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The mechanism mediating the activation of acetyl-coenzyme A carboxylase-α gene transcription by the liver X receptor agonist T0-901317

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Abstract **In birds and mammals, agonists of the liver X receptor (LXR) increase the expression of enzymes that make up the fatty acid synthesis pathway. Here, we investigate the mechanism by which the synthetic LXR agonist, T0-901317, increases the transcription of the acetyl-coenzyme A carboxylase-α (ACCα) gene in chick embryo hepatocyte cultures. Transfection analyses demonstrate that activation of ACCα transcription by T0-901317 is mediated by a *cis*-acting regulatory unit (−101 to −71 bp) that is composed of a liver X receptor response element (LXRE) and a sterol-regulatory element (SRE). The SRE enhances the ability of the LXRE to activate ACCα transcription in the presence of T0-901317. Treating hepatocytes with T0-901317 increases the concentration of mature sterol-regulatory element binding protein-1 (SREBP-1) in the nucleus and the acetylation of histone H3 and histone H4 at the ACCα LXR response unit. These results indicate that T0-901317 increases hepatic ACCα transcription by directly activating LXR•retinoid X receptor (RXR) heterodimers and by increasing the activity of an accessory transcription factor (SREBP-1) that enhances ligand induced-LXR•RXR activity.—**Talukdar, S., and F. B. Hillgartner. **The mechanism mediating the activation of acetyl-coenzyme A carboxylase-α gene transcription by the liver X receptor agonist T0-901317. *J. Lipid Res.* 2006.** 47: **2451–2461.**

Supplementary key words fatty acid synthesis • sterol-regulatory element binding protein • thyroid hormone • chicken • histone acetylation

The first committed step of the fatty acid synthesis pathway is the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA. This reaction, catalyzed by acetyl-coenzyme A carboxylase-α (ACCα), constitutes a key control point in the synthesis of long-chain fatty acids from carbohydrate (1, 2). Malonyl-CoA serves as a donor of C_2 units for the synthesis of palmitate catalyzed by fatty acid synthase. Malonyl-CoA is also a substrate of specific elongases involved in the chain elongation of fatty acids to very-long-chain fatty acids (3). The essential role of ACCα in lipid biosynthesis has been confirmed by studies demonstrating that knockout of the ACCα gene disrupts embryonic development before day 7.5 (4).

In lipogenic tissues of birds and mammals, transcription of the ACCα gene is regulated by nutritional and hormonal factors. For example, ACCα transcription is low in livers of starved chicks, and feeding a high-carbohydrate, low-fat diet stimulates an 11-fold increase in ACCα transcription (5). Diet-induced changes in ACCα transcription are mimicked in primary cultures of chick embryo hepatocytes by manipulating the concentrations of hormones and nutrients in the culture medium (6). Incubating chick embryo hepatocytes with the active form of the thyroid hormone 3,5′,3-triiodothyronine (T3) stimulates a 5- to 7-fold increase in ACCα transcription. The mechanism by which T3 increases ACCα transcription involves multiple processes. First, T3 interacts with the nuclear 3,5′,3-triiodothyronine receptor (TR) bound to a 3,5′,3-triiodothyronine response element (T3RE) on the more downstream promoter (promoter 2) of the ACCα gene (7). This T3RE (−101 to −86 bp) is composed of two hexameric half-sites arranged as direct repeats with 4 bp separating the half-sites (DR-4 element). Second, T3 treatment increases the binding of TR•retinoid X receptor (RXR) heterodimers to the ACCα T3RE. The mechanism for this effect has not been defined. Third, T3 treatment increases the binding of sterol-regulatory element binding protein-1 (SREBP-1) to a sterol-regulatory element (SRE) (−80 to −71 bp) located immediately downstream of the ACCα T3RE (8). SREBP-1 directly interacts with TR•RXR heterodimers and enhances the ability of this complex to activate ACCα transcription in the presence of T3 (9).

In our studies analyzing the regulation of ACCα transcription by T3, we observed that the ACCα T3RE not only bound protein complexes containing TR•RXR hetero-

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dimers but also bound protein complexes containing liver X receptor (LXR)•RXR heterodimers (7). LXRs are nuclear hormone receptors that are bound and activated by naturally occurring oxysterols (10, 11). Two isoforms of LXR, designated LXRα and LXRβ, have been identified in birds and mammals. LXRβ is expressed in a wide variety of tissues, whereas LXRα is selectively expressed in liver, adipose tissue, intestine, and macrophages (12, 13). LXRs play a key role in regulating cholesterol excretion by mediating the stimulatory effects of oxysterols on the transcription of genes involved in reverse cholesterol transport and bile acid synthesis. For example, naturally occurring oxysterols and synthetic, nonsteroidal LXR agonists activate the transcription of a battery of genes involved in cholesterol efflux (ABCA1, ABCG1, ABCG5, and ABCG8), cholesterol clearance (cholesteryl ester transfer protein and apolipoprotein E), and cholesterol catabolism (cholesterol 7α-hydroxylase) (14–16). For each of these genes, regulation of transcription by LXR agonists is conferred by a liver X receptor response element (LXRE) that binds LXR•RXR heterodimers. Because oxysterols are produced in proportion to cellular cholesterol content, LXRs have been proposed to function as sensors in a feed-forward pathway that stimulates reverse cholesterol transport and cholesterol excretion in response to high cholesterol levels in the diet. Consistent with this proposal, mice lacking the LXRα and/or LXRβ gene exhibit diminished cholesterol excretion and increased cholesterol levels in the blood and liver when fed a high-cholesterol diet (17, 18).

The ability of LXR agonists to activate genes involved in cholesterol excretion has led to an evaluation of the atheroprotective properties of these compounds in murine models of atherosclerosis. Oral administration of the synthetic LXR ligand/agonist, T0-901317, to mice lacking the LDL receptor or apolipoprotein E causes an increase in blood HDL levels and reverses the formation of atherosclerotic lesions in the aorta (19, 20). LXR agonists also improve insulin sensitivity in murine models of type 2 diabetes (21, 22). These exciting findings are tempered by the observation that treatment with T0-901317 also causes hypertriglyceridemia and the development of a fatty liver in rodents and chickens (23–26). These undesired effects of T0-901317 are caused by alterations in the expression of enzymes that make up the fatty acid synthesis pathway. For example, T0-901317 treatment increases the hepatic expression of the mRNAs encoding ACCα, FAS, ATP-citrate lyase (ATP-CL), and stearoyl-coenzyme A desaturase-1 (SCD1) (25–27). The aim of this study was to determine the mechanism by which T0-901317 regulates the expression of ACCα in avian liver. We show that T0-901317 acts directly on the liver to increase the expression of ACCα and that the extent of this effect is modulated by the presence of insulin and T3. We further show that T0-901317 increases ACCα transcription by activating LXR•RXR heterodimers bound to the ACCα LXRE/T3RE and that SREBP-1 interacts with LXR•RXR to enhance the stimulatory effects of T0-901317 on ACCα transcription.

EXPERIMENTAL PROCEDURES

Cell culture and analytical assays

Hepatocytes were isolated from livers of 19 day old chick embryos as described previously (28). Cells were incubated in serum-free Waymouth's medium MD752/1 containing penicillin (60 μg/ml) and streptomycin (100 μg/ml) on untreated Petri dishes at 40°C in a humidified atmosphere of 5% CO_2 and 95% air. Hormone and other additions were as described in the figure legends. The triacylglycerol concentration of the culture medium was measured using an enzymatic kit (Sigma).

Isolation of RNA and quantitation of mRNA levels

Medium was removed and RNA was extracted from hepatocytes by the guanidinium thiocyanate/phenol/chloroform method (29). Total RNA (15 μg) was separated by size on 0.9% agarose, 0.7 M formaldehyde gels and then transferred to a Nytran membrane (Schleicher and Schuell) using a vacuum blotting apparatus (Pharmacia Biotechnology). The RNA was cross-linked to the membrane by ultraviolet light and baked at 80°C for 30–60 min. RNA blots were hybridized with ^{32}P-labeled DNA probes labeled by random priming (30). Hybridization and washes were as described (31). Membranes were subjected to storage phosphor autoradiography. Hybridization signals were quantified using ImageQuant software (Molecular Dynamics). cDNAs for chicken ACCα (5), FAS (32), SCD1 (33), SREBP-1 (34), ATP-CL (35), ABCA1 (25), LXRα (36), and malic enzyme (37) have been described.

Plasmids

Reporter plasmids are named by designating the 5′ and 3′ ends of the ACCα DNA fragment relative to the transcription start site of promoter 2. A series of 5′ deletions and 3′ deletions of ACCα promoter 2 in the context of p[ACC−2054/+274] chloramphenicol acetyltransferase (CAT) have been described (7). An ACCα promoter construct containing a mutation of the SRE between −79 and −72 bp in the context of p[ACC−108/+274]CAT has been described (9). p[ACC−108/−66]TKCAT, p[ACC−84/−66]TKCAT, and pTKCAT constructs, containing mutations in the −108 to −66 bp ACCα fragment, have been described (9).

Transient transfection

Chick embryo hepatocytes were transfected as described by Zhang, Yin, and Hillgartner (7). Briefly, cells were isolated and incubated on 60 mm Petri dishes. At 6 h of incubation, the medium was replaced with one containing 10 μg of lipofectin (Invitrogen), 1.5 μg of p[ACC−2054/+274]CAT or an equimolar amount of another reporter plasmid, and pBluescript KS(+) to bring the total amount of transfected DNA to 1.5 μg per plate. At 18 h of incubation, the transfection medium was replaced with fresh medium with or without T0-901317 (6 μM). At 66 h of incubation, chick embryo hepatocytes were harvested and cell extracts were prepared as described (38). CAT activity (39) and protein (40) were assayed by the indicated methods.

Western blot analysis

Nuclear extracts were prepared from chick embryo hepatocytes as described (9). The proteins of the nuclear extract were subjected to electrophoresis on 10% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes (Millipore) using an electroblotting apparatus (Bio-Rad Laboratories, Hercules, CA). Immunoblot analysis was carried out using

a mouse monoclonal antibody against SREBP-1 (IgG-2A4; American Type Culture Collection, Manassas, VA). Antibody/protein complexes on blots were detected using enhanced chemiluminescence (Amersham Biosciences). Chemiluminescence on the blots was visualized using a FluorChem 8000 imager (Alpha Innotech Corp.), and signals for the mature form of SREBP-1 were quantified using FluorChem V200 software.

Histone acetylation

The extent of histone acetylation on ACCα promoter 2 was measured using a chromatin immunoprecipitation (ChIP) assay. The procedure for this assay was as described by Yin et al. (41). ChIP assays were carried out with antibodies against acetyl-histone H3 (06-599) and acetyl-histone H4 (06-866; Upstate Biotechnology). Precipitated DNA was analyzed by PCR using Taq DNA polymerase (New England Biolabs) and primers specific for the ACCα and SCD1 promoters. The cycling parameters were as follows: 1 cycle of 95°C for 4 min; 30 cycles of 95°C for 1 min, 61°C for 1 min, and 72°C for 1 min; and 1 cycle of 72°C for 10 min. The forward primer of the ACCα gene was 5′-TCCCCTCCGTCAGCAGCCAATGGG-3′; the reverse primer was 5′-ATCCCCGGTCCCGCCCTCGGCTCC-3′. The forward primer of the SCD1 gene was 5′-AGCGAACAGCAGATTGCGGCAG-3′; the reverse primer was 5′-TCTCGGCGTGCCAGAAGGGAGGT-3′. Amplified products were subjected to electrophoresis on 2% agarose gels and visualized by ethidium bromide staining.

Statistical methods

Data were subjected to ANOVA, and statistical comparisons were made with Dunnett's test or Student's *t*-test. Statistical significance was defined as $P < 0.05$.

RESULTS

LXR activation increases the expression of ACCα in primary cultures of chick embryo hepatocytes

Oral administration of T0-901317 to chickens and rats causes a 2- to 3-fold increase in hepatic ACCα mRNA levels (25, 26). To investigate whether this phenomenon was attributable to a direct effect of T0-901317 in the liver, we determined whether T0-901317 regulated the expression of ACCα in primary cultures of chick embryo hepatocytes. Incubating hepatocytes with T0-901317 in the absence of other hormones for 24 h caused a 2.4-fold increase in the abundance of ACCα mRNA (**Fig. 1**). Treatment with the RXR agonist 9-*cis* retinoic acid had no effect on ACCα mRNA abundance in the absence or presence of T0-901317. Thus, LXR agonists but not RXR agonists regulate ACCα expression in hepatocyte cultures.

In chick embryo hepatocytes, insulin has no effect by itself but amplifies the increase in ACCα transcription caused by T3 (6). This observation prompted us to investigate whether insulin modifies the effects of T0-901317 on ACCα expression. Incubating hepatocytes with T0-901317 stimulated a greater increase in ACCα mRNA abundance in the presence of insulin (3.5-fold) than in the absence of insulin (2.4-fold) (Fig. 1). Thus, as observed for T3 regulation of ACCα, insulin enhances the stimulatory effects of T0-901317 on ACCα expression.

In previous work, we showed that both TR and LXR bind the ACCα T3RE as heterodimers with RXR (7). This observation raised the possibility that a common *cis*-acting regulatory sequence is involved in mediating the effects of T0-901317 and T3 on ACCα transcription. As a first step in investigating this possibility, we determined the effects of T0-901317 on the expression of ACCα in the presence of T3. Incubating hepatocytes with T3 and insulin caused a 4.6-fold increase in the abundance ACCα mRNA abundance (Fig. 1). Addition of T0-901317 in the presence of T3 and insulin caused a further increase in ACCα mRNA abundance, although the magnitude of this effect (39%) was substantially smaller than the effect of T0-901317 on ACCα expression in the presence of insulin alone (350%). Addition of T0-901317 in the presence of T3, insulin, and 9-*cis* retinoic acid had no effect on ACCα mRNA abundance. The nonadditive effects of T0-901317 and T3 on ACCα mRNA abundance support the proposal that a common *cis*-acting sequence(s) mediates the actions of T0-901317 and T3 on ACCα transcription.

We also investigated the effects of T0-901317 on the expression of other lipogenic enzymes. In hepatocytes incubated in the absence and presence of insulin, addition of T0-901317 to the culture medium increased the abundance of mRNAs encoding FAS, SCD1, and ATP-CL (Fig. 1). T0-901317-induced expression of FAS, SCD1, and ATP-CL was higher in the presence of insulin than in the absence of insulin. In hepatocytes incubated with T3 and insulin, addition of T0-901317 caused a small increase (32%) in the abundance of FAS mRNA but had no effect on the abundance of SCD1 mRNA or ATP-CL mRNA. Incubating hepatocytes with 9-*cis* retinoic acid in the absence or presence of T0-901317 had no effect on FAS, SCD1, and ATP-CL mRNA levels. Thus, regulation of the expression of FAS, SCD1, and ATP-CL by agonists of LXR and RXR is similar to that of ACCα.

In contrast to ACCα, FAS, SCD1, and ATP-CL, the ability of T0-901317 to increase the expression of malic enzyme was substantially lower than that of T3. Treatment with T0-901317 and insulin caused a 2.9-fold increase in malic enzyme mRNA abundance, whereas treatment with T3 and insulin caused a 35-fold increase in malic enzyme mRNA abundance (Fig. 1). These observations are consistent with previous work demonstrating that the major T3RE mediating T3 regulation of malic enzyme transcription lacks the ability to bind LXR•RXR heterodimers (7).

To determine whether the T0-901317-induced increase in lipogenic enzyme expression was associated with an increase in triacylglycerol production, the triacylglycerol concentration in the culture medium was monitored in hepatocytes treated with or without T0-901317. The triacylglycerol concentration of the culture medium increased progressively during a 48 h incubation period (**Fig. 2**). The extent of the increase in triacylglycerol concentration was higher in cells treated with T0-901317 relative to cells not receiving T0-901317. An increase in hepatic lipogenic enzyme expression and triacylglycerol secretion likely contributes to the hypertriglyceridemia observed in animals treated with T0-901317 (23, 24, 26).

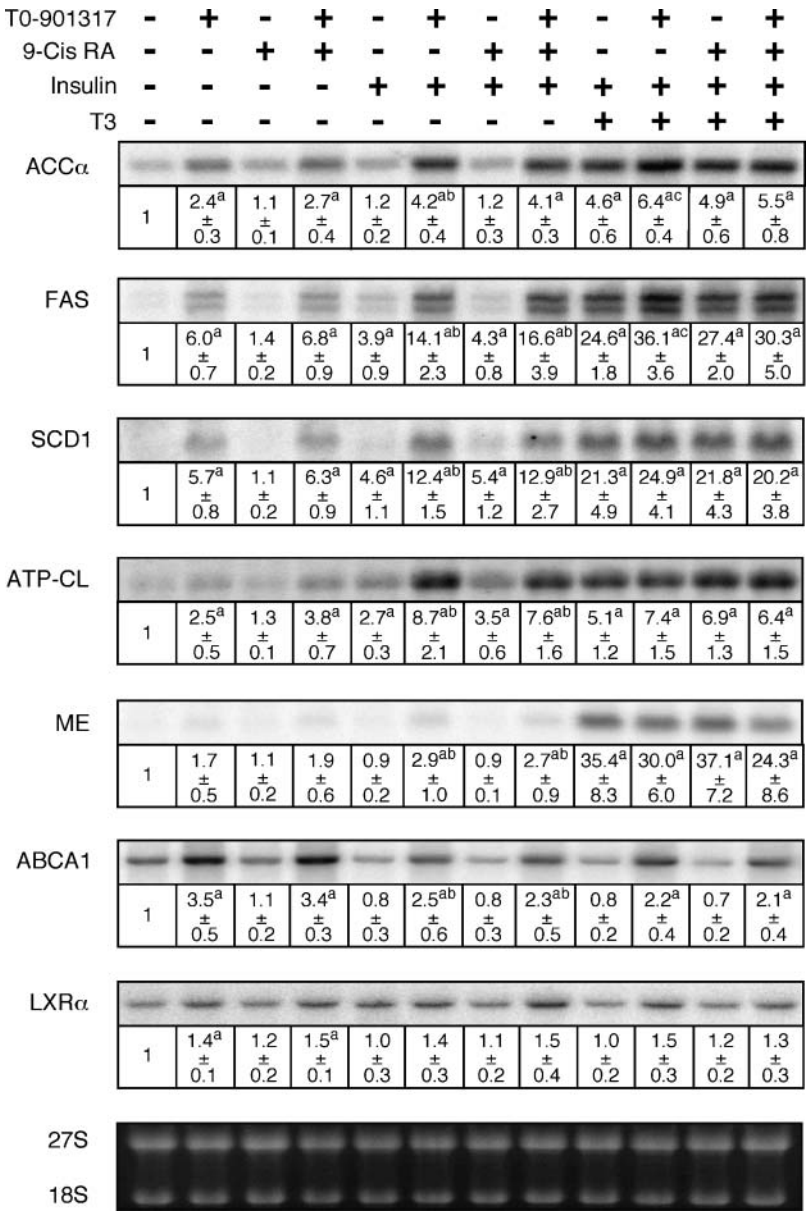

Fig. 1. The liver X receptor (LXR) agonist T0-901317 increases the expression of acetyl-coenzyme A carboxylase-α (ACCα) and other lipogenic enzymes in primary cultures of chick embryo hepatocytes. Hepatocytes were isolated as described in Experimental Procedures and incubated in serum-free Waymouth's medium. At 18 h of incubation, the medium was changed to one of the same composition supplemented with or without T0-901317 (6 μM) in the absence or presence of insulin (50 nM), insulin and 9-*cis* retinoic acid (9-Cis RA; 100 nM), insulin and 3,5′,3-triiodothyronine (T3; 1.5 μM), or insulin, T3, and 9-*cis* retinoic acid. After 28 h of treatment, total RNA was isolated and the abundance of mRNA encoding ACCα, FAS, stearoyl-coenzyme A desaturase-1 (SCD1), ATP-citrate lyase (ATP-CL), malic enzyme (ME), ABCA1, and LXRα was measured by Northern analysis. Levels of mRNA in cells treated without T0-901317, 9-*cis* retinoic acid, insulin, and T3 were set at 1, and the other values were adjusted proportionally. Values are means ± SEM of four experiments. Hybridization signals from a representative experiment are shown for each mRNA. Ribosomal subunits (27S and 18S) stained with ethidium bromide are shown as controls for RNA loading. [a] Mean is significantly ($P < 0.05$) different from that of cells treated without T0-901317, 9-*cis* retinoic acid, insulin, and T3; [b] mean is significantly ($P < 0.05$) different from that of cells treated with insulin; [c] mean is significantly ($P < 0.05$) different from that of cells treated with insulin and T3.

Identification of a LXR response unit that mediates the effects of T0-901317 on ACCα transcription

Previous studies have shown that T3 regulation of ACCα transcription is mediated by a 23 bp region (−101 to −71 bp) in promoter 2 of the ACCα gene (7). This region contains a DR-4 element (−101 to −86 bp) that binds heterodimers composed of TR•RXR and LXR•RXR and a SRE (−82 to −71 bp) that binds SREBP-1. To determine the role of these sequences and other sequences in the ACCα gene in mediating the stimulatory effects of T0-

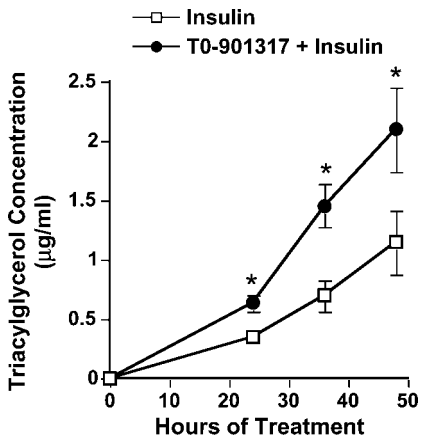

Fig. 2. Effect of T0-901317 on the accumulation of triacylglycerols in the culture medium of chick embryo hepatocytes. Hepatocytes were plated on 90 mm Petri dishes (1×10^7 cells/dish) in Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition supplemented with or without T0-901317. After 0, 24, 36, and 48 h of incubation with T0-901317, the concentration of triacylglycerol in the culture medium was measured using a spectrophotometric assay. Data are means ± SEM of three experiments. * Mean is significantly ($P < 0.05$) different from that of cells incubated with insulin for the same time period.

901317 on ACCα transcription, transient transfection experiments were performed using reporter constructs containing portions of ACCα promoter 2 linked to the CAT gene. In chick embryo hepatocytes transfected with a reporter construct containing 2,054 bp of 5′-flanking DNA, T0-901317 caused a 2.9-fold increase in promoter activity (**Fig. 3**). 5′ deletion of ACCα sequences to −391, −136, and −108 bp had no effect on T0-901317 responsiveness. Deletion of ACCα sequences containing the DR-4 element (−108 to −84 bp) abolished the stimulatory effect of T0-901317 on ACCα transcription. Further deletion to −41 bp had no effect on T0-901317 responsiveness. Mutation of the SRE (−80 to −71 bp) in the context of 108 bp of 5′-flanking DNA caused a 49% reduction in T0-901317 responsiveness. These results indicate that the DR-4 element (LXRE) is required for T0-901317 regulation of ACCα transcription and that the SRE enhances the ability of the LXRE to activate ACCα transcription in the presence of T0-901317.

To determine whether the functional interaction between the ACCα LXRE and SRE required the presence of additional *cis*-acting sequences, hepatocytes were transfected with constructs containing fragments of the ACCα gene linked to the minimal promoter of the herpes simplex virus thymidine kinase (TK) gene. The TK promoter alone was unresponsive to T0-901317 (**Fig. 4**). When a DNA fragment containing both the ACCα LXRE and the ACCα SRE (−108 to −66 bp) was linked to the TK promoter, treatment with T0-901317 caused a 5.5-fold increase in promoter activity. Mutation of the ACCα SRE in the context of the ACCα −108 to −66 bp fragment caused a 49% decrease in T0-901317 responsiveness. When a DNA fragment containing the ACCα SRE alone (−84 to −66 bp) was appended to the TK promoter, T0-901317 treatment had no effect on promoter activity. These data demonstrate that the ACCα SRE can function alone to enhance T0-901317 regulation conferred by the ACCα LXRE. Thus, a region of the ACCα gene containing a LXRE and a SRE is responsible for mediating the effects of T0-901317 on ACCα promoter 2 activity. We refer to this region as the ACCα liver X receptor response unit (LXRU).

LXR activation increases the abundance of mature SREBP-1 in chick embryo hepatocytes

In our studies analyzing the regulation of the ACCα gene by T3, we showed that T3 treatment increased the abundance of the mature, active form of SREBP-1 in chick embryo hepatocytes and that this effect contributed to the activation of ACCα transcription by T3 (8). This observation prompted us to ask whether T0-901317 regulated SREBP-1 levels in chick embryo hepatocyte cultures. The time course of the effects of T0-901317 on the abundance of mature SREBP-1 protein, SREBP-1 mRNA, and ACCα mRNA was determined in hepatocytes cultured in the presence of insulin. Treatment with T0-901317 for 6 h caused a 1.5-fold increase in mature SREBP-1 concentration (**Fig. 5**). A larger increase in mature SREBP-1 concentration (2.2-fold) was observed after 24 h of treatment with T0-901317. In contrast to the time course for mature SREBP-1, the T0-901317-induced stimulation of ACCα mRNA levels occurred at a later time point (between 24 and 48 h of treatment). This observation is consistent with the proposal that alterations in SREBP-1 levels play a role in mediating the regulation of ACCα transcription by T0-901317. Treatment with T0-901317 increased the abundance of SREBP-1 mRNA, and this effect was maximal (1.9- to 2.1-fold) at or before 2 h of incubation. Thus, T0-901317-induced changes in mature SREBP-1 concentration appear to be mediated by a pretranslational mechanism.

Effect of LXR activation on histone acetylation of the ACCα gene

The T0-901317-induced increase in ACCα transcription may be mediated in whole or in part by alterations in histone acetylation. Previous studies have shown that the activation of LXR•RXR heterodimers by LXR ligands/agonists triggers the recruitment of coactivator complexes containing histone acetyltransferase activity (42, 43). Increased histone acetylation causes a chromatin decondensation that enhances the accessibility of the basal transcriptional machinery and other transcription factors to the target promoter. To investigate the role of histone acetylation in mediating the activation of ACCα transcription by T0-901317, ChIP experiments were performed in chick embryo hepatocytes incubated in the absence or presence of T0-901317. Hepatocytes were treated with 1% formaldehyde to cross-link DNA to associated proteins. Protein-DNA complexes were immunoprecipitated with an antibody against acetylated histone H3 or an antibody against acetylated histone H4. Immunoprecipitated DNA

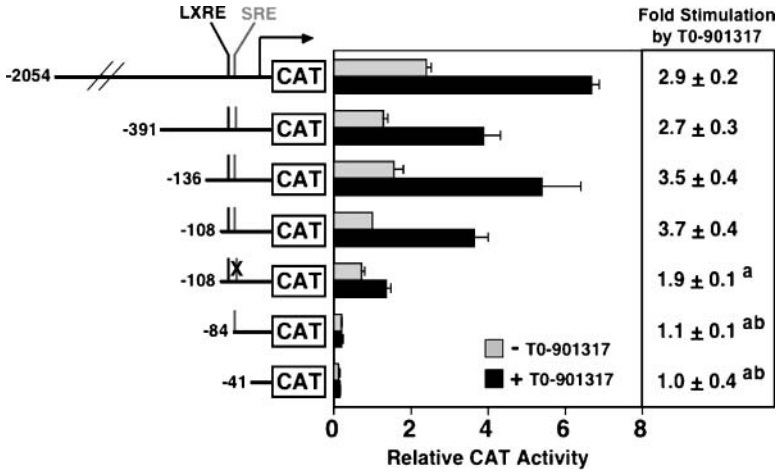

Fig. 3. Effect of mutations of the 5′-flanking region of ACCα promoter 2 on transcriptional activity in the absence and presence of T0-901317. Chick embryo hepatocytes were transiently transfected with p[ACC-2054/+274]chloramphenicol acetyltransferase (CAT) or equimolar amounts of other plasmids as described in Experimental Procedures. After transfection, cells were treated with or without T0-901317 for 48 h. Cells were then harvested, extracts were prepared, and CAT assays were performed. Left: The constructs used in these experiments. The number at the left of each construct is the 5′ end of the ACCα DNA in nucleotides relative to the transcription initiation site of promoter 2. The 3′ end of each construct was +274 bp. The locations of the liver X receptor response element (LXRE) (−101 to −86 bp) and the sterol-regulatory element (SRE) (−80 to −71 bp) are indicated by vertical lines. A block mutation of the SRE is indicated by an X through the vertical line. Right: The CAT activity of cells transfected with p[ACC-108/+274]CAT and treated with T0-901317 was set at 1, and the other activities were adjusted proportionally. The fold stimulation by T0-901317 was calculated by dividing the CAT activity for hepatocytes treated with T0-901317 (+ T0-901317) by that for hepatocytes not treated with T0-901317 (− T0-901317). The fold responses were calculated for individual experiments and then averaged. The results are means ± SEM of six experiments. [a] The fold stimulation by T0-901317 is significantly ($P < 0.05$) lower than that of p[ACC-108/+274]CAT; [b] the fold stimulation by T0-901317 is significantly ($P < 0.05$) lower than that of p[ACC-108/+274]CAT containing a block mutation of the SRE.

was analyzed by PCR using primers that flanked the ACCα LXRU. In hepatocytes incubated in the absence of T0-901317, acetylation of histone H3 and histone H4 was detected at the ACCα LXRU. Addition of T0-901317 to the culture medium stimulated a rapid increase (≤2 h) in the acetylation of histone H3 and H4 (**Fig. 6**, **Table 1**). Histone acetylation remained high for 6 h of T0-901317 treatment and then declined between 6 and 24 h of T0-901317 treatment. These results support the proposal that an increase in histone acetylation plays a role in mediating the effects of T0-901317 on ACCα transcription.

We also used ChIP to assess the effects of T0-901317 treatment on histone acetylation in an uncharacterized region of the SCD1 promoter using a primer set that amplified SCD1 sequences between −369 and −127 bp. In contrast to the data for the ACCα gene, treatment with T0-901317 had no effect on histone acetylation of this region of the SCD1 gene (Fig. 6). This observation indicates that the effects of T0-901317 on histone acetylation are sequence-specific.

DISCUSSION

In previous work analyzing the effects of T3 on ACCα transcription in avian hepatocytes, we identified a T3RE that conferred T3 regulation on ACCα promoter 2 (7). Interestingly, this T3RE not only bound protein complexes containing TR•RXR heterodimers but also bound protein complexes containing LXR•RXR heterodimers. In this report, we provide functional evidence that LXR•RXR heterodimers regulate ACCα transcription. A specific ligand/agonist of LXR (T0-901317) activates ACCα transcription, and this effect is mediated by the LXRE/T3RE in ACCα promoter 2. We also demonstrate that SREBP-1 is an accessory factor that enhances the ability of LXR•RXR to increase ACCα transcription in the presence of T0-901317 and that LXR activation by T0-901317 increases the concentration of the mature, active form of SREBP-1 in chick embryo hepatocytes.

How does SREBP-1 enhance the stimulatory effects of T0-901317 on ACCα transcription? One possibility is that SREBP-1 facilitates the recruitment of coactivators to T0-901317-bound LXR•RXR complexes. LXRα, LXRβ, and SREBP-1 interact with several coactivator proteins, including CREB binding protein (CBP) and the TRAP/ARC/DRIP complex (42–47). We postulate that the presence of SREBP-1 on ACCα promoter 2 provides additional coactivator interaction sites that stabilize the binding of CBP, TRAP/ARC/DRIP, and other coactivators to T0-901317-bound LXR•RXR. In support of this model, SREBP-1 and nuclear hormone receptors interact with separate peptide se-

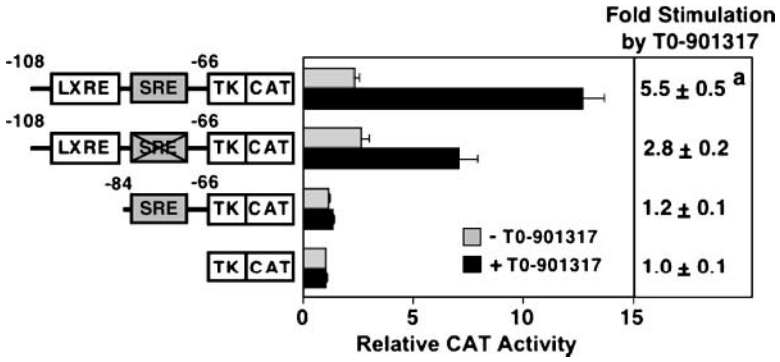

Fig. 4. The ACCα SRE alone enhances the transcriptional activity of the ACCα LXRE in the presence of T0-901317. Fragments of the ACCα gene containing the LXRE and/or SRE were linked to the minimal thymidine kinase (TK) promoter in TKCAT. Chick embryo hepatocytes were transiently transfected with these constructs and treated with or without T0-901317 as described in the legend to Fig. 3 and in Experimental Procedures. Left: The constructs used in these experiments. Numbers indicate the 5′ and 3′ boundaries of the ACCα DNA relative to the transcription initiation site of promoter 2. A block mutation of the SRE is indicated by an X across the box representing the SRE. Right: The CAT activity in hepatocytes transfected with TKCAT and treated without T0-901317 was set at 1, and the other activities were adjusted proportionally. The fold stimulation by T0-901317 was calculated as described in the legend to Fig. 3. The results are means ± SEM of five experiments. [a] The fold stimulation by T0-901317 for p[ACC-108/-66] TKCAT is significantly ($P < 0.05$) higher than that of any other construct.

quences on CBP and separate subunits of the TRAP/ARC/DRIP complex (48–51).

SREBP-1 also enhances the ability of TR•RXR to activate ACCα transcription in the presence of T3 (9). In analyzing the mechanism mediating this effect, we showed that SREBP-1 interacted physically with TR and that binding of T3 to TR enhanced this interaction. We postulated that SREBP-1•SREBP-1 homodimers formed a tetrameric complex with TR•RXR heterodimers and that tetrameric complex formation enhanced the recruitment of coactivators to ACCα promoter 2. We also showed in these studies that LXR•RXR heterodimers do not interact physically with SREBP-1. Thus, in contrast to the mechanism by which SREBP-1 enhances TR•RXR activity, direct interactions between SREBP-1 and LXR•RXR do not play a role in mediating the stimulatory effects of SREBP-1 on LXR•RXR activity.

The mechanism by which LXR agonists regulate transcription has been analyzed for other lipogenic genes. In human hepatoma cells, T0-901317 activation of FAS transcription is mediated by a single LXRE in the FAS promoter (52). In primary rat hepatocyte cultures, the T0-901317-induced increase in SREBP-1c transcription is mediated by two LXREs in the SREBP-1c promoter (53, 54). Both the FAS promoter and the SREBP-1c promoter contain one or more SREs that are located ~110–540 bp downstream of the LXRE(s). In cells that express physiological levels of LXR, these SREs enhance basal transcription but have little or no effect on the regulation of transcription by T0-901317. In contrast to these findings, the SRE in ACCα promoter 2 enhances the T0-901317 regulation of transcription but has no effect on basal transcription in chick embryo hepatocytes (Figs. 3, 4). These gene-specific differences in SRE activity may be attributable to variations in the proximity of the SRE relative to the LXRE and other *cis*-acting regulatory sequences. For example, the close association of the SRE with the LXRE/T3RE in ACCα promoter 2 may facilitate interactions between LXR•RXR and SREBP-1 and the ability of SREBP-1 to enhance T0-901317 responsiveness, whereas the wide separation of the SREs and LXREs in the FAS and SREBP-1c promoters may impede interactions between LXR•RXR and SREBP-1 and the ability of SREBP-1 to enhance T0-901317 responsiveness. Previous studies have shown that the SREs in the FAS and SREBP-1c promoters are closely linked to a binding site for nuclear factor-Y (NF-Y) and that SREBP-1 activity is dependent on interactions between SREBP-1 and NF-Y (53–56). These interactions enhance the ability of SREBP-1 to stimulate basal transcription. In contrast, the SRE in ACCα promoter 2 is not closely associated with binding sites for NF-Y. The lack of interaction of SREBP-1 with NF-Y on ACCα promoter 2 may explain why the ACCα SRE is not effective in modulating basal transcription.

Promoter 2 of the human ACCα gene and the rat ACCα gene contains two closely spaced SREs that mediate the effects of sterol depletion on ACCα transcription (57, 58). In contrast to promoter 2 of the chicken ACCα gene, a sequence resembling a DR-4 element is not present in the region flanking the SREs in the human and rat ACCα promoters. Thus, the role of SREBP-1 in mediating the stimulatory effects of T0-901317 on ACCα transcription may vary depending on the class of animals.

Another finding of this study is that LXR plays a permissive role in mediating the actions of insulin on ACCα transcription in chick embryo hepatocytes. Insulin stimulates ACCα expression in the presence of T0-901317 but has no effect on ACCα expression in the absence of T0-901317 (Fig. 1). In rat hepatocytes, insulin enhances

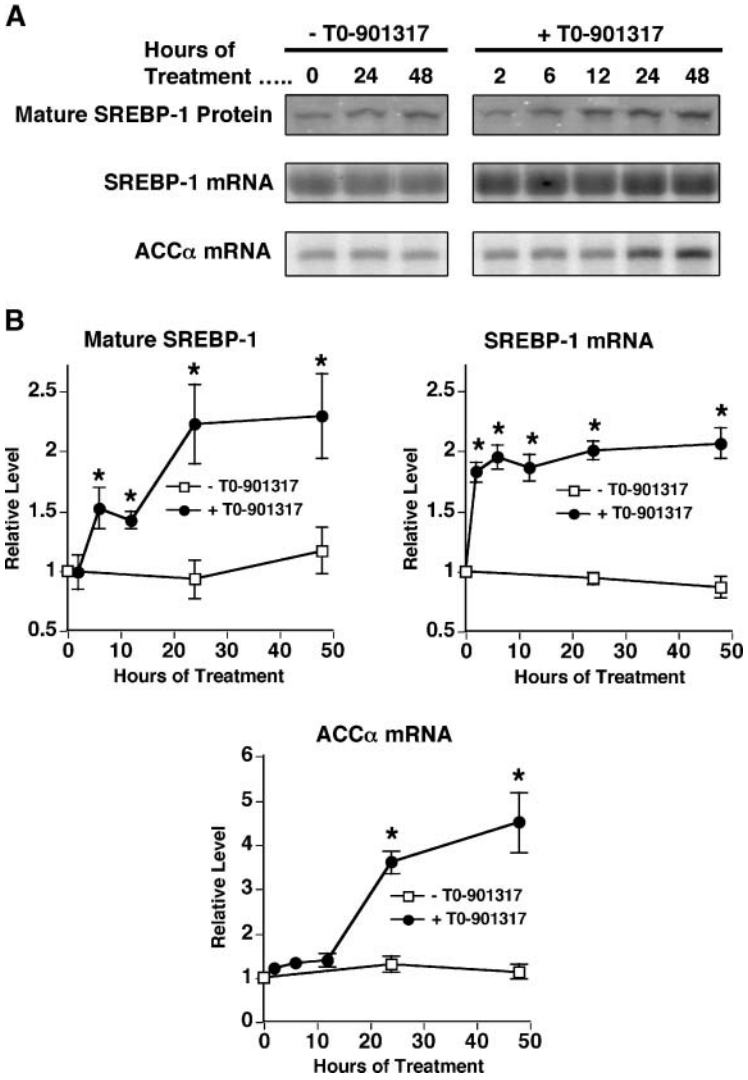

Fig. 5. T0-901317 increases the concentration of mature sterol-regulatory element binding protein-1 (SREBP-1) in chick embryo hepatocyte cultures. Chick embryo hepatocytes were isolated and incubated in serum-free Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition. T0-901317 was added at this time. After 2, 6, 12, 24, and 48 h of T0-901317 treatment, cellular extracts or total RNA were prepared as described in Experimental Procedures. A: The abundance of mature SREBP-1 in nuclear extracts was measured by Western analyses. The abundance of SREBP-1 mRNA and ACCα mRNA was measured by Northern analysis. These data are from a representative experiment. B: Signals for mature SREBP-1 protein from Western analyses and SREBP-1 mRNA and ACCα mRNA from Northern analyses were quantitated. Levels of mature SREBP-1 protein, SREBP-1 mRNA, and ACCα mRNA in hepatocytes treated with T0-901317 for 0 h were set at 1. Values are means ± SEM of four experiments. * The mean is significantly ($P < 0.05$) different from that of cells incubated with T0-901317 for 0 h or without T0-901317 for 24 or 48 h.

the ability of T0-901317 to increase mature SREBP-1 concentration as a result of a stimulatory effect of insulin on the processing of precursor SREBP-1 to mature SREBP-1 (59). We have confirmed this finding in chick embryo hepatocytes (data not shown). Because SREBP-1 enhances the LXR activation of ACCα transcription in chick embryo hepatocytes, we postulate that alterations in mature SREBP-1 abundance mediate the stimulatory effects of insulin on ACCα transcription. In support of this hypothesis, insulin does not increase the expression of ABCA1 in the presence of T0-901317 (Fig. 1). Previous work has shown that the ABCA1 gene is not a target of SREBP-1 (60).

Previous studies performed in rat hepatocytes indicate that insulin induces ACCα expression by increasing the activity of LXR. For example, Tobin et al. (61) have shown that insulin stimulates a 10-fold increase in the expression of LXRα mRNA and that ablation of the LXRα gene abolishes the stimulatory effect of insulin on ACCα expression. Others have shown that insulin increases the transcription of SREBP-1c and that this effect is mediated by two LXR•RXR binding sites in the SREBP-1c promoter (53, 54). SREBP-1c is a key factor mediating the effects of insulin on ACCα transcription in rat hepatocytes (27, 62). These findings contrast with our data indicating that LXR plays a permissive role in mediating the effects of insulin on ACCα transcription in chick embryo hepatocytes. The reason for the differences between birds and rodents in the mechanism by which insulin regulates ACCα transcription is not clear. They may reflect subtle class-dependent differences in the role of insulin in the control of lipogenesis and/or other metabolic processes in liver.

The ACCα LXRE/T3RE enhances ACCα transcription in both the absence and presence of T0-901317 and T3, with a greater activation observed in the presence of T0-901317 and T3 (Figs. 3, 4) (7). We previously hypothesized that the enhancer activity of the ACCα LXRE/T3RE in the absence of T0-901317 and T3 was mediated by LXR•RXR heterodimers, as the primary protein complexes that bind the ACCα LXRE/T3RE in the absence of T0-901317 and T3 contain LXR•RXR heterodimers (7). Recent studies have shown that the unliganded form of LXR•RXR represses gene transcription as a result of its ability to interact with corepressor proteins and that the addition of LXR ligand increases gene transcription by

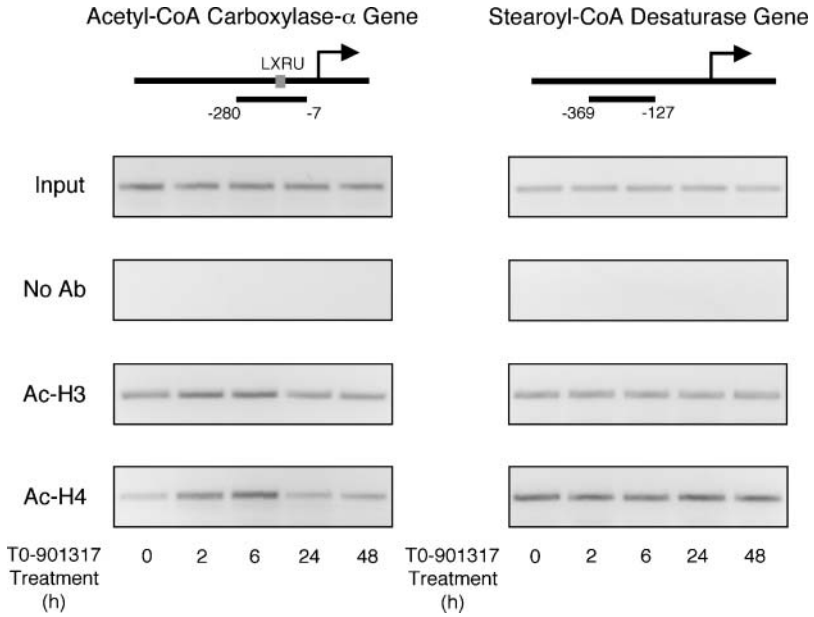

Fig. 6. Treatment of chick embryo hepatocyte cultures with T0-901317 causes a transient increase in histone acetylation at the ACCα liver X receptor response unit (LXRU). Chick embryo hepatocytes were isolated and incubated in serum-free Waymouth's medium containing insulin. At 18 h of incubation, the medium was changed to one of the same composition. T0-901317 was added at this time. After 2, 6, 24, and 48 h of T0-901317 treatment, the association of acetylated histone H3 (Ac-H3) and acetylated histone H4 (Ac-H4) with ACCα and SCD1 genomic sequences was measured. Chromatin immunoprecipitation assays were performed as described in Experimental Procedures. Immunoprecipitates were analyzed by PCR using primers that flanked the LXRU of ACCα promoter 2 and an uncharacterized region of the SCD1 promoter. The regions of the ACCα and SCD1 genes that were amplified by PCR are indicated at top. Chromatin samples that were processed in parallel without the application of primary antibody served as controls (No Ab). The input lanes show the results of PCR using chromatin samples taken before the immunoprecipitation step. Results are representative of three independent experiments.

causing the release of corepressors and the recruitment of coactivators to LXR•RXR (63). In view of these observations, we further hypothesize that LXR•RXR complexes associated with the ACCα LXRE/T3RE in the absence of T0-901317 and T3 are bound by endogenous LXR and/or RXR ligands. Several lines of evidence support this proposal. First, treatment of chick embryo hepatocytes with naturally occurring agonists of LXR [22-(*R*)-hydroxy cholesterol] and RXR (9-*cis* retinoic acid) has little or no effect on ACCα mRNA abundance (Fig. 1 and data not shown). Second, the ACCα LXRE/T3RE is not associated with the corepressor protein, nuclear receptor corepressor, in the absence of T0-901317 and T3 (41). Last, the ACCα LXRE/T3RE is associated with acetylated histone H3 and acetylated histone H4 in the absence of T0-901317 and T3 (Fig. 6). The ability of T0-901317 to increase the transcriptional activity and histone acetylation of the ACCα LXRE/T3RE is likely attributable to the fact that this synthetic agonist is more effective than endogenous LXR agonists in stimulating the recruitment of histone acetyltransferase-containing coactivators to LXR•RXR (42).

TABLE 1. Effect of T0-901317 treatment on the acetylation of histones at the acetyl-CoA carboxylase-α liver X receptor response unit

	T0-901317 Treatment				
Histone	0 h	2 h	6 h	24 h	48 h
Acetylated histone H3	1.0	1.4 ± 0.1[a]	1.8 ± 0.1[a]	1.1 ± 0.2	1.1 ± 0.1
Acetylated histone H4	1.0	1.7 ± 0.1[a]	2.2 ± 0.2[a]	1.0 ± 0.1	1.1 ± 0.1

Signals from chromatin immunoprecipitation assays using the indicated antibodies were quantitated. The data are expressed as fold differences relative to samples from hepatocytes treated with T0-901317 for 0 h. Values are means ± SEM of three experiments. Results from a representative experiment are shown in Fig. 6.

[a] Significantly different ($P < 0.05$) from cells treated with T0-901317 for 0 h.

In summary, we show that T0-901317 activates ACCα transcription by increasing the activity of LXR•RXR and SREBP-1 and the acetylation of histone H3 and histone H4 on ACCα promoter 2. The identification of small molecules that selectively inhibit one or more of these processes represents a potential strategy to enhance the utility of T0-901317 in the treatment of atherosclerosis and type 2 diabetes. The feasibility of this approach is supported by recent studies demonstrating that suppression of ACCα expression reverses hepatic steatosis and insulin resistance in rats fed a high-fat diet (64).

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