Hormonal regulation of the human sterol 27-hydroxylase gene CYP27A1

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The mitochondrial sterol 27-hydroxylase (CYP27A1) is a multi-functional cytochrome P450 enzyme that catalyses important hydroxylations in the biosynthesis of bile acids and bioactivation of vitamin D₃. Previous results [Babiker, Andersson, Lund, Xiu, Deeb, Reshef, Leitersdorf, Diczfalussy and Björkhem (1997) *J. Biol. Chem.* **272**, 26253–26261] suggest that CYP27A1 plays an important role in cholesterol homeostasis and affects atherogenesis. In the present study, the regulation of the human CYP27A1 gene by growth hormone (GH), insulin-like growth factor-1 (IGF-1), dexamethasone, thyroid hormones and PMA was studied. HepG2 cells were transfected transiently with luciferase reporter gene constructs containing DNA fragments flanking the 5'-region of the human CYP27A1 gene. GH, IGF-1 and dexamethasone increased the promoter activity by 2–3-fold, whereas thyroxine (T₄) and PMA repressed the activity significantly when measured with luciferase activity expressed

in the cells. The endogenous CYP27A1 enzyme activity in the cells was stimulated by GH, IGF-1 and dexamethasone, whereas T₄ and PMA inhibited the activity. Experiments with progressive deletion/luciferase reporter gene constructs indicated that the response elements for GH may be localized in a region upstream to position – 1094 bp. The putative response elements for dexamethasone were mapped to positions between – 792 and – 1095 bp. The – 451 bp fragment of the human CYP27A1 gene was found to confer the activation by IGF-1, and the inhibition by T₄ and PMA. Results of the present study suggest that CYP27A1 is regulated in human cells by hormones and signal-transduction pathways.

Key words: bile acid biosynthesis, cholesterol homeostasis, growth hormone, PMA, thyroid hormone.

INTRODUCTION

Mitochondrial CYP27A1 (sterol 27-hydroxylase, P450c27 and mitochondrial vitamin D₃ 25-hydroxylase) is a multifunctional cytochrome P450 enzyme that catalyses hydroxylations in bile acid biosynthesis, cholesterol homeostasis and bioactivation of vitamin D₃ [1–5]. In the neutral pathway of bile acid biosynthesis from cholesterol, CYP27A1 initiates degradation of the C₂₇-steroid side chain. In the acidic pathway, it catalyses the initial 27-hydroxylation of cholesterol [6]. CYP27A1 is one of the two hepatic P450 enzymes that catalyses the 25-hydroxylation of vitamin D₃ [7,8]. Both hepatic and extrahepatic CYP27A1 are active in hydroxylations of C₂₇ sterols and vitamin D₃. In addition to bile acid biosynthesis and bioactivation of vitamin D₃, the enzyme has been found to play an important role in the regulation of cholesterol homeostasis by forming oxysterols and also in the elimination of cholesterol from cells [9].

The genes for rat and human CYP27A1 have been isolated [10,11]. The rat CYP27A1 gene contains a cryptic TATA box (TTTAAA) located 24 nt upstream of transcription initiation [10]. In contrast, sequence analysis of the human CYP27A1 gene did not reveal canonical TATA and CAAT boxes in the immediate 5'-flanking region [11]. Functional assays have shown that the – 217/– 10 nt region from the translational start site contains all the elements for basal transcription [12].

Some information is available on the regulation of CYP27A1. Most of these studies have been performed with rat as a model [13–16]. It has been reported that glucocorticoids, such as dexamethasone, probably increase post-transcriptionally sterol 27-hydroxylase mRNA in rat hepatocytes. They also increase

its protein mass and decrease its rate of protein degradation [14]. A recent study indicates that dexamethasone stimulates transcriptional activity of the human CYP27A1 gene [17]. 1,25-Dihydroxyvitamin D₃ has been found to suppress CYP27A1 mRNA levels in rat kidney and intestine [15,18]. Measurement of enzymic activity and mRNA levels in rat liver and primary cultures of rat hepatocytes had suggested that there is a slow regulation of CYP27A1 by bile acids in the same way as CYP7A1 (cholesterol 7 α -hydroxylase) [13,14,16]. However, in human beings, mouse and rabbit, there appears to be no feedback regulation by bile acids [19–22]. There are only a few reports on the regulation of the human CYP27A1 gene [17,19,23].

Hormones like pituitary growth hormone (GH), thyroid hormones, and the protein kinase C stimulator PMA are known to regulate some enzymes in cholesterol metabolism such as the cholesterol 7 α -hydroxylase (CYP7A1), the rate-limiting enzyme in the classical pathway for bile acid synthesis in liver. GH has been reported to stimulate the enzyme activity of CYP7A1 in rats [24–26]. Thyroid hormones and PMA have been reported to repress the promoter activity of the human CYP7A1 gene [27,28]. There is no information about the effects of these hormones or PMA on the activity of the human CYP27A1 gene promoter or the CYP27A1 enzyme activity.

The objective of the present study was to examine the effects of GH, insulin-like growth factor-1 (IGF-1), dexamethasone, thyroid hormones and PMA on the activity of the human CYP27A1 gene promoter. The effects on CYP27A1 enzyme activity in the cells were also examined. The promoter sequence was analysed and progressive deletion/luciferase constructs were made in an attempt to localize the elements mediating the effects.

Abbreviations used: AP, activator protein; CYP, cytochrome P450; GAS, γ -interferon-activated sequence; GH, growth hormone; IGF-1, insulin-like growth factor-1; NF- κ B, nuclear factor κ B; T₃, 3,3',5-tri-iodothyronine; T₄, thyroxine.

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The nucleotide sequence data reported in this paper will appear in EMBL Nucleotide Sequence Database under the accession number AJ 544720.

Figure 1 Schematic diagram and basal promoter activity of human CYP27A1/luciferase chimaeric gene constructs

HepG2 cells were transfected transiently with deletion constructs of the CYP27A1 gene ligated in pGL2 vector (4.5 kb-CYP27A1-Luc, 1094 bp-CYP27A1-Luc, 792 bp-CYP27A1-Luc and 451 bp-CYP27A1-Luc) as described in the Experimental section. The luciferase activity was normalized to β -galactosidase transfection efficiency and protein content for each sample. Results are given as relative luciferase activity compared with the activity of the 4.5 kb-CYP27A1-Luc construct (100%). The activity after transfection with pGL2 basic luciferase vector without insert (negative control) was < 2%. Data represent the means of two experiments.

EXPERIMENTAL

Materials

Materials used for culturing of cells were obtained from Gibco (Invitrogen Corporation, Paisley, U.K.). pGL2 luciferase reporter vectors and Psv β -galactosidase were from Promega (Madison, WI, U.S.A.). The luciferase assay system was also from Promega. Human GH (somatotropin), IGF-1, dexamethasone, L-thyroxine (T_4), 3,3',5-tri-iodothyronine (T_3) and PMA were from Sigma-Aldrich. HepG2 (liver, hepatoblastoma and human) cells were obtained from A.T.C.C. (Manassas, VA, U.S.A.). The Superfect transfection reagent and plasmid purification kit were obtained from Qiagen (Valencia, CA, U.S.A.).

Construction of plasmids for transient transfection

A 4.5 kb DNA fragment of human CYP27A1 upstream of the translation-initiation codon [11] was a gift from Professor Eran Leitersdorf (Hadassah University Hospital, Jerusalem, Israel). The DNA fragment was inserted into a Bluescript vector at the *SacI* site. This plasmid was digested with *SacI* enzyme to obtain the 4.5 kb DNA fragment. The DNA fragment was inserted into the pGL2 basic luciferase vector, which had been digested with *SacI*, to give the CYP27A1-4.5 kb-luciferase plasmid (4.5 kb-CYP27A1-Luc). Three progressive deletion constructs were prepared harbouring fragments of 1094, 792 and 451 bp upstream of the CYP27A1 translation start (Figure 1). The 1094 bp fragment was obtained by digestion of the 4.5 kb DNA fragment with *SmaI* enzyme, giving three fragments approx. 0.6, 1.2 and 2.6 kb in size. Sequence analysis showed that the 1.2 kb fragment was located immediately upstream of the ATG. The fragment was inserted into the *SmaI* site of the pGL2 basic vector to give the 1094 bp-CYP27A1-luciferase plasmid (1094 bp-CYP27A1-Luc). The 792 and 451 bp fragments were prepared by PCR with the 1094 bp-CYP27A1-Luc plasmid as template. The primers used were 5'-TGCGGGTACCATGTTTTTAACTT-3' as forward primer for the 792 fragment and 5'-TCAAGGTACCAGGCCATTGGGAG-3' as forward primer for the 451 fragment. The primers contained a *KpnI* site. The reverse primer, containing a *HindIII* site, was 5'-TCGCAGAAGCTTCCTCAGCC-3'. The 792 and 451 bp fragments were ligated in the *KpnI*-*HindIII* sites of the pGL2 basic vector. The plasmids containing DNA inserts and pGL2b (without insert) were enriched in Luria-Bertani medium containing 100 mg/ml ampicillin.

DNA transfection and treatment of HepG2 cells

The pGL2 basic luciferase vectors with and without the insert were transfected transiently into HepG2 cells. The plasmid DNA used in transfection experiments was prepared by the Qiagen plasmid purification method. HepG2 cells were seeded at 10^6 cells/60 mm tissue culture dish in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) foetal calf serum. After 24 h, the cells were transfected with 5 μ g of plasmid DNA and 1.5 μ g of β -galactosidase (Psv β -galactosidase) by the Superfect transfection method. After 24 h, the medium was replaced by a serum-free medium and the transfected cells were treated with GH, IGF-1, dexamethasone, thyroid hormone or PMA for the indicated time of treatment. The cells were then harvested and luciferase activity was assayed using the TD-20/20 luminometer (Turner Designs, Sunnyvale, CA, U.S.A.). The luciferase activity was normalized to β -galactosidase activity and protein concentration. Transfected cells without treatment (but with the proper vehicle added) were used as controls. All experiments were performed in triplicate.

Assay of CYP27A1-mediated enzyme activity in treated and untreated cells

In some transfection experiments, the effect of added dexamethasone, T_4 or PMA on the endogenous 27-hydroxylase activity was also measured. 3 H-labelled 5 β -cholestane-3 α ,7 α -diol was added to the cells directly after the addition of the compound used for treatment. In other experiments, non-transfected cells were treated with GH or IGF-1 for 48 h before addition of the substrate. The medium and the cells were extracted with acidified diethyl ether or trichloroethane/methanol (2:1, v/v), and analysed for 27-hydroxylated products [19].

RESULTS

GH, IGF-1 and dexamethasone stimulate the human CYP27A1 promoter activity

The CYP27A1-luciferase plasmid harbouring the 4.5 kb fragment of the 5'-flanking sequence (4.5 kb-CYP27A1-pGL2b) was first used for studies on the possible regulation of human CYP27A1 gene by hormones and PMA. The promoter activity, as measured with luciferase activity, was studied in a transient transfection assay in HepG2 cells. Table 1 summarizes the effects of GH, dexamethasone, T_4 , T_3 and PMA on the luciferase activity driven by the CYP27A1 promoter.

Table 1 The effect of GH, dexamethasone, T_4 , T_3 and PMA on the relative CYP27A1-luciferase activity in HepG2 cells

HepG2 cells were transiently transfected with the 4.5 kb fragment of the CYP27A1 promoter ligated in pGL2 (4.5 kb-CYP27A1-luc) as described in the Experimental section. The cells were then treated with dexamethasone (1 μ M) or PMA (1 μ M) for 6 h or with GH (0.02 i.u./ml), T_3 (1 μ M) or T_4 (1 μ M) for 48 h. The luciferase activity was normalized to β -galactosidase transfection efficiency and protein content for each sample. Results are given as relative luciferase activity compared with that in untreated transfected cells (100%). Data represent means $\pm$ S.D. from six experiments. Data with T_3 are presented as the mean with a range from two experiments. Statistically significant difference compared with control value. * P < 0.01.

Treatment	Relative CYP27A1-luciferase reporter activity (%)
GH	225 $\pm$ 15*
Dexamethasone	310 $\pm$ 55*
T_4	39 $\pm$ 5*
T_3	36 (33-39)
PMA	19 $\pm$ 5*

Table 2 Effect of PMA, dexamethasone, T₄, GH and IGF-1 on the endogenous CYP27A1 activity in HepG2 cells

In experiments on the effect of treatment with PMA (1 μ M) or dexamethasone (1 μ M) for 6 h and T₄ (1 μ M) for 24 h on the endogenous 27-hydroxylase activity, ³H-labelled 5 β -cholestane-3 α ,7 α -diol was added to the transfected cells directly after addition of PMA, dexamethasone or T₄. In experiments with GH (0.01–0.03 i.u./ml) or IGF-1 (50 nM), untransfected cells were treated for 48 h with the respective compound. ³H-labelled 5 β -cholestane-3 α ,7 α -diol was then added and incubated for 6 h. The medium and cells were extracted and analysed for 27-hydroxylated products as described in the Experimental section. Data represent means $\pm$ S.D. from 3 to 7 experiments. Statistically significant difference compared with control value. * P < 0.01, ** P < 0.02 and *** P < 0.05.

Treatment	Formation of 5 β -cholestane-3 α ,7 α ,27-triol [nmol · (mg of protein) ⁻¹ · h ⁻¹]
Control (6 h)	3.3 $\pm$ 0.4
PMA (6 h)	0.5 $\pm$ 0.05*
Dexamethasone (6 h)	5.8 $\pm$ 0.3**
Control (24 h)	0.77 $\pm$ 0.18
T ₄ (24 h)	0.43 $\pm$ 0.10***
Control (48 h)	0.23 $\pm$ 0.04
GH (48 h)	0.37 $\pm$ 0.08*
IGF-1 (48 h)	0.48 $\pm$ 0.12*

Dexamethasone and GH increased the promoter activity. Induction by dexamethasone was dose- and time-dependent. Maximal induction was found when the cells were treated with 1 μ M dexamethasone and harvested after 6 h of treatment. As shown in Table 1, treatment with 1 μ M dexamethasone for 6 h increased the activity by approx. 3-fold compared with that in the untreated control cells.

The induction by GH was time-dependent and the promoter activity increased up to at least 48 h. Treatment with 0.01–0.03 i.u./ml human GH (specific activity, 2 i.u./mg of protein) for 48 h resulted in approx. 2-fold higher activity when compared with that in controls (Table 1).

Since many biological effects of GH are mediated by IGF-1, we also considered it of interest to study the influence of IGF-1 on the CYP27A1 promoter activity. In contrast with the 2-fold induction observed with GH (cf. Table 1), the luciferase activity driven by the 4.5 kb fragment of the CYP27A1 promoter was not affected by IGF-1 (results not shown). However, when the 1094 bp-CYP27A1-Luc construct harbouring a shorter promoter sequence was used, a significant stimulation was found (cf. Table 3). Interestingly, no significant effect of GH was found with the luciferase construct harbouring the shorter promoter sequence.

Thyroid hormones and PMA repress the human CYP27A1 promoter activity

Table 1 shows that T₄, T₃ and PMA decreased the activity of the CYP27A1 promoter. In separate experiments, the most marked repression was found when the cells, transfected with 4.5 kb-CYP27A1-Luc, were treated for 48 h with thyroid hormones and for 6 h with PMA (results not shown). In the presence of 1 μ M T₄ or T₃, the promoter activity was 30–40% when compared with the activity in untreated, transfected control cells. PMA treatment resulted in an even more marked repression (Table 2).

It has been reported that the combined addition of thyroid hormone and dexamethasone stimulates bile acid formation in primary rat hepatocytes, but tends to decrease bile acid formation in primary human hepatocytes [29]. In the present study, a separate set of experiments was performed with the combined addition of dexamethasone and T₄ or T₃ (1 μ M for 24 h). Dexamethasone

with T₄ and dexamethasone with T₃ suppressed the promoter activity to 75 and 79% when compared with control cells respectively.

Effects of PMA, dexamethasone, T₄, GH and IGF-1 on the endogenous CYP27A1 activity in HepG2 cells

Experiments were also performed to correlate the regulation of the transfected chimaeric gene and the endogenous gene in the HepG2 cells. The HepG2 cells catalysed the 27-hydroxylation of 5 β -cholestane-3 α ,7 α -diol. To study the effect of PMA, dexamethasone and T₄ on the endogenous CYP27A1 activity, 5 β -cholestane-3 α ,7 α -diol was added to the transfected cells directly after the addition of these compounds to HepG2 cells. The cells were treated for 6 or 24 h. The medium was extracted, and the amount of 27-hydroxylated product formed during the period of treatment was analysed. As shown in Table 2, PMA and T₄ decreased the rate of formation of 27-hydroxylated product to 15 and 55% compared with that in the control respectively. Dexamethasone increased the endogenous CYP27A1 activity by almost 2-fold. Treatment of non-transfected cells with GH or IGF-1 increased the CYP27A1 activity by approx. 2-fold. These results are in agreement with the effects observed on the promoter activity.

Experiments with progressive deletion constructs of the human CYP27A1 promoter

To identify regions of the human CYP27A1 promoter responsive to potential regulators, the human CYP27A1 upstream 5'-flanking region was linked to the luciferase reporter gene of pGL2 vector and the 5'-progressive deletion constructs were made for transient transfection assays in HepG2 cells. Figure 1 shows a comparison of the luciferase activities of the chimaeric reporter gene constructs harbouring fragments of 4.5 kb, 1094 bp, 792 bp and 451 bp upstream of the CYP27A1 translation start. The luciferase activity was decreased when sequences upstream of nt –835 were removed, and the activity increased when sequences upstream of nt –793 were removed. This suggests that a suppressor is located between nt –1094 and –793. The longest construct, 4.5 kb-CYP27A1-Luc, had an activity approximately as high as the shortest construct, 451 bp-CYP27A1-Luc (Figure 1).

The luciferase vectors, harbouring the fragments of 1094, 792 and 451 bp upstream of the CYP27A1 translation start, were tested for responsiveness to IGF-1, dexamethasone, T₄, PMA and GH after transfection of HepG2 cells. As shown in Table 3, IGF-1 increased the promoter activities of all constructs. IGF-1 increased the activity of 451 bp-CYP27A1-Luc by 2-fold. Dexamethasone increased the activity of the 1094 bp-CYP27A1-Luc, but not the constructs harbouring shorter fragments. T₄ and PMA repressed the activity of all constructs and also the activity of 451 bp-CYP27A1-Luc, harbouring the shortest fragment, by 55 and 68% respectively. GH had no significant effect on the activity of the constructs containing the shorter promoter fragments shown in Table 3.

Results summarized in Table 3 indicate that the response elements for IGF-1, T₄ and PMA are located in the nt –451/+42 fragment and suggest that the response elements for dexamethasone are located in the nt –1095/–792 fragment. As mentioned above, GH increased the activity of the 4.5 kb-CYP27A1-Luc (cf. Table 1) but not that of constructs with shorter promoter fragments (Table 3). This finding suggests that the response elements for GH are located in a region upstream to nt –1094.

Table 3 Effect of treatment with IGF-1, dexamethasone, T_4 , PMA and GH on the relative luciferase reporter activity in HepG2 cells transfected with progressive deletion fragments of the human CYP27A1 promoter

HepG2 cells were transiently transfected with deletion constructs of the CYP27A1 gene ligated in pGL2 vector (1094 bp-CYP27A1-Luc, 792 bp-CYP27A1-Luc and 451 bp-CYP27A1-Luc) as described in the Experimental section. The cells were then treated with dexamethasone (1 μ M) or PMA (1 μ M) for 6 h or with IGF-1 (50 nM), T_4 (1 μ M) or GH (0.02 i.u./ml) for 48 h. The luciferase activity was normalized to β -galactosidase transfection efficiency and protein content for each sample. Results are given as relative luciferase activity compared with that in untreated transfected cells (100%). Data represent means $\pm$ S.D. from three experiments. Statistically significant difference compared with control value. * $P < 0.01$.

Treatment	Relative CYP27A1-luciferase reporter activity (%)
IGF-1	
1094 bp-CYP27A1-Luc	168 $\pm$ 28*
792 bp-CYP27A1-Luc	154 $\pm$ 5*
451 bp-CYP27A1-Luc	213 $\pm$ 16*
Dexamethasone	
1094 bp-CYP27A1-Luc	238 $\pm$ 27*
792 bp-CYP27A1-Luc	110 $\pm$ 7
451 bp-CYP27A1-Luc	112 $\pm$ 8
T_4	
1094 bp-CYP27A1-Luc	42 $\pm$ 7*
792 bp-CYP27A1-Luc	51 $\pm$ 8*
451 bp-CYP27A1-Luc	45 $\pm$ 8*
PMA	
1094 bp-CYP27A1-Luc	37 $\pm$ 1*
792 bp-CYP27A1-Luc	42 $\pm$ 4*
451 bp-CYP27A1-Luc	32 $\pm$ 3*
GH	
1094 bp-CYP27A1-Luc	99 $\pm$ 25
792 bp-CYP27A1-Luc	101 $\pm$ 4
451 bp-CYP27A1-Luc	115 $\pm$ 13

Analysis of the human CYP27A1 5'-flanking sequence

The 1431 bp region upstream from the +1 site (ATG, the translation-initiation site) was analysed for putative response elements considered to be of relevance for the observed hormonal effects using Transcription Element Search Software (<http://www.cbil.upenn.edu/>) and the associated Transfac DATABASE (Computational Biology and Informatics Laboratory, University of Pennsylvania, PA, U.S.A.). GC-rich sites and sequences resembling or exhibiting similarity to activator protein (AP)-1, AP-2, cAMP-response element, γ -interferon-activated sequence (GAS), glucocorticoid response element, hepatocyte nuclear factor-1 ('HNF/LF-B1'), nuclear factor κ B (NF- κ B), peroxisome-proliferator-activated receptor, Sp1 and thyroid hormone response element regulatory sequences were identified (Figure 2). The factors binding to these sequences may be of relevance for the observed hormonal effects (cf. Discussion section).

DISCUSSION

Previous studies on the regulation of CYP27A1 at a molecular level have been performed mostly with the rat as model and focused on the possible feedback regulation by bile acids [13,14,16]. There are only a few reports on the regulation of the human CYP27A1 gene, and the roles of hormones, other than dexamethasone, have not been explored [17,19]. In the present study, luciferase reporter constructs containing DNA fragments of the 5'-flanking region of the human CYP27A1 gene have been examined in human hepatoblastoma (HepG2) cells. Results demonstrate that, besides dexamethasone, several other hormones and PMA affect the activity of the human CYP27A1 promoter.

-1431 ATCTGAGGTCAGGAGTTTGAGACACGCTGGCCACATGGCAAAACCCCTA
TRE/PPAR/CRE(rev)
-1381 TCTTTACTAAAAATACAAAAATTAGCCGGCGTGTGGGGCACTCTA
GAS-like
-1331 ATTCAGCTACTCTGGAGGCTGAGACCGGAGAATTGCTTGAACCGGGAGG
-1281 CGGAGGTTCAGCGAGCCAGGATTGCACCACTGCACCTCCAGCTGGAACGA
-1231 CAGGGTGAGACTCTGGGAGTGGGGAGGAGAGAATTAAACAACTATATGA
AP-2 HNF-1
-1181 CACAAAGGAACAAAAATACACAAGACAGGATAAGAGAGAGGAAGGATACA
GRE
-1131 GGGAGAGCTAACATACATATAGCAGTTTGAAGTCCCGGAAAGGAGAAAA
-1081 AAATGGGGAGGAGCAATATTTGAACAGATAATATCTGAGAACTCCCCAG
AP-2
-1031 ACTGATCAAGACATCCAGTCACAGAGTCAAGAACTAGGAGTCTAGAA
AP-1
-981 TAAATAAGTAGAAAGCCACACCTAGCAACTCAGCTGTGAGCTAATTGAA
GRE
-931 CTTAATATGCCCTTTGTTGTTCTGGGCTGCCTGTAATACCAATTTTTC
GRE
-881 AAATGCTGTAGAACCCAGCTGTACCATCTGCTGGGTCTTCATCTCTG
-831 GCTTGAATGTGATCTTTGACCTGTATTTATTTATCTTGGCTGAGTTA
GRE AP-1 AP-1
-781 TGTTTTTAACTTTTGGACAACTATCTTTGAATATACATGAACATAGG
-731 ATTCATAAGACAATCTTTTTTTTCTCTCTGCTGTACGTTTTCCTGACT
GRE
-681 ATGTTACTCTTTCCATTTTATTAATAATTTGTCGCCATCATCTACGAA
-631 AGCTGGTCTCAACCTGCAGTTTGAATATCTGCTTGGCAGACGCGGCA
-581 GGGAGGTGCGTGGAGGGAGAATTGAATTAATAAAAAACAAACACAGC
-531 AATATTAGATTTCTTTGTTTCAGCGCCGCCCTCCAGGGATCAGATGACT
AP-1
-481 GGCCGCCCTCGCTCGAATCTGACTCGGGATCAATCCGGAAGGCCATTGG
AP-2 AP-1
-431 GAGAAGCGGAGGCGAGCTTAGCCACGCGCGTTCCTGCTCCAGGAC
Sp1
-381 GCGAGGGTGGCTTGGTGGGAAACCGGACCGGGGAGGACTATCC
AP-2
-331 GGTGTGGGGCTTCCCGATTTTGAAGAATCTGCTGCAACCCCGCCAGCA
NF- κ B GC-rich/Sp1
-281 GTTCAAGCCAAAGCGAAAAATTATTTGAGAGCGCTCGGGCGCGGGGTAG
TRE-like HNF/LF-B1 Sp1
-231 GAGTGTGCGGAGGCGCTTGGTGGGGCGCGGTGATATCCCGAGTCAC
Sp1
-181 CGCGTCCCTCTCTGACGCTCCCGCTGCTGGGAGGAGCGAGGAGCGA
Sp1
-131 GCGGAAGGGTCTAGCTGGCTTTGCTCGGCCCTCCCGAGCGCCCGGCTT
AP-2
-81 TGAACCCCGCTGCACTGCTGTCTGGGGGTGTCGGGGGACTCAGCACTGG
Sp1 GC-rich/Sp1
-31 ACCCAAGGTGCAAGGCGGCGGAGCACAAACCATGCTGCTGCGCTGGGCTGG
Met
+20 CGAGGCTGAGGTGGGCGCTGCGAGGGGCGCGCTGCGCTGCTGCCCCAC
+70 GGGGCGAGAGCCAAAGGCGCGATCCCTGCGGCCCTCCCTCGGACAAGGC
+120 CACCGGAGCTCGTA

Figure 2 Nucleotide sequence of the 5'-flanking region of the human CYP27A1 gene

Analysis of the 1431 bp region upstream from the +1 site (ATG, translation-initiation site) was performed using Transcription Element Search Software (<http://www.cbil.upenn.edu/>) and the associated Transfac DATABASE (Computational Biology and Informatics Laboratory, University of Pennsylvania). Several putative regulatory elements and transcription factor binding sites are underlined and labelled. CRE, cAMP-response element; GRE, glucocorticoid response element; HNF/LF-B1, hepatocyte nuclear factor-1; PPAR, peroxisome-proliferator-activated receptor; TRE, thyroid hormone response element.

The finding that dexamethasone, PMA, T_4 , GH and IGF-1 also affected the endogenous CYP27A1 enzyme activity in the cells supports a physiological relevance for the regulation observed by these compounds.

There have been different explanations for the mechanism by which dexamethasone increases the CYP27A1 mRNA levels [14,17]. From studies with rat hepatocytes, Stravitz et al. [14] concluded that the glucocorticoid appears to increase post-transcriptionally, e.g. stabilize, sterol 27-hydroxylase mRNA and protein levels in rat hepatocytes. On the other hand, a recent study of the human CYP27A gene by Segev et al. [17] reported

that CYP27A1 is up-regulated by dexamethasone and that the dexamethasone-responsive element is located between 1087 and 678 bp upstream of the putative ATG. Our results indicate that dexamethasone-responsive elements may be located in the –1095/–792 region, and analysis of the nucleotide sequence identified glucocorticoid response element half-sites in this region (cf. Figure 2).

GH has an important role in the regulation of hepatic low-density lipoprotein receptor expression, and in the control of cholesterol 7 α -hydroxylase activity and bile acid biosynthesis [24–26,30]. It has been reported from studies using Northern-blot hybridization and immunoblot analysis that the steady-state level of rat CYP27A is under the control of pituitary-regulated steroids and GH [31]. The presence of receptors for human GH on HepG2 cells has been demonstrated previously [32]. Results of the present study show that GH increased both the endogenous CYP27A1 enzyme activity and the activity of the human CYP27A1 promoter in HepG2 cells. Results indicate that the response elements for GH may be located in a region upstream to position –1094 bp. Effects of GH on the transcription of genes are known to be mediated by the binding of members of the signal transducers and activators of transcription to an element resembling a GAS [33,34]. Interestingly, a site that resembles the GAS consensus sequence, TTNCNNNA [34], is located between nt –1378 and –1370 (cf. Figure 2).

Many of the actions of GH are mediated by the GH-dependent production of IGF-1, and in the therapy of growth disorders, IGF-1 can fully substitute for GH [35]. Considering this, it is interesting that IGF-1 was found to increase the activity of the human CYP27A1 promoter as well as the endogenous CYP27A1 enzyme activity. Results from promoter deletion analysis indicate that the –451 fragment of the human CYP27A1 gene confers activation by IGF-1. IGF-1-stimulated gene expression is considered to be mediated by transcription factors that bind to GC-rich domains of genes [36,37]. In agreement with this, the –451 fragment of the human CYP27A1 gene was found to contain several GC-rich regions (cf. Figure 2). The finding that human GH and IGF-1 increase both the endogenous CYP27A1 enzyme activity and the promoter activity of the human CYP27A1 gene in HepG2 cells, suggests that GH and IGF-1 may play a role in the regulation of CYP27A1 in human beings. It is also significant that the levels of GH and growth factors like IGF-1 are lowered in patients with osteoporosis [38,39]. These patients also have decreased levels of 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃, metabolites formed by CYP27A1 [4,7].

Thyroid hormones are known to affect the mRNA levels and promoter activities of sterol 12 α -hydroxylase (CYP8B1) and cholesterol 7 α -hydroxylase (CYP7A1) in the rat, and CYP7A1 in human beings [27,28,40]. In the present study, thyroid hormones were found to suppress the activity of the human CYP27A1 promoter markedly and also decreased the sterol 27-hydroxylase activity in HepG2 cells. Results indicate that the response elements may be localized in the –451 bp fragment of the CYP27A1. It is not known how thyroid hormone exerts the effect on CYP27A1 promoter activity, since no well-characterized thyroid hormone-responsive elements (such as AGGTCA), which could explain the suppressing effect, were found in the 451 bp sequence upstream of the translational start. A DNA sequence (AGTTCA), which is present in the human CYP7A1 gene promoter and suggested to be involved in thyroid hormone receptor α -binding [41], was identified between nt –282 and –277 in the CYP27A1 promoter (cf. Figure 2). Analysis of the nucleotide sequence upstream of nt –451 identified a thyroid hormone-responsive element (AGGTCA) between nt –1426 and –1421.

PMA, a known activator of protein kinase C, strongly repressed the promoter activity and the sterol 27-hydroxylase activity in the cells. PMA is known to affect the transcription of several genes via phosphorylation of transcription factors, such as NF- κ B, AP-1 and AP-2 [27,41–44]. Experiments with progressive deletion/luciferase reporter gene constructs suggest that PMA-responsive elements are located in the region 451 bp upstream of the translational start. Sequence analysis of the –451 bp fragment of the human CYP27A1 gene revealed that this region contains putative AP-1, AP-2 and NF- κ B-binding sequences.

Results of the present study suggest that CYP27A1 is regulated in human cells by hormones and signal-transduction pathways. Many effects of physiological regulators (e.g. IGF-1, phorbol ester and thyroid hormones) seem to be mediated by response elements located in the region downstream of nt –451. It appears that this proximal promoter may contain a domain that regulates the effects of multiple signals.

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