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Growth hormone mediates zone-specific gene expression in liver

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The *CYP2B1/2* gene pair is an example of many liver genes that exhibit a characteristic zonated expression pattern in the liver. The factors governing this zonation are poorly understood. We observed that after hypophysectomy the expression of CYP2B1/2 protein and mRNA in the normally silent periportal (upstream) region was high, in both male and female rats. Treatment with growth hormone counteracted the effect of hypophysectomy, limiting expression to the perivenous (downstream) region, completely in females and partially in males. This shows that a hormone zone-specifically regulates gene expression in the liver.

Hypophysectomy; Rat; Growth hormone; Cytochrome P450 expression; Liver zonation; Perivenous

1. INTRODUCTION

The mechanisms responsible for the spatial pattern of gene expression in different organs are not well understood. In the mature mammalian liver the majority of the genes investigated so far have been observed to exhibit a zonated expression pattern within the acinus, the microcirculatory unit of the liver [1,2]. Bloodbourne factors, including oxygen, nutrients, and hormones, all of which form gradients as a result of the unidirectional blood flow, have been suggested to be responsible for establishment of this zonation. However, the possible role of specific hormones has to date received little attention. Members of the cytochrome P450 (*CYP*)* gene superfamily serve as good models to address this question, since the expression of most of these genes seems to be particularly zonated, their expression being restricted to the perivenous (centrilobular) region [3–5]. In addition, the expression of several members of the CYP family has been found to be regulated by pituitary-dependent hormones. Thus the ethanol-inducible CYP2E1 is increased by hypophysectomy and decreased by treatment with growth hormone (GH) [6]. The expression of the male-specific CYP2C11 form is stimulated by the male-type episodic GH secretion [7] but suppressed by the female-type more continuous GH secretion, which in turn leads to stimulation of the female-specific CYP2C12 expression [8]. We studied the expression of the phenobarbital-inducible *CYP2B1/2* gene pair which is also increased by hypophysectomy, decreased by growth hormone [9], and constitutively expressed in the perivenous region [10]. We hypothesized that growth hormone may affect not only the overall expression of CYP2B1/2 but also regulate the acinar distribution. We studied the effect of hypophysectomy and of GH administration on zonation both by immunohistochemistry and by analysis of periportal and perivenous cell lysates. The lysates were obtained by a zone-restricted digitonin pulse during in situ liver perfusion and analysed for their content of CYP2B1/2 apoprotein and mRNA by immunoblotting and by reverse transcription of RNA followed by PCR analysis [11]. Hypophysectomy was found to permit and GH treatment to prevent the expression of CYP2B1/2 in the periportal region, suggesting that the regulation of CYP2B1/2 expression by GH is zone-specific.

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*The nomenclature for cytochrome P450 proposed by Nebert et al. in (1991) DNA Cell Biol. 10, 1–14, is used.

Abbreviations: EGF, epidermal growth factor; GH, growth hormone; hGH, human recombinant growth hormone.

2. EXPERIMENTAL

2.1. *Animals*

Hypophysectomized and sham operated male and female Sprague–Dawley rats were obtained from Møllegaard, Ejby, Denmark. The animals were hypophysectomized at the age of 5 weeks, transferred to our laboratory at the age of 6 weeks, and were then allowed to stabilize for 1 week. All animals were fed a commercial R3 laboratory diet (Ewos, Södertälje, Sweden) and water ad libitum. The hypophysectomized rats were given human recombinant growth hormone (hGH; Norditropin, Nordisk Gentofte A/S, Denmark), 0.01 IU/h, or saline by continuous infusion for 7 days. For the continuous GH infusion, osmotic minipumps (Alzet 1701, Palo Alto, CA, USA) were implanted subcutaneously on the backs under halothane anesthesia. The effect of GH treatment was monitored as weight increase. The experiments were approved by the local committe for animal experiments.

2.2. *Immunohistochemical localization of CYP2B1/2*

Liver tissue was fixed, sectioned and treated as previously described

[5]. The rabbit antiserum against rat CYP2B1/2 and the purified protein were generously donated by Dr. Magnus Ingelman-Sundberg.

2.3. *Collection of periportal and perivenous cell lysates*

Zone-specific cell lysis was achieved during in situ perfusion by a modified dual digitonin pulse technique [12]. Briefly, periportal cells were lysed by infusing 6.7 ml/kg b.wt. of 3.5 mM digitonin (ICN, Cleveland, OH, USA) via the portal vein, and the lysate was 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. The zone-specificity of the cell lysates was verified by alanine aminotransferase (ALAT, EC 2.6.1.2.) assay as in [13].

2.4. *Analysis of CYP2B1/2 apoprotein by immunoblot*

Cell lysate proteins (30 or 60 μg) were separated in 8.7% SDS-polyacrylamide gels and transferred electrophoretically to nitrocellulose filters. The filters were blocked with bovine serum albumin and probed with rabbit polyclonal IgG to rat CYP2B1/2 (Oxygen, Dallas, TX, USA). Bands were stained with ProtoBlot AP system (Promega, Madison, WI, USA) and assayed using a UVP GDS 2000 videodensitometer (Ultraviolet Products, Cambridge, UK) and purified CYP2B1/2 as internal standard. The linearity of the response within the protein range used was checked.

2.5. *Isolation of total RNA and first strand cDNA synthesis*

Total RNA from 0.5 ml of digitonin eluates (which are virtually devoid of nuclear material) was isolated as described by Chomczynski and Sacchi [14] except that 0.25 ml of both phenol and chloroform was used and extraction was performed twice with phenol/chloroform and once with 0.5 ml of chloroform. The amount of RNA was determined spectrophotometrically at 260 nm, purity by nucleic acid/protein ratio (A_{260}/A_{280}), and integrity by RNA electrophoresis in 2.2 M formaldehyde/1.25% agarose gels First strand cDNA was produced from 2 μg RNA in an 80 μl reaction volume using Promega's Reverse Transcription System and random hexanucleotide primers. The response was linear under the conditions used.

2.6. *Determination of CYP2B1/2 mRNA in lysates*

This was performed using a PCR-based semiquantitative method [11]. For PCR of cDNA from reverse-transcribed RNA, we used primers specific to both isozyme forms: 5'-CCA TGA CCC ACA GTA CTT TGA CC-3' (sense) and 5'TGC CAC TCT CCT TGG GCG TG-3' (antisense) Primers were from exons 8 and 9, and PCR produced an expected 242 bp fragment 10 μl of cDNA was amplified in 100 μl reaction volume containing 2 U *Taq* DNA polymerase, 1 × PCR buffer (both from Promega), 50 pmol of both primers, 0.2 μM each dNTP, and 2 mM $MgCl_2$. Amplification in the programmable thermal controller (MJ Research, Inc. Watertown, MA, USA) consisted of 30 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min. In the last cycle the elongation step was extended to 5 min. 20 μl of amplification product was run in 4% NuSieve GTG (FMC BioProducts, Rockland, ME, USA) agarose gel electrophoresis. Gels were stained with ethidium bromide, video photographed under UV illumination, and video scanned as immunoblots. The curvilinearity of amplification was validated by varying the amount of cDNA and number of cycles Samples to be compared were always run together. The within-assay reproducibility of the reverse transcription reaction and of PCR amplification were 100 ± 7 (n = 16) and 100 ± 16 (n = 20) (mean ± S D.).

3. RESULTS AND DISCUSSION

Immunohistochemical examination of liver sections revealed that CYP2B1/2 staining, normally restricted to the perivenous region, was markedly changed after hypophysectomy. In the liver of female rats, CYP2B1/2 staining had spread towards the terminal portal veins, leaving only the most affluent areas unstained (Fig. 1). Hypophysectomized male animals exhibited corresponding changes in their distribution pattern.

The most conspicuous effect of hypophysectomy on the acinar distribution of CYP2B1/2 protein was seen by comparing the periportal and perivenous cell lysates. In periportal samples from control females, no CYP2B1/2 protein could be detected and in males only a faint band was seen (Fig. 2). In marked contrast, after hypophysectomy, the amount of CYP2B1/2 protein in periportal samples had increased at least 50-fold (Fig. 3), to the same level as in perivenous lysates. The same effect was seen in males. Semiquantitative determination of CYP2B1 and 2B2 mRNA revealed that the acinar distribution and the effect of hypophysectomy faithfully followed that of the protein distribution. Thus, while in female controls no periportal mRNA signal, and in males only a very weak signal, could be detected after hypophysectomy, periportal samples exhibited a strong mRNA signal in both sexes (Figs. 2 and 3). In congruence with the effect on the CYP2B1/2 protein, hypophysectomy not only increased the total expression, but also completely abolished the original difference between periportal and perivenous mRNA expression.

Hypophysectomized animals were next treated with GH to study the direct involvement of this hormone. Female hypophysectomized rats that had been treated for 7 days with hGH by osmotic infusion exhibited an almost completely normalized 2B1/2 distribution (Figs. 2 and 3). The expression of the CYP2B1/2 protein in the periportal region was extinguished, while the amount of protein in the perivenous lysates was only slightly affected. GH treatment also markedly reduced the periportal mRNA signal, but did not, however, fully extinguish it. This suggests that GH acted not only at the pre-translational level but also at the translational or post-translational level. The effects of GH treatment in hypophysectomized males were less distinct. GH infusion reduced the 2B1/2 apoprotein to one-seventh in periportal samples, but only to one-half in perivenous lysates. This resulted in partial restoration of the normal perivenous expression pattern. However, in the hypophysectomized males, GH did not appear to have a zone-specific effect at the mRNA level. Both periportal and perivenous CYP2B1/2 mRNA was reduced to about one-half and no acinar gradient was established.

These results provide strong evidence that growth hormone represses the expression of CYP2B1/2 in the periportal region. The finding that GH treatment counteracted hypophysectomy more efficiently in females than in males could be due to the continuous minipump infusion of GH, which mimicks the female GH secretion pattern. On the other hand, continuous GH infusion has been found to be more efficient than intermittent injections in repressing not only CYP2B1/2 [9] but also

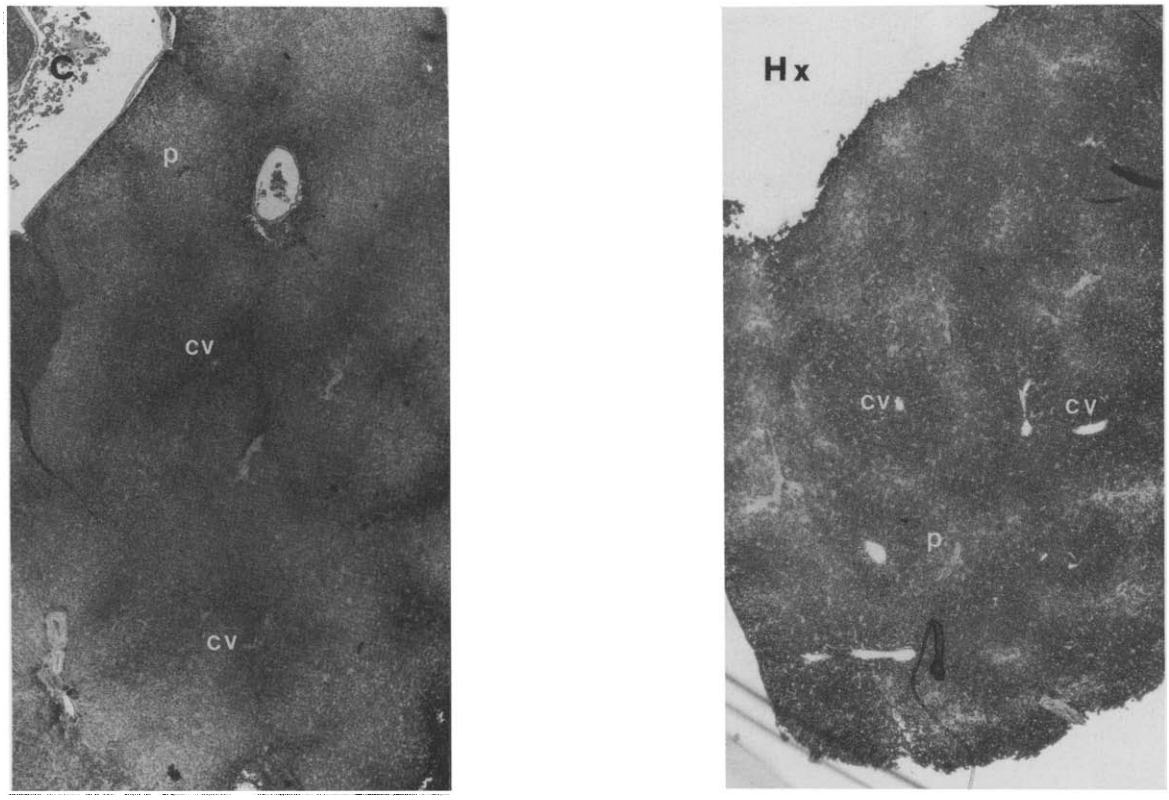

Fig. 1. Effect of hypophysectomy on the distribution of the CYP2B1/2 in liver sections. Immunohistochemical staining was performed as described in section 2. C, control female (left panel); Hx, hypophysectomized female (right panel); cv, central vein area; p, portal vein area.

several male-specific CYP forms [15,16]. Other pituitary regulated factors, such as thyroid hormones and glucocorticoids, also regulate the expression of CYP2B1/2 [17,18]. These factors could play a more prominent role in males, thus explaining their weaker GH response.

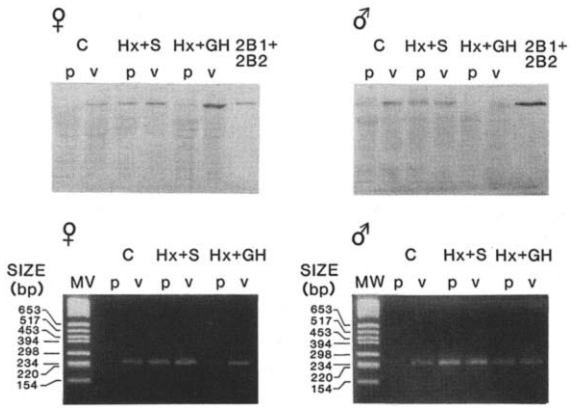

Fig. 2. Effect of hypophysectomy and GH treatment on CYP2B1/2 protein and mRNA in periportal (p) and perivenous (v) cell lysates. For immunoblots (upper panel) 60 μg protein was applied for control (C) and hypophysectomized + GH-treated rats (Hx + GH) and 30 μg for hypophysectomized + saline-treated (Hx + S) rats. For CYP2B1/2 mRNA determination 10 μl of cDNA produced from total RNA from cell lysates was amplified with PCR (lower panel). Boehringer DNA molecular weight marker VI was co-electrophoresed.

Growth hormone could repress gene expression, specifically in the periportal region, by several alternative mechanisms, all of which would be based upon gradients along the portocentral axis in the sinusoid. To our knowledge, there is as yet no information on the steepness of the GH gradient in the liver or on GH receptor zonation in the liver. Such gradients have been reported for EGF and for several hormones, including insulin, glucagon and thyroxine (for a review, see [2]). Interestingly, zonation of the EGF receptor has been described, with high expression of high-affinity receptors in periportal cells [19].

The majority of the P450 enzymes, as well as many other liver enzymes, share the centrilobular expression pattern of the *CYP2B1/2* gene pair. We suggest that GH is involved in the low or absent expression of some of these proteins in the periportal region. In support of this suggestion, we recently observed that hypophysectomy increases the periportal expression of another gene, carbonic anhydrase II, that is normally found mainly in the perivenous region [20] (T. Oinonen and K. Lindros, unpublished). On the other hand, GH probably regulates the expression of other proteins in the opposite way, thus allowing or stimulating their expression in the periportal region.

In conclusion, our observation of the GH-induced repression of CYP2B1/2 isoenzymes in the periportal

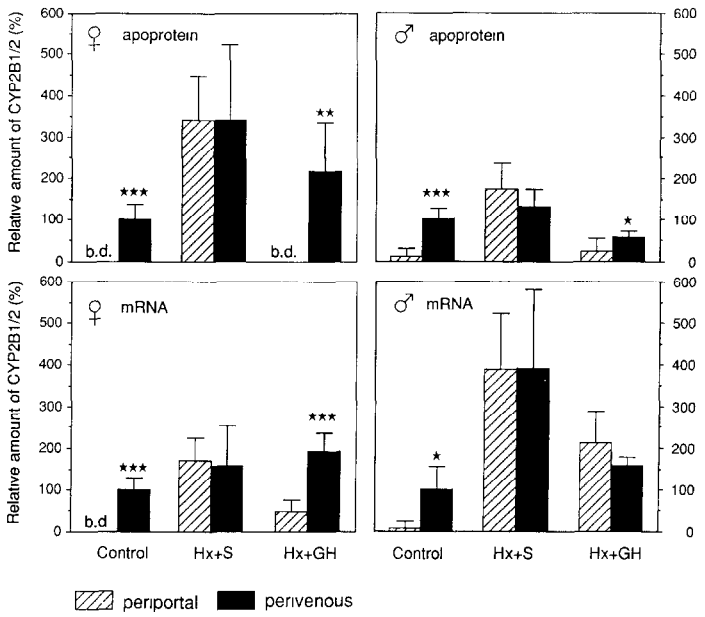

Fig. 3. Videodensitometric quantitation of CYP2B1/2 apoprotein and mRNA. The figures are means ± S.D. from 4 or 5 pairs of periportal (p) and perivenous (v) eluates from control, hypophysectomized (Hx + S) and hypophysectomized GH-treated (Hx + GH) female and male rats. 100% = perivenous control value *, ** and *** = $P < 0.05$, 0.01 and 0.001, respectively, for statistical significance (Student's t-test) of p-v difference within each group. b.d, below detection limit.

region demonstrates that a hormone-mediated signal can be restricted to one zone, either allowing or preventing the expression of a gene product. We suggest that the importance of GH, and probably also of other hormones, in zonated liver gene expression has previously been underestimated.

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