Growth Hormone and Bile Acid Synthesis

Key Role for the Activity of Hepatic Microsomal Cholesterol 7α-hydroxylase in the Rat

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Abstract

Growth hormone (GH) has an important role in the regulation of hepatic LDL receptor expression and plasma lipoprotein levels. This investigation was undertaken to characterize the effects of GH on hepatic cholesterol and bile acid metabolism in the rat.

In hypophysectomized (Hx) rats, the activities of the rate-limiting enzymes in cholesterol and bile acid biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) and cholesterol 7α-hydroxylase (C7αOH), were reduced by 71 and 64%, respectively. HMG CoA reductase mRNA levels were reduced by 37%, whereas C7αOH mRNA was increased by 81%. LDL receptor expression was reduced by 18% in Hx rats, without any change in the LDL receptor mRNA levels. Although the normal diurnal variation of C7αOH activity was preserved in Hx rats, the activity of C7αOH was much reduced both at midday and midnight. Total hepatic cholesterol was increased by 14% in Hx animals whereas microsomal cholesterol was unchanged. The rate of cholesterol esterification was enhanced (by 38%) in liver microsomes from Hx rats.

Stepwise hormonal substitution of Hx rats showed that GH, but not thyroid hormone or cortisone, was essential to normalize the enzymatic activity of C7αOH. GH also normalized the altered plasma lipoprotein pattern in Hx rats, and increased the fecal output of bile acids. The latter effect was particularly evident when GH was combined with cortisone and thyroid hormone. Also in normal rats, GH stimulated C7αOH activity.

In conclusion, GH has an essential role to maintain a normal enzymatic activity of C7αOH, and this, at least in part, explains the effects of GH on hepatic cholesterol metabolism. GH is also of critical importance to normalize the altered plasma lipoprotein pattern in Hx rats. (*J. Clin. Invest.* 1997. 99:2239–2245.) Key words: acyl coenzyme A:cholesterol acyltransferase • cholesterol 7α-hydroxylase • 3-hydroxy-3-methylglutaryl coenzyme A reductase • low density lipoprotein receptor • somatotropin

Introduction

The liver is a key organ in regulating the metabolism of cholesterol, controlling synthesis, clearance from plasma, and excretion from the body, both as biliary cholesterol and after conversion to bile acids (1–4). Disturbances of hepatic cholesterol metabolism are of critical importance in the pathogenesis of several common disease entities, such as hyperlipidemia, atherosclerosis, and gallstone disease (1–8). Our understanding of the integrated control of the activity of critical structures involved in cholesterol metabolism has improved considerably during the last few years. In particular, the mechanisms for the regulation of rate-determining steps in lipoprotein uptake and cholesterol synthesis, such as LDL receptors and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase),[1] by the cellular demand for cholesterol have been clarified (9–11). The regulation of the enzyme controlling the conversion of cholesterol to bile acids, cholesterol 7α-hydroxylase (C7αOH), is not understood as well, although the transcriptional regulation of activity through feedback inhibition by bile acids is well documented (12–14).

In addition to regulation by sterols, hepatic cholesterol metabolism is also under hormonal influence (1–4, 6). For example, it has been shown that estrogen, thyroid hormone, insulin, and glucagon may stimulate LDL receptor expression and lower plasma LDL cholesterol (2, 15–19). Of particular interest is the recent demonstration that growth hormone (GH) is important for the maintenance of normal cholesterol homeostasis (20–23). Thus, in contrast to normal rats, hypophysectomized (Hx) rats are highly sensitive to dietary cholesterol, and hepatic LDL receptors are strongly suppressed when cholesterol is introduced into their food (21, 22). Furthermore, the pronounced stimulatory response to estrogen on hepatic LDL receptors is abolished in Hx rats (21, 23). In both of these situations, GH has been shown to be essential for the reinstitution of normal hepatic LDL receptor expression (20–23). These studies could not determine whether the LDL receptor was the primary target for GH action. However, the data indirectly suggested that hepatic excretion of cholesterol or bile acids was probably altered after hypophysectomy (23). In agreement with this concept, studies performed three decades ago have indicated that bile acid synthesis is dependent on an intact pituitary function (24, 25). Both in vitro and in vivo studies have suggested that the activity of C7αOH may be under hormonal control (12, 14, 25–28). The present studies were undertaken to identify a possible target structure for the in vivo action of GH on hepatic cholesterol metabolism. In particular,

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Received for publication 12 December 1996 and accepted in revised form 19 February 1997.

J. Clin. Invest.

0021-9738/97/05/2239/07 $2.00
Volume 99, Number 9, May 1997, 2239–2245

1. *Abbreviations used in this paper:* ACAT; acyl coenzyme A:cholesterol acyltransferase; C7αOH, cholesterol 7α-hydroxylase; C, cortisone; GH, growth hormone; HMG CoA reductase; 3-hydroxy-3-methylglutaryl coenzyme A reductase; Hx, hypophysectomized; T_4, L-thyroxin.

our experiments were focused on the regulation of the activity of C7αOH.

Our results show that the activity of several structures of importance in hepatic cholesterol metabolism is reduced in Hx rats, and that the fecal excretion of bile acids is diminished. The activity of C7αOH is severely reduced after hypophysectomy, whereas its mRNA level is increased to levels observed in cholesterol-fed normal rats. GH substitution alone is essential to maintain a normal enzymatic activity of C7αOH and a normal plasma lipoprotein pattern, but the fecal excretion of bile acids is only normalized when substitution with GH is combined with cortisone (C) and L-thyroxin (T_4).

Methods

Materials. Osmotic mini pumps (model 2001, delivering 1 μl solvent per hour) were from Alza Corp. (Palo Alto, CA). Recombinant human GH (Genotropin®) and cortisone (Solu-Cortef®) were obtained from Pharmacia & Upjohn (Stockholm, Sweden). Thyroid hormone was from Sigma Chemical Co. (St. Louis, MO). [Oleoyl-^{14}C] oleoyl coenzyme A and [cholesteryl-1,2,6,7-^{3}H] cholesteryl oleate were purchased from New England Nuclear, (Boston, MA). All other materials were obtained from previously described sources (23, 29–31).

Animals and experimental procedure. Altogether, 113 male Sprague Dawley rats were used in five separate experiments. Hx rats and controls were obtained from A/S Møllegaard Breeding Centre (Skensved, Denmark). Animals were 7 wk old at the time of hypophysectomy, and body weight was monitored to verify failure to gain weight. Animals were kept under standardized conditions with free access to water and chow. The light cycle was from 6 a.m. to 6 p.m., if not stated otherwise. Between 10 and 11 a.m., rats were rapidly anesthetized with ether and killed by cervical dislocation after blood was drawn by cardiac puncture. The livers were immediately removed and one piece (~ 1 g) of fresh liver was taken for preparation of microsomes and subsequent assay of enzyme activities as described below. The remaining organ was immediately frozen in liquid nitrogen.

Hormonal substitution was given as continuous infusions, using osmotic mini pumps that were implanted surgically under light ether anesthesia (21, 23). If not otherwise stated, GH was administered at a rate of 1.2 mg/kg per d, C at 400 μg/kg per d, and T_4 at 42 μg/kg per d. Each hormone was delivered by a separate pump. Animals that did not receive any hormonal substitution were sham operated.

In one experiment, animals were kept in metabolic cages allowing for the collection of feces as described below.

Activities of HMG CoA reductase, C7αOH, and acyl coenzyme A: cholesterol acyltransferase (ACAT). Microsomes were prepared by differential ultracentrifugation of liver homogenates in the absence of fluoride as described previously (29–31). Microsomal HMG CoA reductase was assayed by determining the conversion of [^{14}C]HMG CoA to mevalonate, and expressed as picomoles formed per milligram protein per minute (29). The activity of C7αOH was determined as the formation of 7α-hydroxycholesterol (picomoles per milligram protein per minute) from endogenous microsomal cholesterol using isotope dilution – mass spectrometry as described (30). For ACAT activity, two assay systems were used, one without and one with the addition of 50 nmol of exogenous cholesterol (31). In both assay systems, the incorporation of [^{14}C]oleoyl coenzyme A into cholesteryl esters (picomoles per milligram protein per minute) was determined using 0.1–0.2 mg of microsomal protein (31). All enzyme assays were carried out in duplicate.

Hepatic cholesterol. Liver homogenates and hepatic microsomes were prepared as described (30, 31). The lipid fraction was obtained by extraction with chloroform/methanol (2:1, vol:vol) and dried under a stream of nitrogen. The cholesterol content was assayed using a commercially available method (Boehringer Mannheim, Mannheim, Germany).

Ligand blot assay of LDL receptors. This was performed using ^{125}I-labeled rabbit β-migrating VLDL, as described previously (23). Gels (6% polyacrylamide) were loaded with the indicated amounts of membrane protein, and pictures of the blots were generated and 120-kD bands were quantitated (arbitrary units) with a Fujix Bio-imaging analyzer (BAS 2000; Fuji Photo Film Co., Tokyo, Japan). Background values obtained by counting irrelevant filter pieces of the same area have been subtracted from the data.

Quantitation of mRNA. Hepatic total RNA was isolated by ultracentrifugation on Cs Cl after homogenization of tissues in guanidium isothiocyanate, and the mRNA levels for the LDL receptor, HMG CoA reductase, and C7αOH were quantitated by a solution hybridization titration assay using mouse cRNA-probes (32). The mRNA abundance was expressed as copies of mRNA molecules per cell, assuming 15 pg of RNA per cell; this is not an absolute quantification.

Fecal bile acid excretion. This was measured by the method of Beher (33) as modified by Wolle et al. (34). Briefly, feces were collected for two consecutive days and homogenized in two volumes (vol/wt) of water. Aliquots (corresponding to 1 g of feces) were incubated for 30 min at 70°C, after addition of 7 ml of ethanol. The mixture was filtered through a paper filter that was then rinsed once with 6 ml of 70°C ethanol. After drying a 4-ml aliquot under nitrogen, 2 ml of 3 M NaOH was added and samples were hydrolyzed by incubation at 100°C for 2 h. After adjusting pH to 9, the bile acid concentration was measured in a 70-μl aliquot using a fluorescence system based on resazurin (33).

Size-fractionation of lipoproteins by fast protein liquid chromatography (FPLC). Equal volumes of plasma from every animal of each group were pooled (5 ml), and the density was raised to 1.21 g/ml with KBr. After ultracentrifugation at 10^5 *g* for 48 h, the supernatant was removed and adjusted with 0.15 M NaCl, 0.01% EDTA, 0.02% sodium azide, pH 7.3, to 2.66 ml, and 1 ml of this solution (corresponding to 2.5 ml of plasma) was injected on a 54 × 1.8 cm Superose 6B column after filtration through a Millipore 0.45 μm HA filter; 2-ml fractions were collected (19, 35).

Statistical methods. Data are presented as means and SEM. Significances of differences between data of normal and Hx rats in Figs. 1, 2, and 3 were evaluated by two-tailed Student's *t* test (36). When multiple groups were analyzed simultaneously, one-way analysis of variance was used, followed by post-hoc comparisons according to the method of Tukey (36).

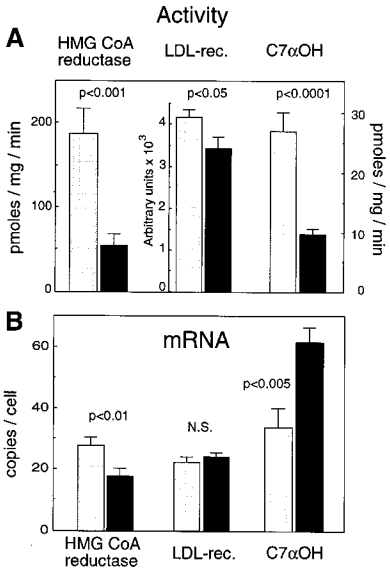

Figure 1. Enzymatic activities (picomoles of product formed per milligram microsomal protein per minute) for HMG CoA reductase and C7αOH, and LDL receptor (*LDL-rec.*) binding activity (*Arbitrary units*) in nine normal (*open bars*) and nine Hx (*filled bars*) rats (*A*). The respective mRNA levels, assayed by solution hybridization using cRNA probes, are shown in *B*. Thin bars represent SEM.

Results

Pronounced differences in hepatic cholesterol metabolism were found when comparing Hx with normal rats (Fig. 1). Assay of the enzymatic activity of HMG CoA reductase (Fig. 1 *A*) showed that Hx rats had clearly reduced levels (29% of controls; $P < 0.001$). The expression of hepatic LDL receptors, assayed by ligand blot, was slightly but significantly suppressed (by 18%; $P < 0.05$). The enzymatic activity of C7αOH, the rate-limiting step in bile acid synthesis, was strongly reduced (36% of controls; $P < 0.0001$). Quantitation of mRNA abundancies by solution hybridization (Fig. 1 *B*) showed that HMG CoA reductase mRNA levels were reduced by 37% in Hx rats ($P < 0.01$), while mRNA levels for the LDL receptor were not significantly changed. In contrast, the mRNA levels for C7αOH were markedly increased in Hx animals (+ 82%; $P < 0.005$).

In Hx animals (Fig. 2), total hepatic cholesterol was increased by 14% ($P < 0.02$) and the enzymatic activity of ACAT was increased by 38% in Hx rats ($P < 0.01$) when assayed with endogenous microsomal cholesterol as substrate. However, although total activities were increased severalfold when exogenous cholesterol was added to the assay tubes, the difference in cholesterol esterification between the two groups was abolished.

The activity of hepatic C7αOH is subject to a marked diurnal variation, with peak activity after midnight (12, 37–39). To determine whether hypophysectomy had different effects in relation to the diurnal phase, we studied four groups of rats (two normal and two Hx). One normal and one group of Hx rats were killed 5 h after onset of light, and the remaining two groups of intact and Hx rats were killed 5 h after onset of darkness. C7αOH activity was twofold higher during the dark period, both in intact and in Hx animals (Fig. 3 *A*). At both time points, however, the Hx rats had clearly lower activities than normal animals (50% reduction). Thus, the diurnal variation of C7αOH was still present in Hx rats, although enzyme activities were reduced in these animals overall. When microsomal free cholesterol was determined in the four groups of animals (Fig. 3 *B*), the levels during the dark phase were not statistically different from normal and Hx rats, respectively. The pronounced changes in C7αOH activity which occurred after hypophysectomy, prompted us to determine which particular hormonal axis was critical to maintain the enzymatic activity of

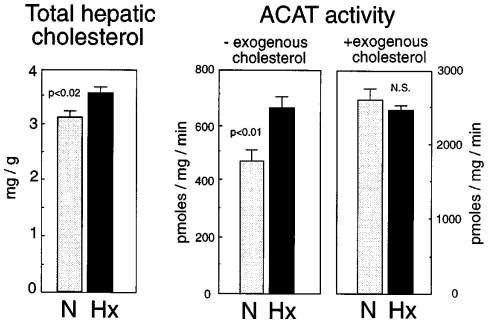

Figure 2. Total hepatic cholesterol concentrations and ACAT enzyme activities in nine normal (*N*) and nine Hx rats (*Hx*). ACAT activity was determined on freshly prepared liver microsomes in the absence and presence of exogenous cholesterol as described in Methods. Thin bars represent SEM.

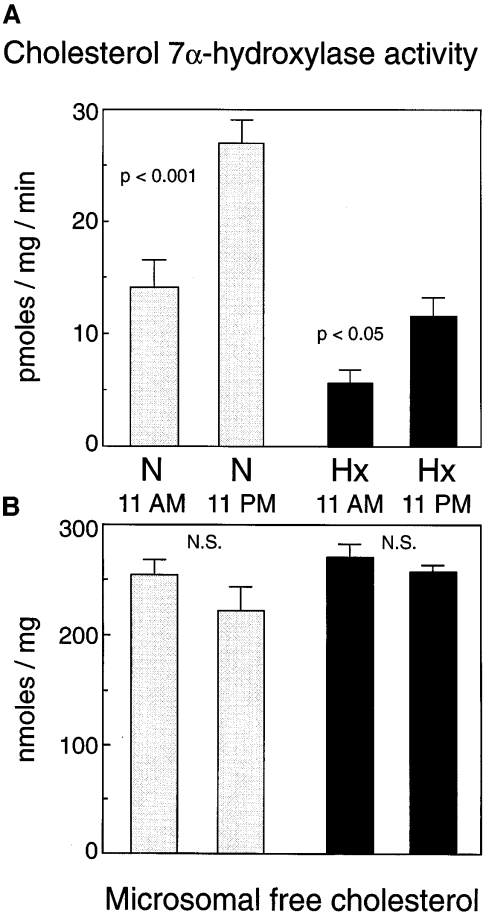

Figure 3. Lack of effect of hypophysectomy on the diurnal variation of C7αOH activity in rats. Groups of four rats were housed in normal and reversed light cycle. Animals were killed at times of the day corresponding to the indicated hours, with light cycle between 6 a.m. and 6 p.m. (*A*) C7αOH activity was immediately assayed using freshly prepared liver microsomes. (*B*) Free cholesterol in liver microsomes. Thin bars represent SEM. *N*, normal rats; *Hx*, hypophysectomized rats.

C7αOH. For this purpose, Hx rats were hormonally substituted for 6 d by continuous subcutaneous infusions using mini pumps. Groups of Hx rats received only C, T_4, or GH, combinations of C + GH or C + T_4, or full hormonal substitution with C + T_4 + GH. Normal rats served as reference. Subsequent determination of the activity of microsomal C7αOH (Fig. 4) showed that only the groups of animals where GH was included in the substitution displayed enzyme activities statistically indistinguishable from those seen in normal controls. Animals receiving only C or T_4, or C + T_4, had significantly reduced enzyme activities that were in the range previously found in Hx animals. Thus, substitution with GH was of critical importance to normalize the enzymatic activity of C7αOH in Hx rats.

After hypophysectomy, fecal bile acid excretion is diminished in the rat (25), and the plasma lipoprotein pattern is altered so that a prominent LDL cholesterol peak emerges (22). Our demonstration of an important role for GH in maintaining the enzymatic activity of C7αOH also raised the question of whether the reduced fecal excretion of bile acids and the severely altered plasma lipoprotein pattern in Hx rats could be

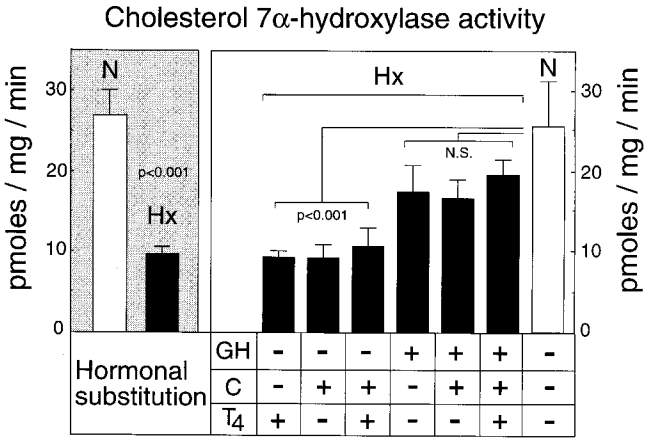

Figure 4. Effect on the enzymatic activity of C7αOH of stepwise hormonal substitution of Hx animals (*right panel*). Hx rats received the indicated hormonal substitutions (*GH*, 1.2 mg/kg per d; *C*, 400 μg/kg per d; T_4, 42 μg/kg per d) for 6 d, by continuous mini pump infusion. The experiment was staggered, so that two animals from each group were killed at 11 a.m. for three consecutive days. Enzyme activity was determined after incubation of freshly prepared liver microsomes. Normal animals served as controls, each group consisted of six animals. The shaded left panel represents the data from nine normal (*N*) and nine Hx animals obtained in the previous experiment (Fig. 1). Thin bars represent SEM. In the statistical analysis, each group was compared with the normal control group.

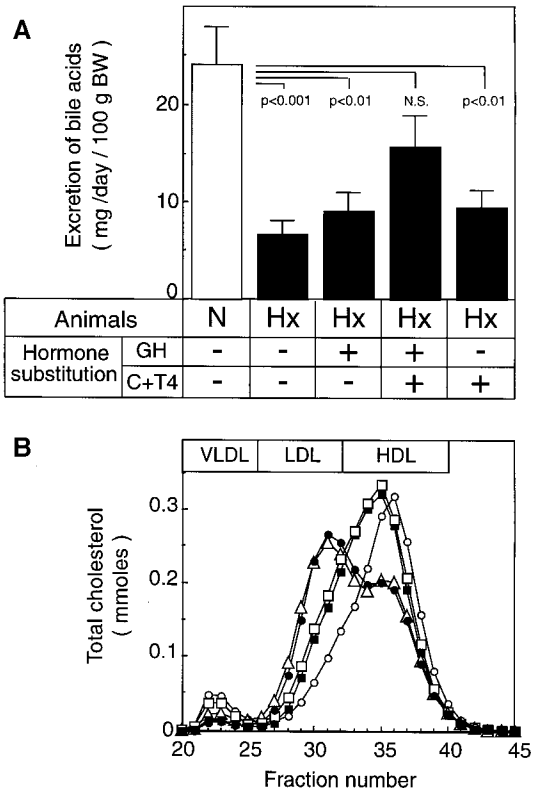

Figure 5. Effect of hormonal substitution of Hx rats on fecal excretion of bile acids (*A*). Hx rats received the indicated hormonal substitution for 6 d before time of killing. *N*, normal rats; *Hx*, Hx rats; *GH*, growth hormone; *C* + T_4, cortisone and thyroid hormone. GH was administered at a dose of 2.4 mg/kg per d. Data are means (five rats per group) and SEM (error bars). (*B*) Cholesterol contents of plasma lipoproteins after FPLC-separation of plasma from the same groups. Normal rats (○), Hx rats (●), and Hx rats receiving GH (■), C + T_4 (Δ), C + T_4 + GH (□). 5 ml of pooled plasma was ultracentrifugated, the concentrated lipoproteins were separated by FPLC, and fractions were assayed for cholesterol. The respective lipoprotein classes are indicated.

normalized by GH substitution. To address this question, groups of Hx rats were substituted with continuous infusions of only GH, or the combinations of C + T_4, or C + T_4 + GH. Nonsubstituted Hx rats served as controls, and normal rats were studied as reference. All animals, housed in metabolic cages, received standard rodent chow, and were killed after 6 d of infusion. Feces were collected during the last 2 d before time of killing.

In agreement with the previous substitution experiment, assay of the activity of hepatic microsomal C7αOH showed that the reduced enzyme activity in Hx rats was clearly increased by GH treatment, to levels indistinguishable from those of normal controls (not shown). Full substitution with C + T_4 + GH increased the C7αOH activity to levels even higher than observed in normal control rats. When the fecal excretion of bile acids was determined (Fig. 5 *A*), it was found that substitution with GH or C + T_4 slightly increased (by 30%) the output of bile acids, representing a less impressive stimulation of in vivo bile acid synthesis. However, when substitution with all three hormones was given, the fecal bile acid output increased by ~ 150%, reaching a level not statistically different from that of normal rats.

Analysis of plasma lipoprotein cholesterol after separation by FPLC showed, as expected (22), the presence of a clear LDL peak among Hx rats; this was not observed in normal rats (Fig. 5 *B*). However, there was virtually no effect of C + T_4 on the plasma lipoprotein pattern. In contrast, substitution with only GH, or full substitution with all three hormones, abolished the typical LDL peak present in Hx animals so that the plasma lipoprotein pattern was almost normalized.

Finally, we wondered if GH could stimulate the activity of C7αOH in normal rats with an intact pituitary function as well. During dietary challenge with cholesterol, the activity of C7αOH is normally stimulated in the rat, whereas this response is suppressed upon the addition of bile acids to the diet (37). To evaluate the effect of GH, we studied four groups of rats receiving, respectively: regular chow (controls); 2% dietary cholesterol; 2% dietary cholesterol + 0.5% sodium cholate; and 2% dietary cholesterol + 0.5% sodium cholate + GH (10 μg/h). Animals were challenged with these diets for 14 d, and GH was infused during the last 6 d. The rats were then killed, and samples of liver tissue were pooled and assayed for C7αOH activity (Fig. 6). As expected, dietary cholesterol increased the activity of C7αOH, whereas further addition of sodium cholate to the diet resulted in a return of enzyme activity to levels seen in animals on normal chow. However, when GH was administered to animals receiving cholesterol and sodium cholate, the activity of the enzyme increased by threefold. Thus, treatment with GH could stimulate C7αOH activity in normal rats as well.

Discussion

This investigation identified several changes in hepatic cholesterol metabolism in the Hx rat, and five major new findings

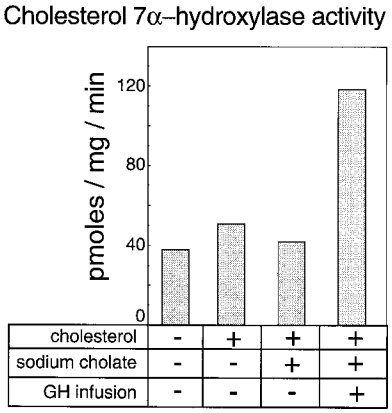

Figure 6. Effect of GH infusion into normal rats during dietary challenge with 2% cholesterol and bile acid (0.5% sodium cholate). Rats received the indicated diet for 2 wk; on day 8 one group of animals received subcutaneous mini pumps that delivered GH at a rate of 10 μg/h. 6 d later, on day 14, all animals were killed at 11 a.m. and liver tissues were pooled for assay of C7αOH activity on freshly prepared microsomes. Each group consisted of three animals.

should be particularly emphasized. First, the activity of C7αOH is highly dependent on GH. Second, a pronounced nontranscriptional regulation of C7αOH activity can occur in vivo. Third, GH has a permissive effect on the hepatic excretion of bile acids when given in combination with C and T_4. Fourth, GH is important to normalize the plasma lipoprotein pattern in Hx rats, and this effect does not appear to be closely linked to the activity of C7αOH or to the excretion of fecal bile acids. Fifth, although the enzyme activity is lowered, the diurnal variation of C7αOH activity is maintained in Hx rats as well.

Together with recent information on the regulatory mechanisms of sterol-regulatory element binding proteins (9–11), these data form the basis for a plausible explanation to the observed overall effects of GH on HMG CoA reductase, C7αOH, and LDL receptor expression, and the resulting increase of cholesterol content in the liver of Hx rats. As a consequence of hypophysectomy, the GH deficiency will result in a reduced enzymatic activity of C7αOH and a diminished synthesis of bile acids, leading to a strongly reduced fecal excretion of bile acids. The reduced cellular concentration of bile acids will in turn derepress the transcription of the C7αOH gene and increase the C7αOH mRNA levels, as was also found. The reduced synthesis of bile acids will, on the other hand, lead to an accumulation of hepatic cholesterol that will suppress the expression of HMG CoA reductase and the LDL receptor. The finding of an unaltered LDL receptor mRNA level, but a clearly reduced LDL receptor expression suggests that cholesterol may also reduce LDL receptor expression by a posttranscriptional mechanism. Evidence for such a posttranscriptional suppression of the LDL receptor by cholesterol has in fact been shown previously in vitro in cells transfected with a LDL receptor gene lacking the steroid responsive element (40).

On the other hand, the increased hepatic cholesterol will stimulate the enzymatic activity of ACAT, an enzyme that has been shown to be driven by substrate (cholesterol) availability (31, 41). In our study, ACAT activity was increased in Hx rats and, furthermore, when excess cholesterol was added to the assay tubes, there was no longer any difference between normal and Hx animals, although the enzymatic activity increased severalfold, as expected (31). These results confirm previous findings on ACAT regulation (31, 41) and suggest that the activity of ACAT is driven by substrate availability also in the current experimental situation.

From current general concepts of hepatic cholesterol homeostasis, together with our present findings in the Hx rat, it therefore seems plausible that one important, and perhaps primary, effect of hypophysectomy would be a reduced enzymatic activity of C7αOH. The fact that C7αOH mRNA was not influenced by the overexpression of truncated SREBP-1 (11) would also suggest that this enzyme is regulated independently of HMG CoA reductase and LDL receptor expression. Interestingly, we could demonstrate that of three hormonal axes controlled by the pituitary (cortisol, thyroid hormone, and GH), only GH was of major importance to normalize the enzymatic activity of C7αOH. In addition, we could show that a pronounced stimulation of C7αOH activity occurred in response to GH also in normal rats during challenge with dietary cholesterol and bile acids.

The enzymatic activity of C7αOH peaks at the middle of the dark phase and is believed to be driven by transcriptional regulation (38, 39). Previous studies have also reported that the diurnal rhythm of C7αOH activity is attenuated or abolished in Hx rats (42, 43). This finding could not be confirmed in the present study, however. We compared the activity of C7αOH in groups of intact and Hx rats killed at the same hour during the light and dark phase, respectively. As expected, the activity of C7αOH was increased in normal rats during the dark phase. In Hx rats killed during the light phase, the activity of C7αOH was reduced (65%) compared with controls. However, the activity of C7αOH was clearly increased during the dark phase compared with the light phase also in Hx rats. This would suggest that the diurnal variation of C7αOH is intact after hypophysectomy, although the absolute level of the activity of the enzyme is clearly reduced. This finding is in agreement with recent data demonstrating that DBP elements in the C7αOH promoter drive the diurnal rhythm of this enzyme (44, 45).

The fact that GH may function as an important regulator of hepatic C7αOH activity has, to the best of our knowledge, not been shown previously. However, it may be of interest to note that Pandak et al. (46) recently observed that the activities of hepatic C7αOH and HMG CoA reductase were increased by twofold after partial nephrectomy in the rat, without any change in steady state mRNA levels. Since this procedure leads to an increased GH secretion, as well as an altered GH surge pattern, which are believed to be of importance for the contralateral renal growth that follows (47), it may be an interesting model to further study the potential importance of GH for bile acid metabolism.

It is interesting to consider whether the present results, observed in vivo in the rat, may also be of relevance to human physiology. In the literature, one clinical study of hypopituitary patients has indicated that pituitary GH may be important for bile acid formation (48). In contrast, studies in normal young males (with intact pituitary function) have not shown any effect of supraphysiological doses of GH on bile acid production (49). In our study, we show that GH has a clear effect on the enzymatic activity of C7αOH in both Hx and normal rats. However, the effect of GH on fecal bile acid excretion was not as pronounced, and it was only when GH was combined with C and T_4 that the fecal output of bile acids was clearly increased. The presence of differences between rats and humans with regard to the transcriptional regulation of

C7αOH has been shown recently by Wang et al. (50) for hormones such as glucocorticoids and insulin. If there are important species differences in the C7αOH response to GH between rats and humans, they will have to be elucidated by further studies. Compared with humans, the basal turnover of hepatic cholesterol appears to be increased several times in the rat (Parini, P., unpublished observations). Furthermore, the possible influence of age on hormone responsiveness should also be considered. Thus, there is a considerable reduction in bile acid synthesis with increasing age in normal humans (51). It may be hypothesized that this phenomenon is related to the known decrease in GH secretion which occurs with normal aging (52). Further clinical studies on the effects of GH treatment in elderly subjects, and in adults with GH deficiency, will be of great importance to further understand the relevance of GH for bile acid synthesis in humans.

The severely altered lipoprotein pattern in Hx rats was normalized in a striking way after GH administration. This effect was evidently not the exclusive consequence of the activation of C7αOH, or fecal bile acid excretion. This finding is in agreement with the fact that mice where the C7αOH gene has been knocked out have a normal plasma lipoprotein pattern (53). Thus, effects of GH on other aspects of lipoprotein metabolism, such as LDL receptors, apoB editing, and lipid synthesis (20, 54, 55), are probably of greater importance for the development of the abnormal plasma lipoprotein pattern observed in Hx rats. In this context, it is also of interest to note that in GH-treated normal young adults, the clearance of plasma LDL is profoundly stimulated (Angelin, B., unpublished observations) and LDL cholesterol is lowered (49, 56), without any concomitant change in bile acid production (49).

In conclusion, our studies have demonstrated that GH plays an important role in the control of C7αOH activity and bile acid synthesis in the rat. The magnitude of regulation is very powerful, since Hx animals had one-third of normal C7αOH activity in spite of an 80% increase in mRNA levels. An important question for further study will be how GH controls the enzymatic activity of C7αOH. Possible levels could involve translation efficiency at the ribosomal level, or modification of the protein itself, e.g., through phosphorylation. Regardless of the mechanism responsible, it is clear that the identification of this presumably new regulatory process will be of great interest.

Acknowledgments

We thank Mrs. Ingela Arvidsson for expert technical assistance and Dr. Ingemar Björkhem for kindly providing deuterated 7α-hydroxycholesterol as well as constructive criticism of the manuscript.

This work was supported by grants from the Medical Research Council (03X-7137), the Swedish Society for Medical Research, The Nordic Insulin Fund, the Widengren, Thuring, Osterman, Jeansson, Ax:son Johnson, Ruth and Richard Julin and Lundström Foundations, the Foundation of Old Female Servants, the Swedish Heart-Lung Foundation, "Förenade Liv" Mutual Group Life Insurance Co., Stockholm, Sweden, and from the Karolinska Institute.

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