Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ

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The liver X receptors (LXRs) are members of the nuclear hormone receptor superfamily that are bound and activated by oxysterols. These receptors serve as sterol sensors to regulate the transcription of gene products that control intracellular cholesterol homeostasis through catabolism and transport. In this report, we describe a novel LXR target, the sterol regulatory element-binding protein-1c gene (SREBP-1c), which encodes a membrane-bound transcription factor of the basic helix-loop-helix-leucine zipper family. SREBP-1c expression was markedly increased in mouse tissues in an LXR-dependent manner by dietary cholesterol and synthetic agonists for both LXR and its heterodimer partner, the retinoid X receptor (RXR). Expression of the related gene products, SREBP-1a and SREBP-2, were not increased. Analysis of the mouse SREBP-1c gene promoter revealed an RXR/LXR DNA-binding site that is essential for this regulation. The transcriptional increase in SREBP-1c mRNA by RXR/LXR was accompanied by a similar increase in the level of the nuclear, active form of the SREBP-1c protein and an increase in fatty acid synthesis. Because this active form of SREBP-1c controls the transcription of genes involved in fatty acid biosynthesis, our results reveal a unique regulatory interplay between cholesterol and fatty acid metabolism.

[Key Words: LXR; RXR; cholesterol; fatty acids; SREBP-1; nuclear receptor]

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The liver X receptors [LXRs] were originally identified as orphan members of the nuclear hormone receptor superfamily of transcription factors, as their cognate ligands were unknown. LXRα [NR1H3, also known as RLD-1] is highly expressed in liver, intestine, kidney, and adipose (Willy et al. 1995; Repa and Mangelsdorf 2000). LXRβ [NR1H2, also known as UR, NER1, OR-1, and RIP15] is found in nearly all tissues examined (Teboul et al. 1995; Repa and Mangelsdorf 2000). Early studies revealed that LXRs bind DNA as obligate heterodimers with retinoid X receptors [RXRs], a family of nuclear receptors that are activated by their endogenous ligand 9-cis retinoic acid and highly specific synthetic agonists, called rexinoids (Boehm et al. 1995). The RXR/LXR heterodimer belongs to a subclass of nuclear receptor heterodimers that are permissive to ligand-induced transactivation of target genes by the RXR partner, which facilitated the early characterization of this orphan receptor heterodimer (Willy and Mangelsdorf 1997). RXR/LXR binds with high affinity to a DNA sequence comprised of two direct repeats of a hexanucleotide motif [AGGTCA] separated by four nucleotides, now commonly referred to as an LXRE of the DR4 type.

A major advance in the study of LXRs came with the identification of their ligands as a specific class of naturally occurring oxysterols, most arising as metabolic derivatives of cholesterol (Janowski et al. 1996, 1999; Lehmann et al. 1997). The most potent physiologic ligands include 24(S),25-epoxycholesterol, which is present in liver; 22(R)-hydroxycholesterol, which is found in abundance in adrenal gland; and 24(S)-hydroxycholesterol, also known as cerebrosterol because of its high levels in brain. In a variety of assays, these ligands were found to bind and activate LXRα and β in a specific, saturable manner at concentrations consistent with those found in tissues in which cholesterol metabolism and LXR expression are high (Janowski et al. 1999; Repa and Mangelsdorf 2000, Schroepfer 2000). Recently, a synthetic nonsteroidal LXR agonist has also been described (Repa et al. 2000; Schultz et al. 2000).

The pattern of expression of LXRs and their oxysterol ligands first suggested a role for LXRs in cholesterol metabolism. Indeed, the first target gene identified for LXRα was cholesterol 7α-hydroxylase [CYP7A1], which encodes the rate-limiting enzyme in the classic pathway of bile acid biosynthesis (Lehmann et al. 1997; Peet et al. 1998). Mice subjected to high cholesterol diets or synthetic agonists of LXR showed marked increases in
CYP7A1 expression, resulting in increased production and fecal elimination of cholesterol-derived bile acids [Peet et al. 1998; Repa et al. 2000]. Recently, the ATP-binding cassette transporters, ABC1 and ABC8, which are implicated in the flux of cellular free cholesterol, have been found to be under the transcriptional regulation of the RXR/LXR heterodimer [Costet et al. 2000; Repa et al. 2000; Venkateswaran et al. 2000]. Cholesterol ester transfer protein (CETP), which has been implicated in reverse cholesterol transport has also been reported to be a direct LXR target gene [Luo and Tall 2000]. These findings suggest that LXRs may serve as sterol sensors by responding to elevated oxysterol concentrations and up-regulating the expression of key genes responsible for enhancing cholesterol catabolism and transport.

The identification of oxysterol-regulated genes dependent on RXR/LXR has been greatly facilitated by studies in mouse strains devoid of LXRα and/or LXRβ [Peet et al. 1998; Repa et al. 2000; Venkateswaran et al. 2000], and the availability of synthetic RXR and LXR agonists [Boehm et al. 1995; Repa et al. 2000]. The initial characterization of the LXRα-null mouse strain had previously shown that liver mRNA levels for several genes involved in fatty acid synthesis are reduced compared with levels in control mice [Peet et al. 1998]. In addition, we and others have noted that one of the major pharmacologic responses of animals treated with rexinoids or LXR agonists is a significant increase in serum triglyceride levels [Miller et al. 1997; Schultz et al. 2000].

In this report, we reveal the mechanism responsible for the RXR/LXR-dependent regulation of lipogenesis in mice. We show that sterol regulatory element-binding protein-1c (SREBP-1c), a transcription factor known to regulate the expression of lipogenic enzymes [Kim and Spiegelman 1996; Shimano et al. 1997a; Horton and Shimomura 1999; Shimano et al. 1999], is a direct target gene of RXR/LXR. In mice lacking the LXR genes, the basal expression level of liver SREBP-1c mRNA is significantly reduced and, in parallel, so are the mRNA levels of lipogenic enzymes known to be affected by SREBP-1c. In addition, feeding diets containing high cholesterol, rexinoids or LXR agonists results in an increase in SREBP-1c mRNA and protein expression in wild-type mice, but not in Lxrα/−/− mice. Finally examination of the 5′-flanking region of the mouse SREBP-1c gene revealed an LXRE motif necessary for the RXR/LXR-mediated up-regulation of expression. These studies establish mouse SREBP-1c as a target gene of the oxysterol receptor, LXR, and suggest a novel convergence of homeostatic mechanisms for cholesterol and fatty acid metabolism.

Results

SREBP-1 expression and fatty acid synthesis in LXR-deficient mice

As observed previously, the expression of liver SREBP-1 mRNA was reduced in LXRα null mice fed a standard rodent diet [Fig. 1; Peet et al. 1998]. No such change was seen in mice lacking only the LXRβ gene, but a profound reduction in SREBP-1 mRNA was shown by Lxrα/−/− mice [Fig. 1]. When fed a high cholesterol diet for seven days, mice harboring at least one LXR gene showed a nearly threefold increase in SREBP-1 mRNA relative to chow-fed animals of a similar genotype. A modest increase in liver SREBP-1 mRNA levels was also observed within the first day of feeding wild-type mice a 2% cholesterol diet (Y. Zhang and D.J. Mangelsdorf, unpubl.).

![Figure 1. Effect of dietary cholesterol on the liver expression of SREBPs and lipogenic enzymes in wild-type and Lxr-knockout mice.](image-url)
Mice lacking both LXRα and LXRβ, however, failed to show this dietary cholesterol-mediated induction in SREBP-1 expression.

Examination of several lipogenic target genes of SREBP-1 revealed similar changes in expression (Fig. 1). Stearoyl CoA desaturase (SCD) mRNA was coordinately reduced in $Lxra^{-/-}$ and $Lxra/\beta^{-/-}$ mice fed a basal diet and modestly induced by cholesterol feeding in all mice except the $Lxr$ double-knockout strain. The SCD cDNA probe used in these northern analyses does not distinguish SCD-1 and SCD-2. However, liver from mice fed standard basal diets is reported to express SCD-1 exclusively [Kaestner et al. 1989], and only under conditions in which elevated levels of the nuclear, active SREBPs are present in mouse liver does SCD-2 mRNA become barely detectable (Korn et al. 1998). Therefore, the changes observed in SCD expression most likely reflect alterations in SCD-1 mRNA levels. The expression levels of fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC) mRNA were not elevated by cholesterol feeding but continued to show a relative decrease in $Lxra^{-/-}$ and $Lxra/\beta^{-/-}$ mice fed either low- or high-cholesterol diets. In agreement with the observed changes in RNA levels of lipogenic enzymes, determination of fatty acid synthesis rates in organs of chow-fed $Lxra^{-/-}$ mice and their wild-type littermates by a whole animal in vivo [$^3$H]water incorporation technique (Shimano et al. 1996) revealed that fatty acid synthesis was reduced 75% in liver, 25% in kidney, and 35% in small intestine compared with wild-type mice. Furthermore, these studies showed that the incorporation of C18:1 and C16:1 fatty acids into phospholipids and triglycerides was significantly reduced (J.D. Horton, D.J. Peet, and D.J. Mangelsdorf, unpubl.). SREBP-2 mRNA levels were reduced by cholesterol feeding as previously reported (Shimano et al. 1996) and modestly induced by cholesterol feeding in all mice receiving these agonists. However, LG268 and T0901317 were ineffective in inducing SREBP-1 in the $Lxr$ double-knockout mice, except for a modest increase by LG268 in the small intestine. Interestingly, the basal level of SREBP-1 mRNA in $Lxra/\beta^{-/-}$ mice receiving the vehicle control was reduced in liver but slightly elevated in adipose and intestine.

**SREBP-1c mRNA and protein are regulated by RXR/LXR**

Two SREBP-1 isoforms are derived from a single gene by the use of two distinct transcriptional start sites under the control of separate promoters and are alternatively spliced to yield the SREBP-1a isoform (containing 29 amino acids derived from exon 1) or the SREBP-1c isoform (containing only five amino acids encoded by exon 1) [Yokoyama et al. 1993; Hua et al. 1995], which is also called ADD1 [Tontonoz et al. 1993]. Northern analyses such as those depicted in Figures 1 through 3 do not distinguish SREBP-1a and SREBP-1c. Therefore, a previously described RNase protection assay [Shimomura et al. 1997b] was used to differentiate between the two mouse SREBP-1 mRNA isoforms [Fig. 4A; see Materials and Methods]. Oral administration of the RXR agonist for 16 to 18 h resulted in the induction of only SREBP-1c expression in liver, intestine, and white fat of wild-type mice (Fig. 4A,B) to a degree similar to that observed previously by Northern analysis. No change in the mRNA levels of SREBP-1a were detected. Kidney SREBP-1c mRNA was also increased by LG268 or T0901317 by threefold in wild-type mice, but not in $Lxra/\beta^{-/-}$ mice [data not shown].

![Figure 2. Effect of nuclear receptor agonists on SREBP-1 mRNA expression.](image-url)}
SREBs are synthesized as ∼120 kD precursor proteins that are bound to the endoplasmic reticulum and nuclear envelope (Brown and Goldstein 1997). Under low cellular sterol conditions, two sequential proteolytic cleavage reactions occur to liberate an ∼70-kD amino-terminal fragment that translocates to the nucleus to activate gene transcription (Brown and Goldstein 1999). Immunoblot analyses of membrane and nuclear extracts of liver and intestine of LG268-treated mice revealed that the increase in SREBP-1c mRNA by the RXR/LXR heterodimer is reflected in the amount of the active nuclear form of SREBP-1c protein that serves as a transcription factor (Fig. 5A). This elevation in the nuclear active form of SREBP-1c would account for the up-regulation of expression for lipogenic enzymes regulated by SREBP-1 (Fig. 1). The relative increase in the nuclear form of SREBP-1 protein mirrored the change observed previously in the mRNA levels in these tissues from mice receiving rexinoids as measured by Northern analysis. The similar increase in the levels of SREBP-1 mRNA and the processed, nuclear form of SREBP-1 protein suggests that the enzymatic activities of the SREBP proteases are not rate-limiting under these experimental conditions, since the amount of the unprocessed, precursor form of SREBP-1 does not change. SREBP-2 protein levels were unaffected in these mice (Fig. 5B).

Identification of an LXRE in the mouse SREBP-1c promoter

To investigate the molecular mechanism of RXR/LXR-mediated regulation of mouse SREBP-1c the 5′-flanking region of the mouse SREBP-1c promoter was cloned and examined for the presence of an LXRE. The 11032 ∼ 130 bp fragment from the 5′-flanking region of the mouse SREBP-1c promoter (Fig. 3) was ligated into the XbaI/MluI site of the luciferase reporter vector and the reporter construct was transiently transfected into NIH3T3 cells. The luciferase activity of the reporter construct was significantly increased in the presence of the RXR/LXR agonists LG268 and T0901317, indicating the presence of an LXRE in the mouse SREBP-1c promoter.

Figure 3. Regulation of SREBP-1 mRNA by RXR and LXR agonists in liver (A), white adipose tissue (B), and small intestine (C). Male wild-type and Lxrαβ−/− mice were fed diets containing 0.2% cholesterol plus vehicle (open bars), 30 mgk LG268 (hatched bars), or 50 mgk T0901317 (black bars) for 12 h. Northern analysis was performed on mRNA individually (A) or as a pooled sample consisting of three to four animals per group (B,C). Relative mRNA levels in liver (A) are expressed as the mean ± SEM, and a representative Northern blot is shown above. Small intestine mRNA was isolated from the mucosa of the duodenum (D), jejunum (J), and ileum (I).

SREBP-1c is regulated by rexinoid. [A] Three mice per group were fed diets containing 0.2% cholesterol plus vehicle (−) or 30 mgk LG268 (+) for 16–18 h. Pooled RNA was isolated from liver, intestine, and epididymal white adipose depot of each group. Aliquots of the pooled RNA (20 µg) were subjected to RNase protection assays using a 32P-labeled cRNA probe that can distinguish between 1a and 1c isoforms of SREBP-1 (BP1). RNA samples from two independent studies were examined in the same blot. Relative mRNA levels in liver (A) are expressed as the mean ± SEM, and a representative Northern blot is shown above. Small intestine mRNA was isolated from the mucosa of the duodenum (D), jejunum (J), and ileum (I).
region of this gene was sequenced and analyzed (Fig. 6A). A 6.5-kb genomic fragment of the mouse SREBP-1c promoter, including the transcription start site, was placed before a luciferase reporter gene to test for RXR/LXR responsiveness in a cell-based reporter assay (Fig. 6B). When this reporter plasmid was introduced into HEK293 cells, luciferase activities were increased three- to fourfold on incubating the cells with either LG268 or T0901317 and greater than 10-fold when both synthetic agonists were present. Similar results were observed in primary hepatocytes transfected with the pBP1c6500 luciferase reporter and treated with LG268 and T0901317 [data not shown]. Serial 5’ truncations of the SREBP-1c promoter revealed that the majority of ligand-induced responsiveness in a cell-based reporter assay (Fig. 6B). This effect was more pronounced when additional LXRs were provided to the HEK293 cells [Fig. 8B]. These in vitro assays (Figs. 6–8) in conjunction with the whole animal analyses (Figs. 1–5) show that mouse SREBP-1c is a direct target gene of the RXR/LXR heterodimer.

Figure 5. Regulation of SREBP-1 protein expression by rexinoid. Immunoblot analysis of SREBP-1 and SREBP-2 was performed on fractionated cell extracts from liver and intestine of wild-type male mice fed diets containing 0.2% cholesterol plus vehicle (−) or 30 mpk LG268 (+) for 18 h. P and N denote the precursor and cleaved nuclear forms of SREBP, respectively, and the asterisk indicates a nonspecific band. The filters for liver and intestine were exposed to film at room temperature for 15 sec and 45 sec, respectively.

Discussion

The work presented here describes the first report of nutrient-mediated regulation of SREBP-1c transcription defined at a molecular level. Cholesterol-derived oxysterols activate the RXR/LXR heterodimer to induce expression of the mouse SREBP-1c gene through an LXRE located in its proximal promoter. This finding was confirmed by experiments using RXR- and LXR-specific synthetic agonists and mouse strains of defined Lxr genotype, and by the identification and characterization of the LXRE in the 5’-flanking region of the mouse SREBP-1c gene responsible for this transcriptional regulation.

SREBP-1c regulation of fatty acids

Numerous reports of nutrient regulation of SREBP-1c have appeared in the recent literature (for review, see Osborne 2000). Investigators have identified a reduction in SREBP-1c mRNA in mouse liver and adipose with fasting and a restoration or overshoot of SREBP-1c mRNA levels on refeeding mice a high-carbohydrate diet (Horton et al. 1998; Kim et al. 1998). This overshoot in SREBP-1c expression may be caused by a recently identified SRE in the promoter of mouse SREBP-1c that would allow for a feedforward autoregulation of this gene on SREBP-1c induction [Amemiya-Kudo et al. 2000]. Interestingly, when Lxrβ−/− and wild-type mice are subjected to this fasting/refeeding regimen, SREBP-1c mRNA levels are similarly affected in both mouse strains, indicating that LXRs are not involved in this regulatory pathway [data not shown].

Insulin/glucose have been strongly implicated as the mediators of the refeeding-induced up-regulation of SREBP-1c transcription, although their precise molecu-

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lar mechanism of action has not yet been defined. When isolated rat adipocytes or hepatocytes are treated with insulin, a transcriptional increase in SREBP-1c/ADD1 is observed (Kim et al. 1998; Foretz et al. 1999). Likewise, when rats are made diabetic by the administration of streptozotocin, liver SREBP-1c mRNA levels are reduced and can be restored with insulin administration (Shimomura et al. 1999b).

Specific polyunsaturated fatty acids or fish oil have also been used in animal feeding studies, with variable results.

Figure 6. RXR and LXR agonists activate mouse SREBP-1c promoter. (A) The sequence of the mouse SREBP-1c 5'-flanking region (~1.3 kb) is shown with the DR4 LXRE motif denoted by arrows. The putative transcription start site at ~63 is boxed. (B) RXR and LXR agonists (LG268 and T0901317) synergistically activate a luciferase reporter driven by the SREBP-1c promoter. A 6.5-kb fragment of the SREBP-1c promoter was linked to pGL3 basic luciferase reporter. The resulting plasmid pBP1c(6500)-Luc was cotransfected into HEK-293 cells with a control reporter plasmid pCMV-βGal as described in Materials and Methods. Twenty hours after transfection, cells were treated with vehicle [DMSO and/or ethanol], LG268 [1 µM], T0901317 [10 µM], or LG268 [1 µM] plus T0901317 [10 µM]. Normalized luciferase activity of cells treated with vehicle is arbitrarily defined as 1, and relative luciferase activities from different treatments are shown as “fold increase”. Each value represents data from four independent transfection experiments (each in duplicate). (C) Deletions of the ~6500 kb mouse SREBP-1c promoter were assayed as described in B. Data shown are the average of three independent transfection experiments (each in duplicate) in the upper panel and two independent experiments in the lower panel. The 100% value for basal activity corresponds to the normalized luciferase activity obtained with the pBP1c(6500)-Luc promoter construct in the absence of LG268 and T0901317. Individual values for fold-activations were: upper panel 8.1 (4.9, 7.4, 12), 4.5 (3.8, 4.8, 5.0), 5.0 (2.4, 5.5, 7.0), 0.73 (0.4, 0.8, 1.0); lower panel 7.8 (5.1, 10.5), 0.85 (0.6, 1.1), 4.4 (4.8, 4.8, 0.98) (0.95, 1.0).
results, suggesting that these nutrients may reduce SREBP-1 mRNA levels or affect SREBP-1 protein processing (Kim et al. 1999; Yahagi et al. 1999). These latter data have recently been confirmed by demonstrations that unsaturated fatty acids repress both SREBP-1 transcription and proteolytic processing in HEK293 cells (V.C. Hannah, J. Ou, A. Luong, J. Goldstein, and M.S. Brown, in prep.).

Hypertriglyceridemia and RXR/LXR agonists: the role of SREBP-1c

The consequence of SREBP-1c up-regulation has been extensively investigated in cells and mice that overex-

press the active, nuclear form of this transcription factor [Kim and Spiegelman 1996; Shimano et al. 1997a; Horton et al. 1998b; Pai et al. 1998]. Increased production of either the SREBP-1a or SREBP-1c transcription factor results in the enhanced expression of genes encoding enzymes involved in lipogenesis [for review, see Horton and Shimomura 1999; Osborne 2000]. SREBP-1c, which contains a shorter, less negatively charged activation domain, is not as active as SREBP-1a [Shimano et al. 1997a]; however, SREBP-1c is the predominant isoform found in most tissues [Shimomura et al. 1997b]. Ultimately, the result of SREBP-1c production is an increase in fatty acid synthesis and development of a fatty liver [Shimano et al. 1997a; Shimomura et al. 1999a].

Further evidence that SREBP-1c is integral to increased liver fatty acid synthesis was shown by the failure of SREBP1−/− mice to up-regulate the expression of lipogenic enzymes when subjected to a fasting/refeeding treatment (Shimano et al. 1999). Consistent with an LXR-mediated up-regulation of SREBP-1c, liver triglyc-

![Figure 7](image1.png)

**Figure 7.** The LXRE in mouse SREBP-1c promoter is a high affinity binding site for RXR/LXR heterodimer. (A) Sequences of the LXRE derived from the mouse mammary tumor virus LTR (ΔMTV-LXRE), wild-type mouse SREBP-1c promoter region (1c-WT), and mutant mouse SREBP-1c promoter region (1c-MUT). For comparison, the LXRE sequences of the bottom strands of 1c-WT and 1c-MUT are aligned with the top strand sequence of ΔMTV. (B) Electrophoretic-mobility shift assays were performed as described in Materials and Methods. The 32P-labeled 1c-WT LXRE probe was incubated with in vitro synthesized human LXRα and RXRα proteins as indicated. For antibody supershift experiments, anti-RXRα antibody (αRXR) or a non-specific antibody (ns) were used. Free probe, RXR/LXR complex, and antibody-supershifted RXR/LXR complex are denoted by arrows. Nonspecific bands are indicated by asterisks. (C) Competition gel mobility-shift assays were performed as described in Materials and Methods using 32P-labeled 1c-WT LXRE as input probe and unlabeled oligonucleotides as competitors at 10- and 50-fold molar excesses. Autoradiography was performed for 16 h at −80°C with an intensifying screen.

![Figure 8](image2.png)

**Figure 8.** Mutation of the LXRE sequence abolishes activation of mouse SREBP-1c promoter by RXR/LXR. (A) Schematic diagrams of pBP1c(6500)-Luc wild-type and mutant reporter plasmids. Differences between wild-type and mutant plasmids (nucleotides −300 to −280) are shown for comparison. (B) Activation of wild-type, but not mutant, LXRE reporter plasmids. Wild-type and mutant pBP1c(6500)-Luc were cotransfected into HEK293 cells. For each 60 mm dish 0.5 µg pBP1c-Luc, 25 ng, CMV-βgal and CMX-LXRα or pCDNA3 were used. Three hours after transfection, cells were switched to medium A containing vehicle (DMSO) or 10 µM T0901317, and assayed as described in Figure 6. Data represent three independent transfection experiments (each in duplicate).
eride concentrations are elevated in normal mice following long-term cholesterol feedings or when treated with rexinoids and LXR agonists (Schultz et al. 2000). This accumulation of triglyceride by the feeding of cholesterol or RXR/LXR acting drugs is absent in mice lacking the LXRs (Peet et al. 1998; Schultz et al. 2000) or mice that harbor a hepatocyte-specific deletion of RXRα (Wan et al. 2000). Thus, the RXR/LXR-mediated up-regulation of fatty acid synthesis through SREBP-1c provides an explanation for the hypertriglyceridemia seen in animals and patients receiving rexinoid therapy (Miller et al. 1997). Interestingly, in one study in hamsters (Shimomura et al. 1997a) dietary cholesterol did not lead to an increase in SREBP-1, suggesting that the response to endogenous LXR ligands is weak or absent in this species. Consistent with this hypothesis we note that the hamster also fails to up-regulate CYP7A1, another known LXR target gene, in response to dietary cholesterol [Horton et al. 1995]. Nevertheless, administration of synthetic RXR or LXR agonists to hamsters results in potent hypertriglyceridemia, suggesting that the LXR-mediated SREBP-1 induction of lipogenesis is conserved in this species [Schultz et al. 2000]. The basis for this species variation is currently under investigation.

Model of LXR action as a cholesterol sensor

Stearoyl CoA desaturase-1 is a target gene of SREBP-1c (Shimano et al. 1997a; Tabor et al. 1999). This enzyme is responsible for the Δ9-cis desaturation of stearoyl-CoA and palmitoyl-CoA, converting them to oleoyl-CoA and palmitoleoyl-CoA, respectively. Early studies of cholesterol-fed rats suggested that SCD expression was enhanced by an indirect means [Garg et al. 1986]. The current studies would suggest that cholesterol feeding results in LXR-mediated up-regulation of SREBP-1c and subsequent SREBP-1c-directed induction of SCD-1. The role of SCD-1 in cholesterol homeostasis was recently shown in mice harboring a null mutation in this gene. SCD1−/− mice showed impaired production of oleic acid and a diminished capacity to esterify cholesterol for hepatic storage and packaging into VLDL for export to other tissues [Miyazaki et al. 2000]. This notion is consistent with unpublished data demonstrating C18:1 fatty acid synthesis is significantly reduced in LXR knockout mice [J.D. Horton, D.J Peet, and D.J. Mangelsdorf, unpubl.]. The benefit of up-regulating SCD-1 would be to increase oleoyl-CoA, the preferred substrate for ACAT-mediated cholesterol esterification (Landau et al. 1997). Thus, under conditions of high cholesterol, LXR would indirectly promote the esterification of free cholesterol to protect the cell from its harmful effects [Fig. 9]. In addition, the increase in fatty acid synthesis under these conditions would promote a more appropriate ratio of cholesterol to other lipids to maintain cell membrane integrity [Fig. 9].

In summary, the identification of SREBP-1c as a direct target of LXR action further solidifies the role of LXRs as sterol sensors that function to ameliorate the effects of high free cholesterol levels [Fig. 9]. In the liver, LXR up-regulates the cholesterol 7α-hydroxylase (CYP7A1) gene to promote production and elimination of bile acids [Peet et al. 1998]. In macrophages and enterocytes, LXR-mediated expression of ABC1 facilitates the efflux of free cholesterol from the cell [Repa et al. 2000]. Finally, in this report it is shown that oxysterol/LXR-mediated up-regulation of SREBP-1c occurs in numerous cell types to promote lipid synthesis to coordinate a homeostatic balance between fatty acids and sterols.

Materials and methods

Animals and diets

All experiments were performed on age-matched, male mice of 2.5 to 4 months of age. Lxr knockout mice were generated as described [Peet et al. 1998; Lobaccaro and Mangelsdorf, unpubl.],
and genotypes were confirmed by Southern and Northern analyses. Animals were housed in temperature-controlled rooms (22°C) with 12-hr light/dark cycles. Mice were fed ad libitum a cereal-based powdered diet (Teklad 7001, Harlan Teklad) supplemented with any of the following: cholesterol (ICN Biomedicals); chenodeoxycholic acid, fenofibrate, or pregnenolone α-carbonitrile [Sigma]. LC268 (Ligand Pharmaceuticals) provided in a vehicle containing 0.9% carboxyl methyl cellulose, 9% polyethylene glycol 400, and 0.05% Tween 80; T0901317 (Repa et al. 2000); or troglitazone [kindly provided by Dr. Roger Unger, University of Texas Southwestern Medical Center, Dallas]. Diets were supplemented with these agents at a level sufficient to provide the appropriate miligrams per kilogram body weight [mpk] dose on consumption of 5 g diet by a 25 g mouse per day. Experiments were performed with the approval of the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

Northern blotting

At the completion of feeding studies, mice were anesthetized and exsanguinated. Liver and epididymal adipose samples were flash-frozen in liquid nitrogen and stored at −80°C until RNA was prepared. Small intestine was removed and flushed with cold phosphate-buffered saline, divided into three equal lengths designated duodenum (proximal), jejunum (medial), and ileum (distal); and the mucosa was carefully removed and placed in a tube for freezing and storage prior to RNA preparation. Total RNA was extracted using RNA STAT-60 (Tel-Test), and mRNA was prepared by pooling equal amounts of total RNA from three to six mice per treatment group or from individual mouse liver total RNA samples. mRNA (5 µg/lane) was prepared; and 30µg aliquots of protein were size fractionated by 8% SDS-PAGE and transferred to Hybond C extra membrane [Amersham] as described [Shimano et al. 1996]. The membrane was incubated with 5 µg/mL of rabbit antiserum SREBP-1 IgG or 5 µg/mL rabbit antimouse SREBP-2 IgG. Immunoblot analysis was performed with the Enhanced Chemiluminescence [ECL] western blotting kit [Amersham], using a horseradish peroxidase-conjugated donkey anti-rabbit secondary IgG [Amersham].

Plasmids

Human LXRα and RXRα expression vectors pCMX-hLXRα and pCMX-hRXRα have been described [Willy et al. 1995]. Isolation of genomic DNA containing mouse SREBP-1 gene has been described [Shimano et al. 1997b]. An Xhol/Ncol fragment (1.3 kb) containing the mSREBP-1c promoter [from −1271 to ATG of mSREBP-1c] was cloned into pGL3 basic vector [Promega] to generate pBP1c(1271)-Luc. The adjacent upstream 5.2-kb SacI fragment containing SREBP-1c promoter from −6500 to −1271 was then cloned into pBP1c(1271)-Luc to generate pBP1c(6500)-Luc. To generate pBP1c(572)-Luc, pBP1c(1271)-Luc was digested with SacI and relocated to create pBP1c(1271)-Luc. The adjacent upstream 5.2-kb SacI fragment containing SREBP-1c promoter from −6500 to −1271 was then cloned into pBP1c(1271)-Luc to generate pBP1c(6500)-Luc. To generate pBP1c(572)-Luc, pBP1c(1271)-Luc was digested with Nhel to release a 700-bp fragment, and the remaining 5.4-kb fragment was relegated to create pBP1c(572)-Luc. To generate pBP1c(170)-Luc, pBP1c(572)-Luc was digested with Nhel/Apal to release a 400-bp fragment, the remaining 5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc. To generate pBP1c(1271)-Luc, pBP1c(1271)-Luc was then digested with EcoRV/Apal to release the 580-bp fragment containing SREBP-1c promoter from −700 to −170. The remaining 5.5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc. To generate pBP1c(1271)-Luc, pBP1c(1271)-Luc was then digested with EcoRV/Apal to release the 580-bp fragment containing SREBP-1c promoter from −700 to −170. The remaining 5.5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc. To generate pBP1c(1271)-Luc, pBP1c(1271)-Luc was then digested with EcoRV/Apal to release the 580-bp fragment containing SREBP-1c promoter from −700 to −170. The remaining 5.5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc. To generate pBP1c(1271)-Luc, pBP1c(1271)-Luc was then digested with EcoRV/Apal to release the 580-bp fragment containing SREBP-1c promoter from −700 to −170. The remaining 5.5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc. To generate pBP1c(1271)-Luc, pBP1c(1271)-Luc was then digested with EcoRV/Apal to release the 580-bp fragment containing SREBP-1c promoter from −700 to −170. The remaining 5.5-kb fragment was then treated with Mung Bean nuclease and relocated to create pBP1c(170)-Luc.
ate pBP1c(6500)-Luc(mut). All constructs were confirmed by sequencing.

Cell culture and transfection assays

Human embryonic kidney-293 (HEK-293) cells were maintained at 37°C in an atmosphere of 8.8% CO₂ in medium A (DME medium containing 100 U/mL penicillin and 100 µg/mL streptomycin sulfate) supplemented with 10% fetal calf serum (FCS). On day 0, HEK-293 cells were plated at 4 x 10⁵ cells per 60-mm dish in medium A. On day 2, the medium was switched to medium A supplemented with 6% MBS (Stratagene) and/or T0901317 as indicated. According to the manufacturer’s protocol. Three hours after transfection, the medium was switched to medium A supplemented with 10% FCS except for the experiment depicted in Figure 8 (see legend). After incubation for 20 h, the medium was changed to medium A containing LG268 and/or T0901317 as indicated. T0901317 and LG268 were added to cells at 10³-fold dilution in medium A containing LG268 and/or T0901317 as indicated. with 10% FCS except for the experiment depicted in Figure 8.

Electrophoretic mobility-shift assays

hlXRx and hRXRx proteins were generated from expression vectors [Willy et al. 1995], using a coupled in vitro transcription/translation system (Promega). Sequences of double-stranded oligonucleotides shown in Figure 7 were synthesized with HindIII overhangs. Oligonucleotide probes were labeled by end-filling, using AMV reverse transcriptase (Boehringer) and Redivue [3²⁵P]dCTP (6000 Ci/mmol, Amersham Pharmacia). Binding reactions were performed in a total volume of 20 µL consisting of 75 mM KCl, 20 mM Hepes (pH 7.4), 2 mM of dithiothreitol, 7.5% [v/v] glycerol, 0.1% [v/v] NP-40, 2 µg of poly[d(I-C)] [Amersham Pharmacia], and 2 µL of nuclear receptors lysates or unprogrammed lysates. Reactions containing lysates were incubated for 10 min at room temperature, followed by the addition of 40 fmol of ³²P-labeled probe, and further incubated for 20 min. Samples were then loaded onto 5% polyacrylamide gels [1:40 crosslink] and run in 0.5x TBE buffer [200 V, 3 h for 15 cm x 20 cm gel at 4°C]. For antibody supershift experiments, 1 µL of anti-hRXRα antibody (Santa Cruz) or non-specific antibody (anti-Myc from Invitrogen) was added to the reaction after the addition of probe and incubated at room temperature for another 10 min before electrophoresis. For oligonucleotide competition experiments, competing cold oligonucleotides were added to the reaction with the radiolabeled probe at 10- or 50-fold molar excesses. After electrophoresis, the gels were dried and autoradiographed with intensifying screens at ~80°C for 16 h.

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