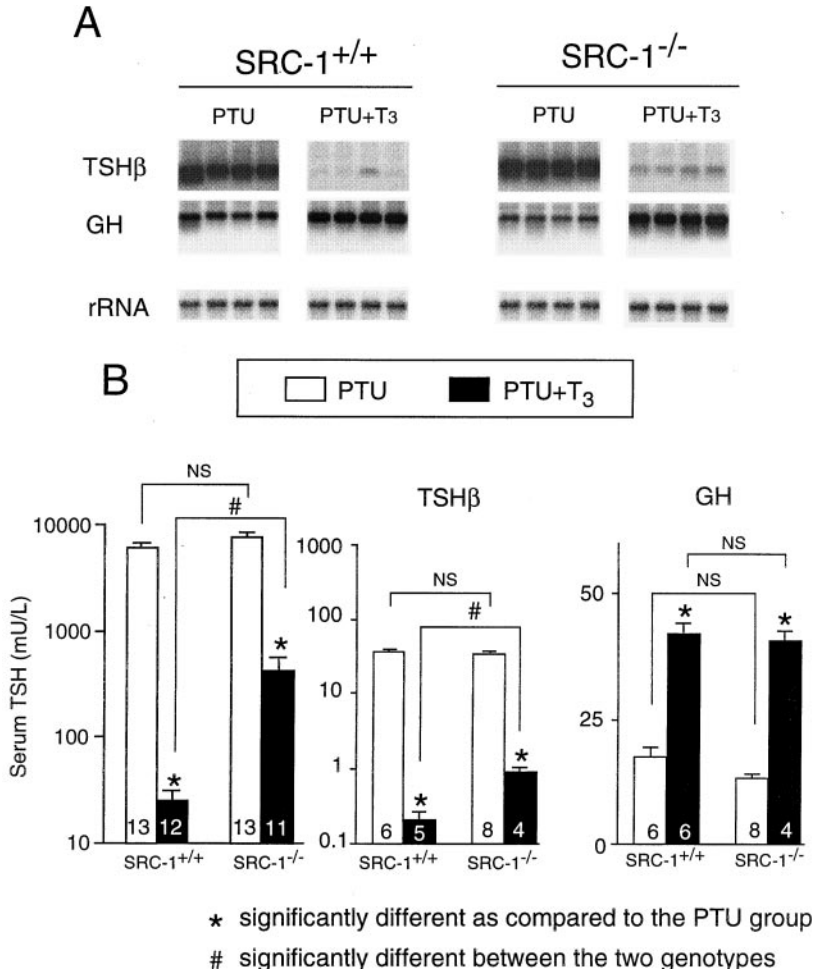

FIG. 1. Northern blot analysis of TSHβ and GH mRNAs from pituitary glands of SRC-$1^{+/+}$ mice (*left panels*) and SRC-$1^{-/-}$ mice (*right panels*). Aliquots of 5 μg total RNA were subjected to analysis. Membranes were reprobed with ^{32}P-labeled cDNA for 18S ribosomal RNA (rRNA). A, Representative autoradiographs of samples from four mice belonging to each treatment group are shown. B, Results of densitometric analysis corrected by the amount of rRNA are expressed as the mean ± SEM in arbitrary units. Differences are statistically significant at $P \leq 0.0083$. The number of mice in each group is indicated in the *bottom* of each bar. Serum TSH concentrations measured in the same groups of animals are shown for comparison. The latter and TSHβ mRNA values are plotted on a logarithmic scale. AU, Arbitrary units.

were comparable after treatment with LoI/PTU only. Administration of L-T_3 resulted in a marked decrease in TSHβ mRNA in both genotypes; however, the levels were more than 4-fold higher in SRC-$1^{-/-}$ compared with SRC-$1^{+/+}$ animals. These results paralleled those of serum TSH and indicate that T_3-dependent suppression of TSHβ mRNA is attenuated significantly in SRC-$1^{-/-}$ mice, suggesting that SRC-1 is involved in enhancing the T_3-dependent down-regulation of TSHβ mRNA expression.

We also examined the expression of GH mRNA, which is known to be regulated positively by T_3 in rats and mice. As shown in Fig. 1, GH mRNA levels increased significantly in response to L-T_3 in both SRC-$1^{-/-}$ and SRC-$1^{+/+}$ animals. However, in contrast to TSHβ mRNA, the magnitude of change was not significantly different between the two genotypes.

Changes in the expression of T_3-responsive genes in liver

Three genes expressed in liver, 5′DI, S14, and ME, have been characterized as being T_3 responsive. Their transcription is up-regulated by T_3 (18–20). We thus examined the involvement of SRC-1 in T_3-dependent transcriptional regulation of these three genes by measuring their respective mRNAs by Northern blotting. As shown in Fig. 2, we could not detect 5′DI mRNA in hypothyroid mice of either genotype, and administration of L-T_3 caused their dramatic increase to the same magnitude. A lesser T_3-dependent increase in ME mRNA was also observed in both genotypes. The levels were not significantly different between SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice whether they were hypothyroid or treated with L-T_3. Thus, inactivation of SRC-1 did not affect the T_3-dependent transcriptional activation of either the 5′DI or ME gene.

In contrast, inactivation of the SRC-1 gene affected transcriptional regulation of the S14 gene in liver. In mice treated with LoI/PTU only, S14 mRNA levels were significantly higher in SRC-$1^{-/-}$ than in SRC-$1^{+/+}$. S14 mRNA increased with L-T_3 treatment only in SRC-$1^{+/+}$ mice, reaching the level found in SRC-$1^{-/-}$ mice, which did not respond to L-T_3 (Fig. 2). Hypothyroid SRC-$1^{+/+}$ mice showed an approximately 6-fold increase in S14 mRNA content from thyroid hormone deprivation to L-T_3 treatment. These results suggest that down-regulation of S14 mRNA during thyroid hormone deprivation is completely abolished in the SRC-1 knockout mouse.

Changes in expression of T_3-responsive genes in heart

The heart is a major target organ for thyroid hormone, and several genes have been shown to be regulated transcriptionally by T_3. Among them are the SERCA2, MHCα, and

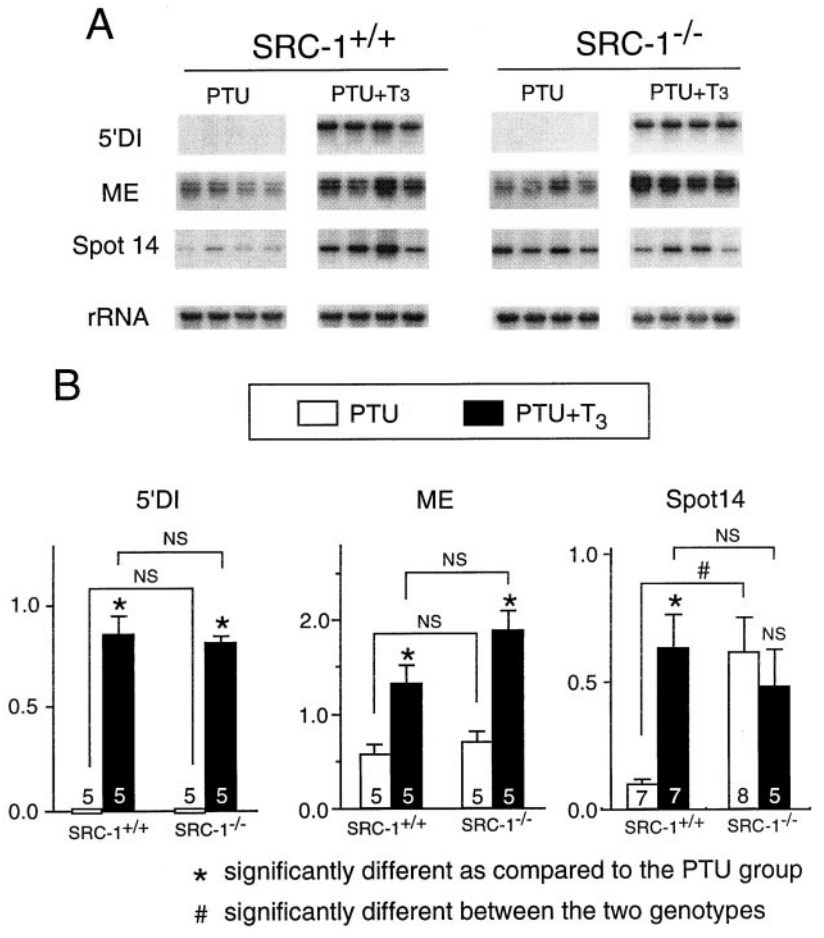

FIG. 2. Northern blot analysis of T_3-responsive genes in liver: 5′DI, ME, and S14 mRNAs. Total RNA was extracted from livers, and aliquots of 20 μg were subjected to the analysis. A, Representative autoradiographs from four SRC-$1^{+/+}$ mice (*left panels*) and SRC-$1^{-/-}$ mice (*right panels*). B, Amounts of mRNA for each experimental group of mice were corrected for the corresponding rRNA content. Differences are statistically significant at $P \leq 0.0083$. Values are expressed in arbitrary units, and *error bars* represent the SEM. The number of mice in each group is given at the *bottom* of each *bar*.

MHCβ genes (21, 22). To study the involvement of SRC-1 in the regulation of expression of these genes by T_3, we examined the effect of thyroid deprivation and supplementation in SRC-$1^{-/-}$ mice.

As shown in Fig. 3, administration of L-T_3 had a similar effect on SERCA2 and MHCα mRNAs in SRC-$1^{-/-}$ and SRC-$1^{+/+}$ mice. Moreover, the mean levels of SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice were not significantly different during hypothyroidism. Therefore, SRC-1 does not seem to modulate the transcriptional regulation of these genes in the presence or absence of thyroid hormone.

To determine whether SRC-1 affects negative transcriptional regulation by T_3 in the heart, we examined changes in MHCβ mRNA expression in SRC-$1^{-/-}$ mice. As reported previously, levels in MHCβ mRNA were high in hypothyroid SRC-$1^{+/+}$ mice (22). This enhanced expression was of similar magnitude in hypothyroid SRC-$1^{-/-}$ mice (Fig. 3). Furthermore, when treated with L-T_3, the clear decline in MHCβ mRNA in hearts of SRC-$1^{-/-}$ mice was not significantly different from that in SRC-$1^{+/+}$ mice. These results indicated that SRC-1 has no effect on the negative transcriptional regulation by T_3 in the heart, principally mediated through TRα.

Changes in serum total cholesterol CK and AP levels

We measured three serum parameters, total cholesterol, CK, and AP, that have been used to assess thyroid status in mice (31, 32). As shown in Fig. 4, total cholesterol levels in mice receiving LoI/PTU were 95 ± 5 and 113 ± 9 mg/dl in SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice, respectively, and the corresponding levels declined to 68 ± 2 and 79 ± 5 mg/dl after administration of L-T_3. These differences were not statistically significant, and the mean decrements were 29% in the SRC-$1^{+/+}$ and 30% in the SRC-$1^{-/-}$ groups. Similarly, there was no significant difference in mean percent changes in serum levels of CK between SRC-$1^{+/+}$ and SRC-$1^{-/-}$ mice, which declined after L-T_3 treatment by 65% in SRC-$1^{+/+}$ and 68% in SRC-$1^{-/-}$. Although the absolute values of all three substances during thyroid hormone deprivation were, on the average, lower in SRC-$1^{+/+}$ mice compared with SRC-$1^{-/-}$ mice, these differences were not statistically significant. However, due to the combination of a lesser decline of AP in SRC-$1^{-/-}$ mice during hypothyroidism and an attenuated increase with L-T_3 treatment, the increment in AP in these mice was only 15% compared with 90% in SRC-$1^{+/+}$ mice. These results suggest that some of the serum markers of thyroid hormone action are modulated by SRC-1.

Discussion

The present study shows that the inactivation of the SRC-1 gene affects T_3-mediated modulation of expression of some T_3-responsive genes, but not all. Expression of GH in the pituitary, of 5′DI and ME in liver, as well as any of three T_3-responsive genes in heart was not affected by inactivation of the SRC-1 gene in either the thyroid hormone-deprived or

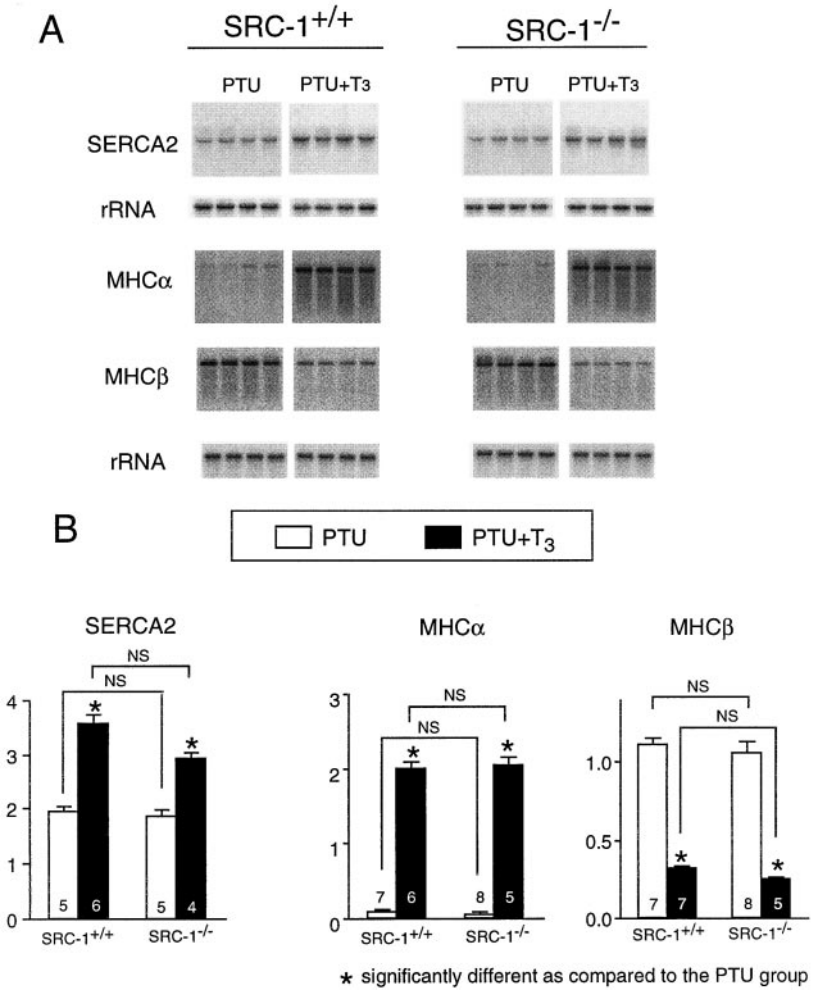

FIG. 3. Northern blot analysis of T_3-responsive genes in heart: SERCA, MHCα, and MHCβ mRNAs. Aliquots of 15 μg total RNA were analyzed for SERCA2 mRNA, and 5 μg were used for the determination of MHCα and MHCβ mRNAs. A, Representative autoradiographs showing results from SRC-$1^{+/+}$ mice (*left panels*) and SRC-$1^{-/-}$ mice (*right panels*). B, Results of densitometric analysis corrected for the amount of 18S rRNA are shown in arbitrary units. Values are expressed as the mean ± SEM. Differences are statistically significant at $P \leq 0.0083$. The number of mice in each group is indicated at the *bottom* of each *bar*.

T_3-replaced state. On the other hand, the expression of TSHβ mRNAs in the pituitary and that of S14 mRNA in liver were modified by SRC-1 deficiency. Although nuclear coactivators, including SRC-1, are widely expressed, their relative abundance varies depending on tissue type (33). Thus, the relative expression pattern may influence the effect of SRC-1 on the expression of T_3-responsive genes among tissues. However, the different effects observed in liver mRNAs suggest that the abundance of nuclear coactivators cannot fully account for the results in SRC-1 knockout mice.

In terms of expression of TR isoforms, liver and heart are in marked contrast. In liver, TRβ1, generated from the TRβ gene (34), is predominantly expressed (35, 36), whereas TRα1, generated from the TRα gene (37), is a dominant isoform in heart (32, 38). Because SRC-1 deficiency did not affect the expression of several T_3-responsive genes in liver as well as in heart, it is unlikely that binding preference of SRC-1 to TR isoforms *in vivo* is responsible for the different modulatory effect of SRC-1 on the transcriptional regulation of T_3-responsive genes. This conclusion is supported by a recent *in vitro* study showing that in the presence of T_3 there is no difference in binding properties to SRC-1 to TRα1, TRβ1, or TRβ2 (39).

The finding that the T_3-mediated suppression of TSHβ gene transcription was attenuated in SRC-$1^{-/-}$ mice is consistent with our previous observation (15) as well as those in the present study of reduced suppression of serum TSH by T_3 in the same animals. These results indicate that SRC-1 also participates in T_3-dependent transcriptional suppression *in vivo*. The mechanism by which T_3 down-regulates transcription of the TSHβ gene is not well understood. Using an *in vitro* transfection system, Tagami *et al.* (40) recently showed that nuclear coactivators strengthened the T_3-dependent repression of TSHα gene transcription and suggested that the negative regulation of a subset of genes by TR involves the active exchange of corepressors and coactivators with intrinsic promoter regulatory elements that normally strongly induce histone acetylation and transcriptional activation. As both TSHα and TSHβ mRNAs are expressed in thyrotrophs and are similarly down-regulated by T_3, the above explanation of the mechanism controlling TSHα expression may also be applied to TSHβ. Therefore, our *in vivo* study would support their hypothesis that nuclear coactivators are necessary for the down-regulation of TSHα or TSHβ genes and indicates that SRC-1 deficiency cannot entirely be compensated for by other members of the SRC family. However, as SRC-1 deficiency did not cause a distinct reduction in the down-regulation of the MHCβ gene by T_3, it also suggests that the mechanism by which T_3 down-regulates the transcription differs in different T_3-responsive genes or accord-

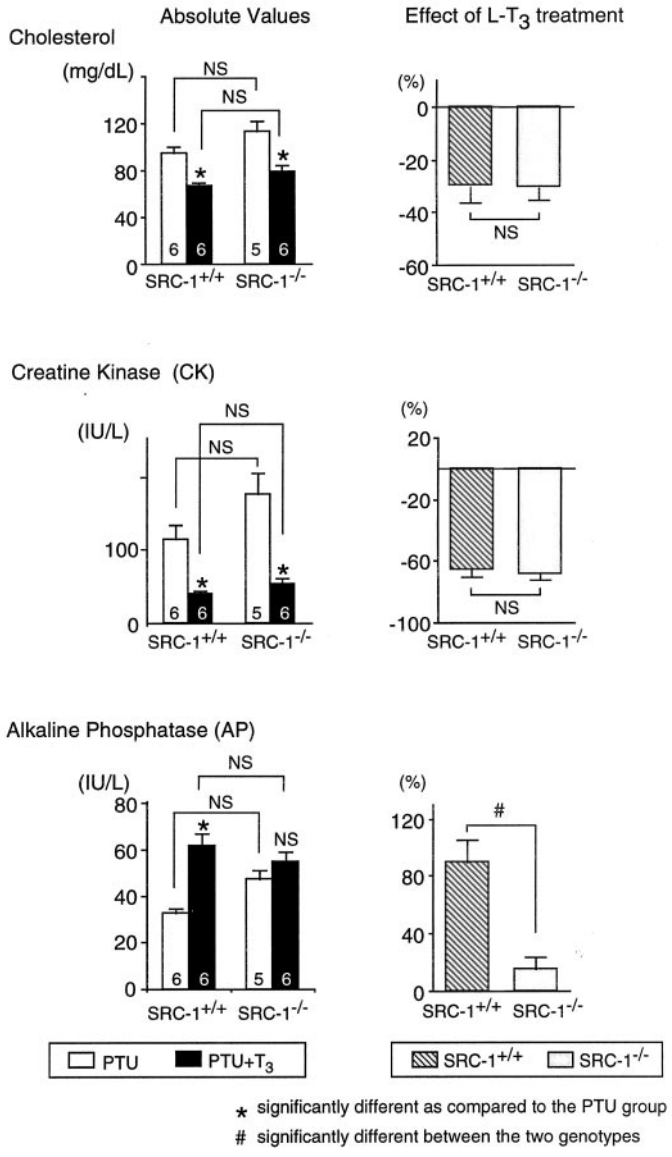

FIG. 4. Effect of thyroid hormone deprivation and L-T_3 treatment on serum parameters: total cholesterol, CK, and AP. Results are shown both in absolute values (*left panels*) and as percent changes produced by L-T_3 treatment (*right panels*). Values are the mean ± SEM, and differences are statistically significant at $P \leq 0.0083$. The number of mice in each group is indicated at the *bottom* of each *bar*.

ing to isoforms, TRβ2 *vs.* TRα1, even if regulated in the same direction.

Surprisingly, down-regulation of the S14 gene by thyroid hormone deprivation was completely prevented in SRC-1 knockout mice, whereas that of other T_3-responsive genes in liver, 5′DI and ME, was strongly maintained. Until now, a number of studies have proposed that unliganded TRs repress the transcription of target genes by binding to corepressors and that T_3 binding to TR causes release of the corepressors and recruitment of coactivators, setting the transcriptional activation in motion (2). This paradigm does not seem to apply in the T_3-mediated regulation of the S14 gene. As the S14 mRNA levels in hypothyroid SRC-$1^{-/-}$ mice were not significantly different from those of T_3-treated SRC-$1^{-/-}$ as well as T_3-treated SRC-$1^{+/+}$ mice, an important mechanism of T_3 affecting S14 gene transcription could be its relative transcriptional suppression in the absence of T_3. This interpretation requires that SRC-1 be involved in the ligand-independent suppression of the S14 gene. A dual effect of SRC-1 in both the absence and presence of ligand is supported by the *in vitro* studies of Oberste-Berghaus *et al.* (39), now shown to occur *in vivo*. S14 has been recognized as a representative T_3-responsive gene in liver, as have been 5′DI and ME. However, the TRE location on the S14 gene is distinct from that on the ME or 5′DI gene. Whereas TREs are found relatively near the transcription start sites of rat ME and human 5′DI genes [approximately −270 and −700 bp, respectively (41, 42)], TREs are located far upstream of the transcription start site of the S14 gene [from −2.5 to 2.8 kb (43)]. Therefore, it could be speculated that the structural difference of the regulatory region of the S14 gene compared with that of the ME or 5′DI gene allows the preferential involvement of SRC-1 in a T_3-independent transcriptional regulation of the S14 gene.

In summary, we examined the effect of SRC-1 on the thyroid hormone-mediated regulation of 8 genes in 3 tissues as well as 4 serum markers *in vivo*. Significant alterations were found in 3 of the 11 markers measured, involving 2 tissues (pituitary and liver) as well as serum, but not heart. Alterations affected both negative and positive modulations by thyroid hormone. Furthermore, the effects were not tissue specific and had variable manifestations, including attenuated or absent response to thyroid hormone or failure of suppression during thyroid hormone deprivation in SRC-1-deficient mice as well as attenuated suppression in hypothyroid mice expressing SRC-1. Some differences in the SRC-1-modulated effects may be TR isoform specific, such as the presence or absence of resistance to thyroid hormone on genes negatively regulated through TRβ2 (pituitary) or TRα1 (heart), respectively.

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