Liver X Receptor Inhibits the Synthesis and Secretion of Apolipoprotein A1 by Human Liver-Derived Cells

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ABSTRACT: The liver X receptor (LXR) agonist TO901317 inhibited the synthesis of apolipoprotein A1 (apo A1) by human liver-derived cells, including the formation of lipid-poor, prebeta-migrating high-density lipoprotein (HDL). Despite activation of the lipid transporter ABCA1 under these conditions, cellular efflux of PL and cholesterol from liver cells was also reduced. By assaying transcription from full-length and truncated promoters and by site-directed mutagenesis, the effect of LXR and its ligand was localized to a binding site for hepatic nuclear factor-4 (HNF4) in the proximal apo A1 promoter (−132/−119 bp). Chromatin immunoprecipitation analysis of apo A1 transcription complexes from control and ligand-activated cells showed an increase in the binding of reported apo A1 transcriptional inhibitor COUP-TF, which competes with HNF4 for DNA binding. It also identified LXR in the apo A1 transcription complex of TO901317-treated cells. Displacement of HNF4 from the −132/−119 bp promoter DNA sequence in the presence of TO901317 was confirmed by gel shift analysis. These data indicate that LXR can be a significant negative regulator of apo A1 transcription and HDL synthesis.

Apolipoprotein A1 (apo A1[1]) is the major protein of plasma high-density lipoprotein (HDL). It is the only protein of a lipid-poor, prebeta-migrating fraction of HDL identified as a major acceptor of cell-derived cholesterol in the reverse cholesterol transport (RCT) pathway (*1*). Prebeta$_1$-HDL, which contains apo A1 and small amounts of phospholipid (PL), is regenerated from mature, spheroidal HDL under the influence of the plasma PL transfer protein (*2*). In addition, particles with properties similar to or identical to those of circulating prebeta$_1$-HDL enter the plasma compartment after the synthesis and secretion of apo A1 (*3*). In mammals, this takes place mainly from hepatic and intestinal cells (*4*−*5*). Most of the PL transferred to newly secreted apo A1 originates from ATP-binding cassette transporter A1 (ABCA1) activity at the cell surface (*6*−*7*).

Transcription of the apo A1 and ABCA1 genes is mediated by different nuclear receptor proteins. That of apo A1 is activated by proteins of the hepatic nuclear factor (HNF) family. These bind to multiple sites within the proximal promoter (*8*, *9*). HNF proteins react as homodimers with direct repeats (DRs) based on a regulatory sequence (AGGTCA) separated by a single nucleotide. A second family of transcription factors, chicken ovalbumin upstream promoter transcription factors (COUP-TFs), inhibits apo A1 transcription competitively (*10*, *11*). Two closely related proteins, COUP-TFI and COUP-TFII, are expressed in liver cells. Apo A1 transcription rates are also strongly influenced by an apo C3 gene enhancer element, part of the apo A1-C3-A4 gene cluster (*12*). Other nuclear receptors, including the farnesoid X receptor (FXR) and peroxisomal proliferation activating receptor-alpha (PPARα) can contribute to apo A1 transcriptional regulation via proximal binding sites in human cells (*13*, *14*).

The most important regulator of ABCA1 transcription is the liver X receptor (LXR). It binds as a heterodimer with the retinoid X-receptor (RXR) to a direct repeat of the AGGTCA motif or a similar sequence, separated by four bases (DR-4 site) (*15*). Although oxysterols are the major natural ligands of LXR, a synthetic agonist (TO-901317) (*16*) has been widely used in cell and animal studies (*17*, *18*). Ligand binding promotes the displacement of co-repressors from DNA-bound LXR/RXR and the recruitment of co-activators (*19*, *20*). Other DR-4 nuclear receptors such as the thyroid receptor (TR), whose ligand is triiodothyronine (T_3), compete with LXR for binding to the ABCA1 DR-4 site (*21*). Overlap between transcription factors binding to the apo A1 and ABCA1 promoters has not been reported, though mechanisms for possible crosstalk have been identified. For example, the transcription of PPARα is LXR-dependent (*22*).

Because they stimulate ABCA1, LXR ligands have emerged as promising drug candidates to increase circulating levels of HDL lipids (*23*). However, their effect on the synthesis and composition of prebeta-HDL, a complex containing both apo A1 and PL, has not been reported. In this study, we found that TO901317 increased ABCA1

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[1] Abbreviations: ABCA1, ATP-binding cassette transporter A1; Apo A1, apolipoprotein A1; COUP-TF, chicken ovalbumin upstream promoter transcription factor; DR, direct repeat; FC, free cholesterol; HDL, high density lipoprotein; HNF4, hepatic nuclear factor-4; LXRa, liver X receptor-alpha; PL, phospholipid; PPARa, peroxisome proliferator activated receptor-a; prebeta1-HDL, prebeta-migrating high density lipoprotein-1; SR, Stokes radius.

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transcription but strongly inhibited both the synthesis of apo A1 and the formation of biologically active prebeta-HDL.

EXPERIMENTAL PROCEDURES

Cell Culture. Human hepatocyte-derived cells (Hep3B, HepG2) were grown to near confluence in 6-well plastic plates in Dulbecco's modified Eagle's medium (MEM) containing 10% fetal bovine serum (FBS). These cell lines secrete comparable levels of apo A1 (*24*). Human intestine-derived (CaCO-2) cells (*5*) were grown under the same conditions. To activate LXR, cells were washed with serum-free DMEM, then incubated in DMEM + 0.2% w/v bovine serum albumin. After 24 h, fresh medium + TO901317 in DMSO (final concentration of 10 μM) was added. Cells treated with the same volume of DMSO without agonist were carried through all experiments. After a total of 48 h of induction, the medium was replaced again and apo A1 secretion assayed by solid-phase immunoassay (*25*). The apo A1 species in the medium were characterized by nondenaturing polyacylamide gel electrophoresis (PAGE) using precast 10−20% gradient gels (Biorad, Richmond, CA). Quantitation from Western blots with polyclonal antibodies to apo A1 (*3*) or apo E (*25*) was performed by scanning densitometry. Determination of apo A1 synthetic rate was measured using cells cultured in a leucine-free medium to which ^{3}H-leucine (NEN/Perkin-Elmer) had been added. After immunoprecipitation with anti apo A1 antibody and protein G agarose, the purified protein was fractionated by SDS−PAGE and tritium label in the apo A1 band determined. In some experiments, cells had been preincubated (24 h) to label PL with ^{3}H-choline or 1,2-[^{3}H]-free cholesterol (FC) (both from NEN/Perkin-Elmer). Lipid bound to secreted HDL was determined after electrotransfer to nitrocellulose membranes. Lipid specific activity was determined as previously described (*3*).

mRNA Quantitation. PCR was carried out with RNA isolated from liver-derived cells using Rneasy kits (Qiagen, Germantown, PA). Reverse transcription was performed with SuperScript II enzyme (In Vitrogen, San Diego, CA) according to the manufacturer's instructions. The reaction was carried out as previously described (*20*). Briefly, 40 ng of original RNA was transcribed. The DNA formed reacted with Taq polymerase with SYBR Green as the fluorophor in the Mx300P real-time PCR system. Beta-actin RNA was the internal control. For beta-actin, the PCR primers were AGAGGGAAATCGTGCGTGAC (forward, f) and CAATAGTGATGACCTGGCCGT (reverse, r). For apo A1, the PCR primers were TCTCGCTGATGAAAGGGCTC (f) and TTGTGGTAGGTTGGGCAGTTC (r).

Luciferase Assays An extended apo A1 promoter construct (−2347 to +397 bp) was obtained by amplifying human genomic DNA. After digestion with HindIII, this fragment was cloned into the pGL3B-basic vector coding for firefly luciferase (Promega, Madison, WI). Shorter promoter fragments were generated from the original construct by using it as a PCR template. Independently, mutations within two major DR-1 nuclear receptor binding sites (−212 to −192 bp; −132 to −119 bp) (*8*) were generated with QuikChange Mutagenesis kits (Stratagene, La Jolla, CA) (Table 1). Plasmids were transfected into HepG2 or Hep3B cells together with the internal control plasmid pRL-TK (Promega), which codes for renilla luciferase under the control of the thymidine kinase (TK) promoter. In some experiments, luciferase assays were carried out with cells that had been transfected with full-length COUP-TF-I or -II cDNA (gift of Dr. M. J. Tsai, Houston, TX). The ability of wild-type and mutant promoter fragments to stimulate transcription was determined by a dual luciferase assay (*21*).

Table 1: Promoter DNA Sequences and Primers[a]

a. Promoter truncations	
PCR Primers:	
-2347/+397 f:	AGCAGTAAGCTTGCTGGGATGGTCAAGTAGGC
r:	CCTGACAAGCTTGGAGGTGGGGGAGAGGG
-1409/+397 f:	AGCAGTAAGCTTATGAGGCCGACCACTCCC
-941/+397 f:	AGCAGTAAGCTTACTTGCCCACGATCTTCC
-372/+397 f:	AGCAGTAAGCTTTGGAGGAGTCCCAGCGTC
-32/+397 f	AGCAGTAAGCTTAATAGGCCCTGCAAG
b. Promoter mutations	
Sequences: -132/-119 wt	TGATCCTTGAACTC
mut	TGAg a t a t c t ACTC
-213/-192 wt	TGAACCCTTGACCCCTGCCCTG
mut	TGAACCg a t a t a a a aTGCCCTG
PCR Primers:	
132/-119 f	CCAGGGACAGAGCTGAGATATCTACTCTTAAGTTCCAC
r	GTGGAACTTAAGAGTAGATATCTCAGCTCTGTCCCTGG
-213/-194 f	CCCGCCCCACTGAACCGATATAAAATGCCCTGCAGCCCC
r	GGGGCTGCAGGGCATTTTATATCGGTTCAGTGGGGGCGGG
c. EMSA Oligonucleotides (-136/- 114):	
GAGCTGATCCTTGAACTCTTAAG, CTTAAGAGTTCAAGGATCAGCTC.	
d. ChIP PCR primers (-302/+12 bp)	
f:	AACTGCCCACACTCCCAT
r:	TCCTTCTCGCAGTCTCTAAGCA

[a] f, forward; r, reverse. The promoter base sequence follows that given by Sastry et al. (*30*).

Electrophoretic Mobility Shift Assay (EMSA). HepG2 cell nuclear extract was obtained from GenTex, San Antonio, TX. Hep3B cell nuclear extract was prepared in the laboratory (*26*). EMSA was carried out using LightShift Chemiluminescent kits (Pierce, Rockford, IL). Biotinylated oligonucleotides corresponding to −140 to −117 bp of the apo A1 promoter sequence were synthesized (Table 1) and complementarily annealed to generate ds-DNA. Unlabeled ds-DNA was prepared in the same way. Labeled DNA (20 fmol) was present in each reaction. A 200-fold excess of unlabeled DNA was included in some tubes. HepG2 or Hep3B nuclear protein was included at 5 μg per reaction. Rabbit polyclonal antibody to human HNF4 (H-171) was from Santa Cruz Biotechnology, CA. Purified recombinant LXRα (ActiveMotif, Carlsbad, CA) was used at 50−250 ng

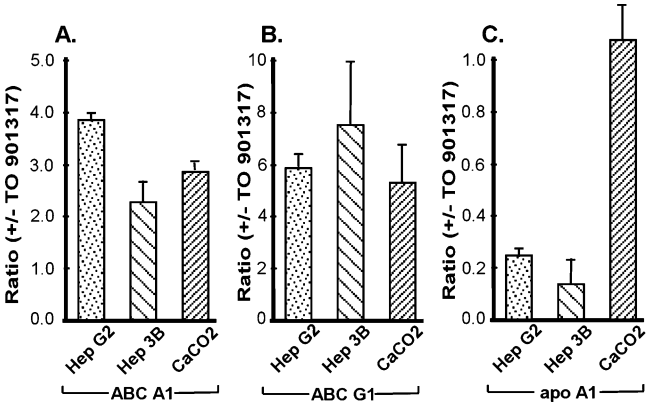

FIGURE 1: Effects of TO901317 on the mRNA levels of the genes of lipid and lipoprotein metabolism. (Panel A) ABCA1 mRNA levels in HepG2 and Hep3B liver-derived cells and CaCO-2 intestine-derived cells. mRNA was assayed as described under Experimental Procedures from cells cultured in the presence or absence of the ligand. (Panel B) ABCG1 mRNA levels under the same conditions. (Panel C) Apo A1 mRNA levels under the same conditions. In each case, mRNA levels in the presence of T0901317 are expressed relative to their levels in the absence of the ligand.

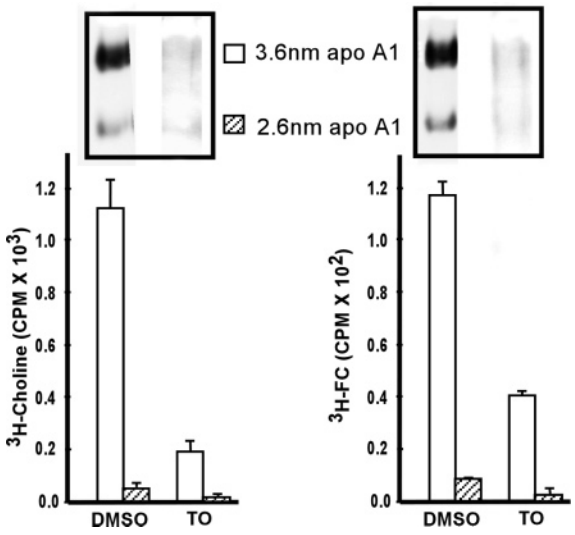

FIGURE 2: Effects of TO901317 on apolipoprotein secretion. Left panel: Effects of the LXR ligand on PL incorporation into HDL in the medium of HepG2 cells labeled with ^{3}H-choline. The medium was collected over 4 h of incubation in serum-free medium; fractionation by nondenaturing PAGE was carried out as described in Experimental Procedures. Insert: Effect on medium levels of the precursor (2.6 nm) and product (3.6 nm) apo A1 species in the same experiment. Left lane, − ligand; right lane, + ligand. Right panel: Effects of the LXR ligand on FC incorporation into HDL in HepG2 cells prelabeled by equilibration with ^{3}H-FC. Insert: effects of the ligand on proportions of secreted apo A1 species in the same experiment. Left lane, − ligand; right lane, + ligand.

per assay. Reaction products were run on 5% polyacrylamide gels in Tris/borate/EDTA buffer (pH 8.4). Gels were electrotransferred to Biodyne-B nylon membranes and UV-cross-linked. DNA and DNA−protein complexes were detected on X-ray film with a chemiluminescent nucleic acid detection module (Pierce Biotechnology, Rockford, IL).

Chromatin Immunoprecipitation (ChIP) Assays. These were carried out as previously described (*27*) except that precipitation from the sheared nucleoprotein complexes (400−500 bp) was carried out using rabbit polyclonal antibodies to human HNF4 (SC8987-X), LXR (SC-13068X), or COUP-TFI/2 (SC-28611-X). Lysed cells were incubated with micrococcal nuclease (2 U) supplemented with 1.3 mmol/L $CaCl_2$. Shearing by sonication was carried out in 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS. After incubation overnight at 4 °C with antibody or nonimmuno IgG (control), cross-linking was reversed with 0.3 M NaCl (4 h, 67 °C). PCR was then carried out to generate a −316/+12 bp apo A1 fragment encompassing the proximal HNF4 promoter binding sites (Table 1).

RESULTS

Effects of the LXR Ligand on ABCA1, apo A1, and HDL Secretion. TO901317 increased ABCA1 mRNA in HepG2, Hep3B, and CaCO-2 cells by 3.8 ± 0.2-, 2.3 ± 0.4-, and 2.8 ± 0.3-fold over control levels (Figure 1A), an effect comparable to that found in previous studies that used HepG2 cells (*23*). An even larger stimulation was measured for mRNA levels of ABCG1 (Figure 1B), consistent with earlier findings (*29*). In contrast, under the same conditions, apo A1 mRNA levels were significantly reduced relative to that of the control in both HepG2 and Hep3B cells treated with TO901317 (−75 ± 2% and −87 ± 8%) (Figure 1C). Apo A1 mRNA levels were unchanged by the same treatment in CaCO-2 cells (+9 ± 15%, NS). These data show that the same agonist had opposite effects on the major genes controlling protein and PL levels in newly synthesized HDL.

We recently showed that apo A1 was secreted from HepG2 and CaCO-2 cells as a PL-free precursor (2.6 nm SR) (*3*). This was converted at the cell surface to a PL-binding product (3.6 nm SR). In response to TO901317, secretion of apo A1 in both 2.6 and 3.6 nm HDL particles was reduced to an extent similar to the decrease in apo A1 mRNA levels in HepG2 cells (Figure 2). Comparable results were obtained with Hep3B cells (data not shown). The incorporation of ^{3}H-leucine into apo A1 was inhibited 95 ± 5% under the same conditions. In contrast, the level of apo E was slightly increased (1.38 ± 0.23 fold) under the same conditions. The effect of TO901317 on the efflux of PL and FC from hepatocytes was also determined. The ligand was without effect on the cellular specific activity of these lipids. Despite the increase in ABCA1 mRNA levels, the secretion of PL (Figure 2, left) and FC (Figure 2, right) to native apo A1 secreted by HepG2 cells was decreased to an extent proportional to that of apo A1. Comparable data was obtained with Hep3B cells. In contrast, lipid efflux from CaCO-2 cells increased 1.9 ± 0.2-fold. TO901317 was without effect on apo A1 protein secretion by CaCO-2 cells (data not shown). These results suggest that the rate of HDL production from liver cells was regulated by the availability of apo A1 and not by PL or FC.

Regulation of Apo A1 Promoter Activity. Sites within the apo A1 promoter that might be responsible for the effect of TO901317 were studied using DNA fragments generated as described under Experimental Procedures. Each was inserted into the pGL3 vector and transfected along with pRL-TK (as internal control) into liver cells. Transcription rates driven by each construct were determined by dual luciferase assay in the presence and absence of the ligand. Luciferase yields from promoter constructs −2347/+397 bp, −1409 /+397 bp, −941/+397 bp, and −372/+397 bp were halved by TO901317 (0.48 ± 0.03 relative to that of the control)(Figure

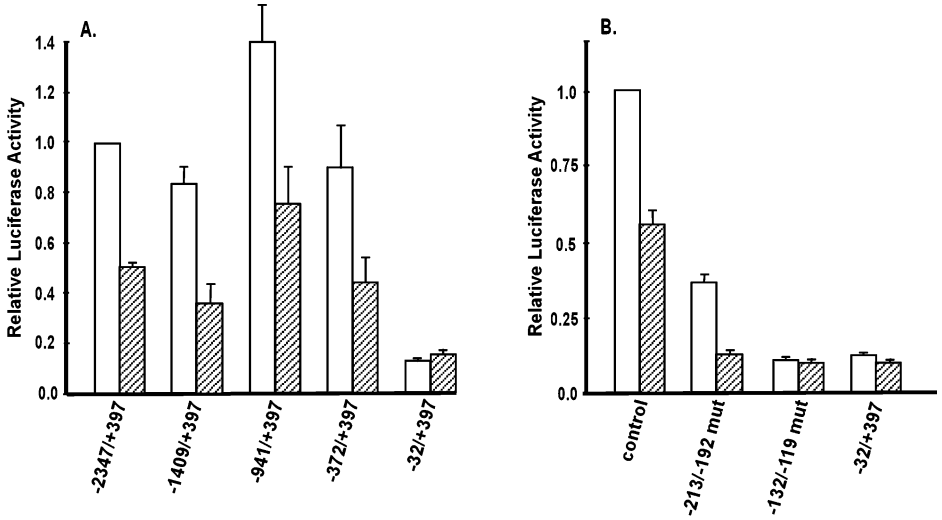

FIGURE 3: Effects of TO901317 on transcriptional activity of wild-type and mutant promoter fragments. Double luciferase assays were carried out as described under Experimental Procedures as the ratio between firefly luciferase linked to apo A1 promoter fragments and renilla luciferase linked to control DNA. (Panel A) Relative luciferase activity of full-length and truncated apo A1 promoter fragments ligated into pGL3: effects of TO901317. The data are expressed relative to the activity of the longest (−2347/+397 bp) DNA fragment in the absence of the ligand. The data represent the means ± one SD for three experiments. Open bars, − TO901317; hatched bars, + TO901317. (Panel B) Effects of mutations in DR-1 sites of the apo A1 promoter: effects of TO901317. The data are expressed relative to the activity with wild-type DNA (−2347/+397 bp)(control). The identities of the open and hatched bars are the same as those in panel A.

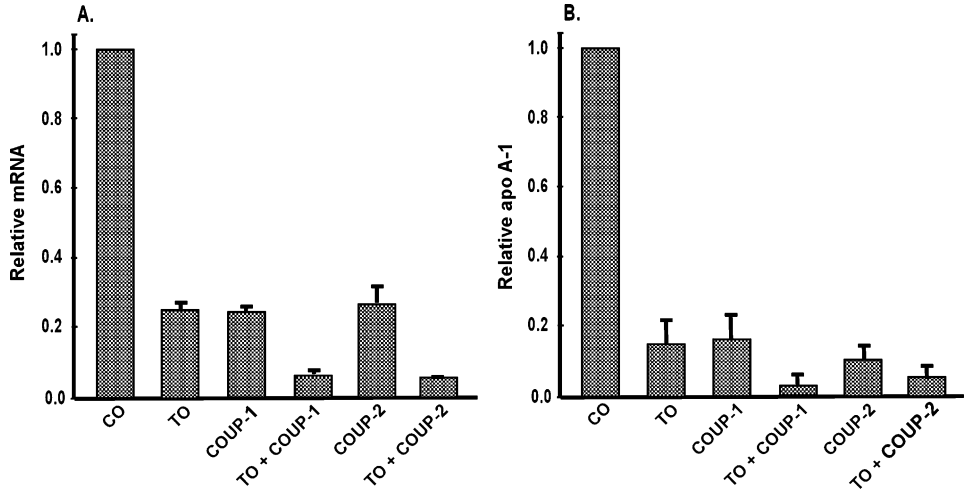

FIGURE 4: Effects of COUP-TF overexpression on luciferase yields in Hep3B cells in the presence and absence of TO901317. CO, sham-transfected control cells; TO, sham-transfected control cells in the presence of TO901317. COUP-1 and COUP-2, cells transfected with full length COUP-TF1 or COUP-TF2 cDNAs, in the absence of TO901317; TO + COUP-1 and TO + COUP-2, transfected cells in the presence of TO901317. (Panel A) Effects on apo A1 mRNA levels. (Panel B) Effects on medium apo A1 levels. In each case, the data is expressed relative to that from control cells in the absence of TO901317.

3A). In contrast, the shortest fragment (−32 /397 bp) was unresponsive to the presence of ligand. This indicated that the major LXR-responsive site was located between −372 and −32 bp in the apo A1 promoter. The shorter promoter fragments also had much reduced activity (compared to wild type) even in the absence of TO901317. This confirmed that promoter elements in the −372 to −32 region were also important in regulating basal transcription rates (*28*).

The −372/−32 bp sequence included major DR-1 sites (−213/−194 bp, −132/−119 bp) of the apoA1 proximal promoter. These were individually mutated in the full-length (−2347/+397 bp) promoter sequence. Both mutations by themselves reduced transcription rates compared to that of the wild type sequence under basal conditions. (Figure 3B). However, although activity from the −213/194 bp mutant, like that of the wild-type promoter, was further reduced (0.50 ± 0.05 relative to that of the control) by TO901317, the ligand had no effect on the −132/−119 bp mutant (0.96 ± 0.05 vs that of the control, NS). These data indicated that the effect of TO901317 on apo A1 was mainly or entirely regulated at the level of transcription and that the −132/−119 bp DR-1 site was of most importance in mediating this effect.

Effects of COUP-TF Proteins on Apo A1. COUP-TF family proteins, by displacing HNF-family proteins from their proximal promoter sites, are major regulators of apo A1 transcription *in vivo* and *in vitro* (*10, 11*). To determine if changes in COUP levels mediated the effects of TO901317, total COUP-TF was first assayed in Hep3B cells by Western blotting in the presence and absence of the ligand, using an antibody detecting both COUP-TF1 and COUP-TF2. No change was detected (data not shown). Overexpression of

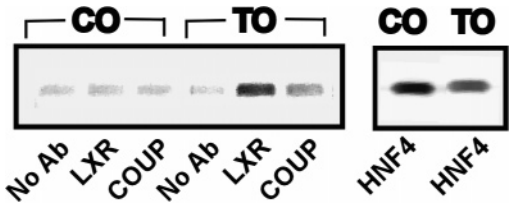

FIGURE 5: ChIP analysis of apo A1 promoter DNA. Following the immunoprecipitation of cross-linked sheared DNA from control and TO901317-treated Hep3B cells using the antibodies shown, the level of DNA was assayed by PCR as described in Experimental Procedures.

either COUP-TF1 or COUP-TF2 in liver-derived cells transfected with a full-length cDNA of either gene led to a significant reduction in both apo A1 transcription rates (Figure 4A) and apo A1 secretion (Figure 4B). However, TO901317 induced an additional decrease in both parameters, proportionate to that seen in cells with baseline COUP-TF levels. These data, although confirming the important regulatory influence of COUP-TF family proteins, indicated that these proteins by themselves did not account for the effects of TO901317 on apo A1 transcription.

Proteins of the Apo A1 Transcription Complex. In ChIP assays, antibodies to transcription factors or co-regulators are used to immunoprecipitate these proteins along with promoter DNA of the same transcription complex. Not only the direct binding of a DNA sequence to a transcription factor but also the indirect binding of co-activators or co-repressors within such transcription complexes is, thus, identified. The signal from DNA precipitated by the HNF4 antibody was reduced 54 ± 3% in cells exposed to TO901317. The signal from DNA precipitated by the COUP antibody was 4.0 ± 1.0-fold higher when the ligand was present. The signal for LXR, which was very low in the absence of TO901317, was easily seen in preparations from ligand-stimulated cells because of a 9.3 ± 2.4-fold increase in signal (Figure 5). These data were consistent with LXR binding to the apoA1 proximal promoter complex, even though this lacks a canonical DR-4 site.

EMSA Assays of the Apo A1 Proximal Promoter. To confirm the predominant role of the −132/−119 bp site in the effect of TO901317 indicated by the luciferase mutation data, EMSA nucleoprotein complexes were characterized from a hepatic nuclear extract incubated with ds-DNA (−140/−117 bp) that encompassed this site. Predictably, excess cold DNA diluted out all signals from DNA−protein complexes. HNF4 binding to the labeled DNA was indicated by the disappearance of a major signal from extracts preincubated with antibody to this protein (Figure 6). TO901317 and recombinant LXR also markedly reduced the signal from HNF4. These data suggest that both factors can help displace HNF4 from its −132/−119 bp binding site. A second, unidentified band, depleted by cold DNA but not by HNF4 antibody, probably reflects the signal from a second nuclear protein at the DR-1 site.

DISCUSSION

The liver is the major source of the HDL proteins in mammalian plasma. The development of LXR ligands for increasing HDL makes their effects on synthesis of particular

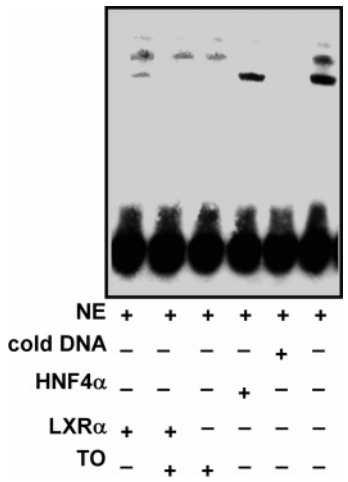

FIGURE 6: EMSA assays of binding of HepG2 nuclear protein with double-stranded biotin-labeled oligonucleotide encompassing the −136 to −114 bp apo A1 promoter site. NE, + nuclear extract from HepG2 cells (4 μg per assay); + cold, with +50-fold excess of unlabeled ds-oligonucleotide; α-HNF4α, preincubated with antibody (2 μg per assay); TO, NE (4 μg) + TO90131 (10 μM); LXR, NE (4 μg) + LXR, 100 ng; TO + LXR, NE (4 μg) + TO901317 and LXR, 100 ng. The arrow identifies the complex of DNA with HNF4, on the basis of its depletion by the antibody. A second unidentified DNA−protein complex is also seen.

interest (*23*). The data reported here show that in human liver-derived Hep3B and HepG2 cells, the LXR ligand TO901317 strongly inhibited apo A1 transcription, mRNA levels, and secretion. Even though mRNA levels of ABCA1, the main source of PL on newly secreted HDL, were raised (*6*), lipid efflux decreased parallel to that of apo A1. It was shown earlier that PL binding by newly formed prebeta$_1$-HDL was limited to ∼2 mol/mol of apo A1 (*3*). This finding, with the present data, suggests that apo A1 levels are rate limiting for ABCA1-mediated PL transfer from liver-derived cells. Secretion of a second HDL protein secreted by HepG2 and Hep3B cells, apo E (*24*), was modestly activated by TO901317. The data indicate that the response of HDL proteins to this ligand is gene-specific.

Transcriptional Regulation of Apo A1. Because intestine-derived CaCO-2 cells were unaffected under the same conditions, it seemed possible that TO901317 might act by blocking a liver-specific enhancer element such as that described by Sastry et al. (*28*). A comparison of full-length and truncated apo A1 promoter constructs and of mutations within the full-length promoter identified the −132/−119 bp sequence as the most significant target of the LXR ligand. This is one of the DR-1 sites normally occupied by HNF proteins in hepatic cells. The conclusion that a LXR ligand had its major effect at this location was unexpected because LXR has been considered to be specific for DR-4 sites (*19*).

LXR-Dependent Inhibition of Apo A1 Transcription. Transcription factors normally bind directly to a consensus DNA sequence, though it was recently shown that they may also act as co-activators, recruiting other proteins to the transcription complex. SREBP1c, a classical DNA-binding transcription factor, is a co-activator of the insulin gene promoter (*30*). The proneural factor Sns acts as either a transcription factor or a co-activator under different conditions (*31*). Agonists including TO901317 can also influence the pattern of co-activator binding (*16*). The EMSA assays

showed that TO901317 mediated the displacement of HNF4 from the −132/−119 bp promoter sequence. The ChIP assays showed that this was coupled to an increase in COUP-TF and LXR binding. COUP-TFs usually bind as homodimers, but heterodimers with retinoic acid receptor RAR (DR-5), TR, or RXR (DR-4) at DR-1 sites have been identified (*32*). The most direct explanation of the current data is that TO901317 may promote the displacement of HNF4 by COUP-TF/LXR heterodimers. LXR could also act as a co-repressor, stimulating the binding of COUP-TF homodimers. Further research will be needed to distinguish these mechanisms. The present data clearly show that TO901317 can have significant negative effects on apo A1 transcription and that these depend, at least in part, on its ability to displace HNF4 from a liver-specific enhancer site in the proximal promoter.

Biological Significance. These data show that in human liver-derived cells, the formation of new HDL is inhibited by a mechanism that can negate beneficial effects on lipid efflux mediated by ABCA1. The pharmacological profile of a successful LXR activator may depend on its net effect on apo A1 production and lipid efflux, which is likely to be specific for human cells and tissues.

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