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Growth Hormone and Vitamin A Induce P4502C7 mRNA Expression in Primary Rat Hepatocytes

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SUMMARY

The effects of growth hormone (GH) and retinoids on P4502C7 mRNA levels were investigated in cultured primary hepatocytes from normal female rats. Northern blot analysis of total nucleic acids from hepatocytes maintained in culture for 90 hr showed low basal levels of P4502C7 mRNA, which were marginally increased after continuous treatment with GH. Retinol treatment gave a slightly higher induction than GH, whereas treatment with all-*trans* retinoic acid alone or with GH in combination with retinol induced P4502C7 mRNA to levels about one-third of those in normal female rat liver. The effects of retinoids on P4502C7 mRNA were dependent on both the dose and type of retinoid used. All-*trans* retinoic acid produced a saturable dose-response curve with a 50% maximal induction of P4502C7 mRNA at 1.5 μM. The isomer 9-*cis* retinoic acid showed a dose-dependent activation of P4502C7 mRNA similar to that of all-*trans* retinoic acid. Retinol gave a 50% maximal response at approximately 5 μM. In the presence of GH, the induction of P4502C7 mRNA appeared additive to the effect of retinol at all concentrations used and to all-*trans* retinoic acid at concentrations up to 1 μM. As determined by a quantitative solution hybridization assay, P4502C7 mRNA levels were induced 3-fold by GH, 5-fold by retinol, and 19-fold by all-*trans* retinoic acid. In the presence of GH, P4502C7 was induced 8-fold by retinol, whereas the induction by saturating concentration of all-*trans* retinoic acid showed no significant additional effect of GH. The importance of vitamin A for the expression of P4502C7 *in vivo* was confirmed by the low abundance of P4502C7 mRNA in vitamin A-deficient animals as compared with vitamin A-adequate control rats. Nuclear run-on experiments performed in cultured primary hepatocytes showed that both GH and retinoic acid exert their effects at the transcriptional level. We conclude that both GH and retinoids can induce P4502C7 mRNA in rat liver hepatocytes, retinoic acid being the dominant inducer.

An important metabolic route for vitamin A (retinol) in the liver is via cytochrome P-450 enzymes. These enzymes constitute a superfamily of membrane-bound mono-oxygenases involved in the oxidative metabolism of both endogenous and exogenous compounds, such as steroids, fatty acids, prostaglandins, carcinogens, and drugs (1). Hepatic P-450 enzymes in the rat are regulated by a large number of factors, including hormonal, dietary, and environmental stimuli.

Human P4502C8, which is expressed in the liver, has been shown to convert retinol and retinoic acid to polar metabolites including 4-hydroxy-retinol and 4-hydroxy-retinoic acid (2). In the rabbit, P4501A2 and the phenobarbital-inducible P4502B4 have high capacity to 4-hydroxylate retinoids (3). These P-450 catalyzed reactions, which lead to more polar metabolites, are believed to be part of the degradative pathway of vitamin A. Studies performed by Leo *et al.* (4) and Leo and Lieber (5) have shown that rat liver microsomes oxidize retinol and retinoic acid to polar metabolites. In one of these studies (4), it was demonstrated that rats fed a diet high in vitamin A had an increased content of total hepatic microsomal P-450, which was associated with an increased metabolism of retinoic acid. Furthermore, electrophoretic analysis of liver microsomes suggested that the P4502C7 protein was induced by vitamin A. These authors also showed that the retinol/retinoic acid metabolism could be reconstituted using phospholipid, cytochrome P-450 reductase, and purified P4502C7 or P4502B1.

We have demonstrated previously that the pattern of GH release from the pituitary gland is a major determinant of sex-specific P-450 expression in the rat (6). From experiments performed in hypophysectomized rats, we and others have concluded that the expression of P4502C7 is dependent upon the mode of GH secretion (7, 8). P4502C7 is expressed in both sexes, with 2- to 3-fold higher levels in females as compared with males. Removal of the pituitary gland leads to low levels of P4502C7 mRNA, whereas continuous treatment with GH reestablishes levels comparable to those found in intact female or male rats.

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ABBREVIATIONS: P-450, cytochrome P-450; GH, growth hormone; IGF-I, insulin-like growth factor I; SDS, sodium dodecyl sulphate; SSC, standard saline citrate; tNA, total nucleic acids; RAR, retinoic acid receptor.

In addition, observations regarding thyroxine regulation of P4502C7 mRNA have also been made (9), and it has been suggested that thyroxine plays a critical role in mediating or facilitating the inductive effect of GH on hepatic P4502C7 expression.

Studies on the regulation of P4502C7 in animals are hampered by the complex hormonal interactions occurring *in vivo*. Therefore, in this study, we have attempted to elucidate the role of GH and vitamin A in controlling P4502C7 mRNA levels by using a system of cultured adult rat primary hepatocytes.

Experimental Procedures

Materials. Collagenase (type XI), all-*trans* retinoic acid, retinol, retinyl-acetate, and insulin (24.4 U/mg) were purchased from Sigma Chemical Co. (St. Louis, MO). 9-*cis* retinoic acid was a generous gift from Hoffmann-La Roche Inc. (Basel, Switzerland). Cell culture dishes were from A/S Nunc (Roskilde, Denmark). Recombinant bovine GH was a generous gift from American Cyanamid Co. (Wayne, NJ). Proteinase K was from Merck (Darmstadt, Germany), glass fiber filters (Whatman GF/C) were from Whatman Ltd. (Madistone Kent, UK), and RNase-A and RNase-T_1 were from Boehringer Mannheim (Mannheim, Germany). Reagents for *in vitro* transcription of cRNA probes and plasmid vector were obtained from Promega Biotec (Madison, WI). GenescreenPlus membranes were from New England Nuclear (Boston, MA), and [^{35}S]UTPαS (1000 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol), and [^{32}P]UTP (400 Ci/mmol) were from Amersham International plc. (Buckinghamshire, UK).

Animals and production of dietary vitamin A deficiency. Female Sprague-Dawley rats (Alab, Stockholm, Sweden) approximately 8 weeks of age were used for preparation of primary hepatocytes. The animals were maintained under standardized conditions of light and temperature, with free access to animal chow and water. Weanling male Wistar rats (3 weeks of age) were obtained from the Department of Animal Care at Westmead Hospital (Westmead, Australia) and were administered a vitamin A-deficient diet (ICN Biochemicals, Seven Hills, Australia) for 10 weeks; vitamin A-adequate controls received the same diet supplemented with 20 IU retinyl acetate/g. At the conclusion of the period of dietary manipulation, rats were fasted overnight and sacrificed under ether anesthesia. For the quantitation of total hepatic vitamin A, hepatic retinol and retinyl palmitate were extracted by the procedure outlined by Azais *et al.* (10). Resolution and quantitation of vitamin A derivatives was by high-performance liquid chromatography using an Ultrasphere ODS column (5 μm, 25 cm × 4.6 mm i.d.; Beckman Instruments Inc., San Ramon, CA) attached to a Waters Associates system. The mobile phase was methanol:acetonitrile 1:1, at a flow rate of 2.5 ml/min; retinol eluted at 2.0 min and retinyl palmitate at 28.0 min.

Hepatocyte isolation and cell-culture. Hepatocytes were isolated by nonrecirculating collagenase perfusion through the portal vein of ether-anesthetized rats and cultured on matrigel in serum-free modified Waymouth medium as described previously (11). Medium was replaced daily, commencing 2 hr after the cells were plated. Treatment of the cells with bovine GH was also initiated 2 hr after plating, and continued until harvesting after 90 hr. All other treatments were from 66 to 90 hr of culture age, i.e., for 24 hr. During the course of the various treatments, we observed no differences in the survival and integrity of the cells. The final hormone concentrations were (unless otherwise stated): bovine GH, 100 ng/ml; retinol, 1×10^{-5} M; and retinoic acid, 1×10^{-5} M dissolved in dimethylsulfoxide (Me_2SO). Equal volumes of Me_2SO were added to GH-treated and control cells. The concentration of Me_2SO in the media never exceeded 0.3%. Cells were harvested after 90 hr in ice-cold phosphate-buffered saline, containing 5 mM EDTA, pH 7.4, collected by centrifugation, and lysed in 4 ml of a buffer consisting of 1% (w/v) SDS, 10 mM EDTA, and 20 mM Tris-HCl, pH 7.5. Cell lysates were stored at −20°C.

Solution hybridization. Total nucleic acids were prepared from the cell lysate from three to five pooled culture dishes and from homogenized liver tissue, with proteinase-K digestion followed by phenol-chloroform extraction as described previously (12). The concentration of the nucleic acids in tNA samples were measured spectrophotometrically, and the DNA concentration was quantified using a fluorometric assay (13). The amount of mRNA for P4502C7 and IGF-I was determined by solution hybridization using [^{35}S]UTP-labeled cRNA probes. The probes were transcribed *in vitro* from cDNA templates essentially according to the method of Melton *et al.* (14). The probe for P4502C7 was a 3′ segment of the cDNA and has been described before (7). The probe and hybridization conditions for IGF-I were as described previously (11). For P4502C7, hybridization of aliquots of tNA samples was performed in 40 μl 0.6 M NaCl, 22 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% SDS (w/v), 1 mM dithiothreitol, and 25% formamide with approximately 20,000 cpm of probe per incubation. Optimal assay temperature was determined to be 75°C. After overnight hybridization, the samples were exposed to RNase-A and RNase-T_1, and the hybrids were precipitated by the addition of 100 μl 6 M trichloroacetic acid. The precipitates were collected on glass fiber filters and the specific activity was determined by liquid scintillation spectrometry. Standard curves were constructed for each assay using tNA extracted from normal rat liver. All determinations of mRNA from experimental samples were performed in triplicates and were within the linear region of the standard curve. An internal standard, consisting of diluted tNA obtained from a normal rat, was used in each assay to allow comparisons of results between assays. Cell experiments were performed at least three times with cells from different rats to ensure reproducibility. Results are expressed as fold induction from vehicle-treated cells, and either one representative experiment or the average of 3–7 experiments is shown.

Northern blot analysis. Total nucleic acids were isolated from three to five pooled culture dishes and normal liver as described above. Twenty micrograms were electrophoresed on 1.2% agarose gels in the presence of 2.2 M formaldehyde and transferred to GenescreenPlus membranes, essentially as described by Sambrook *et al.* (15). The presence of an equal amount of RNA in each lane was verified by the equal intensities of the 18 S and 28 S ribosomal RNA bands after staining with ethidium bromide. The amount of mRNA for P4502C7 was determined using [α-^{32}P]dCTP 3′-labeled cDNA as a probe as described previously (7). Autoradiography was carried out for 18 hr. The P4502C7 probe was removed from the filter in a solution consisting of 0.01% SDS and 0.01 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) at 95°C, and the filter was then reprobed with β-actin-specific cDNA as a control (16).

Nuclear run-on analysis. The isolation of nuclei from hepatocytes

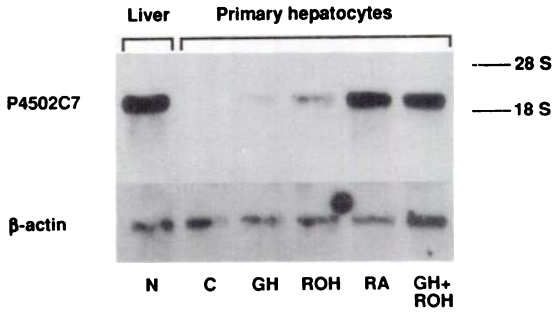

Fig. 1. Effect of GH and retinoids on P4502C7 mRNA levels in primary cultures of female rat hepatocytes as determined by Northern blot analysis. Cells were seeded and treated with hormones (alone or in combination): bovine GH (100 ng/ml), 2–90 hr of culture age; retinol (1×10^{-5} M), 66–90 hr of culture age; all-*trans* retinoic acid (1×10^{-5} M), 66–90 hr of culture age. Cells were harvested and tNA was prepared from five pooled culture dishes. Twenty micrograms of tNA were electrophoresed in each lane on a 1.2% agarose gel in the presence of 2.2 M formaldehyde, transferred to a nylon membrane, and probed with P4502C7 or β-actin-specific sequences. N, normal male liver total RNA; C, vehicle-treated cells; GH, bovine GH; ROH, retinol; RA, retinoic acid.

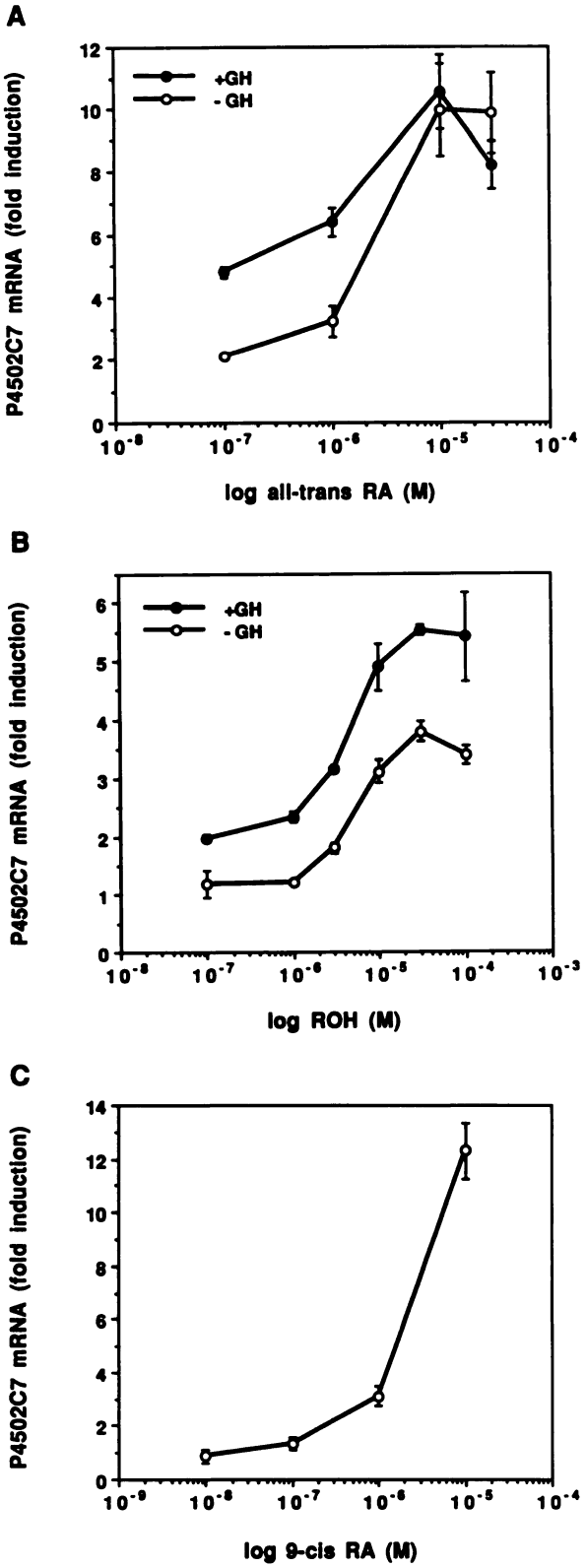

Fig. 2. Dose response of retinoid induction of P4502C7 mRNA levels in primary cultures of female rat hepatocytes as determined by solution hybridization. Cells were seeded and treated with retinoids at 66 hr of culture age in various concentrations as indicated. Bovine GH (100 ng/ml) was continuously present in the medium (where indicated) from 2 hr until harvest at 90 hr of culture age. Cells were harvested and analyzed for P4502C7 mRNA steady state levels. Each point represents the mean ± SD of triplicate determinations of tNA samples from four pooled culture dishes, expressed as fold induction compared with vehicle-treated cells. The level of maximal induction in this set of experiments corresponds to approximately 35–40% of those in normal female rat liver. A, Dose response of all-*trans* retinoic acid in the absence (○) or presence (●) of GH. B, Dose response of retinol in the absence (○) or presence (●) of GH. C, Dose response of 9-*cis* retinoic acid.

has been described in detail elsewhere (17). Ten to 15 culture dishes were used for each treatment and nuclei were prepared from the pooled dishes. For the run-on assay, 5 μg of cDNA for P4502C7 (7), P4502C12 (18), chicken β-actin (16) subcloned into pGem-3Z plasmid, and pGem-3Z without insert were immobilized on GenescreenPlus membranes using a Schleicher and Schuell slot-blot apparatus. The run-on reaction, based on the method by Linial *et al.* (19), was performed as described previously (17), with an equal amount of nuclei in each reaction (20×10^6 nuclei). The obtained transcripts, typically $0.6–1 \times 10^6$ cpm, were hybridized with the filters in 50% formamide, 2% SDS, 0.2 M sodium phosphate (pH 7.2), and 1 mM EDTA, at 42°C for 20 hr. Filters were washed twice at room temperature in 2 × SSC, once at 68°C in 0.5 × SSC/0.2% SDS for 1 hr, and once at 68°C in 0.1 × SSC/0.1% SDS for 1 hr. Autoradiography was carried out for up to 1 week. The run-on analysis was repeated three times with cells from different rats to ensure reproducibility.

Results

Our previous *in vivo* experiments on P4502C7 mRNA expression in the liver showed that this gene is under control of the pituitary in a similar fashion as the female-specific P4502C12, i.e., dependent on continuous GH. As shown in Fig. 1, cells maintained in culture for 4 days expressed low levels of P4502C7 mRNA (lane C) compared with the normal male liver (lane N). Continuous treatment with GH for 4 days gave a small induction (lane GH), whereas treatment for 24 hr (on day 3 to day 4), shown previously to markedly induce P4502C12, was not sufficient for the induction of P4502C7 mRNA (data not shown). On the other hand, treatment for 24 hr (commencing on day 3) with retinol (lane ROH) or all-*trans* retinoic acid (lane RA) resulted in a significant induction of P4502C7 mRNA. Administration of retinol in the presence of GH (lane GH + ROH) as well as all-*trans* retinoic acid treatment alone induced P4502C7 mRNA to levels that were comparable to the level in normal male liver (lane N). We observed no significant difference in the steady state mRNA levels of P4502C7 in untreated cells harvested on days 2, 3, 4, and 5 (data not shown). The effects of GH, retinol, and all-*trans* retinoic acid hence reflect a true induction and not a slowing down in degradation of endogenous P4502C7 mRNA.

In order to further investigate the effects of GH and retinoids on P4502C7 mRNA expression, solution hybridization assays were used to analyze steady state levels in primary hepatocytes in response to increasing concentrations of retinoids in the absence or presence of GH. All-*trans* retinoic acid, retinol, and 9-*cis* retinoic acid resulted in induced P4502C7 mRNA expression in a dose-dependent manner as shown in Fig. 2. All-*trans* retinoic acid treatment alone produced a saturable dose-response curve with a half-maximal induction of P4502C7 mRNA at approximately 1.5 μM (Fig. 2A). The corresponding concentration for retinol alone was approximately 5 μM (Fig. 2B). A maximal effect of GH on induction of P4502C7 mRNA was achieved at a concentration of 100 ng/ml, with levels induced 2- to 3-fold in this set of experiments (data not shown). The retinol concentration needed for a half-maximal induction of P4502C7 mRNA was not significantly changed in the presence

of GH (4 μM instead of 5 μM), and the combined treatment appeared additive over the entire tested concentration range of retinol (Fig. 2B). In contrast, an additive effect of GH and retinoic acid was apparent only at retinoic acid concentrations up to 1 μM (Fig. 2A). The isomer 9-*cis* retinoic acid showed a dose-dependent activation of P4502C7 mRNA similar to that seen with all-*trans* retinoic acid, with a half-maximal induction at approximately 2 μM (Fig. 2C).

The level of P4502C7 mRNA expressed in absolute amounts (cpm/μg tNA) varied somewhat between experiments. However, the effect of treatment expressed as fold induction gave relatively consistent results. Fig. 3 shows average fold inductions of P4502C7 mRNA from three to seven experiments using saturating concentrations of retinoids and/or GH. GH and retinol gave approximately 3- and 5-fold induction of P4502C7 mRNA, respectively, whereas combined GH and retinol treatment resulted in an 8-fold induction. All-*trans* retinoic acid induced P4502C7 mRNA approximately 19-fold without any significant additional effect of GH. The level of induction by all-*trans* retinoic acid in this set of experiments expressed in absolute amounts corresponds to approximately 40% of those in normal female rat liver.

To confirm the importance of vitamin A in the regulation of P4502C7 *in vivo*, the level of P4502C7 mRNA in vitamin A-deficient and control male rats was determined. As shown in Table 1, P4502C7 mRNA expression was reduced significantly in deficient animals. In contrast, the expression pattern of IGF-I mRNA, another GH-regulated gene, was not altered by depletion of vitamin A.

Nuclear run-on analysis (Fig. 4A) showed that the increase in P4502C7 mRNA after treatment with retinoic acid or GH could be attributed to an increased rate of transcription of the P4502C7 gene. P4502C7 transcription was low in vehicle-treated cells (lane C). After treatment with GH (lane GH) for 4 days or with retinoic acid (lane RA) for 24 hr, activation of gene transcription was evident. P4502C12, which was included as a positive control for the effect of GH, was not transcriptionally activated by retinoic acid. Thus the effect of retinoic acid was specific for P4502C7. Since slightly varying amounts of radioactivity were used in the different hybridizations, the autoradiograph was scanned, and the signals were normalized against β-actin transcription. Fig. 4B shows that the P4502C7 mRNA to β-actin mRNA ratio was higher after retinoic acid

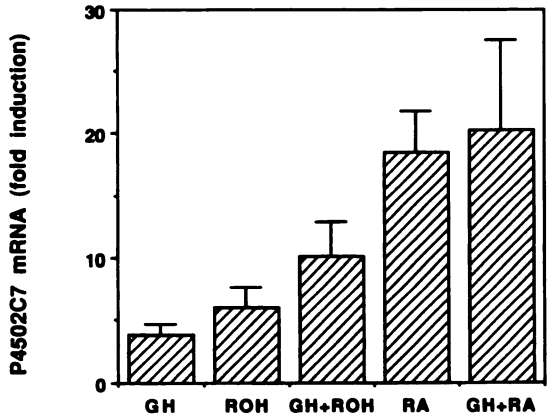

Fig. 3. Effect of GH and retinoids on P4502C7 mRNA expression in primary cultures of female rat hepatocytes as determined by solution hybridization. Cells were seeded and treated with hormones (alone or in combination) as described in Fig. 1, except for the retinol concentration, which was 3×10^{-5} M in this experiment. Results are expressed as fold induction compared with vehicle-treated cells. The level of induction by retinoic acid corresponds to approximately 40% of those found in normal female rat liver. Values are the means ± SE of *n* experiments; GH, *n* = 7; ROH (retinol), *n* = 4; RA (retinoic acid), *n* = 6; GH + ROH, *n* = 3; GH + RA, *n* = 3.

TABLE 1

P4502C7 and IGF-I mRNA expression in control and vitamin A-deficient male rat liver

Male rats were made vitamin A-deficient as described in *Materials and Methods*. Abundance of the mRNA for P4502C7 and IGF-I was determined using solution hybridization with liver tNA samples from six control and six vitamin A-deficient rats. Each value corresponds to the mean ± SD of six individuals.

Diet	P4502C7	IGF-I	Hepatic vitamin A
	(cpm/μg tNA)	*(cpm/μg tNA)*	*(IU/liver)*
Control	138 ± 48	59 ± 10	450 ± 220
Deficient	12 ± 6	60 ± 7	16 ± 15[a]
Percentage of control	9	100	4

[a] Corresponds to the mean ± SD of five animals, since one rat had levels below detection.

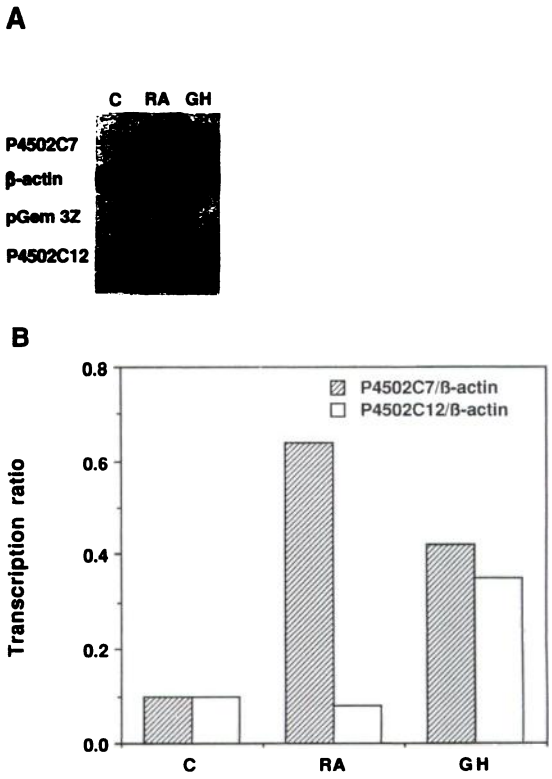

Fig. 4. A, *In vitro* elongation of P4502C7 mRNA as determined by run-on transcription analysis of nuclei from female primary hepatocytes subjected to various treatments. Cells were seeded and treated with hormones. Bovine GH (GH) (100 ng/ml), 2–90 hr of culture age; all-*trans* retinoic acid (RA) (1×10^{-5} M), 66–90 hr of culture age; C, vehicle-treated cells. Nuclei from 15 pooled culture dishes were isolated and allowed to chain-elongate nascent RNA in the presence of [^{32}P]UTP. The RNA was then extracted and hybridized to nylon filters containing excess DNA affixed as slots. The actual cpm used in the hybridizations were as follows: C, 0.75×10^6 cpm; RA, 1×10^6 cpm; GH, 1×10^6 cpm. Unhybridized RNA was washed away, leaving the hybridized RNA to be detected by autoradiography. P4502C7, cytochrome P4502C7 cDNA; β-actin, chicken β-actin cDNA; pGEM 3Z, pGEM 3Z plasmid DNA; P4502C12, cytochrome P4502C12 cDNA. B, Normalization of scanning values to β-actin signals. The data plotted shows the relative transcription ratio between P4502C7 mRNA to β-actin mRNA and P4502C12 mRNA to β-actin mRNA.

than GH treatment, whereas the P4502C12 mRNA to β-actin mRNA ratio displayed the opposite pattern.

Discussion

Our previous *in vivo* studies suggest that P4502C7 is dependent upon GH for its expression, and that the pattern of hormone administration is critical (7). Furthermore, studies by Leo *et al.* (4) have shown that hepatic P4502C7 protein levels are increased in rats fed a diet high in vitamin A. In order to examine the role of GH and retinoids in regulation of P4502C7 mRNA, we have used a system of cultured adult rat primary hepatocytes.

Treatment of hepatocytes with GH for 24 hr at a culture age of 66 hr was insufficient for the induction of P4502C7, whereas the level of induction of P4502C12 following such treatment was comparable to what has been reported before (data not shown) (11). P4502C7 was, however, induced after continuous GH treatment for 4 days. This slow effect on P4502C7 mRNA expression in response to GH is in agreement with results from time course experiments performed in hypophysectomized animals (7) and indicates that synthesis of protein factors mediating the GH action might be required. However, the fold induction of P4502C7 mRNA by GH in hepatocyte cultures was low compared with the induction seen in GH-treated hypophysectomized animals. This discrepancy can possibly be explained by an indirect effect of GH on the expression of P4502C7 in the animal. The previous studies by Leo *et al.* (4) suggesting an autoregulation of P4502C7 by retinoid substrates and our demonstration that retinoids, in particular retinoic acid, have a profound effect on the expression of P4502C7 mRNA may perhaps indicate that at least some of the effect of GH on P4502C7 is mediated via retinoids.

Chylomicron remnants containing almost all absorbed vitamin A, in the form of retinyl esters, are taken up by the liver (20), probably via low density lipoprotein receptors, recognizing apolipoprotein E present in the chylomicron. Interestingly, both low density lipoprotein receptors and apolipoprotein E have been shown to be induced by GH (21, 22). In addition, the rat class I alcohol dehydrogenase, responsible for the initial step of retinol conversion into retinoic acid (23), has been shown to be influenced by GH, both *in vivo* and in primary hepatocytes (24–26). Furthermore, a sex difference in alcohol dehydrogenase activity has been reported by Büttner (27), with higher activity in female than in male rats. Thus, it is not inconceivable that the GH-dependent sex difference in P4502C7 expression, with female rats expressing 2- to 3-fold higher levels than males, is mediated via an effect on alcohol dehydrogenase. In relation to this, it is of interest that the hepatic vitamin A content is higher in normal female rats than in normal male rats (4). GH most likely has a direct effect on the P4502C7 gene (see below), but perhaps GH may also influence uptake and utilization of vitamin A, whereas retinoic acid is the dominant inducer of P4502C7 at the gene level.

The response of P4502C7 in primary hepatocytes to retinoids was dependent on both the dose and type of retinoid used. Addition of all-*trans* retinoic acid induced P4502C7 mRNA to levels comparable to those in normal male liver. Retinol treatment resulted in P4502C7 mRNA levels corresponding to approximately 20% of the level in normal male liver. The female-specific P4502C12 was not induced by retinoic acid or retinol (data not shown), indicating that the effect on P4502C7 was not a general stimulatory effect on P-450 expression. In support of this is our recent report (28) demonstrating specific alterations in rat liver P-450 enzymes in response to vitamin A. This study showed that the total hepatic P-450 content in rats fed a diet high in vitamin A was increased by 26%. More specifically, P4503A2, a male-specific P-450, was increased by 158%, concomitantly with an enhanced level of 6β-hydroxylation of androstenedione and progesterone. On the other hand, androstenedione 16α-hydroxylation and progesterone 21-hydroxylation (mediated by P4502C11 and P4502C6, respectively) were decreased. Any vitamin A metabolism by P4502C7 was, however, not analyzed in this report.

The results obtained in the present study clearly show that maximal induction of P4502C7 mRNA can be achieved by retinoic acid alone. However, at lower concentrations of retinoic acid and at all concentrations of retinol, the effect of GH was additive to the effect of the retinoid. We conclude that both retinoids and GH can induce P4502C7 mRNA; retinoic acid, however, appears to be the dominant inducer in cultured hepatocytes. The fact that P4502C7 mRNA shows very low expression in vitamin A-deficient rats, whereas the expression of IGF-I is not affected by this depletion, further supports that retinoids are of great importance for the expression of P4502C7 *in vivo*.

The run-on analysis showed that GH also has a significant effect on the transcription of the P4502C7 gene. This contrasts with the relatively modest effect of GH on the steady state level of P4502C7 mRNA, perhaps indicating instability of the mRNA after GH treatment. Conversely, retinoic acid might have a positive effect on P4502C7 mRNA stability. However, run-on analysis is a poor quantitative method and the discrepancy may just be a reflection of that. Nevertheless, GH has consistently been found to increase P4502C7 transcription in repeated experiments. Thus, our results demonstrate that both retinoic acid and GH can act at the level of transcription to increase the P4502C7 mRNA content. To our knowledge, this is the first demonstration of transcriptional activation of a P-450 gene in response to retinoic acid.

The effects of retinoids on gene expression are mediated via nuclear receptors that belong to the steroid/thyroid hormone receptor supergene family. The RAR bind to their cognate DNA elements in target genes and activate gene expression in response to nanomolar concentrations of retinoic acid (29). The maximal induction of P4502C7 by retinoic acid was obtained at 10 μM, which seems too high a concentration to suggest involvement of RAR. Recently, another subfamily of retinoid receptors has been characterized that possesses coregulator activity with RAR, namely retinoid X receptors (30–33). Retinoid X receptor was first described as being activated by micromolar concentrations of all-*trans* retinoic acid (34); the natural ligand, however, was later identified as 9-*cis* retinoic acid, which is effective in the nanomolar range (35–37). 9-*cis* retinoic acid did indeed induce P4502C7 mRNA; however, this ligand, like all-*trans* retinoic acid, was also required in the micromolar range for significant induction. In light of this, it is not inconceivable that the transcriptional activation of the P4502C7 gene by retinoids may be conveyed by a metabolite(s) other than all-*trans* - or 9-*cis*-retinoic acid. In this context, it is of interest that the metabolite 4-hydroxyretinoic acid has been demonstrated to activate transcription of a reporter gene in transient transfection experiments, including cotransfection

with RARs and retinoid X receptor (38). Whether metabolites of retinoic acid are involved in the induction of P4502C7 warrants further investigation, and is the focus of our current research.

Acknowledgments

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