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EFFECTS ON CYP27 mRNA EXPRESSION IN RAT KIDNEY AND LIVER BY 1α,25-DIHYDROXYVITAMIN D_3, A SUPPRESSOR OF RENAL 25-HYDROXYVITAMIN D_3 1α-HYDROXYLASE ACTIVITY

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SUMMARY. The production of 1α,25-dihydroxyvitamin D_3 is known to be down regulated by 1α,25-dihydroxyvitamin D_3 itself. It was recently shown that liver mitochondrial sterol 27-hydroxylase CYP27, present also in kidney, catalyzes 1α-hydroxylation of 25-hydroxyvitamin D_3. Treatment of vitamin D-deficient rats with a single i.v. dose of 1α,25-dihydroxyvitamin D_3 resulted in a marked suppression of CYP27 mRNA in kidney. The effects were not as pronounced as for CYP24. Liver CYP27 was not affected to the same extent. The results of the present communication indicate a coordinate regulation of CYP27 mRNA levels and 25-hydroxyvitamin D_3 1α-hydroxylase activity by 1α,25-dihydroxyvitamin D_3 in rat kidney.

In the formation of the hormonally active form, 1α,25-dihydroxyvitamin D_3, vitamin D_3 is first 25-hydroxylated in the liver. 25-Hydroxyvitamin D_3 is then transported to the kidney where it is 1α-hydroxylated. This reaction is catalyzed mainly by mitochondrial cytochrome P450 in kidney. It is assumed that there is an enzyme specific for 1α-hydroxylation (1,2). No specific 1α-hydroxylase has, however, been purified and characterized. It was recently shown that liver mitochondrial sterol 27-hydroxylase CYP27, purified from pig and rabbit, as well as recombinant expressed human CYP27, catalyze 1α-hydroxylation of 25-hydroxyvitamin D_3 (3). The expression of CYP27 is not limited to the liver but mRNA for CYP27 has been found also in several other organs including kidney (4). It has been proposed that also renal 1α-hydroxylation is catalyzed by CYP27 (3). 1α,25-Dihydroxyvitamin D_3 itself is known as an important down-regulator of the 25-hydroxyvitamin D_3 1α-hydroxylase activity in the kidney (5-8). At the same time, 1α,25-dihydroxyvitamin D_3 induces the 25-hydroxyvitamin D_3 24-hydroxylase, CYP24, and the production of 24,25-dihydroxyvitamin D_3(5-9). Of the two dihydroxymetabolites, 1α,25-dihydroxyvitamin D_3 is the active form whereas there remains some controversy about whether 24,25-dihydroxyvitamin D_3 has a physiologic

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role in calcium and bone metabolism (2). Since it is generally considered that the main 1α-hydroxylation of 25-hydroxyvitamin D_3 occur in the mitochondrial fraction of the kidney and that the activity is down regulated by 1α,25-dihydroxyvitamin D_3 we wanted to investigate the effects of 1α,25-dihydroxyvitamin D_3 on the expression of CYP27 in kidney and liver. Rats have previously been used in studies on regulation of 1α- and 24-hydroxylase activities (9) and were therefore chosen as model. They were maintained on a vitamin D-deficient diet with low calcium before administration of 1α,25-dihydroxy-vitamin D_3. The effects of the treatment on CYP27 mRNA levels in kidney and liver were studied.

MATERIALS AND METHODS

Materials- Nylon Hybond-N membrane, Megaprime DNA labeling system and [α-^{32}P]dCTP, specific activity 110 Tbq/mmol, were obtained from Amersham International (Amersham, Bucks., U.K.). Oligo(dT)-Cellulose Type 7 was from Pharmacia. All other chemicals used were of reagent grade. Rat sterol 27-hydroxylase, CYP27, and cholesterol 7α-hydroxylase, CYP7, cDNAs were kindly provided by Dr. Emiko Usui, Department of Biochemistry, Hiroshima University and Professor David W. Russell, Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, respectively. Rat 25-hydroxyvitamin D_3 24-hydroxylase, CYP24, cDNA was a generous gift from Dr. Ylva Pernow and Professor Ingemar Björkhem, Huddinge University Hospital, Karolinska Institutet, Stockholm.

Methods- Male weanling rats (Sprague-Dawley strain) weighing 50 g were maintained for 4 weeks on a vitamin D-deficient diet (0.45% Ca, 0.30% P) and then for additional 2 weeks on a vitamin D-deficient, low calcium diet (0.02% Ca, 0.22% P) and deionized water. 1α,25-Dihydroxyvitamin D_3 dissolved in ethanol (3μg / 15 μl / 100 g body weight), was administrated i.v. to four of the rats 24 h before killing.
Total RNA was isolated from tissue by homogenization (Polytron Disp. 25 InterMed) in a mixture containing 0.12 M LiCl, 0.24 M urea, 0.03% Antifoam A and 0.1% SDS, 20 ml / cm^3 tissue. The mixture was incubated at 0°C for 16 h and rehomogenized. RNA was collected by centrifugation at 16000 x g for 20 min and resuspended in 10 mM triethanolamin, pH 7.5, containing 1 mM EDTA and 0.05% SDS, 3 ml / cm^3 tissue. RNA was extracted once with phenol and once with chloroform / isoamyl alcohol (24:1) and then precipitated with sodium acetate and ethanol. Poly(A)$^+$ RNA was isolated by Oligo(dT)-Cellulose chromatography (10). Northern blot analysis was performed as described previously (9) with the following modifications. Kidney mRNA, 10 μg, and liver mRNA, 5 μg, were used and RNA blots were prehybridized in a mixture containing 50 mM Tris-Cl, pH 7.5, 0.1% sodium pyrophosphate, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 5 mM EDTA, 0.2% bovine serum albumin, 50% formamide, 5 x SSC, 100 μg/ml of denatured salmon sperm DNA and 100 μg/ml of t-RNA. The cDNA probes used in this study were as follows: the full-length cDNAs of the rat CYP27 and CYP7, the 1.7 kb *Bam* HI-*Sal* I fragment of human β-actin and the 0.7 kb *Pst* I fragment of rat CYP24. The probes were labeled with [α-^{32}P]dCTP according to the manufacturers protocol for Megaprime DNA labeling system. Hybridization was performed in the same solution as used for prehybridization at 42°C for 12 h. Filters were washed with 0.1 x SSC, 0.1% SDS at 60°C. The filters were stripped at 65°C in 50% formamide, 2 x SSPE according to Sambrook *et al.*(11). As previously reported by Andersson *et al.* (4) there were two closely spaced hybridizing mRNAs for CYP27 present in kidney. Quantitative analysis of mRNA was carried out by densiometric analysis of the X-ray films at 555 nm using Shimadzu Data Record DR-2. The areas and the ratio between the areas of the peaks corresponding to CYP27 mRNA and β-actin mRNA in each sample were calculated from the scannings. The statistical significance was evaluated by using two-tailed Students t-test.

RESULTS

Effects of 1α,25-dihydroxyvitamin D_3 on CYP27 mRNA levels in kidney

Fig. 1A-D shows the results of Northern blot experiments and mRNA levels of CYP27 and β-actin in kidneys from vitamin D-deficient rats and vitamin D-deficient rats treated with 1α,25-dihydroxyvitamin D_3. The treatment suppressed the CYP27 mRNA levels. Estimation of the CYP27 mRNA levels by analyzing the area of the peaks from densiometric analysis of the X-ray films showed a decrease from 564 ± 30 to 141 ± 25 mm^2. This is a statistically significant decrease ($P < 0.01$). The actin mRNA levels showed some variation between individual rats but the levels for the 1α,25-dihydroxyvitamin D_3-treated group were not significantly decreased compared with those of the untreated group. The ratio between CYP27 and actin mRNA levels were also analyzed and found to be markedly reduced in three of the four 1α,25-dihydroxyvitamin D_3-treated rats. One rat who had a lower actin mRNA level did not show a reduced ratio. In spite of this, the suppression of the CYP27 / actin ratio for the whole treated group compared with the untreated group (2.5 ± 0.9 to 1.2 ± 0.9) was statistically significant ($P < 0.10$). The expression of kidney mitochondrial CYP24 mRNA was determined as control of the treatment. As shown in Fig.2, the levels of CYP24 mRNA increased after treatment with 1α,25-dihydroxyvitamin D_3. This is to be expected since Shinki *et al.* (9) demonstrated that the mRNA levels of 24-hydroxylase, as well as the 24,25-dihydroxyvitamin D_3 serum levels, increase after treatment with 1α,25-dihydroxyvitamin D_3. With the same treatment the serum 1α,25-dihydroxyvitamin D_3 levels were decreased (9). The results of the present communication show that also the CYP27 mRNA levels are suppressed upon 1α,25-dihydroxyvitamin D_3 treatment.

Effects of 1α,25-dihydroxyvitamin D_3 on CYP27 mRNA levels in liver

Fig. 1 E-H shows the results of Northern blot experiments and mRNA levels of CYP27 and β-actin in livers from vitamin D-deficient rats and vitamin D-deficient rats treated with 1α,25-dihydroxyvitamin D_3. The effect of the treatment on CYP27 mRNA levels was less pronounced in liver than in kidney. Estimation of CYP27 mRNA levels by analyzing the area of the peaks from densiometric analysis of X-ray films resulted in a decrease from 430 ± 148 to 207 ± 141 mm^2. This was statistically significant ($P < 0.10$). In contrast to kidney, the actin mRNA levels in liver increased significantly upon 1α,25-dihydroxyvitamin D_3 treatment ($P < 0.05$). This has previously been reported (2). This increase in actin mRNA levels in the livers from 1α,25-dihydroxyvitamin D_3-treated rats resulted in a markedly reduced ratio between CYP27 and actin mRNAs in the treated group (0.69 ± 0.16 to 0.14 ± 0.12). The suppressed ratio was statistically significant ($P < 0.01$). Since actin mRNA levels were affected by the 1α,25-dihydroxyvitamin D_3 treatment, it was considered of interest to use mRNA for another liver enzyme as control. CYP27 mRNA levels were therefore compared with the mRNA expression of cholesterol 7α-hydroxylase, CYP7 (12). Also CYP7 mRNA was found to increase upon 1α,25-dihydroxyvitamin D_3 treatment resulting in a decreased ratio (about 4 times) between CYP27 and CYP7 mRNA levels (data not shown).

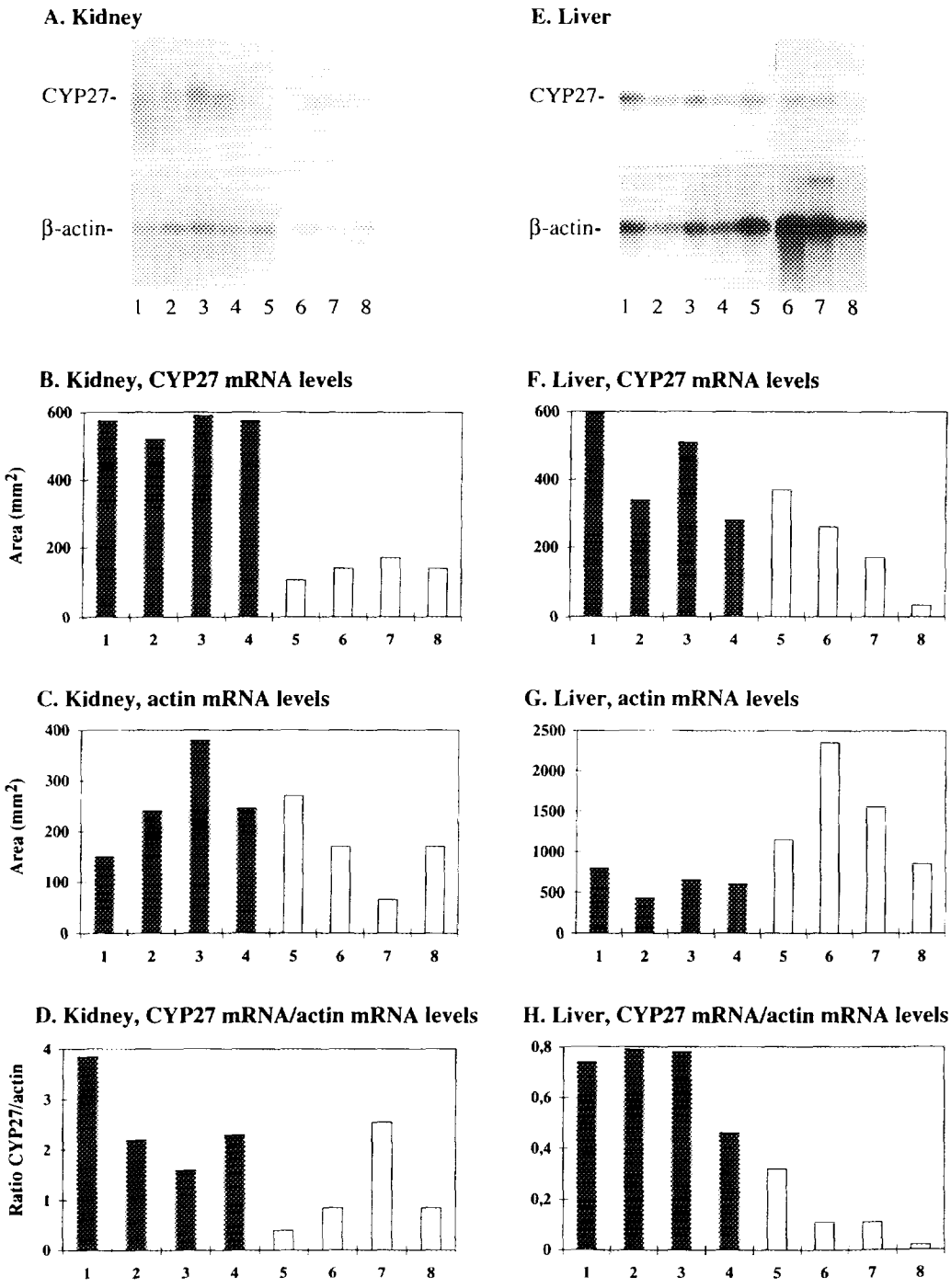

Fig.1. Effects of 1α,25-dihydroxyvitamin D_3 administration on the expression of CYP27 mRNA in kidney (A-D) and liver (E-H).
Rats 1-4 were maintained on a vitamin D-deficient, low calcium diet (filled columns). Rats 5-8 were on the same diet but were given a single i. v. dose of 1α,25-dihydroxyvitamin D_3 (3 μg / 100 g body weight) 24 h before the analysis (open columns). Aliquots of kidney mRNA, 10 μg, and liver mRNA, 5 μg, were subjected to blot analysis. Northern blots were probed with cDNA for CYP27 and β-actin. B,C and F,G show the signal intensity from A and E, respectively, densiometrically analyzed. In D and H the expression of CYP27 mRNA is correlated to the expression of β-actin mRNA.

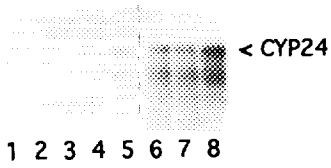

Fig.2. Effects of 1α,25-dihydroxyvitamin D_3 administration on the expression of CYP24 mRNA in kidney.
Northern blots in Fig.1 A were stripped and probed with cDNA for CYP24.

DISCUSSION

The results of the present communication indicate a coordinate regulation of CYP27 mRNA levels and 25-hydroxyvitamin D_3 1α-hydroxylase activity by 1α,25-dihydroxyvitamin D_3 in rat kidney. The results lend support for the proposal that at least part of renal 1α-hydroxylation of 25-hydroxyvitamin D_3 is catalyzed by CYP27 (3). The down-regulation of CYP27 was not as pronounced as the up-regulation of CYP24. If CYP27 were the only renal mitochondrial 1α-hydroxylase it might have been expected that the down-regulation by 1α,25-dihydroxyvitamin D_3 should have been more pronounced. Considering that CYP27 is present in several tissues and involved also in bile acid biosynthesis and 25-hydroxylation of vitamin D_3 it would have been very surprising if the expression should be drastically suppressed by 1α,25-dihydroxyvitamin D_3. The less pronounced down-regulation of CYP27 mRNA in liver indicates that 1α,25-dihydroxyvitamin D_3 affects the expression of CYP27 differently in different tissues. The results of the present communication open up for further studies on the effect of other parameters involved in regulation of vitamin D_3 and calcium homeostasis on CYP27, and the role of this enzyme in bioactivation of vitamin D_3.

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