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Growth Hormone-Regulated Periportal Expression of *CYP2C7* In Rat Liver

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ABSTRACT. Most drug- and steroid-metabolizing cytochrome P450 (CYP) enzymes are expressed in the mammalian liver in a characteristic zonated pattern, with high expression in the downstream perivenous (centrilobular) region. Here, we report that *CYP2C7*, a member of the rat *CYP2* family, is expressed preferentially in the opposite, periportal region. CYP2C7 mRNA, as detected by reverse transcription–polymerase chain reaction, was detected almost exclusively in cell lysates obtained from the periportal region, indicating a very steep acinar gradient. The amount of immunoreactive CYP2C7 protein in periportal cell lysates was also higher than in samples from the perivenous region. This gradient was reversed by hypophysectomy, which markedly and selectively reduced the periportal CYP2C7 protein content. Subsequent growth hormone infusion by osmotic minipumps restored the zonation by selectively increasing the amount of periportal CYP2C7 protein. Although hypophysectomy suppressed CYP2C7 mRNA and growth hormone counteracted it, regulation at this level did not appear to occur in a zone-specific fashion. This indicates that growth hormone-mediated zonal regulation of CYP2C7 protein has additional translational or posttranslational components. Ethanol treatment, which has been shown to affect growth hormone levels, significantly induced CYP2C7 mRNA, but not zone specifically. Our results demonstrate that growth hormone up-regulates the *CYP2C7* gene by enhancing the expression of the protein specifically in the periportal liver region. Growth hormone may up-regulate other periportally expressed liver genes in a similar fashion. BIOCHEM PHARMACOL **59**;5:583–589, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. liver gene zonation; cytochrome P450 expression; growth hormone regulation; hypophysectomy; CYP2C7; ethanol treatment

In the mature mammalian liver, most genes appear to be expressed in an ascending or descending gradient from the portal to the central vein within the acinus, the microcirculatory unit of the liver [1]. As recently described [2], this zonation is particularly prominent for the *CYP*‖ genes (the nomenclature of Nelson *et al.* [3] is used) encoding the drug- and steroid-metabolizing CYP isoforms. The CYP enzymes are typically expressed and induced mainly in hepatocytes located in the downstream perivenous region. Although the factors regulating the zonated expression of *CYP* and other genes are still poorly understood, pituitary-regulated hormones seem to be involved in the regiospecific expression of certain CYP forms. We have previously observed that in hypophysectomized rats there is high expression of *CYP2B* and *3A* in the normally silent periportal region, overturning the constitutive zonation of these forms [4, 5]. The restoration of the constitutive pattern by subsequent GH infusion strongly suggested a GH-mediated down-regulation of both *CYP2B* and *CYP3A* in the upstream periportal region. Such effects were seen both at protein and mRNA levels, demonstrating pretranslational regulation. A zone-specific suppression of the periportal expression of *CYP3A* genes by thyroid hormone (triiodothyronine) has also been described [5]. There are, on the other hand, several examples of other *CYP* genes (*CYP2C11* and *CYP2C12*, *CYP2E1* and *CYP1A2*) that are either up- or down-regulated by GH and other pituitary-dependent hormones in a manner that does not appear to affect zonation [6].

The *P450 2C7* gene belongs to the rat *CYP2C* subfamily. This subfamily is primarily involved in stereospecific steroid metabolism and typically exhibits sexually differentiated expression [7]. In addition, the 2C7 form catalyzes the oxidation of retinol [8] and of retinoic acid [9] to polar metabolites, indicating its involvement in hepatic regulation of vitamin A metabolism. The *CYP2C7* gene is transcriptionally activated just before puberty, and the maximal expression reached in adults is about twofold

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‖ *Abbreviations:* ALAT, alanine aminotransferase; CYP, cytochrome P450; GH, growth hormone; RT-PCR, reverse transcriptase PCR.

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higher in females than in males [10]. The sexually differentiated expression of *CYP2C* forms has been shown to be regulated mainly by the different patterns of GH secretion in male and female rats [7]. The male-type pulsatile secretion allows expression of the main male-specific form CYP2C11, whereas in females the more continuous secretion pattern stimulates the expression of the female-specific CYP2C12 form. Other CYP forms are only partly regulated by the GH secretion pattern. Experiments with hypophysectomized rats have demonstrated that the continuous GH secretion in females allows a higher expression of *CYP2C7* than does pulsatile secretion in males [11–13]. Thus, while hypophysectomy suppresses CYP2C7 mRNA in both male and female rats, and subsequent continuous GH infusion increases it to female levels in both genders, twice daily GH injections to male rats restore CYP2C7 mRNA to the normal male level [13]. The transcription rate is constitutively two times higher in females than in males and is affected both by hypophysectomy and GH infusion [12]. The expression of *CYP2C7* is also stimulated by retinoids, retinol, and retinoic acid, both *in vitro* [14] and in vitamin A-deficient rats [15]. A role for thyroid hormone in the constitutive hepatic expression of CYP2C7 is suggested from experiments with hypophysectomized rats, where thyroid hormone increased the amount of hepatic CYP2C7 mRNA [11]. Moreover, ethanol treatment has been reported to slightly induce the hepatic expression of CYP2C7 (1.3- to 1.7-fold) [16, 17]. Ethanol administration is known to affect circulating GH levels [18, 19] and to alter vitamin A metabolism, resulting in decreased retinoic acid content [20].

The present study was undertaken to investigate the hepatic expression pattern of the *CYP2C7* gene and its regulation. We hypothesized that since we previously had observed that growth hormone zone specifically down-regulated the hepatic expression of the CYP2B and 3A forms, the known up-regulation of total *CYP2C7* expression could also be zonated. Consequently, the hepatic distribution of *CYP2C7* expression was studied both in normal and in hypophysectomized male and female rats, and the effect of subsequent growth hormone administration was evaluated. Moreover, we studied whether the acinar localization of CYP2C7 mRNA is affected by the induction following chronic ethanol treatment.

MATERIALS AND METHODS

Animals

Hypophysectomized and sham-operated male and female Sprague–Dawley rats were obtained from Møllegaard. The animals were hypophysectomized at the age of five or seven weeks, transferred to our laboratory one week after the hypophysectomy, and were then allowed to stabilize for another week. These animals were fed a commercial R3 laboratory diet (Ewos) and water *ad lib*. The hypophysectomized rats were given human recombinant growth hormone (Norditropin®, Nordisk Gentofte A/S; 0.01 IU/hr) by continuous infusion for seven days by osmotic minipumps (Alzet 1701) implanted subcutaneously on the backs under halothane anesthesia. Control rats received saline via the minipump or were implanted with a piece of Teflon tubing. The rats received buprenorphin (Temgesic® 0.3 mg/mL), 0.15 mg/kg s.c., immediately after implantation surgery. The effect of GH treatment was monitored as weight increase. The ethanol induction of CYP2C7 was studied in male Wistar rats, initially weighing 220 to 240 g and housed individually in stainless steel wire cages. For chronic ethanol administration, rats were pair-fed a commercial nutritionally adequate liquid diet (Purina Mills) for 2 weeks, containing 18% (joules) protein, 35% fat, and 47% carbohydrate (controls), or 11% carbohydrate and 36% ethanol. Blood ethanol from tail blood samples (100 μL) was assayed by head-space gas chromatography as described previously [21]. The mean daily ethanol consumption during the two-week chronic ethanol treatment of male rats was 10.8 g/kg body weight. The mean tail blood ethanol level, assayed at 9–13 a.m. on day 9, was 44.7 (27.3–59.6) μmol/mL. All animal experiments were approved by the Institutional Animal Care and Use Committee at Alko Group Ltd.

Collection of Periportal and Perivenous Cell Lysates

Regiospecific cell lysis was achieved during *in situ* perfusion by a modified dual-digitonin-pulse technique [22]. Briefly, periportal cells were lysed by infusing 6.7 mL/kg body weight of 3.5 mM digitonin (ICN) via the portal vein, and the lysate was collected by immediate retrograde flushing. Perivenous cell lysates were obtained by infusing 10 mL/kg body weight digitonin solution via the upper vena cava followed by antegrade flushing. The length of the digitonin-pulse which determines the penetration depth had been empirically determined to allow digitonin to lyse approximately one-fourth to one-third of the cells along the plate in either the proximal or distal part of the sinusoid. The regiospecificity of the cell lysates was verified by ALAT, (EC 2.6.1.2.) assay as in [23].

Immunoblot Analysis of CYP2C7 Protein

Liver samples were prepared using a Potter–Elvehjem homogenizer in 10 mM phosphate buffer, pH 7.2, 0.25 M sucrose, followed by removal of debris by centrifugation at 10,000 *g* for 20 min. Protein determination was done by fluorometry [24]. Digitonin cell lysate proteins (100 μg/well) or liver homogenate proteins (2–3 μg/well) were separated in 8.7% SDS–PAGE and electroblotted to nitrocellulose filters. Rabbit anti-rat CYP2C7 antiserum, (batch 127, [17, 25, 26]), donated by Dr. Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden), was used to probe the filters at a dilution of 1:500. The blot was subsequently treated with a 1:10,000 dilution of goat anti-rabbit antibody linked to horseradish peroxidase and developed using an enhanced chemiluminescence (ECL)

kit. This antibody recognizes purified CYP2C7 and what appears to be a second related CYP2C7 family member (P450EtOH2) of slightly lower mobility, possessing a terminal 12-amino acid sequence differing from 2C7 by 2 amino acids and appearing, like 2C7, to be somewhat inducible by ethanol (2-to-3-fold) [17, 25, 26]. The P450EtOH2 protein is not yet sequenced, but may be an allelic variant of CYP2C7. The antibody does not appear to recognize pure preparations of the closely related CYP2C6, the male-specific CYP2C11, or other members of P450 families 1, 2, 3, or 4 [27, 17]. Autoradiographs were exposed for a range of times to ensure signals within the linear range of the film. Immunoquantitation was accomplished using a GS-700 Imaging Densitometer (Bio-Rad) and Molecular Analyzer 1.4 software. One periportal control sample was included in all the gels run as an internal standard, and data were normalized to this sample. Data were calculated relative to the average control perivenous signal = 100.

Isolation of Total RNA, Synthesis of First Strand cDNA, and RT–PCR

Total RNA from 0.5 mL of digitonin eluates (which are virtually devoid of nuclear material) and from liver samples was isolated using Qiagen's RNeasy kit. The amount of RNA was determined spectrophotometrically at 260 nm, while purity was ascertained by the nucleic acid/protein ratio (A_{260}/A_{280}) and integrity by electrophoresis in formaldehyde-denatured 1.25% agarose gels. First strand cDNA was produced with Promega's Reverse Transcription System either with random hexanucleotide or oligo (dT) primers. Determination of CYP2C7 mRNA was performed using a PCR-based semiquantitative method as described previously [28]. The following primers were used to produce a 242-bp fragment: 5′-GATCTCATTGGTGCAGGG-3′ (sense) and 5′-TCACAGGTCACTGCATGG-3′ (antisense). These primer sequences were carefully chosen to avoid cross-reactivity with any known related CYP form. One to five microliters of cDNA was amplified in a 100-μL reaction volume containing 2 U Taq DNA polymerase, 1 × PCR buffer (both from Promega), 10 pmol of both primers, 0.2 μM of each dNTP, and 2 mM $MgCl_2$. Amplification in the programmable thermal controller (MJ Research, Inc.) consisted of 23 cycles at 94° for 30 sec, 54° for 1 min, and 72° for 1 min, with the last elongation step being extended to 5 min. The relative amounts of the amplification product were determined by anion-exchange HPLC [29] as modified in [5]. The amplification products were also visualized in 20% polyacrylamide PhastGels® (Pharmacia LKB Biotechnology) run in a native buffer system and followed by staining with the PhastGel® Silver kit according to the manufacturer's instructions. The linearity of amplification was validated by varying the amount of RNA and cDNA, as well as the number of cycles. Samples to be compared were always run together. To decrease intra-series variation in the sample sets of the chronic ethanol study, the amount of CYP2C7 transcripts in each sample was normalized to the relative amplification of the same cDNA with β-actin primers, as recently described in [30]. Such normalization could not be performed on samples from the hormonally manipulated rats, because hypophysectomy significantly altered the expression of β-actin mRNA.

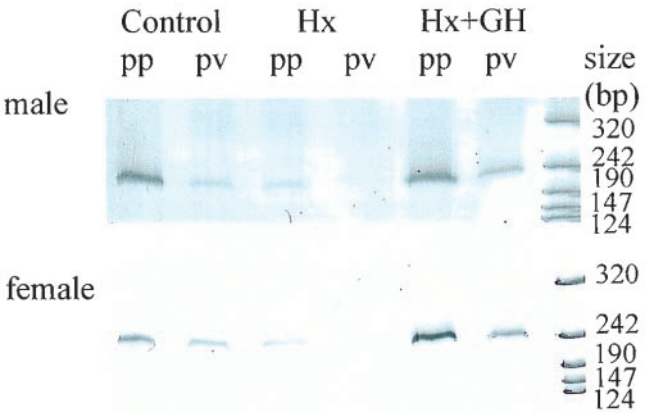

FIG. 1. CYP2C7 mRNA in periportal (pp) and perivenous (pv) liver cell lysates. Lysate mRNA from hypophysectomized (Hx) and growth hormone (GH)-treated male rats was reverse-transcribed. An aliquot of 3 μL of cDNA was amplified with 23 cycles. PCR fragments were run on 20% polyacrylamide gels and stained with silver. A DNA molecular weight marker was co-electrophoresed.

Statistical Analysis

Analysis of variance followed the Student's *t*-test or a non-parametric Mann–Whitney test were used to test for significant differences between treatment groups. The Student's *t*-test for independent samples or a non-parametric Mann–Whitney test was conducted to test for significant differences between periportal and perivenous samples.

RESULTS

During the experiments, the body weight of hypophysectomized animals did not change (average of males 157 g → 152 g; females 181 g → 177 g) in contrast to controls (males 202 → 257 g; females 196 → 204 g) or rats receiving growth hormone (males 157 → 175 g; females 178 → 202 g). To ascertain the acinar origin of the cell lysates, the activity of the periportal marker enzyme ALAT was determined. In the hormonally manipulated males, the activity of ALAT was 8.1 times higher in periportal than in perivenous samples (SEM = 1.0, N = 29), and 21.6 times higher in females (SEM = 3.3, N = 30). In chronically ethanol-treated males, the ALAT activity in periportal samples was eight times greater than in perivenous samples (SEM = 0.7, N = 20). These data are in full agreement with our previous studies [4, 23] and demonstrate the different acinar origin of the cell lysates.

Analysis of periportal and perivenous cell lysates from untreated animals revealed marked zonation of CYP2C7 in both genders. In males, CYP2C7 mRNA (Figs. 1 and 2) and

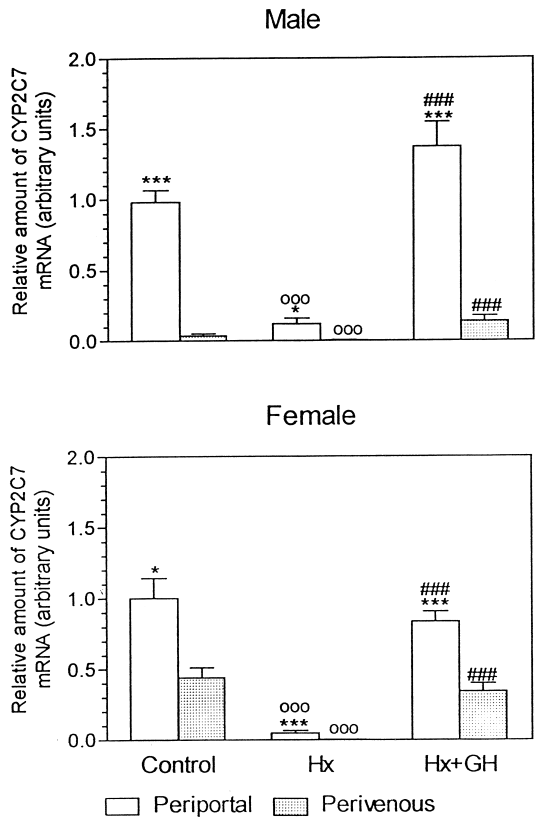

FIG. 2. CYP 2C7 mRNA in periportal and perivenous cell lysates from livers of male and female rats after hypophysectomy (Hx) and subsequent growth hormone (GH) treatment. HPLC quantitation of RT–PCR is presented. The bars are means + SEM from periportal and perivenous eluates from sham-operated, hypophysectomized, or hypophysectomized and GH-infused rats. The value of the mean of control periportal samples was arbitrarily set as 1.0 for both males and females. Significant differences ($P < 0.05$ and 0.001, ANOVA and Student's *t*-test or non-parametric Mann–Whitney test) between periportal and perivenous cell lysates within treatment groups are indicated by * and *, respectively; between controls and hypophysectomized (either periportal or perivenous) by ^{ooo}, $P < 0.001$; and between hypophysectomized and GH-treated by $^{\#\#\#}$, $P < 0.001$.**

apoprotein (Figs. 3 and 4) were detected preferentially in periportal samples, indicating a steep periportal > perivenous CYP2C7 gradient across the liver acinus. A periportally dominated distribution of CYP2C7 mRNA was also observed in females. The female periportal cell lysates contained approximately two times more CYP2C7 mRNA than the perivenous lysates ($P < 0.05$) (Fig. 2). Thus, the decreasing portal > venous gradient for CYP2C7 was steeper in males than in females. In sharp contrast to the distribution of CYP2C7, the slightly slower migrating apoprotein band recognized by the antibody (P450EtOH2) was found to be distributed almost entirely in the pericentral portion of the liver acinus, in congruence with the expression pattern found for most CYP forms (Figs. 3 and 5). The absence of clear staining for P450EtOH2 in the second perivenous sample in Fig. 3 is an exception. It is possible that this represents a polymorphism of expression of P450EtOH2 in the rat similar to that reported for another member of the CYP2C family, CYP2C13 [31].

Analysis of liver samples following hypophysectomy revealed a marked decrease ($P < 0.05$) in CYP2C7 mRNA and apoprotein in the periportal region of male livers. However, whereas perivenous CYP2C7 mRNA virtually disappeared, the level of CYP2C7 apoprotein remained essentially unchanged and the distribution of the apoprotein across the liver lobule equalized. In livers from hypophysectomized females, CYP2C7 mRNA behaved essentially the same as in the male, with significantly decreased steady-state levels in both sectors of the liver lobule ($P < 0.05$). Hypophysectomy altered the distribution of P450EtOH2 in male livers in an opposite manner from that seen for CYP2C7. Significant increases ($P < 0.05$) in expression of this second apoprotein band were observed in both periportal and perivenous regions (Fig. 5). GH replacement by continuous infusion resulted in normalization of the distributions of CYP2C7 mRNA (Fig. 2) and apoprotein (Fig. 4). GH infusion also significantly decreased and thus normalized the periportal expression of P450EtOH2 apoprotein (Fig. 5), but did not significantly affect perivenous P450EtOH2 expression. Some hypophysectomized animals were treated with thyroid hormone (triiodothyronine; daily s.c. injections 50 μg/kg for one week). CYP2C7 zonation was studied because thyroid hormone has been reported to influence whole liver *CYP2C7* mRNA expression [11] and we previously observed that this hormone zone specifically suppressed *CYP3A* expression [5]. However, the triiodothyronine treatment appeared to have no significant effect on either the total hepatic level of CYP2C7 protein and mRNA or their distribution in periportal and perivenous cell lysates (results not shown).

The effect of chronic ethanol administration on the CYP2C7 expression pattern was compared in male rats using a nutritionally controlled model where the pair-fed controls received an equicaloric amount of dextromaltose instead of ethanol. The periportal pattern of zonation was similar to the previous experiment: A more than 30-fold periportal/perivenous CYP2C7 mRNA ratio was observed (Table 1). Ethanol treatment was found to significantly ($P < 0.001$) induce total liver CYP2C7 mRNA. Evaluation of the acinar distribution revealed an increase in CYP2C7 mRNA only in the periportal samples. However, this zone-specificity may be only apparent, due to the very low level of the perivenous CYP2C7 mRNA content.

DISCUSSION

In this study, we have demonstrated that CYP2C7 transcripts are found mainly in the periportal region in rat liver, that the CYP2C7 protein exhibits a similar zonation, and that this expression is governed by growth hormone via mechanisms that seem to involve both pretranslational and translational control. The distribution pattern of CYP2C7 contrasts to most other members of the cytochrome P450

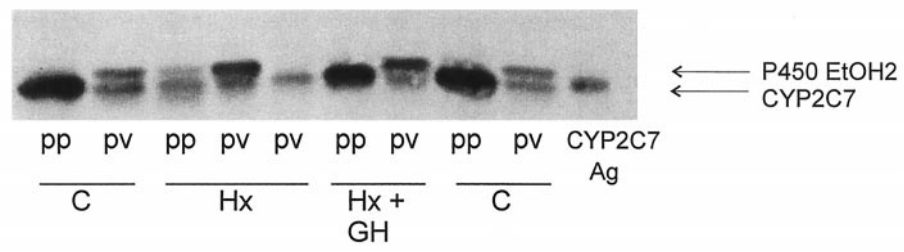

FIG. 3. Western blot analysis of apoproteins immunoreactive to CYP2C7 antibody. One hundred micrograms of cell lysate protein from male livers was separated in 8.7% SDS polyacrylamide gels and electroblotted to nitrocellulose filters which were probed with antiserum to rat CYP2C7. The antibody reacts to the pure CYP2C7 antigen (Ag, lane at right) and to a closely related protein (P450EtOH2) that migrates slightly more slowly. The effect of hypophysectomy (Hx) and treatment with growth hormone (GH) as compared to controls (C) as well as the distribution between periportal (pp) and perivenous (pv) lysates differs completely for these two proteins.

superfamily of genes, which are constitutively expressed and also induced in the downstream perivenous liver region. The factors governing the zonated expression of CYP enzymes have only recently started to be unraveled [2]. Elucidation of these factors is important for understanding the molecular background of drug-induced regional liver damage [32].

As recently reviewed [2], there are a few reported deviations from the predominantly perivenous hepatic expression pattern of CYP enzymes. Interestingly, they all belong to the *CYP2C* subfamily. Thus, a uniform acinar distribution has been demonstrated by *in situ* hybridization for CYP2C6 mRNA [33] and by solution hybridization from digitonin cell lysates for CYP2C12 mRNA [6]. Our observation of the periportal expression of CYP2C7 extends this list, and a common hormonal regulator of the "non-perivenous" expression of these *CYP2C* genes could be envisioned. However, the existence of such a mediator is probably only apparent since: a) in this study the distribution of the closely related P450EtOH2 form and the effect of GH were completely different from CYP2C7; b) *CYP2C6* expression is pituitary-independent [12]; and c) GH allows *CYP2C12* expression in both periportal and perivenous liver regions [6]. We have previously observed that GH is important in the zonated expression of *CYP2B* and *3A* [4, 5]. However, in contrast to CYP2C7, these CYP forms are down-regulated by GH, with this preventing their expression in the upstream periportal hepatocytes. Given that GH was reported to increase total CYP2C7 protein expression [34], a GH-mediated zone-specific up-regulation of CYP2C7 was not completely unexpected. However, GH also appears to act by mechanisms not involving zonation. Several other CYP forms, i.e. CYP2C11, 2C12, and 2E1, are also under pituitary control, albeit not zone specifically [6], and there does not appear to exist a common mechanism explaining the characteristic perivenous expression patterns exhibited by many *CYP* genes.

The acinar gradient of CYP2C7 expression was less steep in females than in males. This difference was clearest in

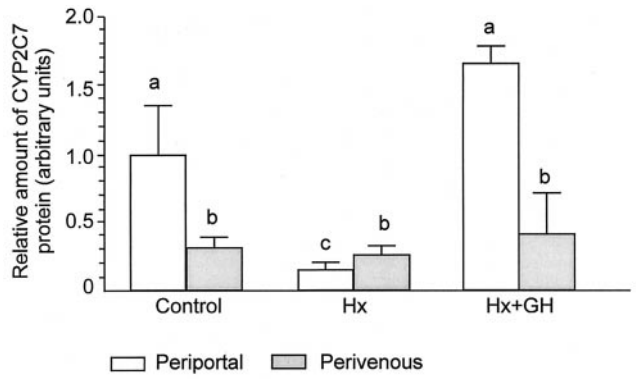

FIG. 4. Apoprotein bands cross-reactive with rabbit polyclonal antibody "127" in Western immunoblot analysis (lower band co-migrating with pure CYP2C7). Cell lysate protein (100 μg) was separated in 8.7% SDS–PAGE and transferred to nitrocellulose. Blots were developed using antibody 127 [17,25,26] and enhanced chemiluminescence as described in Methods. Data based on male animals are presented as means + SEM for 3–5 samples for each liver region and treatment. Hx = hypophysectomized. The lettered superscripts indicate that the data are significantly different ($P < 0.05$, one-way ANOVA) compared to any other bar with a different letter.

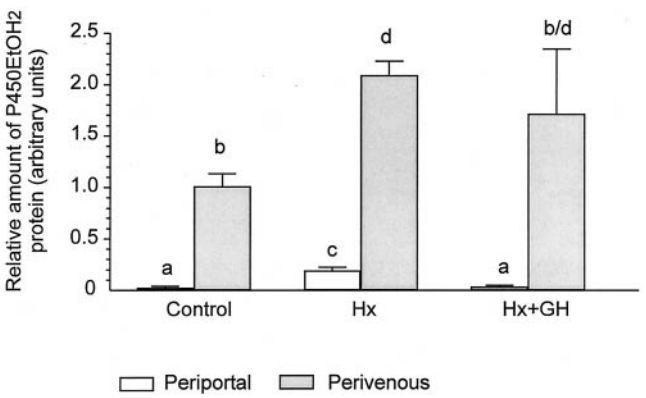

FIG. 5. Western immunoblot analysis of P450EtOH2 apoprotein expression (upper band). One hundred micrograms of cell lysate protein was separated, transferred to nitrocellulose, and blots developed as described in the legend to Fig. 4. Data are from male rats and are presented as means + SEM for 3–5 samples per liver region and treatment. Hx = hypophysectomized. The lettered superscripts indicate that the data are significantly different ($P < 0.05$, one-way ANOVA) compared to any other bar with a different letter.

TABLE 1. Effect of chronic ethanol administration on CYP2C7 mRNA in liver and in periportal and perivenous cell lysates

	Acinar origin	Treatment	
		Untreated	Ethanol
CYP2C7 mRNA	whole liver	100 ± 6 (9)	132 ± 13* (9)
	periportal	100 ± 27 (5)	167 ± 31 (9)
	perivenous	3 ± 3 (5)†	3 ± 2 (9)‡

Mean values ± SEM of 5–9 samples (numbers in parentheses) are given. The CYP2C7 mRNA amounts, as determined by RT–PCR, were normalized by the amount of β-actin in corresponding samples. The means of liver samples from control rats and of periportal control samples were arbitrarily set as 100.

* Denotes significance ($P < 0.001$) compared to corresponding controls and †, ‡ ($P < 0.01$) and ($P < 0.001$, respectively) between periportal and perivenous samples within treatment group.

controls at the mRNA level. In females, the amount of CYP2C7 mRNA in perivenous cells was almost half of that in periportal samples, while in males very low amounts of CYP2C7 mRNA were found in perivenous samples. This gender difference may be regulated by gonadal hormones, possibly via GH signaling. The female-type GH pulse pattern may permit a more efficient CYP2C7 expression that would extend into the perivenous region. Indeed, several studies have observed 2-to-4-fold higher expression of CYP2C7 in females than in males [10, 12, 13, 34] and this is thought to result from a gender difference in the transcription rate of the *CYP2C7* gene [12]. The stronger up-regulation of CYP2C7 by female-type GH secretion as compared to the episodic male pattern is also suggested from the present data: GH infusion to hypophysectomized males was more efficient in re-establishing CYP2C7 than in hypophysectomized females. In controls, a periportal dominance of CYP2C7 was observed both at the protein and mRNA levels. This pattern was opposite that of the perivenous dominance seen earlier both at protein and mRNA levels for the CYP2B and 3A forms [4, 5]. For these CYP forms, hypophysectomy allowed periportal expression whereas subsequent GH treatment prevented it, both at protein and mRNA levels, demonstrating pretranslational and suggesting transcriptional control. In contrast, hypophysectomy significantly reduced CYP2C7 protein only in the periportal region, but mRNA expression was diminished in both acinar regions. GH administration also predominantly increased periportal CYP2C7 protein, but mRNA increased in both regions. GH activates transcription of the *CYP2C7* gene both *in vivo* [12] and *in vitro* [14]. The protein–mRNA discrepancy observed in the present study indicates that in addition to the transcriptional control, GH either increases translation or stability of CYP2C7 protein specifically in the periportal region. A sufficiently steep acinar uptake gradient of GH offers the most evident explanation for the zonated expression observed. Additionally, the receptor itself may exhibit zonation. To our knowledge, no conclusive data on the hepatic uptake gradient of GH have yet been presented. Our own data suggested that the GH receptor protein or the mRNA gradients are probably too shallow to explain much of the GH-mediated zonation effects [6]. Several factors that mediate the GH signal, including the Stat5 protein and a GH-regulated nuclear factor, have been identified [35, 36], but their possible zonation has not been investigated. Vitamin A is an important regulator of *CYP2C7* expression [14, 15] and GH interacts by increasing the uptake of vitamin A [37]. Thus, vitamin A could contribute to establishing the CYP2C7 expression gradient by its own uptake gradient as well as indirectly via a GH-regulated uptake.

Since chronic ethanol treatment is known to reduce circulating levels of GH and to attenuate the episodic secretion pattern towards a more feminine pattern [18, 19], a reduced rather than an increased CYP2C7 expression, particularly in the periportal region, would have been expected. We did not observe this. However, GH levels were not measured. It is possible that chronic ethanol treatment increases CYP2C7 by other mechanisms unrelated to GH, for instance via effects on the hepatic production of retinoids in a way that stimulates CYP2C7 production.

In conclusion, the results of this and of our previous studies [4, 5] demonstrate that the zonated expression of the *CYP 2B*, *2C7*, and *3A* genes is governed by circulating GH that either up-regulates (*CYP2C7*) or down-regulates (*2B* and *3A*) their expression in the periportal region. The zonal up-regulation of CYP2C7 seems to occur both at the translational and posttranslational levels, while the periportal down-regulation of *CYP2B* and *3A* appears to be pretranslational. A sinusoidal gradient in the hepatic uptake of GH or effects of growth hormone on retinoid distribution offers the most likely explanation for these zonal effects. We also postulate that other liver genes known to be periportally expressed are regulated by GH similarly to CYP2C7.

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