Transcriptional regulation of the human cholesterol 7α-hydroxylase gene (CYP7A) in HepG2 cells

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Abstract A stable HepG2 cell line harboring a human cholesterol 7α-hydroxylase (CYP7A) minigene/luciferase reporter gene construct was selected for studying transcriptional regulation of CYP7A gene promoter. Insulin and phorbol 12-myristate-13-acetate (PMA) strongly repressed the promoter activity as measured with luciferase activity expressed in the cells. The promoter activity of the 5′ progressive deletion/luciferase reporter gene constructs was studied in a transient transfection assay in HepG2 cells. PMA represses the promoter activity and the response elements were localized in the -184/-151 and -134/-81 regions. Insulin also represses the promoter activity and response element was mapped in the -298/-81 region. Surprisingly, glucocorticoid receptor (GR) strongly inhibited promoter activity in the presence of dexamethasone, and response elements were localized in the -150/-24 regions. Cotransfection of CYP7A chimeric constructs with an expression vector carrying liver-enriched transcription factor HNF3α stimulated the reporter gene activity, but cotransfection with GR plasmid interfered with the HNF3α-stimulated activity possibly through competition for binding to overlapping GR/HNF3 binding sites. Thus, human cholesterol 7α-hydroxylase gene promoter is strongly repressed by insulin, PMA, and steroid/thyroid hormones and results in the low level of cholesterol 7α-hydroxylase expression in the human liver.—Wang, D-p., D. Stroup, M. Marrapodi, M. Crestani, G. Galli, and J. Y. L. Chiang. Transcriptional regulation of the human cholesterol 7α-hydroxylase gene (CYP7A) in HepG2 cells. J. Lipid Res. 1996. 37: 1831-1841.

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• gene transcription • nuclear receptors • hepatocyte nuclear factor
• HNF 3α • phorbol esters • thyroid hormones • glucocorticoid • insulin

The conversion of cholesterol to bile acids in the liver is a major pathway for disposal of cholesterol from the body (1). Cholesterol 7α-hydroxylase is the first and rate-limiting enzyme in bile acid biosynthetic pathway that converts cholesterol to bile acids in the liver. This enzyme activity is regulated primarily at the level of gene transcription by bile acids returning to the liver via portal vein (1). Hydrophobic bile acids strongly repressed, but hydrophilic bile acids had little effect on the expression of cholesterol 7α-hydroxylase mRNAs (2, 3). Thyroid hormones and glucocorticoid are required for the expression of cholesterol 7α-hydroxylase mRNAs in primary cultures of rat hepatocytes (4, 5). Liver-enriched hepatocyte nuclear factor 3 (HNF 3) strongly stimulated the human CYP7A promoter activity in HepG2 cells (6). The expression of this gene follows a robust diurnal rhythm and is stimulated by DBP, a liver-enriched, diurnally regulated transcription factor (7, 8).

Most studies on the regulation of cholesterol 7α-hydroxylase were carried out in the rat model. However, species differences in bile acid synthesis and cholesterol 7α-hydroxylase activity have been reported (9-11). Cholesterol 7α-hydroxylase activity is increased by cholestyramine and repressed by bile acids in humans (12). As the purified human enzyme has Kₐ and Vₘₐₓ similar to those of the rat enzyme (13), the low cholesterol 7α-hydroxylase activity expressed in the human liver apparently is due to the low level of gene transcription. In spite of the high sequence homology in the proximal promoters among different species, many consensus response elements are not well conserved (11). Therefore, different cis-regulatory elements in the human gene and/or different amounts of transcription factors present in the liver might regulate the human CYP7A gene expression.

Abbreviations: CYP7A, cholesterol 7α-hydroxylase gene, according to the recommended nomenclature (Nelson, D. R., et al. Pharmacogenetics, January, 1996); HNF, hepatocyte nuclear factor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; TR, thyroid hormone receptor; TRE, thyroid hormone response element; Luc, luciferase gene; PMA, phorbol 12-myristate-13-acetate; nt, nucleotide; Dex, dexamethasone; T₄, thyroxine.

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by somewhat different mechanisms. To test this hypothesis, transcriptional regulation of the human CYP7A gene 5′-upstream sequence by physiological regulators needs to be studied in a suitable transfection assay system in culture.

HepG2 cells synthesize and secrete normal primary bile acids (14) and have been adopted as a model for studying transcriptional regulation of cholesterol 7α-hydroxylase gene by transient transfection assay (15, 16). The expression and regulation of cholesterol 7α-hydroxylase mRNAs in HepG2 cells by bile acids, hormones and cholesterol have been reported by several laboratories (2, 14, 15). We found that rat CYP7A gene promoter is stimulated by glucocorticoid, cAMP, and retinoic acids, and repressed by bile acids, insulin and phorbol esters (16). Most response elements conferring the regulation by these physiological regulators in the rat CYP7A promoter are localized in a region from the transcription start site (+1) upstream to about nt 416 (2, 16–19). We suggest that interactions among these positive and negative regulatory factors with overlapping cis-regulatory elements in the rat CYP7A gene promoter determine the level of gene transcription in the liver under different physiological conditions (16).

In the present study, we report the isolation of a stable HepG2 cell line harboring a human CYP7A minigene/luciferase reporter gene for studying transcriptional regulation of the gene by hormones. Promoter/reporter chimeric genes were constructed for transient transfection assay of the promoter activity to identify promoter regions responsive to regulation by thyroid hormones, glucocorticoid, phorbol esters, insulin, and the liver-enriched transcription factor HNF3α. It is evident from our results that the human CYP7A promoter is regulated quite differently from the rat gene in the HepG2 transfection assay system.

MATERIALS AND METHODS

Materials

HepG2 cells were obtained from American Type Culture Collection (ATCC) (Rockville, MD). Dexamethasone (Dex) and L-thyroxine (T4) were from Sigma (St. Louis, MO). Dulbecco’s modified Eagle’s medium (DMEM), Ham’s F-12 salt solution, fetal calf serum, and other tissue culture reagents were from GIBCO/BRL (Grand Island, NY) or Celox (Hopkins, MN). Restriction enzymes and other modifying enzymes were purchased from BRL (Bethesda, MD) or Promega (Madison, WI). [α-32P]-dCTP and [α-35S]-dATP were obtained from DuPont/New England Nuclear (Boston, MA). Luciferase reporter gene vectors pGL2, pGL3, and the reagents for luciferase assays were purchased from Promega. Gene-cLean DNA purification kit was from BIO 101 (La Jolla, CA). Qiagen DNA purification columns were from Qiagen (Chatsworth, CA). Oligonucleotides were synthesized by National Biosciences (Plymouth, MI).

Isolation of stable cell lines harboring human CYP7A minigene

The 2.7 kb Hind III/Bgl II fragment containing sequences from nt -371 (Hind III) to +2377 (Bgl II) site of intron 2 of the human CYP7A gene was removed from a human CYP7A genomic clone pHG7α5.0 (20) by restriction enzyme digestion. The pcDNA3 vector (Invitrogen, San Diego, CA) was modified by removing the Xba I site and digested with Hind III and Bgl II to remove the CMV promoter. The resulting 4.7 kb vector was ligated to the 2.7 kb Hind III to Bgl II fragment of the CYP7A gene. The 2.8 kb Spe I/Sma I fragment containing the luciferase gene and SV40 polyA signal from pFlash I vector (SynapSys, Burlington, MA) was inserted into the Xba I site (+1712) in the exon 2 of the CYP7A/pcdNA3 plasmid. The resulting plasmid pCSH-1 (Fig. 1) contained the human CYP7A minigene and luciferase re-

Fig. 1. Human CYP7A minigene/luciferase chimeric gene construct. The human CYP7A minigene constructed for stable transfection contains sequences from -371 (Hind III site) to +2377 (Bgl II site) of intron 2. The heavy stripped arrow represents the promoter and direction of transcription. The luciferase gene (LUC) and SV40 polyA signal derived from pFLASH I vector are inserted into Xba I site of exon 2. Light lines with solid arrows are derived from pcdNA3 vector. NeoR, neomycin resistant gene; BGHpA, bovine growth hormone polyadenylation signal.
Fig. 2. Effect of insulin on the CYP7A transcription in stable HepG2 cell line. Stable HepG2 cell line 2.2.1 harboring the human CYP7A minigene/luciferase chimeric construct was selected as described in Methods. Confluent cultures of the stable HepG2 cells were treated with insulin at the concentrations indicated. Cells were harvested 40 h after treatment and cell extracts were used to determine luciferase activity (RLU/Abs 280). Representative data shown are the averages of triplicate assays in one experiment. Error bars indicate standard deviation from the mean; (*) indicates statistically significant difference between activities in the control and 0.1 μM insulin-treated cells (\(P \leq 0.01\)).

porter gene linked to the neomycin resistant gene. The pCSH-1 was transfected into HepG2 cells and selected for resistance to G418 essentially according to the method described in Current Protocols of Molecular Biology (21). Confluent HepG2 cells were split 1/15 into 4 × 100 cm dishes containing 12 ml DMEM/F12 medium supplemented with 10% fetal bovine serum, 100 IU penicillin, and 100 μg streptomycin 24 h prior to transfection. Cells were transfected with 25 μg DNA/well using Ca\(^{2+}\) phosphate coprecipitation method and incubated in complete medium for 48 h. Cells were split 1/15 (5 \(\times\) 10\(^5\) cells/plate) into complete medium plus 400 μg G418/ml. Media were changed every 5 days and isolated colonies were transferred to 12-well plates after 17 days. Cultures were maintained at 200 μg/ml G418. The stable cell lines were screened for luciferase activity. A stable cell 2.2.1 that expressed a moderate luciferase activity was selected for testing the effects of bile acids and hormones.

Construction of human CYP7A promoter/luciferase reporter chimeric genes

A 1.9 Kb 5'-flanking region of human CYP7A gene was obtained by polymerase chain reaction (PCR) amplification using a human CYP7A clone pHG7a5.0 as a template (20). The 5'-primer used in PCR reaction was 5'-CGGGGTACCGTTGATGATGGGACACA3', and the 3'-primer was 5'-TGGAAAAAGATTACTGAGTCGCCCAGT-3'. Kpn I (-1877) and Xho I (+24) sites were introduced into the 5'- and 3'-primers, respectively, for generating cloning sites. The 1.9 Kb PCR product (-1877 to +24) was digested with Kpn I and Xho I, and ligated to Xho I-Kpn I-digested luciferase reporter gene vector pGL2basic. The nucleotide sequence of this chimeric construct, pLUC-1877/+24, was confirmed by DNA sequencing. The pLUC-1723 was constructed by restriction digestion of pLUC-1877 with EcoR V and Xho I and ligated into Sma I and Xho I digested pGL2-basic vector. Another construct, pLUC-298, was a spontaneously arising mutant identified by DNA sequencing. The pLUC-785 and pLUC-371 were generated by restriction digestion of pLUC-1877 with Spe I and Hind III, respectively, blunt-ended with Klenow fragment of DNA polymerase I and then digested with Xho I. These fragments were cloned into pGL2-basic vector cut with Mlu I, blunt-ended with Klenow, and digested with Xho I. The construct pLUC-150 was obtained by PCR using a human CYP7A gene plasmid (pHG7a5.0) as the template for amplification using primers to introduce a Kpn I site in the 5'-end (-150) and Xho I site (+24) in the 3'-primer. The PCR product was digested with Kpn I and Xho I, and cloned into pGL2basic plasmid cut...
Fig. 4. Basal activity of the human CYP7A/luciferase chimeric gene constructs. Confluent HepG2 cells were transfected with 2.5 μg of the chimeric gene plasmids indicated. All chimeric constructs were made using pGL3 reporter gene vector as described in Methods. Constructs with (−) mean that the 5′ restriction sites indicated are generated by PCR primer. These constructs were designed for deletion of some putative sequence motifs. The pCL3 promoter vector was included as a reference for SV40 promoter activity in each assay. Transcription activities of chimeric gene constructs are expressed as the luciferase activity expressed in the HepG2 cell extracts (RLU/mU of β-galactosidase activity). Representative data shown are the averages of triplicate assays in one experiment. Error bar represents standard deviation from the mean. Statistics of t-test were done using statistic softwares; (*, ++, or ###) indicates statistically significant differences between two plasmids (all P ≤ 0.001).
Effect of PMA (% of control)

Fig. 5. Identification of the PMA response elements in human CYP7A. Confluent HepG2 cells were transfected with 2.5 μg of chimeric gene constructs in pGL3 vector and treated with 1 μM PMA after glycerol shock. Cells were harvested 16 h after the treatment. Transcription activities were determined and expressed as the percentage of luciferase activity in HepG2 cells without the treatment after glycerol shock. The rationale for choosing this segment of CYP7A for the design of minigene construct are: the Hind III restriction site is conserved in rat, human, hamster, and mouse CYP7A gene; the sequences from transcription start site to Hind III site are highly conserved among different species; most of the response elements conferring regulation by physiological regulators are found in this upstream region; and introns may increase transcriptional efficiency of eukaryotic genes.

Effect of INSULIN (% of control)

Fig. 6. Identification of the insulin response elements in human CYP7A. Confluent cultures of HepG2 cells were transfected with 2.5 μg plasmid of human CYP7A deletion mutants in pGL3 vectors. Cultures were treated with 1 μM insulin and cells were harvested 40 h later. Transcriptional activities are expressed as the percentage of luciferase activities in untreated HepG2 cells (these control values are 9.42, 10.56, 2.94, 4.02, 6.36, 10.89, 1.17, 0.77, 0.64, 0.12, 0.05, 8.27 × 10^6 RLU/mU β-gal for ph-3025Luc, ph-1857Luc, ph-785Luc, ph-319Luc, ph-339Luc, ph-298Luc, ph-185Luc, ph-150Luc, ph-135Luc, and pGL3-promoter, respectively). Representative data shown are the averages of triplicate assays in one experiment. Error bars represent standard deviation from the mean; (*) indicates statistically significant difference between two plasmids (P ≤ 0.006).

RESULTS

Insulin and phorbol esters repress the human CYP7A luciferase reporter gene activity in a stable HepG2 cell line

The CYP7A minigene we constructed contains a 2748 bp gene sequence from nt -371 (Hind III) to nt +2377 (Xba I) in intron 2 (Fig. 1). The rationale for choosing this segment of CYP7A for the design of minigene construct are: the Hind III restriction site is conserved in rat, human, hamster, and mouse CYP7A gene; the sequences from transcription start site to Hind III site are highly conserved among different species; most of the response elements conferring regulation by physiological regulators are found in this upstream region; and introns may increase transcriptional efficiency of eukaryotic genes.

Our criteria for the selection of stable cell line are: the high level of luciferase activity expressed; the response of stable cells to known physiological regulators, such as bile acids, phorbol esters and insulin; and the integration of luciferase and CYP7A minigenes in the genomic DNA of the cells was assessed by Trypan Blue exclusion, which was higher than 95% in all of HepG2 cell cultures treated with different reagents. In each transfection assay, test plasmid (2.5 pg/well) was cotransfected with β-galactosidase plasmid (pCMV β-gal, 5 μg/well) as an internal standard for the adjustment of transfection efficiency. The luciferase activity expressed in HepG2 cells was measured using assay reagents from Promega and expressed as relative light units (RLU)/mU of β-galactosidase activity or per OD280 for the stable cell line (22). Each transfection experiment was performed at least three times using at least two different plasmid preparations. Data presented in each figure are the representation of a typical experiment of triplicated assays of each plasmid.

In co-transfection experiments, appropriate amounts of expression plasmid containing cDNA for glucocorticoid receptor (GRGR, from K. Yamamoto), thyroid hormone receptor (TRα, from R. Evans), or HNF3α (pL-H3α from Dr. W. Chen) was cotransfected with 2.5 μg testing plasmids. The amount of DNA in each assay was kept constant by adding pGL2-basic plasmid.

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The stable cell line HepG2/2.2.1 met all these criteria, although the luciferase activity expressed was intermediate among 12 positive cell lines selected. The luciferase activity expressed under the control of this human minigene in the stable HepG2 cell line was inhibited by hydrophobic bile acids taurodeoxycholic and taurochenodeoxycholic acid in a dose-dependent manner, but less hydrophobic taurocholic acid and hydrophilic tauursodeoxycholic acid had less or no effect on the activity (data not shown). Another cell line expressed about 4-fold higher activity but was not repressed by bile acids.

We demonstrated previously that insulin repressed the transcriptional activity of the rat CYP7A in HepG2 cells, therefore, we further tested the effect of insulin on the human CYP7A transcription in the stable HepG2 cells. As shown in Fig. 2, insulin repressed the human CYP7A/Luc activity by about 50% at a concentration as low as 0.1 μM.

We reported previously that the phorbol ester, PMA, repressed the rat CYP7A transcription in transient transfection assay (16). As shown in Fig. 3, 1.0 μM PMA suppressed the human CYP7A transcription by about 70%, after the treatment of the cells with PMA for 16 h. Prolonged treatment with PMA reduced its effect to about 50%.

**Transient transfection assay of human CYP7A/Luciferase chimeric genes in HepG2 cells**

To identify regions of human CYP7A promoter responsive to potential regulators, the human CYP7A 5'-upstream flanking region was linked to the luciferase reporter gene of pGL3 vector and the 5'-progressive deletion constructs were made for transient transfection assays in HepG2 cells. Figure 4 shows the luciferase activities of these chimeric reporter gene constructs in transient transfection assay in HepG2 cells. The luciferase activities were strongly reduced when sequences upstream of nt -150 were removed. Both negative response elements (nt -339 to -320 and -371 to -340) and positive response elements (nt -1887 to -786, -298 to -151) were localized in the upstream regions. The longer constructs, ph-1887Luc and ph-3025Luc, had activities as high as the shorter construct ph-319Luc. A putative HNF3 site (16) may be located in the -319/-299 fragment. To explain a much higher activity in the ph-319Luc than in ph-298Luc plasmid. This pattern of promoter activity is quite different from that of the rat CYP7A gene in that the rat -344 (Hind III)/+32 fragment has the highest activity and the promoter activities of longer constructs were progressively reduced (16, 17).

**Insulin, PMA, and steroid/thyroid hormones repress the human CYP7A promoter activity**

As shown in Fig. 5, PMA repressed the promoter activities of all constructs except the shortest fragment, ph-80Luc. PMA repressed the ph-135Luc and ph-150 by about 60%. It is apparent that PMA responsive elements...
Effects of glucocorticoid hormones on human CYP7A transcription. Confluent cultures of HepG2 cells were transfected with GR expression plasmids (0.05 µg) and plasmids (2.5 µg) containing deletion constructs of human CYP7A in pGL2 vector. Cells were grown in serum-free media with or without dexamethasone (0.1 µM) as indicated. Cells were harvested 40 h later for the determination of luciferase activity (RLU/mU β-galactosidase). Luciferase activities were expressed as the percentages of the control HepG2 cells which were not treated with Dex but cotransfected with GR; (*) indicate statistic difference of assays with GR+Dex vs. without GR and Dex, all P ≤ 0.003. The promoter activities for non-treated HepG2 cells transfected with chimeric constructs (RLU/mU β-gal activity) are 48.60, 56.84, 49.32, 34.747, 59.17, and 8.39 x 10^3, for pLuc-1877, pLuc-1723, pLuc-785, pLuc-371, pLuc-298, and pLuc-150, respectively.

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We reported previously that retinoic acids strongly stimulated the rat *CYP7A/Luc* gene expression (16), however, we found that retinoic acids did not affect the human *CYP7A/Luc* expression (data not shown).

**Effect of co-transfection of liver-enriched transcription factor HNF3α on human CYP7A promoter activity**

Liver-enriched transcription factors such as HNF1, HNF3, HNF4, DBP, and C/EBP are known to regulate liver-specific transactivation of genes involved in lipoprotein and lipid metabolisms (24). Molowa et al. (6) previously reported that the -432/-220 fragment of the human *CYP7A* gene could confer a 11-fold stimulation of promoter activity by HNF3. These investigators suggested that three putative HNF3 consensus sequences at nt -316, -288, and -255 were HNF3 binding sites as demonstrated by DNase I footprinting assay. They suggested that HNF3 are essential for transactivation of human *CYP7A* promoter. Co-transfection with HNF3α expression plasmid (1:1 mass ratio) stimulated the activity of pLUC-371 by about 10-fold (Fig. 9A), but had very little effect on the pLUC-150 (data not shown).

**Ligand-activated glucocorticoid receptor represses the human CYP7A promoter activity by interfering with HNF3**

As negative GREs are localized in the -298/-151 region and GRE half-sites (TGTTCT, ref. 25) were found to overlap with HNF3 sites (26) in the same region (Fig. 10), we hypothesize that the GR may repress the human *CYP7A* transcription by competing for the overlapping GR/HNF3 binding sites. We studied the effect of cotransfection of GR or HNF3α on the HNF3α or GR-stimulated transcription of the human *CYP7A* promoter, respectively. Figure 9A shows that the luciferase activity of pLUC-371 stimulated by overexpression of HNF3α (plasmid mass ratio of 1:1 of pLUC-371 to HNF3α) could be repressed by cotransfection with GR plasmid in a dose-dependent manner, in the presence of 0.1 μM Dex. As low as 0.1 μg of GR plasmid repressed the activity by about 80%. On the other hand, the luciferase activity of pLUC-371 repressed by overexpression of GR (0.1 μg) could be stimulated by cotransfection with HNF3α plasmid in the amount exceeding 0.1 μg (Fig. 9B).

**DISCUSSION**

The stably transfected HepG2 cell line we isolated responded to known physiological regulators such as insulin and PMA. In stable cell lines, genes are integrated into chromosomes, but in the transient transfection assay, transcription was tested with naked DNA fragments. The chromosomal structures are known to influence gene transcription and regulation. Therefore, this stably transfected HepG2/2.2.1 cell line should be useful for the high throughput screening of drugs affecting the human *CYP7A* gene transcription.

The expression of luciferase activities that reflect the promoter activity of the human *CYP7A/Luciferase* chimeric genes using the luciferase vector pGL3 were
very high in the transient transfection assays in HepG2 cells. This system is more suitable for studying transcriptional repression than the chloramphenical acetyl-CoA transferase (CAT) assay which suffers from low sensitivity and is laborious. The transient transfection assay reported by Molowa et al. (6) using CAT reporter gene showed that the -276/+29 fragment had almost no activity. This is surprising as the proximal promoter is essential for tissue-specific expression of basal transcription activity of the eukaryotic gene. The patterns of promoter activities of the 5'-deletion constructs of the rat and human \textit{CYP7A} genes are quite different, indicating a species difference in the regulation of these two homologous genes. This prompted us to investigate hormonal regulation of the human \textit{CYP7A} gene in detail.

It is somewhat unexpected that glucocorticoid receptor strongly repressed the human \textit{CYP7A} gene but stimulated the rat \textit{CYP7A} gene promoter activity. Analysis of the nucleotide sequences in the -298/-151 region of the human gene identified a perfect GRE half-site TGTTCT (25) and a modified GRE (TGTTGT) in previously identified HNF3 sites (6). Many composite GREs have been identified as negative GREs which have a TGTTCT half-site overlapping with another tranactivator binding site, i.e., AP-1 site (25, 27). The cotransfection results are consistent with the model that GR competes with the HNF3\textalpha for the overlapping binding site, thus represses the transcriptional activity of the gene.

The finding that thyroid hormone inhibits \textit{CYP7A} promoter activity is in contrast to the report that thyroid
hormone stimulates the expression of cholesterol 7α-hydroxylase mRNA in hypophysectomized rats (28, 29). The stabilization of mRNA by thyroid hormone may be more than compensating the down-regulation of gene transcription. TR can function both as a transcription activator or repressor (30, 31). The silencing function of TR may be mediated through its interaction with the general transcription factor TFIIB (32) or by forming a heterodimer with RXR and preventing the activating function of RXR (33). Similar mechanisms may explain the repressive effect of TR on human CYP7A gene expression.

The inhibitory effects of PMA and insulin on human CYP7A promoter were much stronger than on the rat gene. The suppression of CYP7A promoter activity by PMA mimics the inhibitory effect of bile acids which are known to bind and activate protein kinase C isoforms (34, 35). Stravitz et al. (36) recently suggested that bile acids might activate PKC isoforms which presumably repressed CYP7A gene expression by phosphorylation of a transcription factor. The strong repression of human CYP7A promoter activity by insulin may be physiologically significant in the regulation of cholesterol homeostasis. Cholesterol 7α-hydroxylase activity is known to be inhibited by insulin and stimulated in diabetic rats (37, 38). The negative insulin response sequence (IRS, TGTTTTC) is known to bind HNFS which may function as an accessory factor in insulin repression of glucocorticoid stimulated-transcription of phosphoenolpyruvate carboxykinase (PEPCK) and insulin-like growth factor binding protein-1 (IGF BP-1) gene (39, 40). Similar interactions of GR, HNF3, and insulin response factor with the IRS/GR/HNF3 motif in the -293/-273 region may also regulate the CYP7A gene transcription. Inhibition of the CYP7A gene transcription by insulin may lead to hypercholesterolemia in insulin resistance and metabolic syndromes in humans.

It is now apparent that the cholesterol 7α-hydroxylase gene is regulated by a complex mechanism involving multiple factors. The most significant finding of this study is that the human CYP7A gene is resistant to the stimulation by hormones and is more responsive to repression by insulin and PMA. This may explain the much lower level of cholesterol 7α-hydroxylase activity and mRNA expression in the human than in the rat liver. The rat CYP7A gene may be unique in that it can be stimulated by many hormones, including glucocorticoid, retinoic acids, and cAMP. This species difference in the transcription regulation of the CYP7A gene may contribute to different cholesterol metabolism and homeostasis observed in different animals.

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