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Zonation of Cytochrome P450 Enzyme Expression in Rat Liver

ISOZYME-SPECIFIC REGULATION BY PITUITARY DEPENDENT HORMONES

Teija Oinonen, Agneta Mode,† Peter E. Lobie† and Kai O. Lindros*‡*

*BIOMEDICAL RESEARCH CENTER, ALKO GROUP LTD., P.O.B. 350, 00101 HELSINKI, FINLAND, AND
†DEPARTMENT OF MEDICAL NUTRITION, HUDDINGE UNIVERSITY HOSPITAL F60, NOVUM,
S-14186 HUDDINGE, SWEDEN

ABSTRACT. The effect of hypophysectomy and subsequent infusion of growth hormone (GH) or injections of triiodothyronine (T_3) on the acinar expression pattern of four hormonally regulated P450 isozymes was studied to elucidate the involvement of pituitary dependent hormones in regulating the characteristic centrilobular expression pattern of most members of the cytochrome P450 (*CYP*) gene family in rat liver. Hypophysectomy was previously observed to allow high expression of *CYP2B1/2* and *3A1/2* in the normally silent periportal region. In the present study, it had much less effect on the zonation of the ethanol-inducible P450 2E1 form: only a moderate shift of 2E1 staining towards the periportal region was observed by immunohistochemistry. Subsequent injections with T_3 moderately decreased *CYP2E1* expression in the periportal region and no significant countereffect of GH was discerned. T_3 treatment, previously observed to block only the periportal expression of *CYP3A1/2*, counteracted the increased *CYP2B1/2* expression caused by hypophysectomy equally in the periportal and perivenous region. This was true both at the protein and mRNA level, as analysed from cell lysates obtained by *in situ* perfusion of livers by zone-restricted digitonin treatment. Thus, although hypophysectomy and subsequent GH and T_3 treatment affect the total expression of *CYP2B1/2*, *2E1*, and *3A1/2* similarly, the zonal effects were isozyme-specific. In contrast, the perivenous zonation normally seen for the dioxin-inducible P450 1A2 form was steepened rather than diminished by hypophysectomy, both in male and female rats. Administration of GH by the female-type continuous infusion had no effect in male rats, but partially counteracted the effect of hypophysectomy in females, suggesting an involvement of GH. In contrast to other *CYP* genes investigated, the female-characteristic expression of *CYP2C12* was found to be completely non-zonated. Hypophysectomy and continuous GH administration dramatically affected the amount of mRNA of both P450 2C12 and the male-specific 2C11 form, but analysis of periportal and perivenous cell lysates indicated that these effects were not zone-specific. The distribution of the GH receptor was investigated to explain the zonal effects of GH. Immunohistochemically, a moderate perivenous dominance was observed, whereas the mRNA abundance of both GH receptor and GH binding protein was slightly higher in the periportal region. Thus, zonal regulation by GH does not appear to result from a GH receptor zonation; rather, a sinusoidal GH gradient may be involved. These data, combined with our previous results, indicate that pituitary-dependent hormones regulate the zone-specific expression of some P450 forms strongly (i.e. 2B1/2 and 3A1/2), and other forms are moderately regulated (i.e. 1A2 and 2E1), or are affected across the whole acinus (i.e. 2C11, 2C12). BIOCHEM PHARMACOL 51;10: 1379–1387, 1996.

KEY WORDS. cytochrome P450 gene expression; hypophysectomy; growth hormone receptor; triiodothyronine; hepatic zonation; rat liver

Many genes exhibit a zonated expression pattern across the liver acinus. This zonation is thought to result from sinusoidal gradients of oxygen, hormones, metabolites, and other blood-borne factors [1], but the importance of different factors and the molecular mechanisms are, at present, unclear. Zonation is particularly characteristic for the expression of the *CYP*§ family of genes. Their usual expression in the downstream perivenous region in rat liver [2–4] coincides with the area that is more vulnerable to most xenobiotics. Consequently, elucidation of factors regulating the zonated expression of *CYP* genes is important for understanding mechanisms of liver damage.

Hormonal factors, including GH, are known to either upregulate or downregulate the overall hepatic expression of many *CYP* genes, the sex-characteristic forms being par-

‡ Corresponding author. Tel. 358 0 133 27 09; FAX 358 0 133 27 81.

§ *Abbreviations:* *CYP*, cytochrome P450 gene; P450, cytochrome P450 protein; mRNA, nomenclature as proposed in Nelson *et al.*, *DNA Cell Biol* **12:** 1–51, 1993; GH, growth hormone; T_3, triiodothyronine; RT-PCR, reverse transcriptase polymerase chain reaction; GHBP, growth hormone binding protein; RNase, ribonuclease; ALAT, alanine aminotransferase.

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ticularly affected (see Ref. [5] for a review). The episodic male-type or the more continuous female-type pattern of GH secretion has been shown to determine the sex-characteristic expression of the *CYP* genes (see Ref. [6] for a review). In male rats, the pulsatile GH secretion, characterized by high peaks of serum GH every 3–4 hr, upregulates the expression of *CYP2C11*, and, in female rats, the more continuous GH secretion pattern prevents *CYP2C11* expression, but allows the female-characteristic expression of *CYP2C12*. Similarly, the male-type pulsatile GH secretion allows the male-characteristic expression of *CYP2A2, 3A2* [7], and *2C13* [8] whereas, in females, their expression is inhibited by continuous exposure to GH. Thyroid hormones are known to downregulate the expression of *CYP1A2, 2B1, 2B2, 3A1*, and *3A2* [5].

We have previously observed that the zonated expression of *CYP2B1/2* and *3A1/2* genes is hormonally regulated. Thus, after hypophysectomy, high expression of *CYP2B1/2* and *3A1/2* in the normally silent periportal (upstream) region was seen; an effect counteracted by subsequent continuous infusion of GH and, with respect to *CYP3A1/2*, also by administration of T_3 [9, 10].

These observations prompted us to investigate whether or not pituitary controlled hormones regulate the zonated expression of *CYP* genes, in general. Consequently, we investigated the effect of hypophysectomy and subsequent GH and/or T_3 administration on the zonation of four other hormonally regulated P450 forms. The expression of alcohol-inducible *CYP2E1* is predominantly perivenous [11], increased by hypophysectomy, and is suppressed by subsequent administration of GH [12, 13]. The expression of the dioxin inducible *CYP1A2* is also chiefly perivenous [14] and increased by hypophysectomy, an effect counteracted by treatment with T_3 [15]. The expression of *CYP2C11*, which encodes the major male-characteristic testosterone 16α-hydroxylase, P450 2C11, requires a male-type pulsatile GH secretion pattern [16] and is higher in the perivenous region [4]. The zonation of the expression of *CYP2C12* has not previously been studied. This gene encodes a steroid sulfate 15β-hydroxylase, which is the major female-characteristic P450 and positively regulated by female-type continuous GH secretion [17]. The effect of T_3 on the zonation of the phenobarbital-inducible P450 2B1/2 was also studied because, in addition to GH [18], T_3 suppresses 2B1/2 [19]. Zonation of protein was evaluated both by immunohistochemistry and by comparative immunoblot analysis of periportal and perivenous cell lysates obtained by zone-restricted digitonin pulse infusion during *in situ* liver perfusion. In addition, zonation of mRNA species was investigated by analysis of the cell lysates, either by RT-PCR [20] or by Rnase protection analysis in solution.

MATERIALS AND METHODS

Animals

Hypophysectomized and sham-operated male and female Sprague-Dawley rats were obtained from Møllegaard Breeding Center Ltd., Skensved, Denmark. Rats were hypophysectomized or sham-operated at the age of 5 or 7 weeks. A week later animals were transferred to our laboratory and were allowed to stabilize for 1 week. Animals were fed a commercial R3 laboratory diet (Ewos, Södertälje, Sweden) and water *ad lib*. The treatment groups were: 1. control: sham-hypophysectomized, sham-operated for pump implantation (a piece of teflon tubing implanted subcutaneously), and daily i.p. injections with saline; 2. hypophysectomized animals, sham-operated for pump implantation, and daily i.p. injections with saline; 3. hypophysectomized animals treated with human recombinant growth hormone (Norditropin, donated by Nordisk Gentofte A/S, Denmark or Novo Nordisk A/S, Denmark), 0.01 IU/hr by continuous infusion and daily i.p. injections with saline; and 4. hypophysectomized rats, sham-operated for pump implantation, given T_3 (Sigma Chemical Company, St. Louis, MO, U.S.A.) 50 μg/kg i.p. daily for 7 days. GH was administered subcutaneously with osmotic minipumps (Alzet 1701, Palo Alto, CA, U.S.A.) implanted dorsally under halothane anaesthesia. Buprenorfin (Temgesic,™ 0.15 mg/kg s.c.) was given immediately after surgery. The effects of hypophysectomy and GH treatment were monitored by weighing the rats. All animal experiments were approved by the Institutional Animal Care and Use Committee at Alko-Group Ltd.

Collection of Periportal and Perivenous Cell Lysates

Periportal and perivenous cell lysates were obtained during *in situ* perfusion of anaesthetized (phenobarbital 60 mg/kg i.p.) rats by a modified [20] dual digitonin pulsing method [21]. Briefly, periportal cells were lysed by infusion of 6.7 mL/kg b.wt. of 3.5 mM digitonin (ICN Chemicals, Cleveland, OH, U.S.A.) *via* the portal vein and the lysate collected by immediate retrograde flushing. Perivenous cell lysates were obtained by infusing 10 mL/kg b.wt. digitonin solution *via* the upper vena cava followed by antegrade flushing. Digitonin was infused for the period of time determined empirically 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 zonal origin of the cell lysates was evaluated by assay of the periportal marker enzyme alanine aminotransferase (ALAT, EC 2.6.1.2.) in corresponding periportal and perivenous lysates, as described previously [11]. In periportal samples from males, the activity of ALAT was 7.6 times higher than in perivenous samples (SEM = 0.8, n = 39), and from females 20 times higher (SEM = 2.9, n = 40). Thus, the overall periportal/perivenous ratios indicate good separation of cell lysates and are in agreement with our previous studies [9, 11].

Western Blot Analysis

Analysis of cell lysate P450 2B1/2 and 2E1 (50–100 μg protein/well), followed by videodensitometric quantitation of the blots, were performed as recently described [9]. Band

intensities of the samples were normalised to the intensity of a standard run on each gel (purified P450 2B1, or in 2E1 immunoblots, pooled cell lysate, or liver homogenate protein). In P450 2B1/2 immunoblotting, either commercial rabbit immunoglobulins to rat 2B1/2 (Oxygen, Dallas, TX, U.S.A.), or rabbit antiserum [22] were used. The latter was absorbed with microsomal proteins from normal rats to abolish cross-reactivity with an unidentified protein of slightly lower molecular mass than P450 2B1/2. Antisera and P450 2B1 antigen were generously donated by Dr. Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden). A polyclonal rabbit antiserum to rat P450 2E1 was used [22].

Immunohistochemical Localization of P450 2E1 and GH Receptor

Immunohistochemical analysis of P450 2E1 apoprotein in paraffin-embedded liver sections was performed as previously described [4]. The polyclonal rabbit antiserum to P450 2E1 was the same as used for Western blotting. Immunohistochemical localization of GH receptors was performed with polyclonal rabbit antibody specific to the intracellular region of the rat GH receptor [23]. To achieve specific staining with this antibody, it was necessary to incubate the sections with primary antibody overnight at 4°C.

RT-PCR Analysis

The relative amounts of P450 2B1/2, 1A2, GH receptor, and GHBP mRNA in periportal and perivenous cell lysates were analyzed by a PCR-based semiquantitative method as described previously [9, 20]. Total RNA was isolated from digitonin cell lysates essentially as in [24]. First strand cDNA was produced as described in [9]. Conditions and primers for P450 2B1/2 RT-PCR were as described in [9], and for 1A2 in [25]. RT-PCR primers for GH receptor mRNA were chosen from the region coding for the intracellular region of the GH receptor, 5′-CTTTTATGCCCAAGTAAGCG-3′, and 5′-AACCGTGGTGTAGTCTGGG-3′ to amplify a 337 bp fragment. cDNA (4 μL) was amplified in a 100-μL reaction volume containing 2U Taq DNA polymerase, 1 × PCR buffer (both from Boehringer Mannheim), 50 pmol of both primers, 0.2 mM each dNTP (Promega), and 3 mM $MgCl_2$. Mineral oil (100 μL) (Sigma) was added on the top of the reaction mixture, and 26 cycles of PCR were performed. Each cycle consisted of 94°C for 30 sec, 52°C for 1 min, and 72°C for 1 min. The last elongation step was extended to 5 min. The GHBP forward primer, 5′-CGAAGTGCGTGTGAGATCC-3′, was common to both GHBP and GH receptor sequences, but the reverse primer, 5′-AAGCTAGGGATGGCAGATCC-3′, was from the 3′ end of GHBP mRNA that is specific to the GHBP. A 196 bp fragment was amplified from 3–10 μL of cDNA in PCR reaction conditions as described above, except the $MgCl_2$ concentration was 1.5 mM. The linearity of each PCR amplification was ensured by varying the number of cycles and the amount of cDNA in the reaction. Quantitative analysis of amplification products was done by separation of 20 μL of PCR products in 4% (w/v) NuSieve GTG agarose gels (FMC BioProducts, Rockland, ME, U.S.A.), followed by ethidium bromide staining and videodensitometric scanning, or by anion exchange HPLC [26] as in [10].

Solution Hybridization Analysis of P450 2C11 and P450 2C12 mRNA

RNA in cell lysates was extracted [27] and the concentration measured spectrophotometrically. The absence of DNA in the samples was verified fluorometrically [28]. Levels of P450 2C11 mRNA and P450 2C12 mRNA were analyzed using specific [^{35}S]UTP-labeled cRNA probes in solution hybridization assays, as previously described in detail [27, 29]. Quantification of the mRNA species was achieved by comparison with standard curves obtained by hybridizations to liver total nucleic acid (tNA) samples, calibrated to known amounts of *in vitro* synthesized mRNA. Samples were analyzed in triplicate, and the results are expressed as attomols (amole) of specific mRNA per μg total RNA. The interassay variations averaged 10% and were controlled by using internal tNA standards prepared from normal livers.

Statistical Analysis

Male and female rats were treated in two separate experimental series. Immunoblot and RT-PCR results from different series were combined so that, within series, all samples were normalized to the mean of perivenous samples of controls (100%). All results are expressed as mean ± SEM. Comparisons between periportal and perivenous samples within treatment group were conducted using the Student's *t*-test and those between multiple treatment groups with ANOVA, followed by *t*-tests between controls and hypophysectomized, and between hypophysectomized and hormone treatments.

RESULTS AND DISCUSSION

In this study, we have investigated the role of GH and T_3 in regulating the zonated expression of *CYP* genes. We found that the effect on the total expression exerted by hypophysectomy and subsequent hormone administration was zone-specific for some of the investigated genes, but not for all of them. Thus, although the zonation of several P450 isozymes seems to be hormonally controlled, this phenomenon does not appear to be a common denominator for all P450 forms that are expressed in a zonated fashion.

The Effect of Hypophysectomy and T_3 Treatment on Zonation of P450 2B1/2

An important basis for the present study was our previous finding demonstrating the hormonally-controlled zonation

of both P450 2B1/2 and of P450 3A1/2. Hypophysectomy was found to induce the expression of both subfamilies in the normally near-silent periportal region, and this effect was counteracted by subsequent administration of GH [9, 10]. We also had found that subsequent treatment with T_3 resulted in a P450 3A1/2 distribution restricted to a thin rim of 1–3 hepatocytes just surrounding terminal hepatic venules [10]. Here, we confirmed our earlier finding that hypophysectomy completely abolished the zonation of P450 2B1/2 protein in male rat liver (Fig. 1). This was the result of a 13-fold increase in the periportal region as compared to only a 1.7-fold in the perivenous region. Subsequent T_3 treatment, however, reduced P450 2B1/2 protein by about 90% both in periportal and perivenous samples, precluding establishment of perivenous zonation. Thus, T_3 treatment affected the zonation of P450 3A1/2 and 2B1/2 in completely different ways. The zonation pattern of P450 2B2 mRNA was almost identical to that of the apoprotein, demonstrating that the T_3 effect across the whole acinus on the expression of *CYP2B1/2* is pretranslationally regulated (Fig. 1). Corresponding analyses of samples from hormone-

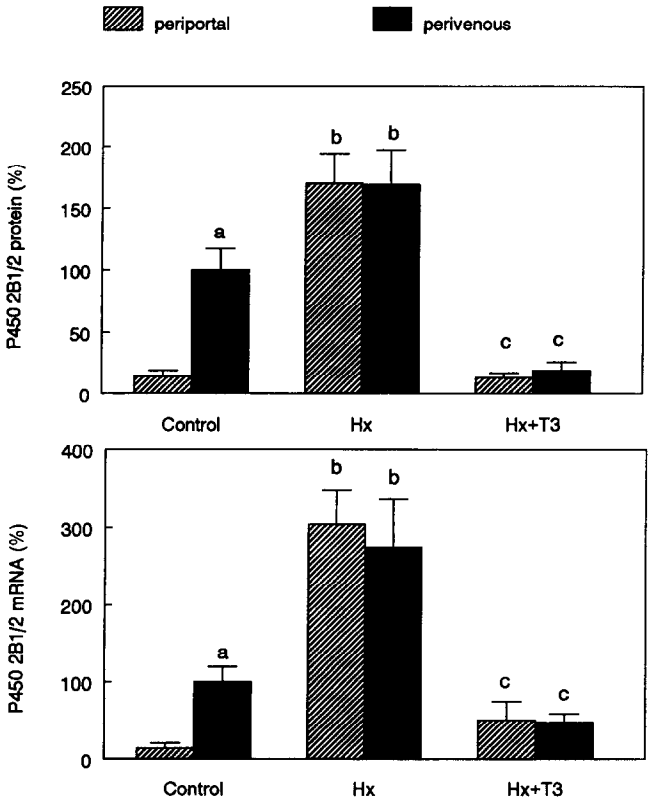

FIG. 1. Effect of hypophysectomy and subsequent T_3 treatment on the distribution of P450 2B1/2 apoprotein and mRNA in periportal and perivenous cell lysates. Videodensitometric quantification of immunoblots and HPLC quantification of RT-PCR products (means ± SEM) from control (n = 10) hypophysectomized (Hx, n = 9), and hypophysectomized plus T_3 treated (Hx + T3, n = 5) male rats is shown. The perivenous control value was arbitrarily set as 100%. P < 0.05 (Student's t-test) a, periportal vs perivenous; b, Hx vs control; and c, Hx + T3 vs Hx.

treated female rats revealed similar effects (results not shown), demonstrating the absence of gender differences with respect to the extent or the distribution of the T_3 effect on *CYP2B1/2* expression.

The Effect of Hormonal Manipulations on P450 2E1 Distribution

Western blot analysis of liver homogenates revealed a 1.7- to 2-fold increase in P450 2E1 protein after hypophysectomy, an effect partly counteracted by GH and T_3 (results not shown). These effects are in agreement with earlier findings [12, 13]. Because hypophysectomy and administration of GH affects the expression of *CYP2E1* much in the same way as that of *CYP2B1/2*, we expected these effects on *CYP2E1* to be zonated. Moderate zonal effects could be discerned by immunohistochemical analysis (Fig. 2). After hypophysectomy, the expression of *CYP2E1*, which normally is restricted to a 4–6 cell layer thick area around the terminal hepatic venules, extended towards the periportal region. However, no significant countereffect of GH infusion on this zonation pattern was seen (results not shown). On the other hand, a peculiar expression pattern was seen in some of the hypophysectomized animals subsequently injected with T_3, with intense P450 2E1 immunoreactivity in cells close to the terminal hepatic veins (Fig. 2). The immunohistochemical changes were, however, not corroborated by analysis of periportal and perivenous cell lysates. Western blot analysis of samples from male rats gave no evidence for any major zonal effects on P450 2E1 protein (Fig. 3). On the contrary, hypophysectomy caused a 2.5-fold increase in the perivenous samples, but there was no evidence for an increase in the periportal region. Neither the administration of GH nor T_3 reduced P450 2E1 protein or affected zonation significantly (Fig. 3). RT-PCR analysis of P450 2E1 mRNA revealed a similar pattern: although hypophysectomy caused a significant increase in P450 2E1 mRNA in periportal samples, the same effect was seen in perivenous samples, and the zonation pattern remained essentially the same (T. Oinonen, unpublished result). Essentially similar data were obtained by analysis of samples from female rats. The reason for the discrepancy between the immunohistochemical data and the Western blot analyses of eluates, which is unique for P450 2E1, is not clear at this time. In our previous studies, essentially similar zonation patterns were seen immunohistochemically and by comparing periportal and perivenous cell lysates [9, 10]. One possible explanation is that digitonin, given to lyse periportal cells, does not penetrate into the more distal periportal regions, where increased immunohistochemical staining of P450 2E1 is seen.

P450 1A2 mRNA Distribution

The P4501A1 and 1A2 forms are induced by dioxin and polycyclic aromatic hydrocarbons and other environmental toxins [30]. Due to their toxicological importance, they have been the subject of extensive studies. In rat liver, only

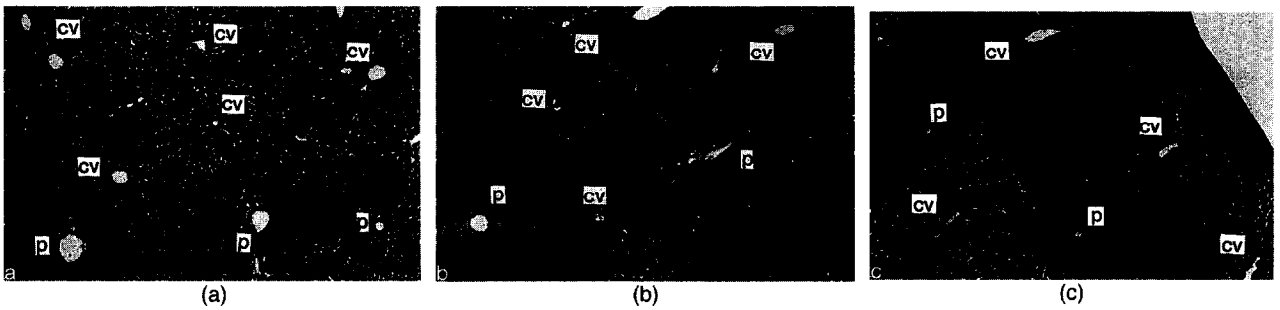

FIG. 2. Comparison of the effect of hypophysectomy and subsequent T_3 injections on the immunohistochemically observed acinar distribution of P450 2E1. T_3 was injected i.p., and liver sections from untreated (a), hypophysectomized (b), or hypophysectomized and T_3-treated (c) male rats were stained with polyclonal antibodies against rat P450 2E1 as described in Materials and Methods. Terminal central venules (cv) and portal venules (p) are indicated in the photographs.

the *CYP1A2* gene is constitutively expressed, predominantly in the perivenous region, where also 1A2 protein [14] and mRNA [25] are found. Because hypophysectomy has been reported to increase the expression of *CYP1A2* and T_3 treatment to counteract this [15], the effect on zonation of these hormonal manipulations was important to establish. Because P450 1A2 protein was not detectable with a specific antibody, zonation of 1A2 mRNA was investigated. A moderate periportal < perivenous gradient of P450 1A2 mRNA was observed by analysis of cell lysates (Fig. 4). In males, but not in females, hypophysectomy resulted in a significant increase in P450 1A2 mRNA in the perivenous samples (Fig. 4). However, in contrast to the effect of hypophysectomy on P450 2B1/2 and 3A1/2, a significant reduction of 1A2 mRNA was seen in the periportal region, both in males and females, resulting in a significant steepening of the perivenous/periportal 1A2 mRNA gradient. In males, both hormones partly counteracted the hypophysectomy-induced increase of *CYP1A2* mRNA in perivenous cells, an effect that was significant in the case of GH. The steepened zonation was not counteracted by T_3 treatment in either gender, and GH had no effect in livers from male animals. Interestingly, in female rats the female-type continuous infusion of GH partly counteracted the suppressive effect of hypophysectomy on periportal P450 1A2 mRNA expression. Although this effect did not reach statistical significance, it contrasts to the

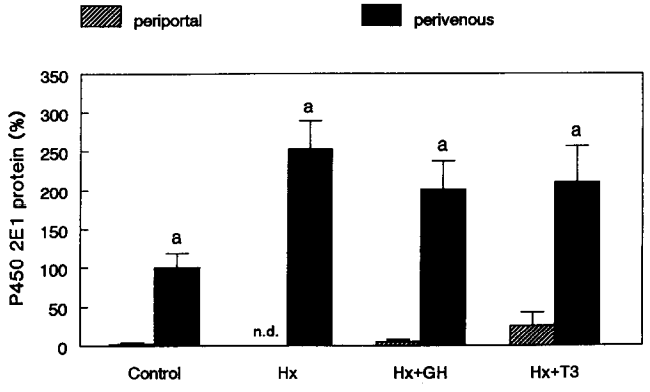

FIG. 3. Effect of hypophysectomy and hormone treatments on the distribution of P450 2E1 apoprotein in periportal and perivenous cell lysates. Videodensitometric quantification of immunoblots and HPLC quantification of RT-PCR products from 4–5 male rats per treatment group is presented (mean ± SEM). Treatment of hypophysectomized (Hx) rats with GH (Hx + GH), or T_3 (Hx + T3) is described in Materials and Methods. The perivenous control value was arbitrarily set as 100%. a = $P < 0.01$ for statistical significance of periportal-perivenous difference in each treatment group by Student's *t*-test. n.d., not detected.

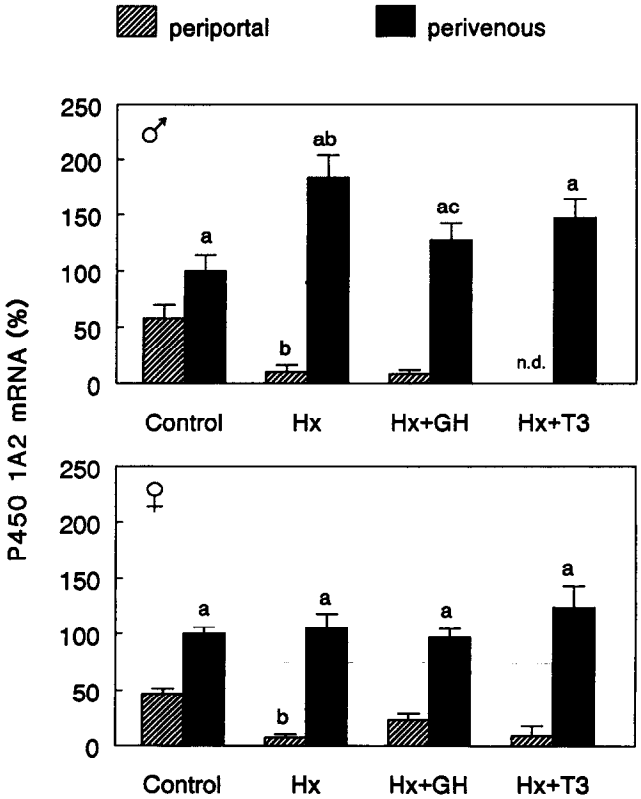

FIG. 4. Effect of hypophysectomy and hormone treatments on the distribution of P450 1A2 mRNA in cell lysates from male and female rats. Videodensitometric quantification of RT-PCR products on ethidium bromide-stained agarose gels is presented as means ± SEM. n = 9 or 10, except for hypophysectomized (Hx), T_3-treated, n = 5. The perivenous control value was arbitrarily set as 100%. $P < 0.05$ (Student's *t*-test) for a, periportal vs perivenous; b, Hx vs control; and c, Hx vs hormone treatments; n.d., not detected.

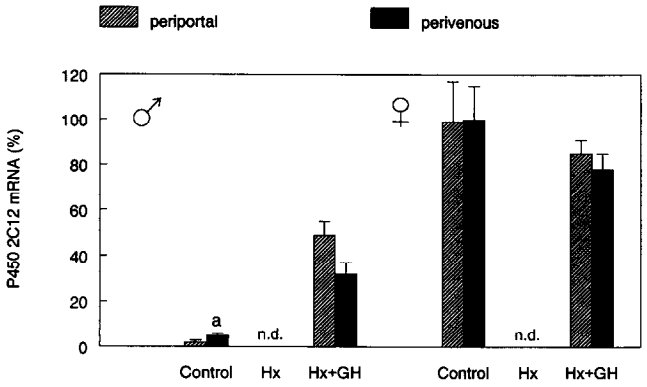

FIG. 5. Effect of hypophysectomy (Hx) and GH treatment on the distribution between periportal and perivenous cell lysates of the female-characteristic P450 2C12 mRNA. P450 2C12 mRNA in periportal and perivenous cell lysates was quantitated using solution hybridization. n = 5 in all groups. The results were normalized to the perivenous control female group arbitrarily set to 100%. a, $P < 0.05$ (Student's t-test) for difference within treatment groups between periportal and perivenous samples; n.d., not detected.

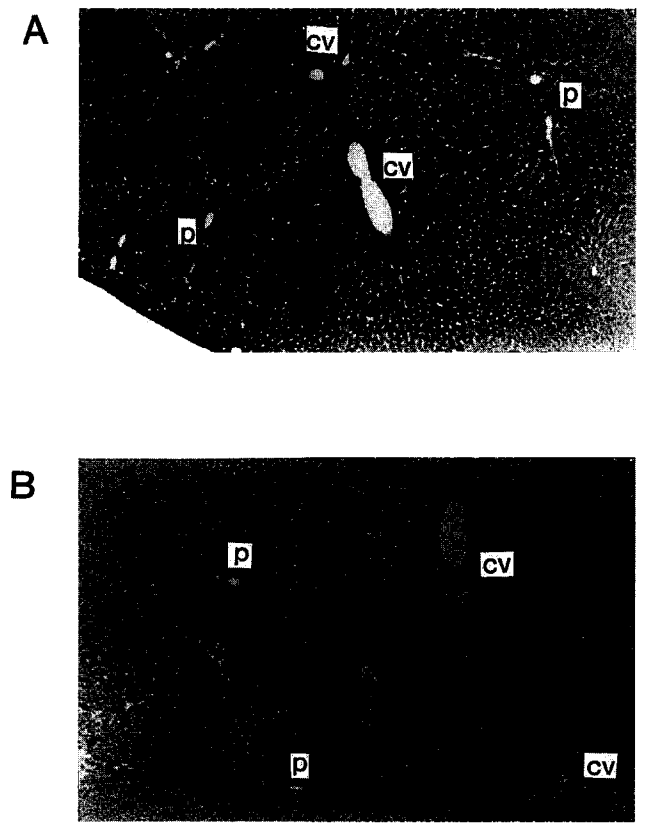

FIG. 6. Immunohistochemical localization of the GH receptor. A polyclonal antibody directed against the intracellular region of the GH receptor was used to stain liver sections (A) from untreated female rat as described in Materials and Methods. Control staining (B) was with nonimmune serum. Note the darker staining in the perivenous areas surrounding the central venules (cv) as compared to the areas around terminal portal venules (p).

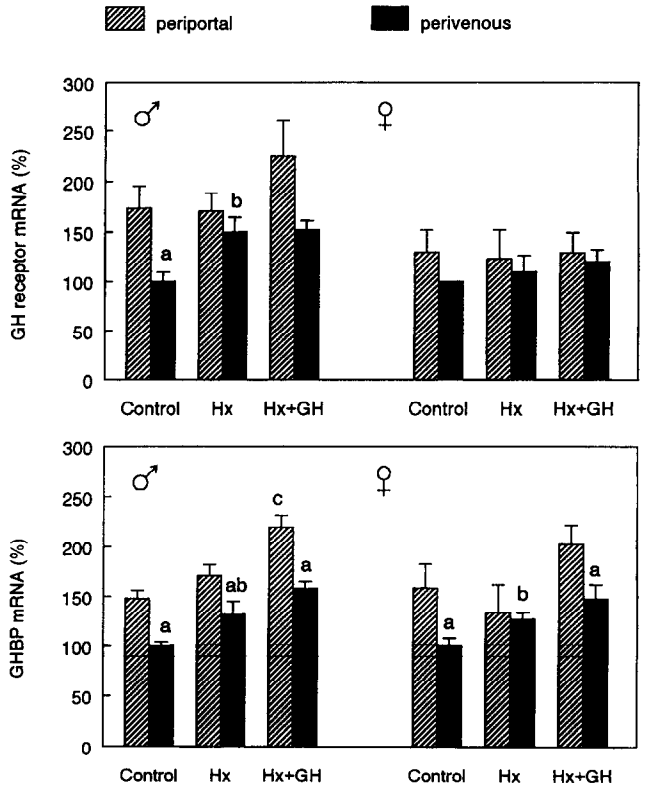

FIG. 7. Distribution of GH receptor and GHBP mRNA in periportal and perivenous cell lysates. HPLC quantification of RT-PCR products from male and female control, hypophysectomized (Hx) or hypophysectomized, and GH-treated (Hx + GH) rats. Means ± SEM of 7–10 animals is given, with perivenous control values arbitrarily set as 100%. $P < 0.05$ (Student's t-test); a, periportal vs perivenous; b, Hx vs control; c, Hx + GH vs Hx.

complete absence of corresponding effect in males. These data suggest, further, that the zonated expression of *CYP1A2* is influenced by the hormones regulated by the pituitary. However, in contrast to the repressive effect of GH on *CYP2B1/2* and *3A1/2* expression in the upstream periportal region, the presence of circulating pituitary hormones seem to be necessary for normal periportal expression of *CYP1A2* transcripts. The absence of any counteref-fect of GH in males may be a consequence of the female-type continuous GH administration because, in females, the periportal expression pattern was partially reestablished by GH. Immunohistochemical analyses are needed, however, to establish whether or not corresponding zonal effects are seen at the protein level.

P450 2C12 and P450 2C11 Distribution

The expression in the liver of the sex-characteristic P450 forms 2C12 (female) and 2C11 (male) is governed by the sex-specific temporal GH secretion patterns in the rat. However, little information on the zonation of these P450 forms is available. In female rats, the female-characteristic P450 2C12 appeared to be quite evenly distributed along

TABLE 1. Summary of the observed hormonal effects on zonation of the P450 forms investigated

P450	Characteristics	Treatment			
		None	Hx	Hx + GH	Hx + T_3
1A2	Dioxin-inducible	pp < pv	pp ≪ pv	pp ≪ pv	pp ≪ pv
2B1/2	Phenobarbital-inducible	pp ≪ pv	pp ≈ pv	pp < pv	pp ≈ pv
2C11	Male-characteristic	pp < pv	pp ≈ pv	–	n.d.
2C12	Female-characteristic	pp ≈ pv	–	pp ≈ pv	n.d.
2E1	Ethanol-inducible	pp ≪ pv	pp < pv	pp < pv	pp ≪ pv
3A1/2	Glucocorticoid-inducible	pp ≪ pv	pp ≈ pv	pp < pv	pp <<< pv

Data in this table is survey of results of this study and of Ref. 9 and 10.

Apoprotein distribution is presented for P450 2B1/2, 2E1, and 3A1/2, and that of mRNA for 1A2, 2C11, and 2C12. < denotes moderate zonation, ≪ marked zonation, and <<< extreme zonation (i.e. staining in only 1–3 cell layers thick region). ≈, no zonation; – not detected; Hx, hypophysectomized; pp, periportal; pv, perivenous; n.d., not determined.

the acinus. Thus, the P450 2C12 mRNA, as analyzed by solution hybridization assay, was similar in periportal and perivenous cell lysates (Fig. 5). This suggests that the female-specific P450 2C12 protein is distributed in the acinus in a pattern diverging from the common predominant perivenous distribution of other P450 forms. After hypophysectomy, no P450 2C12 mRNA could be detected in either periportal or perivenous cell lysates. As expected, female-type GH administration re-established P450 2C12 mRNA, but this occurred to the same extent in both liver regions. P450 2C12 mRNA was also detected in livers from male rats, albeit at very low levels, these levels being significantly higher ($P < 0.05$) in perivenous lysates (Fig. 5). Hypophysectomy completely abolished this expression. The increase in expression by the female-type continuous GH infusion was somewhat higher in periportal than in perivenous samples, suggesting a feminization of the P450 2C12 distribution as opposed to the normally very low, but predominantly perivenous, pattern of this P450 form in males. Pituitary hormones seem to have no zonal effect in females on the expression of *CY2C12*, as expected from the absence of zonation in untreated animals.

Corresponding studies on the effect of hypophysectomy and GH on the acinar distribution of the male-characteristic P450 2C11 mRNA were performed. In male controls, the amount of mRNA was twice as high in perivenous as in periportal lysates (results not shown). The perivenous zonation of mRNA corresponds to an earlier observation on the immunohistochemical distribution of P450 2C11 protein [4]. Hypophysectomy suppressed mRNA levels relatively more in the perivenous region, resulting in a loss of zonation. As expected, subsequent female-type administration of GH by continuous infusion extinguished the P450 2C11 mRNA signal in both periportal and perivenous samples. Episodic administration of GH would be required to rule out the possibility that GH affects P450 2C11 zonation in males.

Distribution of GH Receptor and GHBP

The involvement of GH in the zonation of several P450 subfamilies could occur as a consequence of an acinar gradient of circulating GH or of the GH receptor. To test the second possibility, we studied the distribution of the GH receptor by immunohistochemical staining of liver sections, using a polyclonal antibody to the intracellular region of the GH receptor. In addition, the acinar distributions of both the GH receptor mRNA and GHBP mRNA, which arise from alternative splicing of the same gene [31], were studied by analysis of cell lysate samples with RT-PCR. GH receptor protein was immunohistochemically stained throughout the acinus, but slightly stronger in perivenous and midzonal regions than in the periportal region (Fig. 6). In contrast, analysis of periportal and perivenous cell lysates showed that there was a moderate pp > pv gradient for both the GH receptor mRNA and the GHBP mRNA (Fig. 7). These differences were seen both in males and females, but the differences were clearer in males. Attempts to explain the discrepancy between the immunohistochemically-observed distribution of the GH receptor protein and that of the corresponding mRNA in cell lysates by analysis of GH receptor protein in cell lysates were not successful. Apparently, the receptor protein, being an integral plasma membrane protein, was not released, at least not in an intact form, by digitonin treatment. The conflicting protein and mRNA zonation data could reflect a functional acinar GH gradient in the liver. Binding of GH to its receptor predominantly in the upstream periportal region could lead to internalization of the liganded receptor [32], and subsequent downregulation of the number of GH receptors on the plasma membrane of periportal hepatocytes. Conflicting data on the effect of hypophysectomy and GH administration have appeared. Our results are in accordance with one earlier study [33], indicating that hypophysectomy or subsequent GH treatment have little effect on either the zonation or the total expression of GHR or GHBP mRNA. However, other studies indicate that the hepatic GH receptor mRNA is reduced by hypophysectomy, and increased by GH administration in female rats [34, 35].

Taken together, these and our previous results demonstrate that pituitary-dependent hormones are involved in the regulation of the perivenous expression pattern of a number of *CYP* genes. However, there are major differences between individual P450 forms in their response. Although

the pituitary hormone-mediated regulation of the zonation of the P450 forms 2B1/2, and 3A1/2 appears to be strong, that of the 1A2 and 2E1 forms is only moderate and that of the 2C11 and 2C12 forms minimal or absent (Table 1). Downregulation by GH in the upstream (periportal) region does not appear to result from a GH receptor zonation; rather, a sinusoidal GH gradient may be involved.

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