Hormonal regulation of the zonated expression of cytochrome *P*-450 3A in rat liver

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Most cytochrome *P*-450 enzymes are expressed characteristically in a zonated pattern in the liver. The factors responsible for this heterogenous expression are largely unknown. Here we report how growth hormone and tri-iodothyronine regulate the steroid-hydroxylating cytochrome *P*-450 (CYP) 3A forms, which are constitutively expressed mainly in the perivenous (downstream) liver region. By comparing cell lysates obtained from the periportal and perivenous acinar regions we observed that the elevated CYP3A expression observed after hypophysectomy was due mainly to a dramatic increase in the normally silent periportal region. This effect was particularly strong in females. Treatment with growth hormone re-established the perivenous expression pattern, a finding corroborated by immunohistochemical analysis of liver sections. Analysis of periportal and perivenous mRNA by reverse-transcriptase PCR demonstrated that in males the changes in CYP3A2 mRNA paralleled the changes at the protein level. In females, CYP3A2 mRNA was detected only after hypophysectomy, and the zonal protein changes seemed to be governed by changes in CYP3A1 mRNA levels. Treatment of hypophysectomized animals with tri-iodothyronine also suppressed the expression of CYP3A, both in males and females. However, this occurred almost exclusively in the periportal region. This was observed both at the protein level, as determined by immunoblotting and immunohistochemically, and at the CYP3A1 and 3A2 mRNA level. These results indicate that growth hormone and thyroid hormone regulate the expression of *CYP3A* genes zone-specifically by suppressing their transcription in the periportal (upstream) region of the liver.

INTRODUCTION

The factors regulating the expression of the microsomal cytochrome *P*-450 (CYP) system are the subject of intensive study. It is well established that the expression of individual members of the *CYP* gene superfamily can be strongly induced by endogeneous and especially by exogeneous compounds. It is also becoming evident that both the constitutive and the induced expression of *CYP* genes in the liver is heterogenous [1–3]. In fact, the majority of liver genes appear to be expressed in a zonated fashion. This feature seems, however, to be particularly prominent for the *CYP* genes, which are expressed predominantly in the perivenous (downstream) region of the liver acinus (reviewed in [4]). The heterogenous expression of liver genes has been suggested to be maintained by gradients of hormones, substrates, metabolites, or the O_2/CO_2 tension of sinusoidal blood. We recently observed that growth hormone (GH) suppresses the periportal expression of the rat CYP2B subfamily [5]. This suggested that GH and other hormones regulated by the pituitary could mediate the zonated expression of other *CYP* genes as well. In the present study we have investigated the regulation of the expression of the rat *CYP3A* subfamily, which is constitutively expressed almost exclusively in the perivenous region [3] and, like several other members of the *CYP* superfamily, is regulated by pituitary-dependent hormones (reviewed in [6]).

The *CYP3A* genes code the catalytically active testosterone 6β-hydroxylases and are characteristically induced by pregnenolone 16α-carbonitrile, dexamethasone and phenobarbital (reviewed in [7]). Two members of the family have been well characterized, CYP3A1 and 3A2, which share an 89% amino acid sequence identity [8]. CYP3A1 is not expressed [8] or is expressed at only a low level [9,10] in untreated animals. CYP3A2 is a developmentally regulated, male-specific form [8]. Proteins of an allelic variant of CYP3A1 [10] and a novel CYP3A cDNA clone, bearing 97.8% and 98.4% deduced amino acid similarity to the CYP3A1s mentioned above, have also been documented [11]. Protein data indicate the existence of at least one or two additional CYP3A forms [12–14]. The male-specific CYP3A2 form is induced in both sexes by hypophysectomy, and its expression is counteracted by the administration of GH and thyroid hormones [15–18]. CYP3A2 has been suggested to be suppressed in adult female rats by their continuous pituitary GH secretion profile, but its expression is allowed in males by their pulsatile pattern of GH release [16]. GH also appears to suppress the expression of CYP3A1 in both sexes [17].

In the present study we investigated whether the zone-specific hepatic expression of CYP3A is regulated by pituitary-dependent hormones. Consequently, we studied the effect of hypophysectomy and subsequent GH and tri-iodothyronine (T_3) administration. Zonation was investigated by analysis of periportal and perivenous cell lysates obtained by zone-restricted digitonin pulse infusion during *in situ* liver perfusion [19,20]. Cell lysates were analysed for their content of CYP3A apoprotein by immunoblotting and for their content of CYP3A1 and CYP3A2 mRNA by reverse-transcriptase PCR (RT-PCR) analysis [20]. We also studied the zonal distribution of CYP3A apoprotein by immunohistochemistry on liver sections after different treatments. Our results demonstrate that both GH and T_3 suppress CYP3A expression at the pretranslational level mainly in the periportal region.

EXPERIMENTAL

Animals

Hypophysectomized and sham-operated male and female Sprague–Dawley rats were obtained from Møllegaard, Ejby,

Abbreviations used: ALAT, alanine aminotransferase; CYP, cytochrome *P*-450; GH, growth hormone; i.p., intraperitoneally; T_3, tri-iodothyronine; T_4, thyroxine; RT-PCR, reverse-transcriptase PCR; EGF, epidermal growth factor; the nomenclature for cytochrome *P*-450 in [20a] is used.

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Denmark. Rats were hypophysectomized or sham-operated at the age of 5 or 7 weeks. A week later the animals were transferred to our laboratory, and they were allowed to stabilize for 1 week. The animals were fed a commercial R3 laboratory diet (Ewos, Södertälje, Sweden) and water *ad libitum*. The different treatment groups were: (1) controls, sham-hypophysectomized, sham-operated for pump implantation (teflon tubing of the same size as minipump was implanted under the skin on the back) and daily intraperitoneally (i.p.) injected with saline; (2) hypophysectomized animals, sham-operated for pump implantation and daily i.p. injected with saline; (3) hypophysectomized animals treated with human recombinant GH (Norditropin, donated by Nordisk Gentofte A/S, Denmark or Novo Nordisk A/S, Denmark), 0.01 IU of GH/h by continuous infusion and daily i.p. injected with saline; (4) hypophysectomized rats, sham-operated for pump implantation and given T_3 (3,3′,5-tri-iodo-L-thyronine, Sigma Chemical Company, St. Louis, MO, U.S.A.), 50 μg of T_3/kg i.p. daily for 7 days; and (5) hypophysectomized animals treated with both GH and T_3 as described above. The rats received GH by continuous infusion with osmotic minipumps (Alzet 1701, Palo Alto, CA, U.S.A.) implanted subcutaneously on the backs under halothane anaesthesia. The rats received buprenorphin (Temgesic 0.3 mg/ml) at 0.15 mg/kg subcutaneously immediately after implantation surgery. The effect of hypophysectomy and GH treatment was monitored by weighing the rats. The experiments were approved by the local committee for animal experiments.

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 [19]. Briefly, periportal cells were lysed by infusion of 6.7 ml/kg body 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 body wt. digitonin solution via the upper vena cava followed by antegrade flushing. The length of the digitonin pulse, which determines the penetration depth, was determined empirically to allow sufficient 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 zonal origin of the cell lysates was controlled by assaying the periportal marker enzyme, alanine aminotransferase (ALAT, EC 2.6.1.2.) as in [21].

Immunoblot analysis of CYP3A apoprotein

Immunoblotting and quantification of the blots were perfomed as recently described [5]. Polyclonal rabbit antiserum to rat CYP3A, a generous gift from Dr. Magnus Ingelman-Sundberg (Karolinska Institute, Stockholm, Sweden), was used. With this antiserum, a band of approximately 51 kDa was stained in microsomal samples from pregnenolone 16α-carbonitrile-treated rats or in digitonin eluate samples. Occasionally, an additional band of a lower molecular mass was stained, probably because slight overloading of the gels with cell lysate protein (50–100 μg of protein/well) was necessary in order to detect CYP3A protein in most digitonin eluates. The specificity of the antiserum was ascertained with other antibodies to CYP3A: with a monoclonal mouse antibody 2-13-1 [22] and with a commercial polyclonal rabbit antibody to rat CYP3A (Amersham International, Bucks., U.K.). Liver microsomes (0.1 μg) from pregnenolone 16α-carbonitrile-treated rats were run as standards on each gel. Band intensities of cell lysate samples were normalized to the intensity of the co-migrating single band of the standard.

Immunohistochemical localization of CYP3A

Immunohistochemical analysis of CYP3A apoprotein in paraffin-embedded liver sections was performed as previously described [3]. The same polyclonal rabbit antiserum to CYP3A was used as in immunoblotting.

RT-PCR analysis of CYP3A1 and CYP3A2 mRNA

The relative amounts of CYP3A1 and 3A2 mRNA in periportal and perivenous cell lysates were compared by a PCR-based semi-quantitative method as described previously [5,20]. Total RNA was isolated from digitonin cell lysates essentially as in [23]. First-strand cDNA was produced from 2–4 μg of RNA in a 80 μl reaction volume with Promega's Reverse Transcription System (Promega, Madison, WI, U.S.A.) as described in [5]. Oligonucleotide primers for CYP3A1 and 3A2 RT-PCR were chosen on the basis of the published cDNA sequences [8,24] so that they would differentiate between CYP3A1 and 3A2 forms. The antisense primers were common to both sequences, but the sense CYP3A1 and 3A2 primers had five and six mismatches to CYP3A2 and 3A1 sequences, respectively. For CYP3A1 5′-GATGTTGAAATCAATGGTGTGT-3′ and 5′-TTCAGAGG-TATCTGTGTTTCC-3′ primers were used. RT-PCR produced a single fragment of the expected 289 bp size. Aliquots of 2–10 μl of cDNA was amplified in 100 μl reaction volume containing 2 units of *Taq* DNA polymerase, 1 × PCR buffer (both from Boehringer Mannheim GmbH, Mannheim, Germany), 50 pmol of both primers, 0.2 μM each dNTP (Promega) and 2.5 mM $MgCl_2$. An aliquot (100 μl) of mineral oil (Sigma) was added on the top of the reaction mixture. Either 25 or 26 cycles, consisting of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min, were run in an MJ Research thermal controller (MJ Research Inc., Watertown, MA, U.S.A.). The last elongation step was extended to 5 min. RT-PCR with CYP3A2 primers, 5′-AGTAGTGA-CGATTCCAACATAT-3′ and 5′-TCAGAGGTATCTGTGTT-TCCT-3′, amplified a 252 bp fragment. The conditions for CYP3A2 RT-PCR were as for CYP3A1 except that 50 pmol of CYP3A2 primers and a 54 °C annealing temperature were used. The linearity of the PCR amplification was ensured by varying the number of cycles and the amount of cDNA in the reaction. Qualitative and quantitative analysis of amplification products was done by anion-exhange HPLC [25]. The HPLC system consisted of a 712 Wisp autosampler, a 510 two-pump system, a 486 absorbance detector connected to a System Integrator Module and Baseline 810 software (Millipore Waters, Milford, MA, U.S.A.). For separation of PCR products, a PE TSK DEAE-NPR column (3.5 mm × 4.6 mm, 2.5 μm non-porous particles) and a guard column were used (HPLC Column Kit for PCR Analysis from Perkin-Elmer-Cetus, Perkin-Elmer Corporation, Norwalk, CT, U.S.A.). A 10–40 μl sample of the PCR product was injected. The binary mobile phase was composed of A (25 mM Tris/HCl, pH 9.0) and B (A + 1 M NaCl). The following gradient was employed: 0.5 min 25–45 % B in A, 3 min 45–50 % B in A, 11.5 min 50–62 % B in A , 0.5 min 62–100 % B in A, 3.5 min 100 % B, 0.5 min 100–75 % B in A, 4.5 min 25 % B in A. All gradient segments were linear. The column was operated at 1 ml/min and detection was at 260 nm. These conditions ensured separation of PCR product from dNTPs, primers and possible primer-dimers or extra amplification products.

To ascertain that CYP3A1 sense primer differentiated CYP3A1 and 3A2, PCR fragments amplified with CYP3A1 primers from

cDNA from male and female control rat RNA and from RNA from hypophysectomized female rats were sequenced. Sequencing reactions were performed with a TaqDyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) and the CYP3A1 primers described above and analysed with an ABI 373A DNA Sequenator (Applied Biosystems). There were 15 mismatches between the CYP3A1 and the CYP3A2 sequences [8,24] in the region between the CYP3A1 primers. In the PCR fragments produced with CYP3A1 primers, all of these mismatch bases were found to match with the CYP3A1 sequence, confirming that CYP3A2 was not amplified. This does not exclude the possibility that some other CYP3A mRNA could be detected with CYP3A1 primers, like a novel *CYP3A* form [11], since the mRNA sequences are identical within the amplified region.

Statistical analysis

Male and female rats were treated in two series of experiments. Immunoblot and RT-PCR results from different series were combined so that within series all samples were normalized to the mean of the perivenous samples of the controls. Since CYP3A2 mRNA was not detectable in female control samples, the CYP3A2 RT-PCR results in females were normalized to the mean of the perivenous samples from the hypophysectomized group. Student's *t*-test for independent samples was used to test for significant differences between periportal and perivenous cell lysates: *, ** and *** indicate $P < 0.05$, 0.01 and 0.001, respectively. One-way analysis of variance followed by Student–Newman–Keuls test for multiple comparisons were used to test for significant differences of ALAT activities and periportal/perivenous ratio of ALAT activity between the treatment groups.

RESULTS

Collection of periportal and perivenous cell lysates: ALAT activity in cell lysate samples

The acinar origin of the cell lysates was ascertained by assay of the periportal marker enzyme ALAT. In the whole material, the activity of ALAT in the periportal samples was 13.7 times higher (S.D. = 14.3, $n = 78$) than in corresponding perivenous cell lysates, suggesting complete acinar separation of the samples. However, both gender and the hormonal manipulations influenced the activity of ALAT in periportal and perivenous eluates. In the whole material, the acinar gradient of ALAT appeared to be steeper in females (periportal/perivenous ratio = 20.0 ± 17.8, $n = 39$) than in males (7.6 ± 5.1, $n = 39$, $P < 0.001$), as a result of much lower ALAT activities in perivenous samples from female rats than in respective samples from males (Table 1). In male rats, hypophysectomy increased the ALAT activity, more so in the perivenous (+170%) region than in the periportal region (+50%), resulting in a significant ($P < 0.05$) lowering of the periportal/perivenous ALAT ratio from 9.8 ± 7.2 to 4.0 ± 1.3. Both GH and and combination of GH and T_3 at least partly counteracted these effects, indicating that the changed ALAT zonation pattern was a consequence of the hormonal manipulations on the acinar level and distribution of ALAT. In females the hormonal manipulations appeared to affect the ALAT zonation less: the periportal/perivenous ratio was lowered by hypophysectomy, but this effect was not statistically significant and the effects of subsequent treatment with GH and T_3 were also less pronounced than in males. These data demonstrate that hormonal factors influence both the total activity, and the zonation of ALAT. The effect of hypophysectomy on the zonation of ALAT was undetected in a previous study by Tosh et al. [26], based on comparison of ALAT activities between periportal and perivenous cell populations isolated from hypophysectomized rats.

Immunoblotting and immunohistochemistry

Immunoblot analysis of periportal and perivenous cell lysates revealed that manipulation of the hormonal status had major effects on the acinar distribution of CYP3A apoprotein, in both male and female rats (Figure 1). In control males the content of CYP3A apoprotein in perivenous cell lysates was approximately five times higher than in the corresponding periportal samples, reflecting the previously observed predominantly perivenous distribution pattern of CYP3A [3]. Hypophysectomy increased the cellular content of CYP3A, but mainly in the periportal region, so that the difference between periportal and perivenous lysates almost disappeared. Subsequent GH treatment by continuous infusion for 7 days resulted in markedly reduced CYP3A levels. GH reduced CYP3A by 90% in periportal and by 80% in perivenous eluates thus re-establishing a significantly higher ($P < 0.05$) amount of CYP3A in the perivenous samples. In contrast, when hypophysectomized male rats were treated with T_3 (50 μg/kg, i.p. daily for 7 days), CYP3A was dramatically reduced exclusively in the periportal samples (to 7%) while perivenous CYP3A was practically unchanged. Treatment of hypophysectomized males with both hormones resulted in an almost complete suppression, leaving only trace amounts of CYP3A in a few eluates.

Table 1 The effect of hormonal manipulations on the zonation of liver alanine aminotransferase (ALAT) activity

The values are means ± S.D. of ALAT activity (nmol/min per mg of protein) in periportal (pp) and perivenous (pv) cell lysates from (A) control rats, (B) hypophysectomized (Hx), (C) GH-treated (Hx + GH), (D) T_3-treated (Hx + T_3), and (E) animals treated with both hormones (Hx + GH + T_3). GH was given by continuous infusion and T_3 by i.p. injections, as described in the Experimental section. The two bottom rows give the periportal/perivenous ratios of ALAT activity. The number of animals in each treatment group is given in parentheses. The superscripts ^{a,b,c,d} and ^{e} denote significancies ($P < 0.05$) of differencies between the corresponding treatment groups (A, B, C, D and E).

Sex	Acinar origin	(A) Control ($n = 10$)	(B) Hx ($n = 9$)	(C) Hx + GH ($n = 10$)	(D) Hx + T_3 ($n = 5$)	(E) Hx + GH + T_3 ($n = 5$)
Male	pp	$1077 \pm 227^{b,e}$	$1618 \pm 657^{a,c,d,e}$	$1141 \pm 332^{b,e}$	831 ± 299^{b}	$536 \pm 61^{a,b,c}$
	pv	$153 \pm 90^{b,d}$	$412 \pm 140^{a,c,d,e}$	$129 \pm 47^{b,d}$	$306 \pm 82^{a,b,c,e}$	$60 \pm 14^{b,d}$
Female	pp	1056 ± 214^{c}	$1279 \pm 385^{c,e}$	$808 \pm 284^{b,d,e}$	$1410 \pm 211^{c,e}$	$419 \pm 105^{a,b,c,d}$
	pv	46 ± 31^{d}	114 ± 83	97 ± 61	149 ± 99^{a}	44 ± 49
Male	pp/pv	$9.8 \pm 7.2^{b,d}$	$4.0 \pm 1.3^{a,c,e}$	$10.0 \pm 3.4^{b,d}$	$2.8 \pm 1.1^{a,c,e}$	$9.3 \pm 2.0^{b,d}$
Female	pp/pv	32.4 ± 24.2	18.8 ± 15.5	12.3 ± 7.9	12.9 ± 8.7	19.8 ± 20.0

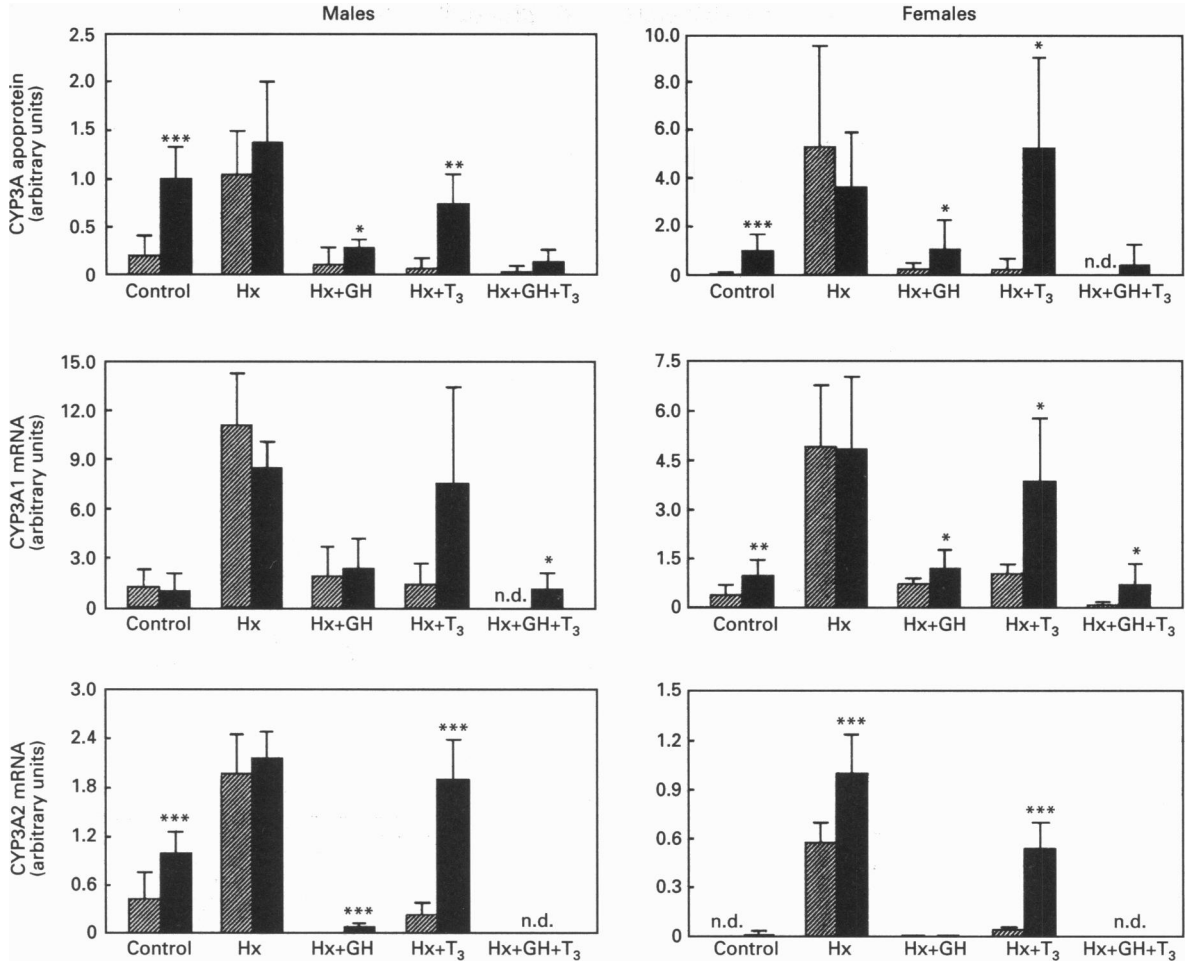

Figure 1 CYP3A apoprotein, CYP3A1 mRNA, and CYP3A2 mRNA in periportal (▨) and perivenous (■) cell lysates from livers of male and female rats after various pituitary hormone manipulations

Videodensitometric quantification of immunoblots and HPLC quantification of RT-PCR are presented. The Figures are means ± S.D. from periportal and perivenous eluates from control ($n = 10$), hypophysectomized (Hx, $n = 8$ or 9), hypophysectomized GH-treated (Hx + GH, $n = 9$ or 10), hypophysectomized T_3-treated (Hx + T_3, $n = 5$) and hypophysectomized GH- and T_3-treated (Hx + GH + T_3, $n = 5$) rats. GH was given by continuous infusion and T_3 by i.p. injections, as described in the Experimental section. Significant differences between periportal and perivenous cell lysates within treatment groups: *, ** and *** indicate $P < 0.05$, 0.01 and 0.001, respectively. Abbreviation: n.d., not detected.

Perivenous digitonin eluate samples from female rats showed immunoreactivity in CYP3A immunoblots while CYP3A was not detectable in almost all periportal samples. The relative increase of CYP3A after hypophysectomy was much stronger in females than in males. In periportal samples this increase was more than 50-fold, while in perivenous samples only a 4-fold increase was observed. This resulted in a complete disappearance of the zonation of CYP3A. The female-type continuous GH treatment almost extinguished the periportal expression, but only reduced the perivenous CYP3A level moderately, resulting in a CYP3A expression pattern resembling that seen in control females. The effect of T_3 treatment was even more zone-selective than in males: in periportal samples CYP3A was suppressed to nearly undetectable levels, whereas the perivenous CYP3A levels remained unaffected. As found in hypophysectomized males, simultaneous treatment of females with both hormones almost completely suppressed the expression of CYP3A apoprotein in both regions.

The effect of the hormonal manipulations on the zonal distribution of CYP3A was also investigated immunohistochemically (Figure 2). In untreated animals, only cells in the perivenous zone were stained with CYP3A antiserum, the expression being even more perivenously restricted in females than in males. Hypophysectomy increased staining across the liver acinus and extended it to the periportal region but the most intense staining was still observed in the perivenous regions. Similar to what was observed by analysis of cell lysates, GH treatment strongly reduced CYP3A apoprotein, restoring the perivenous expression pattern in both sexes. The effect of T_3 treatment was striking and confirmed the cell lysate observations. In both sexes heavy staining was seen exclusively in a layer one to three cells thick surrounding the terminal hepatic venules. In sections from hypophysectomized rats treated with both hormones practically no CYP3A staining was observed, indicating a very strong suppression of CYP3A expression.

RT-PCR

To determine whether the hormonal effects on the zonation of CYP3A expression are pretranslational and to identify the

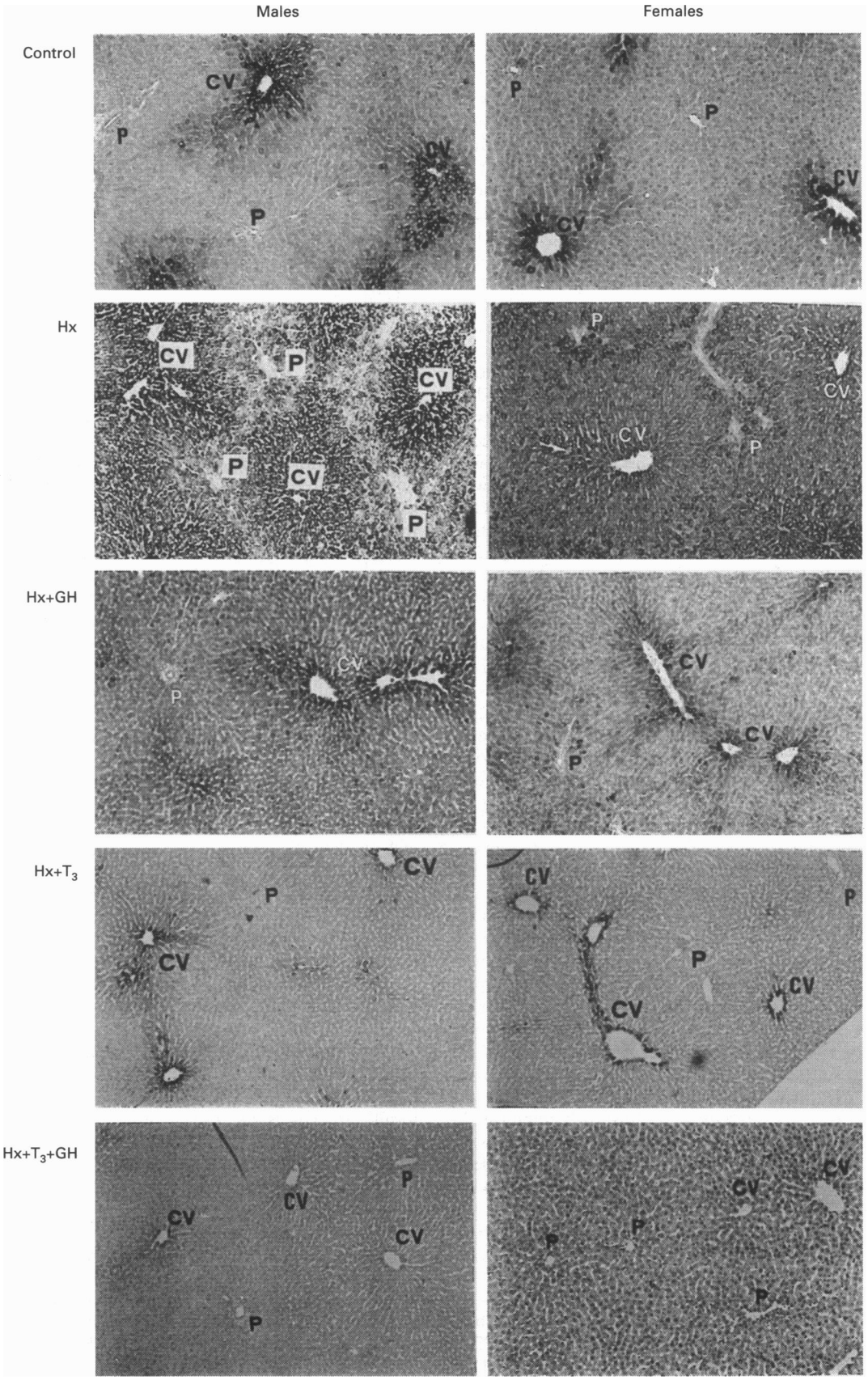

Figure 2 Immunohistochemical localization of CYP3A apoprotein in liver sections, from variously treated male and female rats

Abbreviations: Hx, hypophysectomized; Hx + GH, hypophysectomized, GH-treated; Hx + T_3, hypophysectomized, T_3-treated; Hx + GH + T_3; hypophysectomized, GH- and T_3-treated. GH was given by continuous infusion and T_3 by i.p. injections, as described in the Experimental section. Terminal central venules (CV) and portal venules (P) are indicated in the photographs.

CYP3A forms involved, we analysed the cell lysate CYP3A1 and 3A2 mRNA by RT-PCR. In most cases, analysis of cell lysate mRNAs convincingly demonstrated that changes both in total CYP3A apoprotein and in its zonation were preceded by corresponding CYP3A mRNA changes. In addition, analysis of the relative changes of CYP3A1 and 3A2 mRNA revealed their respective roles in CYP3A apoprotein expression. In control male rats, the amount of CYP3A1 mRNA was rather similar in periportal and perivenous cell lysates while markedly more CYP3A2 mRNA was detected in perivenous samples (Figure 1). Thus the CYP3A2 mRNA distribution correlated with the CYP3A apoprotein distribution and this supports previous studies indicating that CYP3A2 would be the main constitutive CYP3A form in males [8,17]. Hypophysectomy resulted in increased levels of both CYP3A2 and CYP3A1 mRNAs, but the relative increase of CYP3A1 mRNA was more prominent. Periportal CYP3A2 mRNA increased more than the perivenous one, and the portocentral gradient of CYP3A2 mRNA disappeared. After administration of GH, CYP3A1 mRNA was reduced nearly to the control level. GH treatment practically abolished CYP3A2 mRNA expression in periportal samples and reduced it to a very low level in perivenous cell lysates. This was probably a result of the female-type continuous GH administration, which is thought to prevent the constitutive CYP3A2 expression in mature female rats [18]. The zone-specific suppression of CYP3A apoprotein by T_3 was also observed at the mRNA level. In periportal cells, both CYP3A1 and 3A2 mRNAs were reduced while the corresponding perivenous mRNA levels were unaffected. When hypophysectomized males were treated with both GH and T_3, no CYP3A2 mRNA could be detected and CYP3A1 mRNA was only detected at low levels in perivenous samples.

No CYP3A2 mRNA was detected in untreated female rats, with the exception of a negligible amplification in one perivenous sample (Figure 1). CYP3A1 mRNA was found in samples from both regions. However, in contrast to the males, the amount of CYP3A1 mRNA in perivenous samples was found to be significantly higher than in the corresponding periportal samples. The absence of CYP3A2 mRNA suggests that the CYP3A apoprotein detected by immunoblotting and by immunohistochemistry is CYP3A1. Hypophysectomy resulted in a marked increase of CYP3A1 and 3A2 mRNA in periportal as well as in perivenous cell lysates. The perivenous predominance of CYP3A1 mRNA seen in controls was abolished after hypophysectomy, while the amount of CYP3A2 mRNA was higher in perivenous than in periportal samples. The female-type continuous GH treatment completely counteracted the effect of hypophysectomy and suppressed CYP3A1 and 3A2 mRNA to control levels. T_3 treatment had a similar zone-specific effect as in males: periportal CYP3A mRNAs were drastically diminished, while the perivenous levels were not significantly changed. As in males, treatment with both hormones resulted in a complete disappearance of CYP3A2 mRNA and in very low levels of CYP3A1 mRNA, especially in periportal samples.

DISCUSSION

The present study shows that both GH and thyroid hormone are important factors mediating the zonated expression of CYP3A in the liver. Major shifts in the zonation pattern were seen after manipulating the circulating levels of these pituitary-dependent hormones. Parallel changes in the zonation were seen at the apoprotein and mRNA levels, demonstrating that the hormone-mediated signals act pretranslationally.

In untreated animals CYP3A was expressed almost exclusively in the perivenous liver region; the absence of CYP3A in the periportal region was particularly clear in female rats. In agreement with previous reports [15–18] we observed an increased CYP3A expression after hypophysectomy. However, here we demonstrate that this increase was due mainly to a dramatically increased expression in the normally nearly silent periportal region. Consequently, the characteristic acinar expression gradient was abolished. Since this increase in the periportal expression was counteracted and the normal acinar expression pattern re-established by treatment with GH, we advocate a central role for this hormone in suppressing constitutive CYP3A expression in the upstream periportal part of the acinus. This zone-directed GH effect was particularly pronounced in females, most probably because the GH administration from the minipumps was continuous and thus like the female-type GH secretion pattern. The zonal changes after manipulation of circulating GH levels were similar to those recently observed for CYP2B1/2 and suggests that the zonated expression of these genes is regulated by similar GH-sensitive *cis*- or *trans*-acting factors. It is of interest that after treatment with a large dose of phenobarbital the expression of both CYP2B1/2 and CYP3A spreads to the periportal region [3,27], suggesting that phenobarbital could act by antagonizing the GH-mediated suppression.

Treatment of hypophysectomized rats with T_3 also suppressed CYP3A expression but did not restore the normal zonated expression pattern as well as GH. Contrary to GH, while T_3 practically abolished the expression in the periportal region, it hardly affected the perivenous expression at all. Immunohistochemically, the effect of T_3 was seen as a strong staining exlusively in a thin rim of cells around the terminal hepatic venules, resembling the expression pattern of glutamine synthetase [28]. Although the daily T_3 dose was supraphysiological (50 μg/kg i.p.) most of it will rapidly bind to serum proteins and, as demonstrated by others [29], administration of even larger doses of T_3 causes only an approximately 3-fold increase in free T_3. This suggests that normal circulating T_3 levels will regulate the zonation of CYP3A. Our preliminary studies indicate that, contrary to GH, T_3 does not cause a periportal suppression of CYP2B1/2 similar to that observed for CYP3A (T. Oinonen and K. O. Lindros, unpublished work). This could indicate that, in the case of T_3, different transcription factors regulate transcription of *CYP3A* and *CYP2B* genes. The suppression of CYP3A by GH and T_3 seems to be additive since the combined hormone treatment caused stronger suppression than either hormone alone.

Contrary to previous reports [8,17], we observed low but definitive expression of CYP3A in the female rat liver since CYP3A apoprotein and CYP3A1 mRNA were detected. Our results are consistent with more recent studies on CYP3A1 mRNA [10] and CYP3A protein [30]. CYP3A mRNA analysis suggests that the CYP3A apoprotein in control female rats is CYP3A1 or, alternatively, a closely related CYP3A form.

Very little is known at present about how a hormone such as GH or T_3 exerts its zone-specific suppression. It is assumed that hormonal and other blood-borne signals would act zone-specifically as a consequence of their own gradients established along the sinusoidal axis. This presumes efficient extraction of the hormone by the liver. Such gradients have been observed to exist for insulin, glucagon and catecholamines (see [4] for a review). Experiments with perfused liver indicate that while free thyroxine (T_4) is rapidly extracted in the upstream region, T_4 bound to serum proteins is taken up evenly along the hepatic acinus [31]. Serum proteins that bind hormones obviously will dampen or even prevent the establishment of functional hormone

gradients in the liver, but the extent of this buffering capacity will be hormone-specific. Both GH and T_3 could exert their zonal actions via sufficiently steep uptake gradients but there is, to our knowledge, no reliable information on such a functional gradient for either GH or T_3. The regional hormone action could also be exerted via regional expression of the corresponding receptor. Again, there is as yet no reliable data on the acinar distribution of the GH or T_3 receptors nor on the function or zonation of intracellular GH-binding protein [32], an alternatively spliced form of the GH receptor [33]. The periportal expression of the high-affinity epidermal growth factor (EGF) receptor has, however, been described [34] and, interestingly, the expression of this receptor is positively regulated by GH [35]. Thus GH could regulate the zonation of the EGF receptor, too. It is also feasible that neither hormone nor receptor gradients are crucial for the zonated expression, but that regionally expressed *trans*-acting factors operating in a gene-specific manner are involved. For example, CYP2E1 expression is zonated [21], induced by hypophysectomy and suppressed by subsequent administration of GH [36]. However, contrary to CYP2B1/2 and CYP3A, the distribution of CYP2E1 does not seem to change markedly by these treatments (T. Oinonen and K. O. Lindros, unpublished work). This indicates that suppression of *CYP* genes by GH is not necessarily zone-specific and demonstrates the variety of mechanisms involved in the regulation of the expression of different *CYP* genes.

Taken together, the present results and our previous study [5] suggest that GH down-regulates the periportal expression of CYP2B1/2 and CYP3A in the liver and that thyroid hormone also suppresses the expression of CYP3A in the periportal region but, probably not that of CYP2B1/2. Our data on the distribution of ALAT serves as an example of the zonation of another protein that also is hormonally influenced, although less dramatic shifts in the zonation pattern were observed than for CYP3A. On the basis of these observations, we suggest that GH and thyroid hormones play important roles in the zonated expression also of other *CYP* and non-*CYP* genes in the liver.

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