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TRANSCRIPTIONAL CONTROL OF *CYP2E1* IN THE PERIVENOUS LIVER REGION AND DURING STARVATION

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Previous data indicate that the *CYP2E1* gene is transcriptionally activated after birth, but that the expression of ethanol-inducible *CYP2E1* protein, hereafter, is regulated by post-transcriptional mechanisms. The constitutive expression of CYP2E1 protein is restricted to the perivenous region of the liver lobule. Here we present results from *in situ* hybridization and run off experiments indicating that this regioselectivity is caused by a higher rate of gene transcription in the perivenous hepatocytes. We also show that transcription of the *CYP2E1* gene is activated by starvation, indicating that also this P450 gene is under transcriptional control under certain physiological conditions.

The *CYP2E1* gene is known to be transcriptionally activated after birth (1). By contrast, only posttranscriptional mechanisms have been described for the regulation of CYP2E1 # postneonatally. Thus, stabilization of CYP2E1 mRNA appears to be the major mechanism of enzyme protein induction during streptozotocin induced diabetes (2), and CYP2E1 inducing agents like ethanol, acetone, dimethylsulphoxide, isoniazid and imidazole, appear to cause induction of CYP2E1 by posttranslational mechanisms (3-8).

We have previously shown that starvation of rats has a pronounced synergistic effect on the acetone dependent induction of CYP2E1 as

The nomenclature for cytochrome P450 used is as proposed in Nebert, D.W., Gonzalez, F.J., Coon, M.J., Estabrook, R.W., Feyereisen, R., Guengerich, F.P., Gunsalus, I.C., Johnson, E.F., Loper, J.C., Nelson, D.R., Sato, R., Waterman, M. and Waxman, D.J. (1991) DNA Cell Biol, in press.

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well as of CYP2B1/2 (3). Furthermore, we observed that this synergistic increase caused by starvation could be accounted for by a similar increase in the amount of the corresponding mRNAs (3). Starvation alone has also been shown to increase the amount of CYP2E1 mRNA in liver (9).

Both CYP2B1 and CYP2E1 are expressed preferentially in the perivenous region of the liver acinus (10, 11). This is associated with a corresponding distribution of their mRNAs (10, 11). Here, we have investigated whether this regional selectivity in mRNA expression, or the increase seen during starvation, are caused by differences in the rate of gene transcription. The results demonstrate differences in transcriptional activity of the *CYP2E1* gene that might be responsible for the heteroacinar distribution of CYP2E1 protein in the liver as well as for the increase of the protein during certain physiological conditions.

METHODS

Run off experiments. Hepatocytes from the perivenous and periportal rat liver regions were isolated from male rats (180-250g) of the Alko mixed strain by the digitonin-collagenase perfusion technique (12) as previously described (10). Nuclei were prepared by sucrose centrifugation from the isolated cells or from livers of rats treated with acetone (5 ml/kg) once daily for two days, starved for 48 hours, or starved for 48 hours and treated with acetone (cf (5)). Rats treated with acetone were killed 4 hours after the last intragastric injection. The nuclei were incubated with [α-^{32}P]-UTP (Amersham) and labelled trancripts were isolated and hybridized with *CYP2E1* and *CYP2B2* cDNA (kindly provided by Drs F. J. Gonzalez, Bethesda, and B. Scholte, Amsterdam, respectively) essentially as described by Nevins (13). The corresponding amount of apoP450 in the same cell preparations was analyzed by Western-blotting (10).

In situ hybridization. The procedure for *in situ* hybridization is based on the protocol of Simmons et al. (14). Transcription of a riboprobe labelled with [^{35}S]-uridine 5-[α-thio]triphosphate (New England Nuclear, Boston, Ma, USA) was made by using the Riboprobe Gemini II Core System (Promega Biotec, Madison, WI, USA) and a linearized template prepared from a *CYP2E1* cDNA subcloned into the plasmid pGEM4 (Promega Biotec). The probe was diluted to 5 x 10^6 cpm/ml in 50 % formamide (Fluka, FRG), 10 % dextran sulphate (Pharmacia, Sweden), 1 x Denhardts solution (Sigma) 0.3 M NaCl, 1 mM EDTA, 0.5 mg/ml tRNA (Sigma, St Louis, USA) and 100 µM DTT (Sigma).

Male Sprague-Dawley rats (200 g) were anaesthetized with tribromoethanol (0.3 g/kg) and perfused through the heart for 10 minutes with 200 ml 4 % paraformaldehyde and 0.2 % glutaraldehyde in

0.1 M phosphate buffer. The liver was sectioned on a cryostate and the 30 µM thick sections were stored in 4 % paraformaldehyde in 0.1 M phosphate buffer overnight and then mounted on gelatine and poly-L-lysine coated glass slides. Before hybridization the sections were treated with 0.001 % proteinase K in 100 mM Tris, 50 mM EDTA, pH 8.0 for 30 minutes at 37°C, rinsed in the same buffer without proteinase K, and dehydrated in ascending grades of ethanol. About 75 µl of the probe solution was applied to each glass and the sections were covered with a cover glass, sealed at the edges with DPX (Gallard and Schleisenger, NY, USA) and incubated overnight at 65°C. The hybridization solution was removed from the slides and they were washed for 5 minutes each in 4 x SSC. To remove single stranded cRNA, the slides were incubated at 37°C for 30 minutes in 10 mg/ml of RNase A (Sigma) in 0.5 M NaCl, 0.25 M Tris (pH 8.0) and 1 µM EDTA (pH 8.0). Thereafter the sections were desalted in decreasing concentrations of SSC (2x to 0.5x) and washed at high stringency at 65°C for 30 minutes in 0.1 x SSC. Finally, they were dehydrated in ascending grades of alcohol and vacuum dried for 30 minutes. The sections were exposed to autoradiographic emulsion (NTB-2, Kodak) for seven days.

RESULTS AND DISCUSSION

Run-off experiments revealed that nuclei isolated from perivenous hepatocytes expressed a 1.8-fold higher *CYP2E1* gene transcriptional activity compared to cells prepared from the periportal region (Fig 1 and Table I). The difference in amount of CYP2E1 protein between the perivenous and periportal cell preparations used was 2.3 fold. The heterogeneous CYP2E1 distribution was also visualized by *in situ* hydridization (Fig 2). It is apparent that 2E1 mRNA is mainly expressed in the perivenous hepatocytes.

In a previous study, we observed an about 3-fold higher amount of both CYP2E1 protein and mRNA in isolated perivenous hepatocytes as compared to periportal cells (10). However, Western-blot analysis of

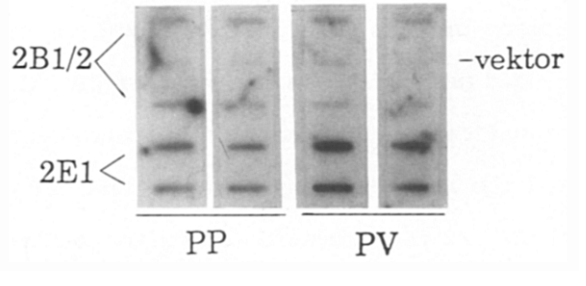

Fig 1. Run-off experiments performed on nuclei prepared from hepatocytes from the perivenous (PV) and periportal (PP) rat liver regions, isolated by the digitonin-collagenase perfusion technique.

Table I. Run off analysis of *CYP2E1* transcription and amount of microsomal apo CYP2E1 in cells isolated from the periportal and perivenous regions of rat liver

Hepatocytes	*CYP2E1* Transcription %	CYP2E1 Apoprotein %
Periportal	100	100
Perivenous	182±18	242±73

The results are expressed in relation to the transcriptional activity or the amount of CYP2E1 observed in the periportal hepatocyte preparations. The data represent mean values of 3 experiments, each with 2 + 2 cell preparations.

cell lysates originating from the very proximal perivenous region or from the distal periportal region, revealed a more than 30-fold difference in the amount of CYP2E1 protein (10). This suggests an incomplete separation of the two types of cells for, in this case, preparation of nuclei aimed for the run off experiments, and indicates that the difference of the *CYP2E1* transcriptional activity between the perivenous and the periportal region of the intact liver lobule is considerably higher than presently found in the incompletely separated cell preparations. This indicates that *cis* or *trans*-acting elements, governing the rate of transcription of the *CYP2E1* gene, are regiospecifically expressed and cause a heterogeneous acinar distribution of the CYP2E1 isozyme.

Experiments with nuclei isolated from variously pretreated rats revealed that starvation caused a 3.6-fold increase in [^{32}P]-labelled CYP2E1 transcript compared to nuclei from control rats (Fig 3 and Table II). Treatment of rats with acetone alone, or in combination with starvation, did not influence the rate of *CYP2E1* gene transcription significantly. In fact, when starved rats were used, acetone depressed the rate of transcription. Run-off experiments carried out with *CYP2B2* cDNA revealed opposite results as compared to *CYP2E1*. Acetone treatment of rats caused a 4-fold activation of the *CYP2B1/2* genes and starvation in combination

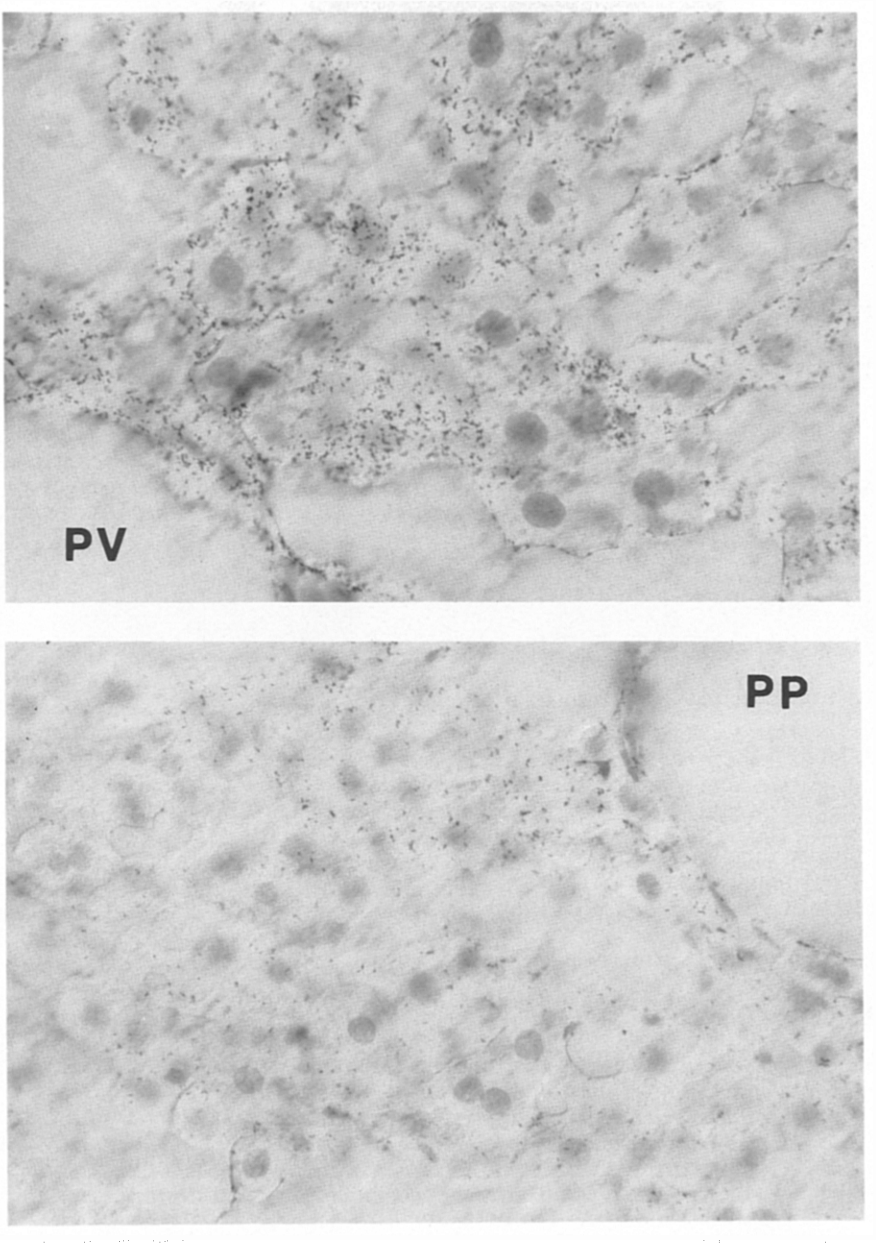

Fig 2. Evaluation of the presence of CYP2E1 mRNA by *in situ* hybridization, using a full length [^{35}S] *CYP2E1* cDNA clone, in a liver from a control rat. The light micrographs are taken from the same liver lobule close to a central vein (PV, upper) and a portal vein (PP, lower). Magnification: 500x.

with acetone caused an 7-fold increase in activity. By contrast, starvation alone decreased the amount of [^{32}P]-labelled 2B1/2 nuclear RNA.

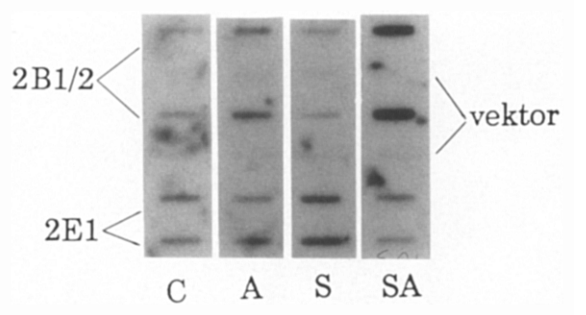

Fig 3. Run-off experiments performed on nuclei isolated from livers of control rats (C), rats treated with acetone (A) once daily for two days, starved for 48 hours (S) or starved for 48 hours and treated with acetone (SA).

The data presented indicate that indeed the *CYP2E1* gene is transcriptionally activated by factors present during starvation and that the regulation of the CYP2E1 enzyme after birth is not only associated with posttranscriptional mechanisms. Interestingly, the *CYP2E1* and *CYP2B1/2* genes were influenced in completely opposite ways in response to starvation, acetone or combinations of these treatments. Thus starvation did not affect the *CYP2B1/2* gene transcriptional rate, whereas acetone effectively did. The relationship between the amount of CYP2B1/2 protein and CYP2B1/2 mRNA previously seen in these type of animals (3), does essentially correspond to the differences in *CYP2B1/2* gene transcriptional rates here seen. Likewise, the increase of CYP2E1 mRNA seen after

Table II. Transcriptional activation of rat liver *CYP2E1* and *CYP2B1/2* by acetone treatment, starvation or a combination of starvation and acetone treatment as determined by run off experiments

Treatment	(n)	*CYP2E1*	*CYP2B1/2*
Control	(5)	100	100
Acetone	(5)	128±74	366±151
Starvation	(6)	364±166	87±21
Starvation+Acetone	(4)	93±32	693±235

The data are related to the amount of radioactivity present in the total transcripts and normalized to the radioactivity found using control nuclei in each experiment. Control experiments showed no significant difference when the values were related to the number of nuclei used in the experiments.

starvation (9), essentially corresponds to the relative difference of *CYP2E1* gene transcriptional rate examined here.

In conclusion, CYP2E1 is mainly regulated at a posttranscriptional level. However, the rate of transcription of the *CYP2E1* gene appears to be activated by factors present under specific physiological conditions such as starvation and by components selectively expressed in perivenous hepatocytes. In the latter case, *CYP2E1* transcription might instead be depressed by factors present in periportal cells. The similar relative acinar distribution of several different forms of hepatic P450s previously reported in a preliminary form (15) and the regional differences in the rate of gene transcription here described, makes it tempting to suggest the existence of specific physiologically controlled perivenous transcriptional factors that bind to a common motif among several different P450 promotors.

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