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PRETRANSLATIONAL INDUCTION OF CYTOCHROME P4501A ENZYMES BY β-NAPHTHOFLAVONE AND 3-METHYLCHOLANTHRENE OCCURS IN DIFFERENT LIVER ZONES

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Abstract—Most of the cytochrome P450 (*CYP*) genes are expressed in an uneven, zonated pattern in the liver. Factors regulating this regionally restricted expression are not well known. In this study we have analysed cell lysates obtained from opposite zones of rat liver by infusing digitonin to the perfused liver to study the zonation of CYP1A1 and CYP1A2 induction. 3-Methylcholanthrene induced CYP1A1 protein in perivenous cells, while a low dose of β-naphthoflavone caused periportal induction. Analysis of CYP1A1 mRNA from cell lysates by reverse transcriptase-coupled polymerase chain reaction (RT-PCR) and *in situ* hybridization experiments both demonstrated that this inducer-specific differently localized effect occurred at the pretranslational level. A corresponding difference in the regional pattern of CYP1A2 induction was seen: induction by β-naphthoflavone reversed the constitutive perivenous pattern into a periportal CYP1A2 mRNA pattern while induction after 3-methylcholanthrene treatment was more panacinar. Attempts to identify the regiospecific factors involved were made by comparing the *in vitro* induction of CYP1A1 by β-naphthoflavone and 3-methylcholanthrene in hepatocytes isolated from the periportal and perivenous region. However, after isolation, induction seemed to be independent of the source of the cells. Our results demonstrate the existence in the liver of regionally acting factors that mediate the induction of CYP1A1 and 1A2 in a local and inducer-specific fashion. These factors could be Ah receptor associated binding proteins operating *in vivo*, but no longer in isolated cells.

Key words: rat liver P450 expression; regional CYP1A induction; 3-methylcholanthrene; β-naphthoflavone; perivenous hepatocytes; Ah receptor

The expression of many liver genes is not uniform, but exhibits a characteristic zonated pattern [see Ref. 1 for a review]. The cytochrome P450 (*CYP*)‡ gene family appears to be particularly unevenly expressed. Most of the *CYP* genes are constitutively expressed and induced mainly in the perivenous region (zone 3) of the liver acinus [2–5]. Knowledge of the factors involved in these processes will increase our understanding of hepatic *CYP* gene expression in general and enlighten why many hepatotoxins activated by specific CYP forms cause regiospecific damage in the liver.

CYP1A1 and CYP1A2 enzymes are induced after exposure to polycyclic hydrocarbons and dioxins [6] and the induced CYP1A1 form may be associated with the development of certain forms of human cancer [7, 8]. Immunohistochemical analysis has shown that in rat liver the constitutive expression of CYP1A forms, mainly CYP1A2, is restricted to hepatocytes close to terminal hepatic venules [9]. *In situ* hybridization studies suggest that in human liver the CYP1A1 and 1A2 forms are also perivenously expressed [10]. In the rat, induction of CYP1A1 by several typical inducers was found to take place mainly in the perivenous region, but was more diffuse after larger doses [5, 11].

The present study was initiated by an observation suggesting that induction of rat liver CYP1A1 by β-naphthoflavone exceptionally occurred in the periportal region, while induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or Arochlor 1254 was observed to take place in the perivenous region [5], where induction of other CYP forms normally occurs [4]. This prompted us to further investigate the zonated expression of the CYP1A subfamily at the pretranslational level. In addition to *in situ* hybridization to CYP1A1 mRNA, we analysed periportal and perivenous cell lysates which we obtained after zone-specific digitonin infusion during *in situ* perfusion [12] for CYP1A1 and CYP1A2 mRNA by RT-PCR. Our results demonstrate that there are inducer-specific and locally acting factors that regulate the expression of *CYP1A1* and *1A2* pretranslationally, resulting in differential zonated expression patterns in the intact animal. However, this difference was not retained in hepatocyte populations isolated from the periportal and perivenous region, suggesting that the inducer- and zone-specific factors were lost after cell isolation.

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‡ Abbreviations: CYP, cytochrome P450, nomenclature as proposed in Nebert *et al.* in *DNA Cell Biol* **10**: 1–14, 1990; Ah receptor, aryl hydrocarbon receptor; RT-PCR, reverse transcriptase-coupled polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing from 190 to 250 g and fed standard R3 diet (Ewos AB, Södertälje, Sweden) were used. The animals received 15 or 100 mg/kg β-naphthoflavone (Aldrich Chemical Co. Inc., Milwaukee, WI, U.S.A.), 5 or 25 mg/kg 3-methylcholanthrene (Sigma Chemical Co., St Louis, MO, U.S.A.) or vehicle only (corn oil, 5 mL/kg), by i.p. injection once a day for three or four consecutive days. The experiments were approved by the local committee for animal experiments.

Immunohistochemistry. Immunohistochemical localization of CYP1A1 in paraffin embedded liver sections with rabbit antibodies against rat CYP1A1 (Oxygen, DA, U.S.A.) was performed as previously described [4]. These antibodies showed no reactivity to liver sections from control rats in immunohistochemistry or in immunoblotting to microsomes, digitonin cell lysates or cell homogenates from control rats, even with high protein loading. After treatment with 3-methylcholanthrene or β-naphthoflavone, a single band of approximately 55 kDa was detected in cell lysates by immunoblotting whereas in microsomes from TCDD-treated rat, an additional band of slightly lower molecular weight, most probably CYP1A2, was faintly stained. We therefore conclude that in the present experiments the antibodies react mainly with CYP1A1 form.

In situ *hybridization.* The protocol described by Traber *et al.* [13] was followed with some modifications. Tissue was embedded in OCT compound (Miles Laboratories, Elkhart, IN, U.S.A.) and frozen in liquid nitrogen. Blocks were cryostat sectioned and the sections placed on slides precoated with 3-aminopropyltriethoxysilane (Sigma) and baked at 250° for 4 hr to destroy ribonucleases. Two serial 8 μm thick sections were placed on each slide for hybridization with test (antisense) and control (sense) probes. The sections were air dried and fixed by immersion in 4% paraformaldehyde in PBS for 5 min and in ice-cold ethanol for 10 min. The fixed slides were hydrated through decreasing concentrations of ethanol (70%, 50%), acetylated with 0.2% acetic anhydride in 0.1 M triethanolamine, pH 8.0, dehydrated through increasing concentrations of ethanol and finally air dried.

To make hybridization probes, the PCR primers described in [14] were used to amplify a 341 bp long fragment of the *CYP1A1* gene from rat liver cDNA. This fragment contains sequences from exons 6 and 7. The fragment was subcloned into the SmaI site of the pGEM-4Z plasmid (Promega, Madison, WI, U.S.A.). The single-stranded RNA probes were synthesized by using the Riboprobe Gemini system II kit (Promega) according to the protocol recommended by the supplier.

For the antisense probe the plasmid was linearized with BamHI restriction enzyme and a 344 bp ^{35}S-labeled single-stranded RNA probe was synthesized using SP6 polymerase. For the sense probe to be used as control, the plasmid was linearized with SacI and a 349 bp ^{35}S-labeled single-stranded RNA probe was synthesized using T7 polymerase. After DNase I treatment and ethanol precipitation the probe was resuspended in solution containing 50% formamide and 10 mM dithiothreitol (DTT).

The sections were hybridized with 1×10^6 cpm/μL of ^{35}S-labeled RNA probe in hybridization buffer (30 μL) composed of 50% deionized formamide, 10% dextransulfate, 0.3 M NaCl, 10 mM Tris/10 mM $NaPO_4$ buffer (pH 6.8), 5 mM EDTA (pH 8.0), 0.02% Ficoll 400, 0.02% BSA fraction 5, 0.02% polyvinylpyrollidone, 10 mM DTT, 1 mg/mL yeast tRNA and 0.1 U/μL RNasin. Prior to addition to the hybridization mixture the probe was boiled for 1 min. The sections were covered with parafilm and the slides were incubated for 16 hr at 63° under humid conditions. Slides serving as negative controls were treated with RNase A (100 μg/mL PBS) for 30 min immediately before acetylation. All the solutions used in fixation and hybridization mixture were treated with 0.1% diethyl pyrocarbonate (DEPC).

After hybridization the parafilm was removed and the slides were washed with gentle agitation in the following solutions: (1) 50% formamide, 2 × SSC at room temperature, 10 min; (2) 2 × SSC at room temperature, 15 min; (3) RNase A (20 μg/mL) in 0.5 M NaCl, 1 mM EDTA at 37°, 30 min to remove unhybridized probe; (4) 0.5 M NaCl, 1 mM EDTA at 37°, 30 min; (5) 2 × SSC, 10 mM β-mercaptoethanol, 55°, 30 min; (6) 0.1 × SSC, 10 mM β-mercaptoethanol, 55° for 30 min, twice. After washing the slides were dehydrated through increasing concentrations of ethanol, dipped in warmed (40–50°) liquid emulsion, Kodak NTB2 (Eastman Kodak Co., Rochester, NY, U.S.A.) diluted 1:2 with distilled water, and exposed in the dark at 4° for 14 days. The slides were developed with Kodak D-19 developer at 14° for 4 min, fixed with Agefix (Agfa-Gevaert, Leverkusen, Germany) at 19° for 5 min, washed in distilled water and counterstained with hematoxylin and eosin. The sections were photographed both in bright-field and dark-field illumination.

Collection of periportal and perivenous cell lysates. Digitonin cell lysates from the periportal and perivenous region were obtained by the dual pulsing principle [15] as modified in [12] and samples for analysis of protein and mRNA treated as recently described [16]. Briefly, periportal cells were lysed by infusion of 6.7 mL/kg body weight 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 weight digitonin solution via the upper vena cava followed by antegrade flushing. The length of the digitonin pulse, which determines the penetration depth, was empirically determined to allow the digitonin to lyse approximately one-third of the cells along the plate in either the proximal or distal part of the sinusoid. The terms periportal and perivenous are therefore arbitrarily used and refer to this model. The zone-specificity of the lysates was verified by assay of alanine aminotransferase as in [17].

Isolation and culture of periportal and perivenous hepatocytes. Intact hepatocytes were isolated from either the periportal or the perivenous region of the liver acinus by using zone-specific destruction with

digitonin infusion as described above, followed by collagenase perfusion and isolation of cells from the intact acinar regions as described in detail before [18, 19]. The washed hepatocyte fractions were plated as a monolayer at a density of 1.4×10^5 cells/cm^2 on plastic dishes (Nunc, Roskilde, Denmark) using MEM medium (Gibco, Paisley, U.K.) supplemented with nystatin (1 μg/mL), gentamicin (50 μg/mL), insulin (80 ng/mL), glucagon (1.8 ng/mL), dexamethasone (0.1 μM), 5% fetal calf serum and 5% newborn calf serum. Medium was changed 4 and 28 hr after plating. 3-methylcholanthrene (0.037 or 3.7 μM), β-naphthoflavone (0.1 or 10 μM), or dimethylsulfoxide as vehicle (final concentration 0.1%), was added at 4 hr after plating and with fresh medium. The cells were removed after 48 hr of culture.

Immunoblotting. The analysis of CYP1A1 apoprotein in periportal and perivenous cell samples was performed by immunoblotting. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was run by the method of Laemmli [20] in 8.7% gels. Proteins were electroblotted on nitrocellulose filters, blocked in 5% milk and probed with rabbit anti rat CYP1A1 immunoglobulins (Oxygen). The filters were stained with the ProtoBlot AP system (Promega) and staining quantified with videodensitometer (Ultraviolet Products, Cambridge, U.K.).

RT-PCR. The relative amount of CYP1A1 and 1A2 mRNA in cell lysates was analysed by semi-quantitative RT-PCR as described in [12, 16]. Total RNA was isolated [21], concentration and purity were determined spectrophotometrically (A_{260} and nucleic acid/protein ratio A_{260}/A_{280}) and integrity ascertained by electrophoresis in formaldehyde denatured agarose gels. 0.5 or 1 μg of total RNA was reverse transcribed using Promega's reverse transcription system and random hexanucleotide primers. The sequences of oligonucleotide primers for rat CYP1A1 PCR were from [14]. The PCR reaction conditions were slightly modified: 10 μL of cDNA was amplified in 100 μL reaction volume containing 100 pmol of both primers, 2 U Taq DNA polymerase, 1 × PCR reaction buffer (both from Promega), 3 mM $MgCl_2$ and 0.2 mM of each deoxynucleotide triphosphate (Promega). Oligonucleotide primers for CYP1A2 PCR were from exons 3 and 6 and specific to CYP1A2. PCR amplification produced an expected 302 bp fragment. 1–10 μL of cDNA was amplified in 100 μL reaction volume containing 2 U Taq DNA polymerase, 1 × PCR reaction buffer (both from Promega), 100 pmol of each primer, 1 mM $MgCl_2$, and 0.2 mM of each deoxynucleotide triphosphate. After heating the reaction mixture for 4 min at 93°, 26 cycles, each consisting of 1 min at 93°, 1 min at 55° and 1 min at 72°, were performed. The last extension reaction was continued for 5 min.

CYP1A1 primers:
5′-CCA TGA CCA CCA ACT ATG GG-3′, sense
5′-TCT GGT GAGCAT CCA GGA CA-3′, antisense.
CYP1A2 primers:
5′-CAGTCA CAA CAG CCA TCT TC-3′, sense
5′-CCA CTG CTT CTC ATC ATG GT-3′, antisense.

PCR products were electrophoresed in 4% NuSieve GTG agarose gels (FMC BioProducts, Rockland, ME, U.S.A.). A Boehringer DNA molecular weight marker VI (containing fragments of 154, 220, 234, 298, 394, 453, 517, 653, 1033, 1230, 1766 and 2176 bp) was co-electrophoresed. Gels were ethidium bromide stained, video photographed on ultraviolet transilluminator and scanned like the immunoblots. The linearity of amplification was validated by varying the amount of cDNA in PCR and RNA in reverse transcription reaction and the number of cycles. The isolated periportal and perivenous RNAs from the same treatment group were always reverse transcribed and the cDNA PCR amplified together to allow semi-quantitative comparison.

RESULTS

Collection of periportal and perivenous cell lysates

The zonal origin of digitonin cell lysates was evaluated by comparing the activity of ALAT (a periportal marker) in corresponding periportal and perivenous lysates. In this study the activity of ALAT in periportal samples was 12.2 ± 5.4 (mean ± SD) times higher than in perivenous samples, which agrees with previous investigations [17] and indicates complete zone selectivity of the cell lysates.

Zonal distribution of CYP1A1 apoprotein

Polyclonal rabbit immunoglobulins to rat CYP1A1 were used for immunoblotting and for immunohistochemical analysis of CYP1A1 apoprotein distribution. These immunoglobulins did not react with eluates or microsomal fraction from untreated normal rats or with liver sections from rats treated with corn oil only (results not shown). After treatment with inducer (15 or 100 mg/kg β-naphthoflavone, or 5 or 25 mg/kg 3-methylcholanthrene) for 3 or 4 days, CYP1A1 induction at the apoprotein level was clearly visible in liver sections by immunohistochemistry (Fig. 1A and B) and in periportal and perivenous cell lysates by immunoblotting (Figs 2 and 3). The zonal distribution of CYP1A1 apoprotein was markedly different after induction with β-naphthoflavone compared to that with 3-methylcholanthrene. Treatment with the low dose of β-naphthoflavone (15 mg/kg) resulted in induction of CYP1A1 in hepatocytes surrounding terminal portal venules (Fig. 1A). In contrast, treatment with the low dose of 3-methylcholanthrene (5 mg/kg) induced CYP1A1 in cells surrounding the hepatic venules (Fig. 1B). A similar reciprocal distribution of induced CYP1A1 apoprotein was seen when periportal and perivenous cell lysates obtained by *in situ* digitonin perfusion were analysed by immunoblotting (Figs 2 and 3). After the low dose of 3-methylcholanthrene, perivenous lysates contained approximately four times more CYP1A1 than periportal lysates. Treatment with the low dose of β-naphthoflavone resulted in the opposite distribution: the content of CYP1A1 protein was significantly higher in periportal than in perivenous lysates.

After rats were treated with high doses of the inducers the zonation disappeared or was less

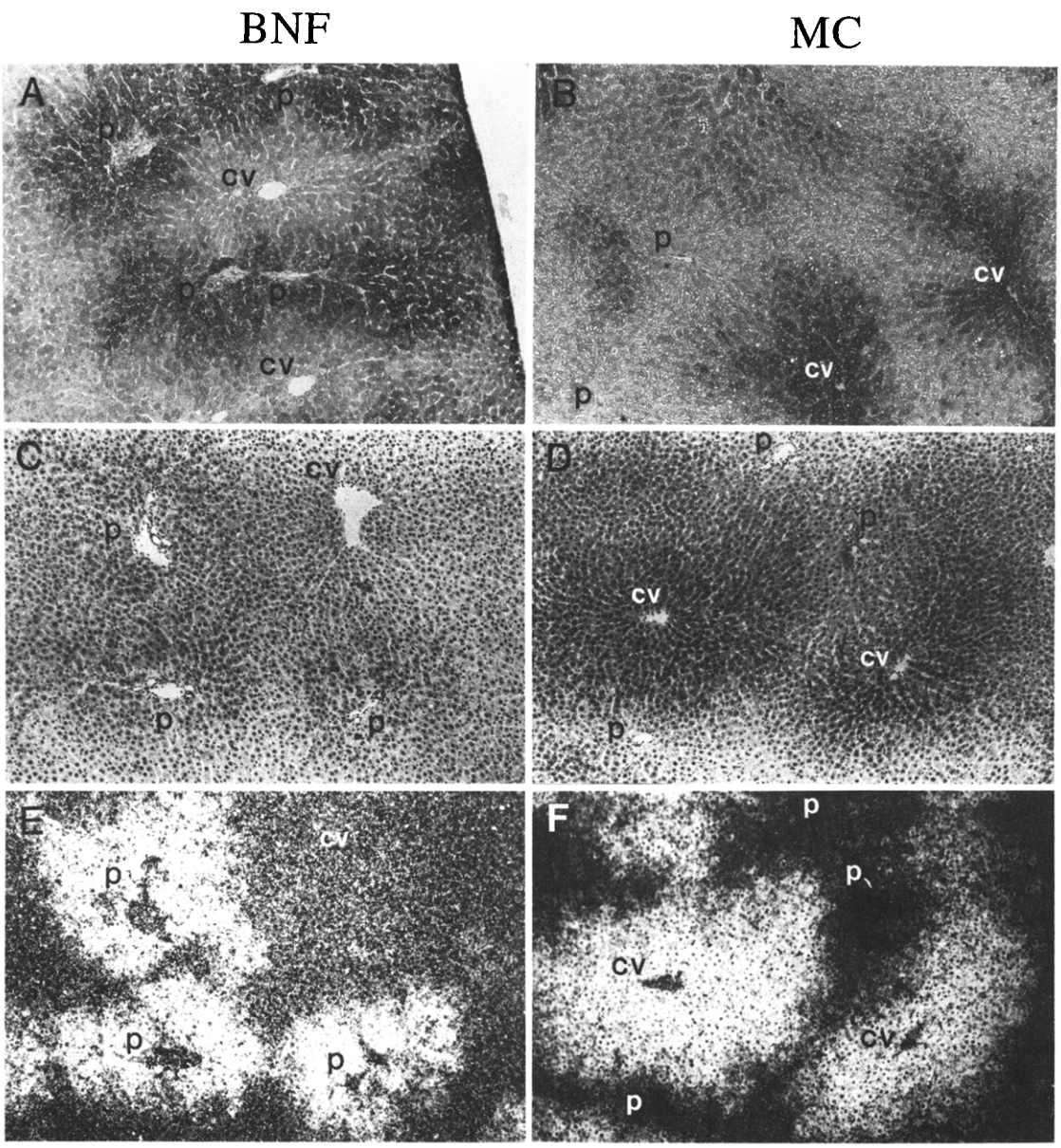

Fig. 1. Localization of CYP1A1 apoprotein by immunohistochemistry and mRNA by *in situ* hybridization in liver sections after treatment with 15 mg/kg β-naphthoflavone (BNF) or 5 mg/kg 3-methylcholanthrene (3MC). Terminal central venules (CV) and portal venules (P) are indicated in each photograph. A and B are immunohistochemical stainings of CYP1A1 after treatment with β-naphthoflavone or 3-methylcholanthrene, respectively. C and E are *in situ* hybridizations of β-naphthoflavone treated rats with the test (antisense) probe in bright-field (C) and dark-field (E) illumination. D and F are liver sections from 3-methylcholanthrene treated rats hybridized with the test probe. Note that as the liver sections from β-naphthoflavone and 3-methylcholanthrene treated animals were analysed in a separate hybridization series, the signal intensities are not directly comparable.

apparent and tended to diminish the difference in spatial induction between 3-methylcholanthrene and β-naphthoflavone. After induction with 25 mg/kg 3-methylcholanthrene, the induction of CYP1A1 was still approximately twice as great in the perivenous than in the periportal region. On the other hand, the high dose of β-naphthoflavone (100 mg/kg) completely abolished the periportal induction predominance and significant zonation was no longer observed. Diminished or abolished zonation after the high inducer doses was also observed immunohistochemically (results not shown).

Zonal distribution of CYP1A1 mRNA

In order to investigate whether the observed reciprocal zone-specific induction by 3-methyl-

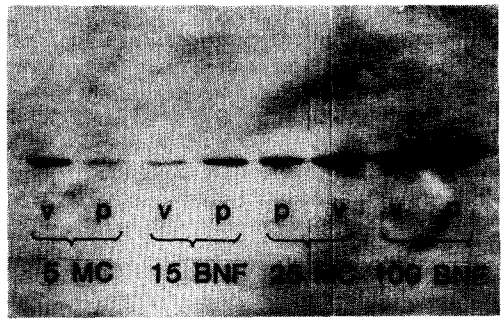

Fig. 2. Immunoblot analysis of CYP1A1 apoprotein in digitonin cell lysates. The lysates were obtained from the periportal (p) or perivenous (v) region of the liver from rats pretreated with 3-methylcholanthrene (MC) (5 or 25 mg/kg), or β-naphthoflavone (BNF) (15 or 100 mg/kg). 30 μg of cell lysate protein was separated by SDS-PAGE and immunoblotted as described in the Methods section.

cholanthrene and β-naphthoflavone would also occur at the pretranslational level, the distribution of CYP1A1 mRNA was investigated by two different approaches. Using *in situ* hybridization with a probe complementary to the CYP1A1 mRNA (antisense probe) we observed that in animals treated with the low dose (15 mg/kg) of β-naphthoflavone, autoradiographical grains were abundant in cells of zones 1 and 2, but especially in the areas surrounding terminal portal venules (Figs 1C and E). On the contrary, after treatment with the low dose (5 mg/kg) of 3-methylcholanthrene, the highest grain densities were observed in the centrilobular areas (Figs 1D and F). Sections from untreated animals hybridized with the test probe were clearly negative (data not shown). The specificity of the hybridization experiments was also verified by hybridizing sections from the 3-methylcholanthrene and β-naphthoflavone treated animals with the control (sense) probe and sections treated with RNase (data not shown). These controls were all negative, showing only faint, uniform background.

Zonation of CYP1A1 mRNA was further investigated by analysing perivenous and periportal digitonin lysates by RT-PCR. The results were in full agreement with the *in situ* hybridization data; treatment with the low dose of 3-methylcholanthrene resulted in a markedly higher accumulation of CYP1A1 mRNA in perivenous cell lysates while after the low dose of β-naphthoflavone the amount was several times higher in periportal lysates than in corresponding perivenous samples (Figs 4 and 5).

Zonal distribution of CYP1A2 mRNA

Since the expression and induction of CYP1A1 and 1A2 is somewhat different, we also investigated the zonation of CYP1A2 mRNA after induction by low dose 3-methylcholanthrene or β-naphthoflavone, to find evidence for different locations of regulatory steps. In corn oil treated control rats, the content of CYP1A2 mRNA was significantly higher in perivenous samples than in cell lysates from the periportal region (Fig. 6A). Induction after the low dose of 3-methylcholanthrene occurred in both periportal and perivenous samples and resulted in zonal equilibration of CYP1A2 mRNA (Fig. 6B). In contrast, the low dose of β-naphthoflavone seemed to cause induction almost exclusively in the periportal region, and resulted in a several fold higher periportal 1A2 mRNA content as compared to that in the perivenous region (Fig. 6C). Thus the treatment of animals with a low dose of β-naphthoflavone reversed the constitutive perivenous CYP1A2 mRNA expression pattern into a periportal expression pattern.

In vitro *induction of CYP1A1*

The *in vitro* induction by β-naphthoflavone

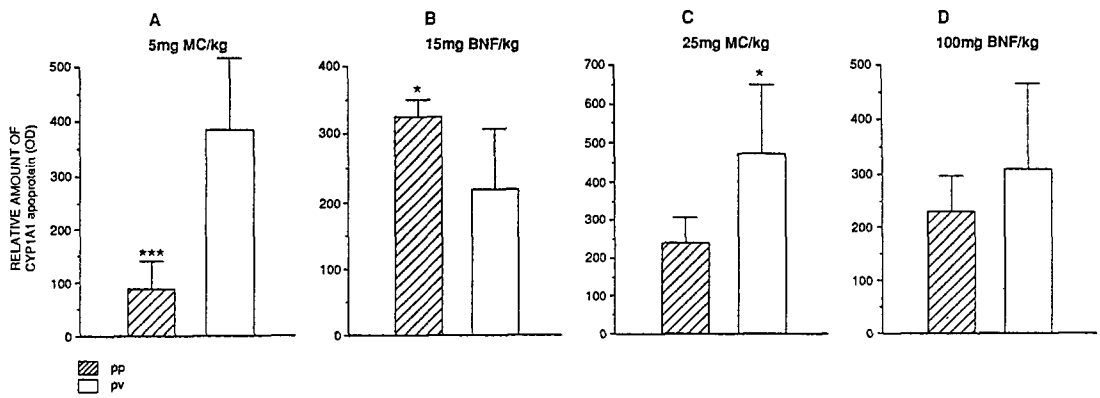

Fig. 3. Videodensitometric quantitation of CYP1A1 immunoblots. The figures are means ± SD of 5–7 pairs of periportal (pp) and perivenous (pv) digitonin eluates from rats treated with 5 (A) or 25 (C) mg/kg 3-methylcholanthrene (MC) or with 15 (B) or 100 (D) mg/kg β-naphthoflavone (BNF). *, **, *** = $P < 0.05$, 0.01 and 0.001, respectively, for statistical significance of pp–pv difference in each treatment group by Student's *t*-test. Since immunoblot analysis and quantitation of the optical densities (OD) of the CYP1A1 bands after each treatment was done in separate runs, absolute values of different treatment groups are not fully comparable.

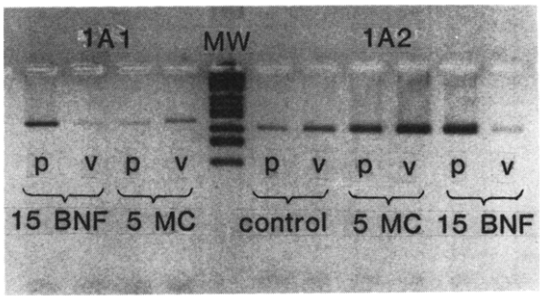

Fig. 4. RT-PCR analysis of CYP1A1 and 1A2 mRNA from periportal (p) and perivenous (v) liver eluates obtained from rats treated with 5 mg/kg 3-methylcholanthrene (MC), 15 mg/kg β-naphthoflavone (BNF) or vehicle only (control). For CYP1A1 PCR 5 or 10 μL and for CYP1A2 PCR 1 or 2 μL of cDNA, reverse transcribed from total eluate RNA, was amplified with PCR. Amplification products were electrophoresed together with the Boehringer DNA molecular weight marker VI in 4% NuSieve GTG and ethidium bromide stained. Staining densities are only comparable in the CYP1A2 RT-PCR control and 3-methylcholanthrene samples, which were run in the same series.

and 3-methylcholanthrene in hepatocyte fractions isolated from the periportal or perivenous region was compared in order to establish whether the factors governing zone-specific induction were retained in isolated cells. ALAT activity in freshly isolated periportal cell preparations was twice as high as in perivenous cells (2.03 ± 0.30, $N = 8$) demonstrating the different acinar origin of the cell populations, in agreement with previous studies [17, 18]. Culture of the hepatocyte populations for 48 hr resulted in low expression of CYP1A1. This was not due to the presence of 0.1% dimethylsulphoxide and was similar in periportal and perivenous cell preparations (results not shown). Induction of CYP1A1 by 0.037 μM and 3.7 μM 3-methylcholanthrene was 6–7 and 25–30 fold, respectively. The corresponding induction by 0.1 μM and 10 μM β-naphthoflavone was 5–6 and 15–20 fold, respectively (Fig. 7). However, the induction was approximately the same in cell populations from the periportal and perivenous regions.

DISCUSSION

The present study demonstrates that two chemicals, both inducing CYP1A1 and 1A2, affect different liver regions. The perivenous-dominated pretranslational induction of CYP1A1 and 1A2 after a low dose of 3-methylcholanthrene seems to represent the normal response, which for CYP1A1 is also observed for other inducers [5, 11] and appears to be characteristic for several other CYP forms as well [4]. The periportal induction observed after a low dose of β-naphthoflavone seems, to our knowledge, to be a unique exception to this pattern.

It does not seem likely that the periportal induction by β-naphthoflavone would be due to its exclusive uptake in this region, since its capacity to diffuse freely into cells should not differ from other lipophilic inducers. Furthermore, the spreading of the induction after a larger dose of β-naphthoflavone (or of 3-methylcholanthrene) suggests a sinusoidal gradient in the sensitivity rather than the spatial uptake of the inducer.

The opposite localisation of the pretranslational CYP1A1 and 1A2 induction by 3-methylcholanthrene and β-naphthoflavone suggests that this zone-specific effect occurs at the level of gene transcription, but

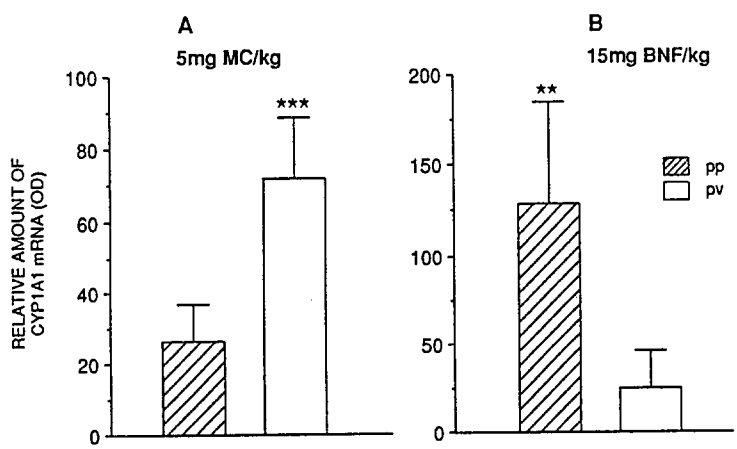

Fig. 5. Videodensitometric quantitation of periportal and perivenous CYP1A1 mRNA. Cell lysates from the periportal (pp) or perivenous (pv) region were subjected to RT-PCR analysis. Results from samples of animals treated with a low dose of 3-methylcholanthrene (A, 5 mg/kg; $N = 5$) or β-naphthoflavone (B, 15 mg/kg; $N = 6$) are shown. **, *** = $P < 0.01$ and 0.001, respectively, for statistical significance of pp–pv difference in each treatment group by Student's t-test. Note that the absolute OD-values of different treatment groups are not fully comparable, since the samples were run in different series. OD = optical density.

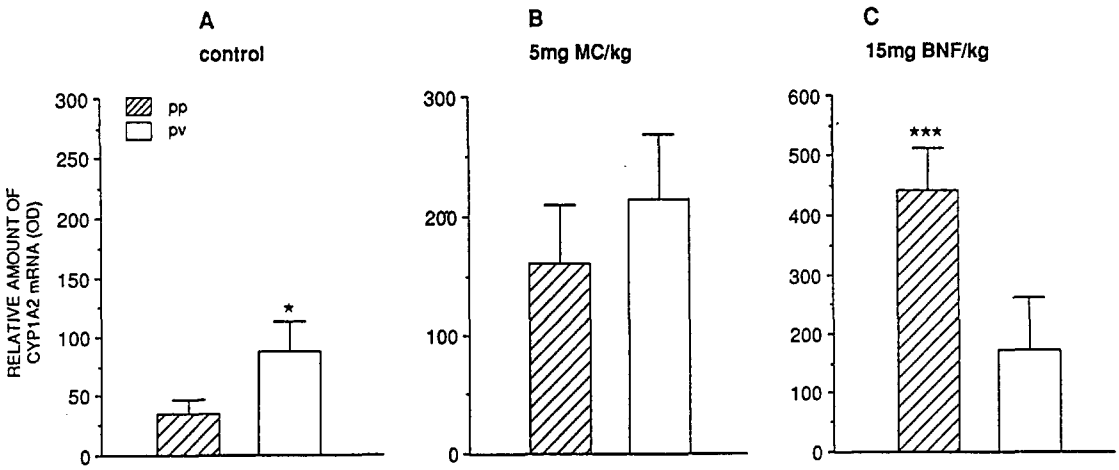

Fig. 6. Videodensitometric quantitation of RT-PCR analysis of CYP1A2 mRNA in periportal (pp) and perivenous (pv) cell lysates from control rats (A, N = 3) and rats treated with 5 mg/kg 3-methylcholanthrene (B, N = 5) or 15 mg/kg β-naphthoflavone (C, N = 5). Samples from the β-naphthoflavone series (C) were analysed separately and are therefore not directly comparable to A and B. *, **, *** = $P < 0.05$, 0.01 and 0.001, respectively, for statistical significance of pp–pv difference in each treatment group by Student's *t*-test. OD = optical density.

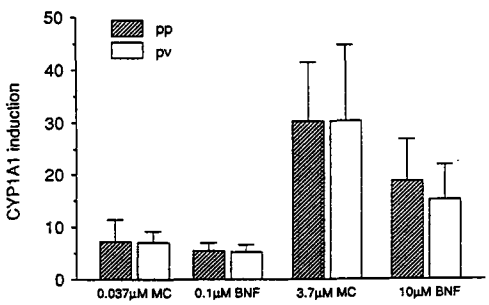

Fig. 7. Comparison of CYP1A1 apoprotein induction by 3-methylcholanthrene and β-naphthoflavone in isolated periportal (pp) and perivenous (pv) hepatocytes. The cells were exposed to 0.037 or 3.7 μM 3-methylcholanthrene (MC) or alternatively to 0.1 or 10 μM β-naphthoflavone (BNF) for 48 hr of primary culture as described in the Materials and Methods. The numbers (mean ± SD) denote fold-induction as compared to cells cultured in the absence of inducers and are calculated from videodensitometric quantitations of immunoblots.

the factors involved are at present unknown. According to present evidence both inducers act by binding to the Ah receptor [for a review see Refs 6 and 22]. To our knowledge there is no information on the possible zonation of the Ah receptor or of its associated regulatory components. To explain the opposite induction response by 3-methylcholanthrene and β-naphthoflavone, the critical factor should be zonated and differently affected by the two inducers studied. The Ah receptor activated process involves transformation of the liganded receptor complex into a DNA binding form, which translocates to the nucleus and interacts with the xenobiotic responsive elements (XREs) in the 5′-flanking region of the *CYP1A1* gene [for a review see Refs 6 and 22]. It is at present unclear to what extent the heat shock protein (hsp90) released from the Ah receptor complex, the Ah receptor nuclear translocator protein (arnt) or other putative regulatory proteins involved are locally expressed and differently regulated by 3-methylcholanthrene and β-naphthoflavone. Available information on the cytosolic 4S ligand binding protein, thought to enhance CYP1A1 induction by binding upstream to a site separate from the XRE binding site [see Ref. 23], does not seem to explain the present findings. While the 4S protein appears to have high affinity towards both 3-methylcholanthrene and β-naphthoflavone, it does not bind TCDD which, however, together with 3-methylcholanthrene cause perivenous CYP1A1 induction, in contrast to β-naphthoflavone. The fact that the induction of CYP1A1 after a large dose of either inducer spreads towards a panacinar pattern, as seen both in the present study and previously [5, 11], suggests that this factor(s) is expressed in a gradient fashion along the sinusoid.

There is evidence from other inducers, i.e. mevinolin and omeprazole, that both *CYP1A1* and *1A2* genes can be activated by Ah receptor-independent mechanisms [24, 25]. Such mechanisms could operate in parallel with Ah receptor-mediated mechanisms for one of the inducers used in this study. For instance, low doses of β-naphthoflavone could derepress *CYP1A1* in periportal cells while high doses could act paniacinarily via the Ah receptor. In addition, distinct isoforms of Ah receptors [26] might exhibit differences in inducer affinity and regional distribution.

The CYP1A inducers may also exert their zone-specific responses via humoral signals. For instance,

it was recently demonstrated that growth hormone represses the periportal expression of *CYP2B1/2* [16], an effect that is probably receptor-mediated. Accordingly, β-naphthoflavone could activate expression by preventing normal regiospecific hormone receptor-mediated suppression. Regionally acting growth factors could also interfere with induction. Indeed, TGFα and EGF have been shown to block 3-methylcholanthrene-mediated CYP1A1 induction [27]. It is therefore feasible that if these and other growth factors were zonated, this could at least partly explain the heterogeneous induction response, which may be inducer-specific. However, at present there is little information on the distribution of these signal factors in the liver.

We were unable to identify regionally acting factors that could explain the inducer-specific effects by comparing the CYP1A1 inducibility in hepatocyte fractions isolated from the periportal or perivenous region. In a previous study we were able to demonstrate that elements responsible for the perivenous induction of CYP2B1/2 by phenobarbital are retained in isolated cells [28]. However, the *in vivo* operating factors that govern the regiospecific induction by β-naphthoflavone and 3-methylcholanthrene obviously were not retained in isolated cells although marked induction of CYP1A1 by both inducers was observed.

In conclusion, this study suggests the existence of factors in the liver that govern the pretranslational induction of CYP1A1 and 1A2 in a zone- and inducer-specific way. They are probably, but not necessarily, associated with the Ah receptor. Identification of these factors will help in understanding not only the mechanisms behind the zone-specific constitutive and induced expression of both *CYP1A* and other *CYP* genes, but ultimately CYP-mediated mechanisms of regiospecific liver damage.

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