High sensitivity of rat hepatic vitamin D₃-25 hydroxylase CYP27A to 1,25-dihydroxyvitamin D₃ administration

CATHERINE THEODOROPOULOS, CHRISTIAN DEMERS, JEAN-LUC PETIT, and MARIELLE GASCON-BARRÉ
Centre de recherche, Hôpital Saint-Luc, Centre Hospitalier de l’Université de Montréal, Département de Pharmacologie, Faculté de médecine, Université de Montréal, Montreal, Quebec, Canada H2X 1P1

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Theodoropoulos, Catherine, Christian Demers, Jean-Luc Petit, and Marielle Gascon-Barre. High sensitivity of the rat hepatic vitamin D₃-25 hydroxylase CYP27A to 1,25-dihydroxyvitamin D₃ administration. Am J Physiol Endocrinol Metab 284: E138–E147, 2003. First published October 1, 2002; 10.1152/ajpendo.00303.2002.—CYP27A is considered the main vitamin D₃ (D₃)-25 hydroxylase in humans. Our purpose was to evaluate the effect of the D₃ nutritional and hormonal status on hepatic CYP27A mRNA, cellular distribution, transcription rate, and enzyme activity. Studies were carried out in normal and in D-depleted rats supplemented with D₃, 25OHD₃, or 1,25(OH)₂D₃. CYP27A exhibited a significant gender difference and was observed throughout the hepatic acinus not only in hepatocytes but also in sinusoidal endothelial, stellate, and Kupffer cells. Neither D₃, nor 25OHD₃ influenced CYP27A mRNA levels. However, 1,25(OH)₂D₃ repletion led to a 60% decrease in CYP27A mRNA, which was accompanied by a 46% decrease in mitochondrial D₃-25 hydroxylase activity. The effect of 1,25(OH)₂D₃ was mediated by a significant decrease in CYP27A transcription, whereas its mRNA half-life remained unchanged. Our data indicate that CYP27A is present in hepatic parenchymal and sinusoidal cells and that the gene transcript is not influenced by the D₃ nutritional status but is transcriptionally regulated by 1,25(OH)₂D₃ exposure.

bile acid biosynthesis; Kupffer cells; stellate cells; hepatocytes; sinusoidal endothelial cells

THE SECOSTEROID VITAMIN D₃ (D₃) of endogenous or exogenous origin has, in its native form, no biological activity. Once in circulation, D₃ is efficiently taken up by the liver (26) and hydroxylated at C-25 by a mitochondrial mixed-function oxidase CYP27A [C₁₇ sterol hydroxylase (EC 1.14.13.15)] (15). In enzymes, the vitamin is presumed to be the only D₃-25 hydroxylase (51). However, a microsomal D₃-25 hydroxylase has also been reported in rodents (11), chickens (12), and pigs (35), but only the porcine enzyme (which has been termed CYP9D25) has been cloned to date (34, 44).

CYP27A is a cytochrome P-450 that catalyzes the first step in the oxidation of the cholesterol side chain in the secondary “acidic” bile acid biosynthesis pathway (13). CYP27A is also able to hydroxylate D₂ and D₂ metabolites at position C-25 (51) as well as at other positions on the secosteroid side chain (29, 54). It has also been reported to be able to catalyze the 1α-hydroxylation of 25-hydroxyvitamin D₃ (25OHD₃), albeit at a much lower rate than the transformation of D₃ into 25OHD₃ (3). However, unlike the tight regulation by the D₃ endocrine system associated with the renal 25-hydroxyvitamin D₃-1α-hydroxylase, the sensitivity of the gene encoding CYP27A to D₃ or to D₃ metabolites has not been characterized. The presence of regulatory mechanisms related to the D₃ status as a modulator of the 25-hydroxylation of the vitamin is, however, a widely accepted notion, which rests on the studies of DeLuca’s group in the early 1970s (Bhattacharyya and DeLuca, Refs. 10, 12). Several laboratories have attempted to evaluate the mechanism(s) involved in the regulation of the activity of the D₃-25 hydroxylase. In the early 1980s, two independent reports (5, 8) raised the possibility that 1,25(OH)₂D₃ might be an inhibitor of the enzyme in rats as well as in humans. Indeed, Bell et al. (8) reported that D₃ administration to human subjects significantly increased mean serum 25OHD₃, whereas the concomitant administration of 1,25(OH)₂D₃ completely prevented the increase in serum 25OHD₃ in response to the same dose of D₃. Subsequent studies revealed that the response of the D₃-25-hydroxylase to various challenges in vitro was greatly influenced by the in vivo calcium and/or D₃ status of the animals (9). However, supplementation with 1,25(OH)₂D₃ was shown to influence the in vivo handling of D₃ by accelerating its biotransformation as well as by increasing the metabolic and biliary clearances of D₃ and/or D₃ metabolites (18, 24, 31, 32). The latter studies suggest the presence of a 1,25(OH)₂D₃-mediated increase in the utilization of the substrate and/or in its turnover but do not, in any way, directly address the effect of 1,25(OH)₂D₃ on the hepatic mitochondrial D₃-25 hydroxylase CYP27A.

To date, studies examining the regulation of the gene encoding CYP27A have focused on its role as a mixed-
function oxidase involved in bile acid biosynthesis (56). The aim of the studies was, therefore, to investigate the influence of the D₃ nutritional and endocrine status on CYP27A as a D₃-25 hydroxylase in the rat liver. We now report that 1,25(OH)₂D₃ is a major regulator of the hepatic CYP27A, which translates into significant decreases in CYP27A steady-state mRNA levels and transcription rate, as well as into a significant decrease in the mitochondrial C-25 hydroxylation of 1α-hydroxyvitamin D₃.

MATERIALS AND METHODS

Experimental Design

The influence of the D₃ endocrine system on the handling of the gene encoding the hepatic mitochondrial D₃-25 hydroxylase CYP27A was evaluated in D-depleted rats and in D-depleted rats following in vivo repletion with physiological concentrations of D₃, 25OHD₃, or 1,25(OH)₂D₃. D depletion and repletion procedures were carried out as previously reported (59). The specific end points of the studies included evaluation of 1) the gender differences and response to cytochrome P-450 inducers on CYP27A mRNA levels, 2) the presence of CYP27A in the main hepatic cell populations, and 3) the effect of 1,25(OH)₂D₃ on CYP27A steady-state mRNA levels, mRNA half-life, transcription rate, and mitochondrial CYP27A hydroxylation activity at C-25.

A CYP27A gene fragment corresponding to base pairs 399–803 of the NH₂-terminal coding region of the rCYP27A sequence of Su et al. (58) (GenBank accession no. M38566) was generated by RT-PCR as previously reported (59). Northern blot analyses of rat livers hybridized with the rCYP27A gene fragment generated consistently revealed a single band of 2.3 kb.

Animals were treated according to the standards of ethics for animal experimentation of the Canadian Council on Animal Care, and all protocols were approved by the local animal ethics committee.

Repletion with D₃, 25OHD₃, or 1,25(OH)₂D₃. Expression of the hepatic CYP27A gene transcript was studied in normal control rats fed a commercial rat Chow diet (Harlan Teklad Normal male rats were exposed to xenobiotics known to induce cytochrome P-450 inducers. Normal male rats were exposed to 1) dexamethasone (two daily ip injections, 100 mg/kg), 2) 3-methylcholanthrene (a single ip injection, 30 mg/kg), 3) β-naphthoflavone (three daily ip injections, 80 mg/kg), 4) acetone (1% vol/vol in drinking water) for a period of 10 days, or 5) phenobarbital (350 mg/ml in drinking water) for a period of 10 days (45).

Experimental Procedures

Determination of circulating Ca²⁺ and D₃ metabolites. Serum Ca²⁺ concentrations were measured with an ICA2 ionized Ca²⁺ analyzer (Radiometer, Copenhagen, Denmark). Serum 25OHD₃ and 1,25(OH)₂D₃ concentrations were measured using the IDS 25OHD₃ and 1,25(OH)₂D₃ assay kits (IDS, Boldon, Tyne and Wear, UK) according to the manufacturer’s instructions.

Hepatic cell isolation. At the time of euthanasia, the livers were flushed with saline and processed for isolation of the individual cell populations. Cells used for RNA analysis were placed in TRizol solution (Burlington, ON, Canada), and RNA was extracted as described by the manufacturer.

Hepatocytes were isolated from nonfasting animals as mentioned elsewhere (25). Rat sinusoidal cells were isolated by the method of Knoop and Sleyster (37) with the following modifications. After Metrizamide density gradient, cells were washed in Gey’s balanced salt solution (GBSS), pH 7.4, at 4°C, resuspended, and introduced in a type J2–21M centrifuge (Beckman Instruments, Palo Alto, CA) equipped with a JE-6B elutriation rotor and a Sanderson chamber. Cells were centrifuged at 2,500 rpm and then were washed out at pump flows of 13, 23, and 42 ml/min to collect stellate, endothelial, and Kupffer cells, respectively, with GBSS, pH 7.4, at 4°C. Cells were centrifuged and counted, and their viability was evaluated. Sinusoidal cells had a viability >95% and were freed of hepatocytes. Cell populations were identified by immunocytochemistry and found to be >88% pure (43).

Cell viability and yield were evaluated by the Trypan blue exclusion test and by counting viable cells in each cell population, respectively.

Northern blot analysis. At the time of euthanasia, the livers were isolated and immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total liver RNA was extracted, blotted onto nylon membranes (Qiagen, Mississauga, ON, Canada), and processed for Northern analyses using the radiolabeled 404-base rCYP27A cDNA probe generated in our laboratory (59).

RT-PCR. After DNase treatment, 1 µg of total RNA from sinusoidal cells was converted in cDNA (First-Strand cDNA Synthesis Kit) using pd(N)₆ as primer; 2.0 µl of RT reaction were amplified for either 25 (CYP27A) or 20 cycles (GAPDH) using specific primers and Taq PCR Master Mix and 0.1 µl of [α-³²P]dCTP (3 000 Ci/mol) in a touchdown Thermal Cycling system (Hybaid, Teddington, UK). Design of primers to generate rCYP27A and GAPDH cDNA fragments was made with the Primers Software of Williamstone Enterprises (http://www.williamstone.com) and the sequences of Su et al. (58) (CYP27A and Tso et al. (62) (GAPDH). cDNA PCR products were loaded and separated onto a nondenaturing 8% polyacrylamide (TBE) gel. The gel was dried and exposed to Kodak X-Omat AR film at −80°C in the presence of an intensifying screen for 4–16 h. Densitometry was performed as described previously (20, 38).

Half-life of the CYP27A gene transcript. Studies on the half-life of the CYP27A gene transcript were achieved in D-depleted rats subjected to intraperitoneal injections of 0.5 mg/kg actinomycin D dissolved in 95% ethanol-saline (1:1 vol/vol), which was administered 6, 12, 18, and 24 h before animals were killed. In studies carried out in 1,25(OH)₂D₃-
injected (12 nmol/kg iv) animals, actinomycin D was first administered half an hour before 1,25(OH)2D3 and subsequently every 6 h over a 24-h period. 1,25(OH)2D3 continued to be administered every 6 h. CYP27A mRNA levels were evaluated as described in Northern blot analysis.

**Nuclear run-on transcriptional assay.** Nuclei were isolated from livers of hypocalcemic D-depleted (D-Ca−) or of 1,25(OH)2D3-repleted rats by the method of Widnell and Tata (67) by use of successive sucrose gradient centrifugations. The rate of CYP27A gene transcription was measured using a previously described nuclear run-on transcriptional assay (50) with the modifications described in Theodoropoulos et al. (59). The labeled RNA was hybridized to nylon membranes on which 300 ng of the 404-bp D3-25 hydroxylase cDNA fragment with 150 ng of 18S ribosomal RNA cDNA fragment (positive control), and 100 ng of pBS (negative control) had been hybridized in 5% SDS, 400 mM NaPO4, pH 7.2, 1 mM EDTA, 1 mg/ml BSA, 50% formamide, and 240 μg/ml salmon sperm DNA. The membranes were prehybridized for 4 h at 52°C in hybridization solution without labeled RNA, and then hybridization was performed at 52°C for 72 h. The membranes were washed and exposed to X-ray films for 7 days, and densitometry was performed as previously described (20, 38).

In situ RT-PCR hybridization. Paraffin liver sections were mounted onto slides pretreated with 3-aminopropyltriethoxysilane (APES), dewaxed in xylene, then rehydrated through a series of ethanol baths, and finally immersed in diethyl pyrocarbonate (DEPC)-treated water. Slides were incubated as described in Gascon Barré et al. (23).

In situ RT-PCR results were performed by the method of Mee et al. (40) with the modifications previously described (23). The reaction was carried out in 25 μl with a OneStep RT-PCR kit and 0.6 μM rCYP27A specific primers (58) with the use of a Hybaid thermal cycler provided with a in situ block (Hybaid). Sections were heated for 30 min at 50°C, 15 min at 95°C, and then 10 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s were performed; finally, slides were heated for 10 min at 72°C. Samples were washed twice in PBS and fixed in 4% paraformaldehyde-PBS for 20 min at 4°C, incubated with 0.25% acetic anhydride in 0.1 TEA for 10 min, and rinsed in 90% ethanol and allowed to dry.

An in situ hybridization was performed using the rCYP27A antisense riboprobes (with linearized CYP27A cDNA as template), which were generated by the single-strand RNA synthesis technique using T7 RNA polymerases and [α-32P]UTP (800 Ci/mmol). Hybridization was performed at 42°C for 16 h with 100 μl of hybridization solution (50% formamide, 2X SSC, 1X Denhardt’s, 0.25M Tris-HCl, pH 7.5, 10% dextran sulfate, 0.5 M Na pyrophosphate, 0.5% SDS, 25 μg/ml denatured salmon sperm DNA, 250 μg/ml yeast tRNA) and 1 X 10^7 cpm/ml of antisense. After a washing, autoradiography was performed with NBT-2 emulsion (Inter- science, Mississauga, ON, Canada). Slides were exposed for 5 days at 4°C, developed with D19 (Interscience) developer, and counterstained with hematoxylin and eosin.

**Mitochondrial D3-25 hydroxylase enzyme activity.** Liver from hypocalcemic D-depleted (D-Ca−) and 1,25(OH)2D3-treated rats (28 pmol/day for 7 days) were homogenized in 10 volumes of 0.25 M sucrose, 1 mM EDTA, 10 mM Tris, 10 M KCl, and 3 U/ml heparin, pH 7.4. Mitochondria were isolated according to Rosenberg and Kappas (48) with the modifications described previously (59). The final mitochondrial pellet was resuspended in 0.25 M sucrose, 1 mM EDTA, 10 mM Tris, 10 M KCl, and 3 U/ml heparin, pH 7.4.

Incubation conditions contained 0.5 mg of mitochondrial proteins suspended in 40 mM potassium phosphate, 0.25 M sucrose, 200 mM EDTA, 20 mM MgCl2, pH 7.4, 0.2 mg bovine serum albumin, 2 μg N,N′-diphenylphenylenediamine (Aldrich Chemicals, Milwaukee, WI), and 10 mM isocitric acid (Sigma Chemicals, St. Louis, MO). The enzyme reaction was started with 20 nmol 1α-hydroxyvitamin D3 (Leo Pharma) and continued for 40 min at 37°C with gentle shaking. Control conditions were carried out using boiled mitochondria. The reaction was terminated with 3.75 ml of chloroform-methanol (1:2 vol/vol), and 6,000 cpm [3H]1α,25(OH)2D3 were added to monitor recovery during the extraction and chromatographic procedures (16). After extraction and evaporation, the residue was dissolved in 150 μl of hexane and injected into a Beckman model 160 fitted with an absorbance detector at 254 nm (Beckman Instruments). A Zorbax-Sil column (4.6 x 250 mm; DuPont Instruments, Wilmington, DE) was used. Elution was done in hexane-isopropanol (9:1 vol/vol) at a flow rate of 2 ml/min. The fractions corresponding to authentic 1α,25(OH)2D3 (retention time 15 min, without overlap from 1αOH3) were collected and counted in a beta spectrometer (Beta LS1801, Beckman Instruments). Identity of the product was further confirmed by a second HPLC on a C18 column eluted with hexane-isopropanol (8:2 vol/vol).

**Statistical Analyses.**

Data are presented as means ± SE. Statistically significant differences between group means were evaluated by ANOVA or the Student’s t-test as indicated in the figure legends. Individual between-group contrasts were evaluated using the Bonferroni test.

**RESULTS.**

**Parameters of the D3 Nutritional and Endocrine Status.**

Table 1 presents the circulating concentrations of 25OHD3, 1,25(OH)2D3 as well as those of the circulating Ca2+ concentrations. Serum 25OHD3 concentrations were significantly increased in D3-supplemented rats compared with the D-depleted, 25OHD3, and 1,25(OH)2D3-repleted rats, but both doses of D3 led to 25OHD3 within the normal physiological range of 28 and 75 nmol/l in animals receiving the low and high doses, respectively. Serum 1,25(OH)2D3 concentrations increased in all repleted groups compared with the D-depleted controls. However, serum 1,25(OH)2D3 concentrations were found to be signifi-
cantly higher in both D₃-repleted groups than in those receiving either 25OHD₃ or 1,25(OH)₂D₃.

Cellular Localization

Investigation of the intrahepatic cellular localization of the gene encoding CYP27A revealed that the transcript was expressed not only in hepatocytes but also in sinusoidal cells. Indeed, the CYP27A gene transcript was clearly found in freshly isolated sinusoidal endothelial, stellate (Ito), as well as Kupffer cells, as illustrated in Fig. 1. However, hepatocytes exhibited the highest abundance of the CYP27A gene transcript with averaged CYP27A mRNA levels of 53, 23, and 9% in sinusoidal endothelial, stellate, and Kupffer cells, respectively, compared with the levels found in hepatocytes (100%).

Gender Differences and Drug Inducibility

Evaluation of the gender differences in CYP27A mRNA levels reveals that female rat livers exhibit 43% higher levels of the gene transcript than their male counterparts (P < 0.01), as illustrated in Fig. 2, A and B. In addition, the hepatic steady-state levels of the CYP27A transcript were found to be significantly induced by the two classical cytochrome P-450 inducers dexamethasone (+45% over basal values) and β-naphthoflavone (+41% over basal values; Fig. 2, B and D). 3-Methylcholanthrene, acetone, and phenobarbital did not significantly affect the steady-state abundance of the CYP27A transcript.

Effect Of D₃, 25OHD₃, or 1,25(OH)₂D₃

CYP27A mRNA levels during long-term exposure to D₃ or 25OHD₃. Repletion of D-depleted rats with either D₃ (low dose) or 25OHD₃ was found not to significantly influence the liver CYP27A mRNA levels following 1, 3, 5, or 7 days of repletion, as illustrated in Fig. 3 for data obtained on day 7 of the repletion protocol. Rats fed the high dose of D₃ exhibited a transient 55% decrease in CYP27A mRNA levels at the 3-day time point but not at any other time points, as illustrated for values obtained following 1 wk of repletion. Serum calcium levels were normalized in all repleted groups, and hypercalcemia was not observed in any of the groups.

CYP27A mRNA levels during long-term exposure to 1,25(OH)₂D₃. By contrast, as illustrated in Fig. 4, the hepatic CYP27A gene transcript was very sensitive to the continuous administration of 1,25(OH)₂D₃, leading to normal circulating Ca²⁺ and 1,25(OH)₂D₃ concentrations as previously reported (59). Indeed, CYP27A mRNA levels progressively decreased throughout the week of 1,25(OH)₂D₃ repletion with a decrease of 23% compared with values observed in D-depleted animals after 1 day of repletion to attain a 60% decrease after 7 days of continuous 1,25(OH)₂D₃ exposure (P < 0.0007).

Intra-acinar localization of the CYP27A gene transcript. Figure 5 presents data on the in situ hybridization of liver specimens obtained from D-depleted, normal D-repleted controls, and 1,25(OH)₂D₃-repleted rats. As illustrated, in liver specimens obtained from D-depleted (Fig. 5, A and B) and from normal controls (Fig. 5, D and E), the CYP27A gene transcript was found to be present throughout the hepatic acinus, with CYP27A mRNA hybridization being observed in both the periportal and the perivenous regions of the acinus. The intensity of the CYP27A mRNA signal was found to be only slightly more intense in liver specimens obtained from D-depleted than in those obtained from normal controls. After 1 wk of 1,25(OH)₂D₃ repletion, however, a clear decrease in the intensity of the CYP27A mRNA hybridization signal was observed in both the periportal and the perivenous regions of the hepatic acinus (Fig. 5, G and H). Negative in situ hybridization controls using the rCYP27A sense riboprobes on hepatic specimens obtained from in D-depleted normal controls and 1,25(OH)₂D₃-repleted rats are presented in Fig. 5, C, F, and I, respectively.

Activity of the mitochondrial D₃-25 hydroxylase. Figure 6 illustrates the effect of 1-wk exposure to 1,25(OH)₂D₃ on the hepatic mitochondrial D₃-25 hydroxylase activity. 1,25(OH)₂D₃ repletion had a significant influence on CYP27A activity, with an averaged
46% decrease in 1α,25(OH)2D3 production following incubation with 1αOH2D3 in liver mitochondria obtained from 1,25(OH)2D3-repleted rats compared with those obtained from D-depleted rats (P < 0.03).

Mechanisms Of 1,25(OH)2D3 Action

CYP27A mRNA half-life. As illustrated in Fig. 7, actinomycin D treatment of D-depleted rats with 0.5 mg/kg progressively decreased CYP27A mRNA levels throughout the 24-h time frame studied to nearly undetectable levels at the 24-h time point (P < 0.0001). Under our experimental conditions, the half-life of the CYP27A gene product was estimated to be 12.7 h. 1,25(OH)2D3 administration was found to influence CYP27A mRNA levels in a manner similar to that observed in animals treated with actinomycin D alone. In addition, over the time period studied, the concomitant administration of the hormone and actinomycin D did not significantly affect CYP27A mRNA levels over those observed with the hormone or actinomycin D alone.

Transcription rate of the CYP27A gene. Nuclear transcription run-on assays were performed on nuclei isolated from livers of D-depleted rats as well as on nuclei obtained from livers of D-depleted animals exposed to a single intravenous 12 nmol/kg dose of 1,25(OH)2D3 6 h before euthanasia (Fig. 8). The 18S ribosomal gene was used as the control gene for both the untreated and treated groups. Quantification for the nuclear run-on assays indicated that, within 6 h of 1,25(OH)2D3 exposure, the transcription rate of the gene encoding the CYP27A was decreased to nearly undetectable levels compared with the level of expression observed in control livers. Nonspecific hybridization, estimated by hybridization to pBS plasmid DNA, did not account for the observed CYP27A mRNA decrease in transcription rate.

DISCUSSION

Our data show for the first time the presence of the CYP27A gene transcript in hepatic sinusoidal cells. Although hepatocytes were found to harbor the highest level of the transcript, the observation indicates that cell populations other than hepatocytes may also be involved in the production of 25OHD3 in the normal rodent liver. These data indicate that CYP27A is more widely distributed than originally thought, as the intestine, kidney, calvaria, long bones, lung, spleen, adrenals, epidermis, and central nervous system have also been shown to express the CYP27A gene transcript (14, 36, 55, 59, 60). Circulating macrophages and vascular endothelial cells are also known to harbor the CYP27A gene product and to hydroxylate cholesterol at C-25 as a means of excreting cholesterol (14). These observations indicate that, although the liver is the main 25OHD3 production site under normal physiological circumstances, many organs and cell types also harbor the enzyme and, hence, possess the capacity to...
metabolize D₃ to 25OHD₃, as has been clearly illustrated in previous studies on the rat duodenum and human fetal jejunum and colon (59, 60).

The present studies also reveal a significant gender difference in the expression of the gene, with 43% higher steady-state mRNA levels in female than in male rat livers. The latter observation is in agreement with the data reported by Andersson and Jornvall (2) and Saarem and Pedersen (52), where the activity of CYP27A was found to be higher in female than in male rats, as well as data of Addya et al. (1), who reported that the enzyme was highly influenced by sex hormones. This observation is also in line with the higher expression of the gene in normal human liver specimens obtained from women compared with those obtained from men (23). Interestingly, data on the intracinar distribution of the CYP27A gene transcript show that in D-depleted as well as in normal rats, CYP27A mRNA was widely distributed within the liver parenchyma with a rather diffuse distribution all along the hepatic acinus. 1,25(OH)₂D₃ repletion significantly decreased the CYP27A gene transcript as evidenced by both Northern blot analysis and in situ hybridization. The decrease in CYP27A mRNA was observed in both the periportal and the perivenous regions of the hepatic acinus and translated into a significant reduction in mitochondrial C-25 hydroxylation activity.

Hepatic CYP27A has previously been shown to be transcriptionally regulated by glucocorticoids, growth hormone, cholic acid, cyclosporin A, insulin, and the physiological state of the animal (42, 56, 58, 64–66). In the present studies, the gene, as expected (57), was found to be induced by dexamethasone, a known inducer of the CYP3A family of cytochrome P-450s (28). In addition, CYP27A was shown to be upregulated by β-naphtoflavone, a known inducer of CYP1A1 and -1A2 (17). On the other hand, other classical cytochrome P-450 inducers (phenobarbital, acetone, ethanol) did not influence the abundance of the CYP27A gene transcript. Our data, however, clearly show that 1,25(OH)₂D₃ led to a significant decrease in the transcription rate of the CYP27A gene without significantly affecting the stability of its message, as evidenced by a similar decrease in CYP27A abundance in D-depleted animals and in animals exposed to 1,25(OH)₂D₃. The almost complete inhibition of CYP27A transcription within 6 h after a single intravenous 1,25(OH)₂D₃ dose suggests that the action of the hormone involves receptor interactions. The normal rat liver has been shown to harbor a low abundance of the nuclear vitamin D₃ receptor (VDRn) (53), and the presence of a membrane receptor (VDRm) (7) has previously been suggested in rat hepatocytes (4, 6). However, the participation of either of
these receptors in the regulation of the gene encoding CYP27A still remains to be demonstrated. Indeed, to date, the mode of action, most particularly at the nuclear level, of the putative VDRn still remains to be demonstrated, whereas the CYP27A gene promotor has not been shown to harbor the vitamin D receptor element direct repeat-3 consensus sequence [(G/A)GGT(G/C)A] (21, 56). Interestingly, however, 1,25(OH)2D3 has lately been shown to influence the expression of the gene encoding CYP3A4 by a mechanism that did not involve a direct VDRn-RXR-VDRE interaction but involved rather a VDRn-retinoid-X-receptor-pregnane-X-receptor element interaction through an everted repeat-6 motif, indicating that 1,25(OH)2D3 signaling can also be mediated through complexed nuclear cross talk with several response motifs (61).

The long CYP27A mRNA half-life as well as the slight, albeit not significant, 1,25(OH)2D3-mediated increase in its mRNA half-life in the presence of a significant inhibition in the expression of the gene transcript may explain the rather slow and progressive decrease in CYP27A mRNA levels observed over the 7-day period studied. These later observations, combined with the known long half-life (several weeks) of serum 25OHD3, suggest that exogenously adminis-

Fig. 5. Representative photomicrographs of rat liver sections obtained after in situ hybridization with an rCYP27A antisense riboprobe. A 10-cycle RT-PCR amplification was used. Liver sections were obtained from D-depleted rats (A and B), normal control rats (D and E), and 7-day 1,25(OH)2D3-repleted rats (G and H). Negative in situ hybridization control sections with the rCYP27A sense riboprobe are presented in C, F, and I for livers obtained from D-depleted, normal, and 1,25(OH)2D3-repleted rats, respectively.

Fig. 6. Effect of 1,25(OH)2D3 on the hepatic mitochondrial C-25 hydroxylation activity. Hepatic mitochondrial proteins from hypocalcemic D-depleted and 1,25(OH)2D3-repleted rats (28 pmol/day for 7 days) were incubated with 20 nmol 1H251OHD3. The product of the reaction of 1H251,25(OH)2D3 was evaluated by HPLC. Data are presented as means ± SE. Statistically significant differences between group means were analyzed by Student’s t-test; n = 8–11 animals/group, P < 0.03.
indicate that the D₃ nutritional status has no signi-
ificant impact on the regulation of CYP27A, affecting the production of 25OHD₃, as illustrated in a
model scenario. Moreover, the sensitivity of the hepatic CYP27A to 1,25(OH)₂D₃ was also shown to be higher
(with a 60% decrease in CYP27A mRNA) than that found in the intestine (40% decrease). The reasons for
the observed differences between the two organs in the regulation of the gene encoding CYP27A by the nutri-
tional status may rest in the fact that the intestine, but not the liver, harbors the 1α-hydroxylase, allowing
intestinal cells to locally produce 1,25(OH)₂D₃ following D₃ or 25OHD₃ administration. On the other hand,
the influence of 1,25(OH)₂D₃ on the CYP27A mRNA half-life as well as on the CYP27A transcription rate
was also shown to be much more pronounced in liver than in intestine. It is postulated that the high sensitivity
of the liver to 1,25(OH)₂D₃ administration may be due to a combination of factors, such as the efficient hepatic
capture of exogenously administered 1,25(OH)₂D₃ (27) combined with the absence of the D₃-24 hydroxylase,
which is also postulated that D₃ or 25OHD₃ may compete
ting re -
flaction of the hormone and that the protective effect
concentration is not a good predictor of the hepatic
action of the hormone and that the protective effect
against the downregulatory effect on CYP27A may rest
on the circulating or cellular levels of D₃ or 25OHD₃. It
is also postulated that D₃ or 25OHD₃ may compete
with 1,25(OH)₂D₃ for uptake by the liver. In fact, the
season of the year (summer/fall) or the circulating
concentrations of 25OHD₃ and the hepatic mRNA lev-
els of CYP27A have already been reported in human
subjects, whereas no correlation was observed between
the circulating 1,25(OH)₂D₃ concentration and the
CYP27A gene transcript (23).

Interestingly, we recently reported a negative regu-
lation of the intestinal CYP27A following repletion
with D₃, 25OHD₃, and 1,25(OH)₂D₃ (59), indicating
differences between the two organs in the overall reg-
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system. Moreover, the sensitivity of the hepatic
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than in intestine. It is postulated that the high sensitivity
of the liver to 1,25(OH)₂D₃ administration may be due to
a combination of factors, such as the efficient hepatic
capture of exogenously administered 1,25(OH)₂D₃ (27)
combined with the absence of the D₃-24 hydroxylase,
servation indicates that the circulating 1,25(OH)₂D₃
concentration is not a good predictor of the hepatic
action of the hormone and that the protective effect
against the downregulatory effect on CYP27A may rest
on the circulating or cellular levels of D₃ or 25OHD₃. It
is also postulated that D₃ or 25OHD₃ may compete
with 1,25(OH)₂D₃ for uptake by the liver. In fact, the
season of the year (summer/fall) or the circulating
concentrations of 25OHD₃ and the hepatic mRNA lev-
els of CYP27A have already been reported in human
subjects, whereas no correlation was observed between
the circulating 1,25(OH)₂D₃ concentration and the
CYP27A gene transcript (23).

Interestingly, we recently reported a negative regu-
lation of the intestinal CYP27A following repletion
with D₃, 25OHD₃, and 1,25(OH)₂D₃ (59), indicating
differences between the two organs in the overall reg-
ulation of CYP27A by the D₃ nutritional/endocrine
system. Moreover, the sensitivity of the hepatic
CYP27A to 1,25(OH)₂D₃ was also shown to be higher
(with a 60% decrease in CYP27A mRNA) than that
found in the intestine (40% decrease). The reasons for
the observed differences between the two organs in the
regulation of the gene encoding CYP27A by the nutri-
tional status may rest in the fact that the intestine, but
not the liver, harbors the 1α-hydroxylase, allowing
intestinal cells to locally produce 1,25(OH)₂D₃ follow-
ing D₃ or 25OHD₃ administration. On the other hand,
the influence of 1,25(OH)₂D₃ on the CYP27A mRNA
half-life as well as on the CYP27A transcription rate
was also shown to be much more pronounced in liver
than in intestine. It is postulated that the high sensitivity
of the liver to 1,25(OH)₂D₃ administration may be due to
a combination of factors, such as the efficient hepatic
capture of exogenously administered 1,25(OH)₂D₃ (27)
combined with the absence of the D₃-24 hydroxylase,

Exogenously administered low-dose D₃ [which led,
however, to the normalization of serum Ca²⁺ and the
secondary hyperparathyroidism (data not shown)] or
25OHD₃ administration was found not to significantly
affect CYP27A mRNA. A higher dose of D₃ led only to
a transient decrease in CYP27A, which promptly re-
turned to D-depleted values following 1 wk of repletion
despite elevated serum Ca²⁺ concentrations. The data
indicate that the D₃ nutritional status has no signifi-
cant effect on the total hepatic CYP27A. Paradoxically,
evaluation of the D₃ endocrine status revealed that the
circulating 1,25(OH)₂D₃ concentrations and circulating
Ca²⁺ were significantly higher in D₃-repleted animals
compared with 1,25(OH)₂D₃-repleted animals. This ob-
fig. 7. in vivo influence of 1,25(OH)₂D₃ on the half-life of CYP27A
mRNA. Animals received doses (ip) of actinomycin D (0.5 mg/kg)
every 6 h, and they were either kept untreated or received 12
nmol/kg (iv) 1,25(OH)₂D₃ every 6 h. Animals were killed 6, 12, 18, or
24 h after actinomycin D administration. Data are presented as
means ± SE; n = 4–5 animals/group. Statistically significant differ-
ences between group means were analyzed by ANOVA, with individu-
able contrasts evaluated by the Bonferroni post hoc test. No signifi-
cant differences were observed among the different groups.

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which is, however, present in intestine (20, 49) and which could contribute to the intestinal catabolism of exogenously administered 1,25(OH)2D3, as well as in differences in cellular life span, with hepatocytes having a life span of several months whereas the turnover of intestinal cells is only a matter of days.

The present studies thus clearly show that, in rodent liver, the gene encoding CYP27A is expressed not only in hepatocytes but also in all sinusoidal cells. They also illustrate a significant gender difference in steady-state CYP27A mRNA levels and show that 1,25(OH)2D3 administration significantly influences the transcription of the CYP27A gene, which was accompanied by a significant decrease in the mitochondrial C-25 hydroxylation of the model D3 compound 1αOH-D3. Our data also illustrate that 1,25(OH)2D3 also affects the synthesis of bile acids via a downregulation of the secondary “acidic” pathway.

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