Regulation of Cholesterol 7 α-Hydroxylase Gene Expression in Hep-G2 Cells

EFFECT OF SERUM, BILE SALTS, AND COORDINATE AND NONCOORDINATE REGULATION WITH OTHER STEROL-RESPONSIVE GENES*

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Regulation of cholesterol 7 α-hydroxylase mRNA level in Hep-G2 cells was studied and compared with that of two other sterol-responsive genes, those for the low density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. In culture medium containing 10% fetal bovine serum (complete medium) for up to 24 h, the mRNA for cholesterol 7 α-hydroxylase gradually increased to 2-fold of the time 0 control. The addition of β-migrating very low density lipoprotein (β-VLDL) (40 μg/ml) and 25-hydroxycholesterol (5 μg/ml) prevented the increase in mRNA level for the LDL receptor, and HMG-CoA reductase mRNA levels and the 10-26% of the control at 8 h. The effect with β-VLDL was sustained for 24 h. With 25-hydroxycholesterol, both LDL receptor and HMG-CoA reductase mRNA returned to base line by 24 h. In contrast, β-VLDL increased cholesterol 7 α-hydroxylase mRNA levels above the serum-free control within 8 h (+32%), and this was sustained for 24 h (+47%). There was a slight induction of cholesterol 7 α-hydroxylase mRNA levels by 25-hydroxycholesterol at 8 h (+18%); but by 24 h, its level was below that of the control (-47%). There was no induction of cholesterol 7 α-hydroxylase mRNA levels by β-VLDL or 25-hydroxycholesterol when the cells were grown in complete medium. As determined by nuclear run-on assay, the increase in the transcriptional rate of the cholesterol 7 α-hydroxylase gene in cells grown in serum-free medium (3.9-fold of the rate in complete medium) and incubated with β-VLDL (+68% above serum-free control) at 8 h, was comparable with the increase in mRNA levels (3.5-fold and +32%, respectively). When bile salts were added to serum-free medium and cells cultured for up to 24 h, chenodeoxycholate and glycochenodeoxycholate caused a marked suppression of the level of cholesterol 7 α-hydroxylase mRNA, while cholate and its conjugates did not. The chenodeoxycholate effect was dose-dependent and was apparent within 2 h. It occurred at the level of transcription, as judged by decreased nuclear run-on. These bile salts did not appear to affect cholesterol efflux from the cells, their viability, the rate of transcription of several housekeeping genes or albumin secretion. These results demonstrate that Hep-G2 cells will be useful for elucidating the molecular events in the regulation of the cholesterol 7 α-hydroxylase gene in a human liver system. In Hep-G2 cells grown in the absence of serum, the cholesterol 7 α-hydroxylase gene responds to sterols, hormones, and bile salts.

Three metabolic pathways in liver play major roles in determining whole body cholesterol homeostasis. The first is cholesterol biosynthesis through mevalonate, in which the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase catalyzes the rate-limiting step. Second is the uptake of exogenous and endogenous cholesterol via the low density lipoprotein (LDL) receptors. Third is the degradation and elimination of cholesterol through the bile acid biosynthetic pathway. Cholesterol 7 α-hydroxylase is the initial and rate-limiting enzyme in the conversion of cholesterol to bile acid.

Cholesterol 7 α-hydroxylase has been reported to be regulated in vivo by a number of factors (1), including feedback by bile acids (2-4), hormones (5, 6), a diurnal rhythm (7), and dietary factors (8). Interruption of the return of bile acid to the liver by a bile fistula (9) or by administration of anion exchange resin to absorb bile acids in intestine induces cholesterol 7 α-hydroxylase activity (10) and steady-state mRNA levels (8). Conversely, infusion or dietary administration of bile acids decreases the enzyme's activity (11) and steady-state mRNA level (9). Feeding of cholesterol induces cholesterol 7 α-hydroxylase activity in several species, including humans (12). Administration of an inhibitor of HMG-CoA reductase to bile fistula rats reduced cholesterol 7 α-hydroxylase activity and bile acid synthesis (11), suggesting that cholesterol, in some way, regulates cholesterol 7 α-hydroxylase and bile acid synthesis.

Davis and co-workers (13) reported that cholesterol or the cholesterol precursor mevalonate increased bile acid synthesis in isolated cultured rat hepatocytes. However, an effect of bile acids was not observed in early studies (13, 14) in primary hepatocyte culture. Addition of fetal bovine serum and dexmethasone is necessary to maintain cholesterol 7 α-hydroxylase activity in rat (15) or pig (16) hepatocyte monolayers. Down-regulation of bile acid synthesis and cholesterol 7 α-hydroxylase gene expression in Hep-G2 cells was studied and compared with that of two other sterol-responsive genes, those for the low density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. In culture medium containing 10% fetal bovine serum (complete medium) for up to 24 h, the mRNA for cholesterol 7 α-hydroxylase gradually increased to 2-fold of the time 0 control. The addition of β-migrating very low density lipoprotein (β-VLDL) (40 μg/ml) and 25-hydroxycholesterol (5 μg/ml) prevented the increase in mRNA level for the LDL receptor, and HMG-CoA reductase mRNA levels and the 10-26% of the control at 8 h. The effect with β-VLDL was sustained for 24 h. With 25-hydroxycholesterol, both LDL receptor and HMG-CoA reductase mRNA returned to base line by 24 h. In contrast, β-VLDL increased cholesterol 7 α-hydroxylase mRNA levels above the serum-free control within 8 h (+32%), and this was sustained for 24 h (+47%). There was a slight induction of cholesterol 7 α-hydroxylase mRNA levels by 25-hydroxycholesterol at 8 h (+18%); but by 24 h, its level was below that of the control (-47%). There was no induction of cholesterol 7 α-hydroxylase mRNA levels by β-VLDL or 25-hydroxycholesterol when the cells were grown in complete medium. As determined by nuclear run-on assay, the increase in the transcriptional rate of the cholesterol 7 α-hydroxylase gene in cells grown in serum-free medium (3.9-fold of the rate in complete medium) and incubated with β-VLDL (+68% above serum-free control) at 8 h, was comparable with the increase in mRNA levels (3.5-fold and +32%, respectively). When bile salts were added to serum-free medium and cells cultured for up to 24 h, chenodeoxycholate and glycochenodeoxycholate caused a marked suppression of the level of cholesterol 7 α-hydroxylase mRNA, while cholate and its conjugates did not. The chenodeoxycholate effect was dose-dependent and was apparent within 2 h. It occurred at the level of transcription, as judged by decreased nuclear run-on. These bile salts did not appear to affect cholesterol efflux from the cells, their viability, the rate of transcription of several housekeeping genes or albumin secretion. These results demonstrate that Hep-G2 cells will be useful for elucidating the molecular events in the regulation of the cholesterol 7 α-hydroxylase gene in a human liver system. In Hep-G2 cells grown in the absence of serum, the cholesterol 7 α-hydroxylase gene responds to sterols, hormones, and bile salts.

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‡ The abbreviations used are: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low density lipoprotein; FBS, fetal bovine serum; β-VLDL, β-migrating very low density lipoprotein; G6P, glucose-6-phosphate dehydrogenase; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; kb, kilobase(s).
yrase mRNA by bile acids was demonstrated in hepatocytes cultured under these conditions (15, 16).

Hep-G2 cells have been used as a model for studies of cholesterol metabolism, including HMG-CoA reductase (17, 18) and LDL receptor (19, 20) regulation, apolipoprotein synthesis (21-23), and bile acid synthesis (24-27). In a previous study, we examined several parameters of bile salt metabolism and reported that cholesterol 7 \( \alpha \)-hydroxylase mRNA could be easily measured in this cell line and that changes in its level correlated with changes in bile salt secretion (28, 29). The purpose of this study was to identify factors that regulate cholesterol 7 \( \alpha \)-hydroxylase in this cell line, to ascertain if the regulation is coordinate with that of the genes for the LDL receptor and HMG-CoA reductase, and to determine whether such regulation occurs at the level of transcription.

**EXPERIMENTAL PROCEDURES**

**Materials**—\( \alpha \)P\( ^32 \)PdCTP and \( \alpha \)P\( ^32 \)PGTP (3000 Ci/mmole) were purchased from Amersham Corp. (Chicogen, IL), 250-cholesterol and progesterone from Steraloids (Wilton, NH), and bile salts from Sigma.

**Cell Culture**—Hep-G2 cells were grown in 25 or 75 cm\(^2\) tissue culture flasks, as previously described (21, 22). Confluent monolayers of cells were split (day 0) into Eagle's minimum essential medium containing 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 \( \mu \)g/ml streptomycin, and supplemented with 0.3 mg/ml a-glutamine. Medium was changed on day 3, and experiments were started on day 4 or 5. \( \beta \)-mitigating very low density lipoprotein (\( \beta \)-VLDL) (40 \( \mu \)g/ml), 25-cholesterol (5 \( \mu \)g/ml), progestrone (10 \( \mu \)g/ml), dexamethasone (1 \( \mu \)M), and ethanol vehicle alone (0.1%) were added to the medium, and the cells were allowed for up to 24 h.

**Preparation of \( \beta \)-VLDL**—\( \beta \)-VLDL were prepared from animals fed an atherogenic diet, as previously described (30).

**RNA Preparation and Northern Blot Analysis**—RNA was isolated from Hep-G2 cells by guanidine thiocyanate-phenol-chloroform extraction (31). Total RNA (20 \( \mu \)g) was electrophoresed on 1% agarose gel in solution containing 6 \( \times \) SSC, 0.1% SDS, and then transferred to nitrocellulose membrane (Nitroplus, Micron Separations Inc., Westborough, MA) with sodium chloride (1.5 M)/sodium citrate (0.15 M) (10 \( \times \) SSC) as the transfer buffer. The membrane was baked (80 °C, 2 h) and incubated at 42 °C in solution containing 6 \( \times \) SSC, 2 \( \times \) Denhardt's, 50% formamide, 0.1% SDS, and 100 \( \mu \)g/ml salmon sperm DNA. The cDNA probe for human cholesterol 7 \( \alpha \)-hydroxylase (1.65 kb long containing the coding region of 10-1652 base pairs) was obtained as a gift from Dr. David Russell of the University of Texas Southwestern Medical School. The human LDL receptor (EcoRI-BglII fragment of pLDR3) and hamster HMG-CoA reductase (2.9- \( \beta \) XbaI fragment of pEBD277), glucose-6-phosphate dehydrogenase (G6PD) (1.5-kb EcoRI fragment of pGD-P-25A1), and human glyceroldehyde-3-phosphate dehydrogenase (G3PDH) (0.5-kb XbaI-HindIII fragment of pHGAP) were obtained from American Type Culture Collection, were subcloned into pBluescript II KS(+/-), and were labeled with \( \alpha \)P\( ^32 \)PdCTP (3,000 Ci/mmole) by the random primer method (32). Hybridization was carried out at 42 °C in the prehybridization solution. The membranes were washed with 0.1 \( \times \) SSC, 0.1% SDS at room temperature for 15 min twice, followed by washes of 15 min at 60 °C in 0.25 \( \times \) SSC/0.1% SDS and 0.1 \( \times \) SSC/0.1% SDS and then exposed to x-ray film.

**Nuclear Run-on Transcription Assay**—Nuclei were prepared and nuclear run-on experiments performed exactly as previously described (33).

**Albumin Secretion**—Albumin secretion in serum-free media was measured by enzyme-linked immunosorbent assay as previously described (17).

**Cholesterol Content**—Cholesterol content was determined by gas liquid chromatography, using an adaptation of the method of Ishikawa et al. (34), as previously described (35).

**Detection of Cholesterol Efflux**—Hep-G2 cells were grown to confluence in T150 flasks containing minimum essential medium and 5% FBS. The medium was changed to serum-free medium with 100 \( \mu \)Ci mevalonate (specific activity, 30.3 Ci/mmole) and incubated for 24 h. The cells were washed thoroughly, and serum-free medium with or without 100 \( \mu \)m cholesterol was added. The cells were incubated for an additional 8 h at 37 °C, and the medium and cells were separated. An aliquot of the supernatant medium was taken immediately; a second aliquot (0.25 ml) was extracted by the method of Folch et al. (36), and the radioactivity in the organic and aqueous phases was counted. The density of the supernatant medium was adjusted to 1.25 g/ml with KBr and centrifuged at 100,000 \( \times \) g for 48 h. A 1.6-m1 fraction was removed from the top of the tube and designated the total lipoprotein fraction. Aliquots of this and the infranate were counted; another aliquot of each was extracted by the method of Folch et al. (36), and radioactivity in the organic and aqueous phases was determined.

The cells were washed and suspended in phosphate-buffered saline. One aliquot was used for cholesterol determination and the others for protein content by the method of Lowry et al. (37).

**RESULTS**

**Time Course of the Expression of mRNAs for Cholesterol 7 \( \alpha \)-Hydroxylase, LDL Receptor, and HMG-CoA Reductase**—In previous work (28, 29), we demonstrated the expression of the mRNA of cholesterol 7 \( \alpha \)-hydroxylase in Hep-G2 cells and reported a potentially complex regulation that correlated with the rate of bile salt synthesis. To begin to elucidate the primary regulatory factors of this gene, the effect of culture conditions on the level of its mRNA was examined. Hep-G2 cells were cultured in serum-free medium or medium containing 10% fetal calf serum (complete medium). Total RNA was isolated and analyzed by Northern blot analysis, and levels were quantified by densitometric scanning of the autoradiogram and normalized for G6PDH and G3PDH mRNA levels. Normalization for G6PDH or G3PDH provided similar results (data not shown). The same nitrocellulose strips were used to probe for the mRNAs for cholesterol 7 \( \alpha \)-hydroxylase, the LDL receptor, and HMG-CoA reductase. The 3.7-kb size of the cholesterol 7 \( \alpha \)-hydroxylase is similar to that of the rat message (7, 38, 39). When cells were cultured in complete medium, the level of the three mRNAs changed in a reciprocal manner. mRNA levels for LDL receptor and HMG-CoA reductase decreased to half of the time 0 control after 24 h of incubation in complete medium, while that for cholesterol 7 \( \alpha \)-hydroxylase gradually increased to about 2-fold (Fig. 1A), although only the 2-h point was statistically different from the initial value. When the cells were grown in serum-free medium, the mRNA for both LDL receptor and HMG-CoA reductase were increased 5- and 6-fold compared with the time 0 control, respectively (Fig. 1B), as would be expected for cells grown in the absence of sterols. Somewhat surprisingly, there was a comparable induction of the mRNA level for cholesterol 7 \( \alpha \)-hydroxylase (Fig. 1B). When lipoprotein-deficient serum was used instead of serum-free medium, the mRNA levels for the LDL receptor and HMG-CoA reductase increased severalfold but that for cholesterol 7 \( \alpha \)-hydroxylase did not change compared with complete medium (Table I). These results demonstrated that regulation of the expression of these genes is not always coordinate and that cultivation of these cells in the presence of serum represses the cholesterol 7 \( \alpha \)-hydroxylase gene.

**Time Course of the Effect of \( \beta \)-VLDL on mRNA Expression**—It is believed that the induction of the LDL receptor and HMG-CoA reductase in serum-free medium is due to sterol depletion caused by the absence of lipoprotein. To confirm this and assess the role of lipoprotein cholesterol in regulating cholesterol 7 \( \alpha \)-hydroxylase, Hep-G2 cells were cultured in serum-free medium containing \( \beta \)-VLDL for various time periods. As expected for the LDL receptor and HMG-CoA reductase, the presence of \( \beta \)-VLDL not only prevented the induction by serum-free medium but suppressed the levels well below baseline line. The mRNA levels for LDL receptor and HMG-CoA reductase were 26 and 29% of the control at 8 h (Fig. 2A). The effect was sustained for 24 h. In contrast, the addition of \( \beta \)-VLDL to serum-free medium resulted in a further increase in cholesterol 7 \( \alpha \)-hydroxylase mRNA level above the serum-free medium control within 8 h (+32% as compared with culture in serum-free medium alone), and this was sustained for 24 h (+47%). This suggests that cholesterol taken up through the LDL receptor pathway in-
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A

B

Fig. 1. Time course of the expression of mRNA for cholesterol 7 α-hydroxylase, LDL receptor, and HMG-CoA reductase in Hep-G2 cells in complete medium or serum-free medium. Hep-G2 cells were grown to confluence; they were then washed and either complete (10% FCS) (panel A) or serum-free medium (panel B) was added. After various times in culture, the cells were harvested and the total RNA isolated. After electrophoresis on agarose gel, the RNA (20 μg) was transferred to nitrocellulose membranes. The nitrocellulose strips were hybridized with 32P-labeled cDNA. mRNA levels were quantified by densitometric scanning of the autoradiogram and normalized for the standard. 7α-OH, cholesterol 7 α-hydroxylase; LDL−R, LDL receptor; HMG-CoA, HMG-CoA reductase. Data are presented as mean percent of time 0 control (n = 3 experiments with duplicate determinations). S.E. are less than 20%, except mRNA levels for cholesterol 7 α-hydroxylase at 8 and 24 h, LDL receptor at 8 and 24 h, and HMG-CoA reductase at 24 h whose S.E. are 20–40%. * statistically significant with at least 95% confidence.

Table I

mRNA levels of cholesterol 7α-hydroxylase, HMG CoA reductase, and LDL receptor in cells grown in various media

Hep-G2 cells were grown to confluence, the medium removed, and the cells washed. Fresh medium, containing 10% fetal bovine serum (complete medium), 10% fetal bovine serum from which the lipoprotein had been removed by ultracentrifugation at d = 1.25 for 48 h (lipoprotein-deficient serum), or no fetal bovine serum (serum-free medium) was added. The cells were cultured for an additional 8 h at which time the cells were harvested and the mRNA isolated. This was analyzed as described in the legend to Fig. 1.

|                         | Cholesterol 7α-hydroxylase | HMG-CoA reductase | LDL receptor |
|-------------------------|-----------------------------|-------------------|--------------|
| Complete media          | n = 2                       | n = 3             | n = 3        |
| Serum-free media        | 100                         | 100               | 100          |
| Lipoprotein-deficient serum | 352                       | 210               | 789          |

duces the mRNA level for cholesterol 7 α-hydroxylase in Hep-G2 cells.

In contrast, when the cells were cultured in complete medium, mRNA levels for the LDL receptor and HMG-CoA reductase were suppressed to 20–50% of the control within 8 h by β-VLDL (Fig. 2B), but stimulation of cholesterol 7 α-hydroxylase mRNA was much less apparent, with a marginally significant increase of 13% at 24 h (Fig. 2B). Thus, culture in the

Fig. 2. Time course of the effect of β-VLDL on the expression of mRNA for cholesterol 7 α-hydroxylase, LDL receptor, and HMG-CoA reductase in Hep-G2 cells. Hep-G2 cells were cultured in serum-free (panel A) or complete medium (panel B) containing β-VLDL (40 μg/ml). After various times in culture, the cells were harvested, and total RNA was isolated. This was analyzed, as described in the legend to Fig. 1. 7α-OH, cholesterol 7 α-hydroxylase; LDL−R, LDL receptor; HMG-CoA, HMG-CoA reductase. Data are presented as mean percent of the control at each time point (n = 3 experiments with duplicate determinations). S.E. are less than 10%, except mRNA levels for cholesterol 7 α-hydroxylase at 2 h in serum-free medium and 8 h in complete medium, LDL receptor, and HMG-CoA reductase at 8 and 24 h in serum-free medium, and 2, 4, and 24 h in complete medium whose S.E. are 15–30%. Panel C, representative Northern blot from experiment (panel B). *, statistically significant with at least 95% confidence.
Expression of 25-hydroxycholesterol for cholesterol 7 α-hydroxylase as it does for the LDL receptor, and HMG-CoA reductase were suppressed to 34-47% of the control and deplete the hepatocyte of cholesterol.

By 25-hydroxycholesterol at 4 and 8 h and then returned to the base line as in serum-free medium (Fig. 3B). However, there were no significant changes of the mRNA level of cholesterol 7 α-hydroxylase by 25-hydroxycholesterol in Hep-G2 cells grown in serum containing medium, with an insignificant increase at 4 h (Fig. 3B) followed by a gradual decrease (-25% at 8 h; -30% at 24 h).

**Cholesterol 7 α-Hydroxylase Gene Transcription**—To determine whether changes in the mRNA levels for cholesterol 7 α-hydroxylase were due to changes in the transcriptional rate, nuclear run-on transcription assays were carried out using nuclei isolated from Hep-G2 cells incubated in the presence or absence of FBS, with and without β-VLDL for 8 h. Hybridization of run-on RNA for cholesterol 7 α-hydroxylase, G3PDH, and the vector alone to the appropriate single-stranded DNA fixed to nitrocellulose was performed, and the transcriptional rate was quantified by densitometric scanning of the autoradiogram and corrected for G3PDH gene transcription. The transcriptional rate of cholesterol 7 α-hydroxylase in serum-free medium was 3.9-fold higher than that in complete medium (Fig. 4). This increase is comparable with that of the cholesterol 7 α-hydroxylase mRNA level at 8 h (3.5-fold). P-VLDL induced the transcriptional rate of cholesterol 7 α-hydroxylase by a further 68% compared with the 8 h control (Fig. 4). This induction is also comparable with that of the increase in mRNA level (32%). These results indicate that the increased mRNA level of cholesterol 7 α-hydroxylase seen in Hep-G2 cells with these regulators is the result of transcriptional regulation.

**Effect of Steroid Hormone on mRNA Expression**—Dexamethasone is known to increase cholesterol 7 α-hydroxylase activities and mRNA level in primary rat hepatocytes (15, 16). Dexamethasone (1 μM) increased cholesterol 7 α-hydroxylase mRNA level in Hep-G2 cells grown in serum-free medium by 39% compared with the control (not shown). The mRNA level...
of LDL receptor was not affected (~2.5%), and HMG-CoA reductase mRNA level was reduced insignificantly compared with the control. Thus, the response of the cholesterol 7 α-hydroxylase gene to dexamethasone in Hep-G2 cells is similar, but of a lesser magnitude to that in cultured hepatocytes. The mRNA level of cholesterol 7 α-hydroxylase was suppressed to 24% of the control by progesterone (10 μg/ml). The mRNA levels for LDL receptor and HMG-CoA reductase displayed a noncoordinate response. The former was induced 3-fold, but the latter was suppressed to 34% of the control (not shown).

Effect of Bile Salts on Cholesterol 7 α-Hydroxylase mRNA Level in Hep-G2 Cells—In previous work (28, 29), we demonstrated that bile salts were not actively taken up by Hep-G2 cells but that they could reach significant intracellular concentrations. A representative group of bile salts at a concentration of 100 μM was added to the media of Hep-G2 cells. These were chosen to represent both polar and nonpolar bile salts that were conjugated and free, and primary and secondary. Chenodeoxycholate consistently reduced the level of mRNA for cholesterol 7 α-hydroxylase (Fig. 5A). The more polar-conjugated derivative, glycochenodeoxycholate had a smaller inhibitory effect, while ursodeoxycholate and deoxycholate had marginal effects (Fig. 5A). Neither cholic acid nor taurocholic acid altered the mRNA level of cholesterol 7 α-hydroxylase. The effect of chenodeoxycholic acid was dose-dependent (Fig. 6A). The decrease in mRNA level was discernible within 2 h (Fig. 6B) and at a chenodeoxycholate concentration of 25 μM (Fig. 6A).

For HMG CoA reductase mRNA level, there was a modest increase at 4 h and a significant decrease at 24 h (Fig. 5B and Fig. 6B). This is consistent with other reports that bile salts do not rapidly suppress the activity of this enzyme in cell culture (40) or perfused liver (41). The delayed decrease in the enzyme with culture could be analogous to studies where feeding does affect the enzyme activity (42), perhaps by affecting cholesterol balance through primary effects on the other enzymes.

Chenodeoxycholate and deoxycholate stimulated the level of LDL receptor mRNA (Fig. 5C). This effect occurred at the higher concentration of chenodeoxycholate (Fig. 6B) and, because of a marked variability in its magnitude, did not always achieve statistical significance. Cell viability, as judged by trypan blue exclusion, was unaltered (>97%) in the presence and absence of bile salts. Similarly, albumin secretion was unaffected (not shown).

Effect of Bile Salts on Cholesterol 7 α-Hydroxylase Gene Transcription—To determine whether decreases in the mRNA levels for cholesterol 7 α-hydroxylase in Hep-G2 cells cultured with bile salts were due to changes in the transcriptional activities, the nuclear run-on transcriptional assay was carried out. After 8 h of incubation in serum-free medium containing chenodeoxycholic acid (100 μM), nuclei were isolated, and the transcriptional rate was measured. Chenodeoxycholic acid suppressed the transcriptional rate of cholesterol 7 α-hydroxylase by 48% compared with the control (Fig. 7). Chenodeoxycholate did not appear to affect the transcriptional rate of the control gene.

Effect of Bile Salts on Cholesterol Efflux from Hep-G2 Cells—One possible mechanism for the observed effect of bile salts is that the added bile salts cause a loss of cholesterol from the cells. To study this, Hep-G2 cells were incubated with [3H]mevalonate for 24 h to label cellular cholesterol. The cells were washed and 100 μM chenodeoxycholate added. After an additional 8-h incubation, the medium was collected and the amount of radioactivity measured. The total radioactivity secreted into the medium was the same for control and chenodeoxycholate-incubated cells (Table II). There were no significant differences when the medium was fractionated into total lipoprotein and infranate fractions and into lipid-soluble and water-soluble fractions, although there may have been slightly less radioactivity in lipoprotein lipids in the cells incubated with chenodeoxycholate (Table II). Thus, the effect of bile salts on cholesterol 7 α-hydroxylase mRNA does not seem to be the result of increasing sterol loss from the cell. Similarly, cellular total and free cholesterol content was not affected.

DISCUSSION

Regulation of the cholesterol 7 α-hydroxylase gene in the human hepatoblastoma cell line, Hep-G2 cells, and its relationship to the regulation of the LDL receptor and HMG-CoA reductase genes were investigated. Primary cultured rat hepatocytes express cholesterol 7 α-hydroxylase; however, the activity of the enzyme and the rates of bile acid biosynthesis decrease

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why other investigators in the field have not pursued studies of cholesterol 7 a-hydroxylase in Hep-G2 cells. The cells were cultured in serum-free medium containing various concentrations of chenodeoxycholic acid (panel A) or 100 μM chenodeoxycholate (panel B) for various times. After 8 h of incubation, the cells were harvested, and total RNA was isolated. This was analyzed, as described in the legend to Fig. 1. Data shown are mean ± S.E. was 10% or less for cholesterol 7 a-hydroxylase and 10–30% for LDL receptor and HMG CoA reductase (n = 3 experiments with duplicate determinations). *, statistically significant with at least 95% confidence.

with the time in culture, and the administration of FBS and dexamethasone is necessary to maintain cholesterol 7 a-hydroxylase activity (15) and mRNA expression (16) in these cells. Hep-G2 cells express enough cholesterol 7 a-hydroxylase mRNA to be easily detected by Northern blot analysis. However, when the cells were grown in medium containing fetal calf serum, the level of mRNA was relatively low and did not increase with the addition of dexamethasone. Although cholesterol 7 a-hydroxylase mRNA levels increased 2-fold during a 24-h culture in fresh medium, as it does in primary-cultured rat or pig hepatocytes, it remained relatively low. This may explain why other investigators in the field have not pursued studies of the enzyme in this cell line, which has been extensively used for studies of many other facets of lipid and lipoprotein metabolism (17–20, 22, 23, 43). Somewhat surprisingly, the removal of FBS from the medium dramatically stimulated the mRNA levels for the enzyme. The increase of mRNA levels for cholesterol 7 a-hydroxylase was accompanied by the induction of the transcription rate as measured by nuclear run-on. Further, in the presence of fetal calf serum, while the expected regulation of mRNA levels for the LDL receptor and HMG-CoA reductase to added lipoproteins and cholesterol was observed, there was little regulation of the cholesterol 7 a-hydroxylase gene. This suggests that the regulation of cholesterol 7 a-hydroxylase is not always coordinate with that of other sterol-responsive genes and that some factor or factors can override the sterol regulation. The mechanism of the effect of fetal calf serum is open to speculation. A factor in the serum may be directly repressing expression of cholesterol 7 a-hydroxylase or, perhaps more likely, it may be that when the cells are in a complete medium their growth and replication is rapid relative to mature hepatocytes, and they behave like fetal liver where bile salt synthesis is low. When the fetal serum, and thus the growth and replication stimuli are removed, the cells become more “terminally differentiated”; an intracellular repressor is lost, and this allows increased and regulated transcription of the gene.

In the absence of serum, there was reciprocal and coordinate regulation of the cholesterol 7 a-hydroxylase gene and those for the LDL receptor and HMG-CoA reductase in response to the addition of sterols to the medium. Cholesterol delivered in β-VLDL profoundly decreased the level of mRNA for both the LDL receptor and HMG-CoA reductase, as expected. The observed stimulation of cholesterol 7 a-hydroxylase gene transcription and mRNA is consistent with reports that in rodents (9) cholesterol feeding induces the enzyme's activity by this mechanism and suggests that the sterol effect is a relatively direct one. In humans, there has been some question as to whether cholesterol feeding induces bile salt synthesis. Several reports have documented this phenomenon (12, 44). Apparently, in the monkey, chronic cholesterol feeding decreases the level of the mRNA for cholesterol 7 a-hydroxylase (45). The basis for this response has not yet been explained. The sterol 25-hydroxycholesterol generally induces the same effects as cholesterol but is more potent. Indeed, this sterol also induced rapid and generally coordinate regulation of the three genes under study, although it was a less potent regulator of cholesterol 7 a-hydroxylase than cholesterol delivered in lipoprotein.
However, with prolonged culture in the presence of this compound, a paradoxical response was seen. The levels of LDL receptor and HMG CoA reductase rose to or above base line, and, as we reported earlier (28, 29), the levels of cholesterol 7 α-hydroxylase mRNA and bile salt synthesis actually decreased quite profoundly. The simplest explanation is that the liver metabolizes the 25-hydroxycholesterol, and this leaves it sterol-depleted because of the reduced cholesterol uptake and synthesis and the increased cholesterol catabolism and esterification induced by the oxysterol. This is consistent with the hypothesis that an intracellular pool of sterol is critical in regulating all of these genes.

Even in serum-free medium, the regulation of these three sterol-responsive genes was not always coordinate. Dexamethasone and progesterone affected the genes in a noncoincident manner. More important perhaps were the responses to bile salts. These compounds are known to be physiologic regulators of cholesterol 7 α-hydroxylase (3, 4, 11, 46) and have been reported to have effects on the expression of the other two genes (42, 47, 48). In feeding studies, hydrophobic bile salts are presumed to cause cholesterol 7 α-hydroxylase activity and gene expression (5). In liver cell culture, earlier reports that bile salts affect cholesterol 7 α-hydroxylase activity and gene expression (42, 47, 48) and have been inhibitors of cholesterol 7 α-hydroxylase (3, 4, 11, 46) and have been suppressors of cholesterol 7 α-hydroxylase activity and gene expression (42, 47, 48). In feeding studies, hydrophobic bile salts are relatively less polar molecules may enter the cell more readily than the more polar ones and thus achieve somewhat higher concentrations. Chenodeoxycholate increased LDL degradation, and secretion. Chenodeoxycholate increased LDL secretion or cellular cholesterol content, makes it less likely that this is a primary regulatory response and not one mediated by a sterol-responsive element.

In summary, cholesterol 7 α-hydroxylase gene regulation can be studied in Hep-G2 cells and, in many respects, parallels that in the intact animal. When the cells are cultured in the presence of fetal calf serum, gene expression is low, and regulation is difficult to discern. In the absence of serum, gene expression is quite high and can be regulated. There is a coordinate regulation by cholesterol and oxysterols of the cholesterol 7 α-hydroxylase and LDL receptor and HMG CoA reductase genes, and this may be the molecular basis for the dietary cholesterol-induced increase in bile salt synthesis. However, coordinate regulation was not observed under all conditions. Hormonal effects and the effects of bile salts on cholesterol 7 α-hydroxylase mRNA level appeared to occur independently of effects on the other genes. Together, these results suggest that the cholesterol 7 α-hydroxylase gene will have several regulatory elements that control its expression. Several potential regulatory regions in the 5′-flanking region of the gene have been identified and a number of potential transcription factors proposed (51, 52). Elucidating these could provide considerable insight into how whole body sterol homeostasis is maintained.

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