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Aryl Hydrocarbon Receptor-Associated Genes in Rat Liver

REGIONAL COINDUCTION OF ALDEHYDE DEHYDROGENASE 3 AND GLUTATHIONE TRANSFERASE YA

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ABSTRACT. The tumor-associated aldehyde dehydrogenase 3 (ALDH3) and the glutathione transferase (GST)Ya form are coded by members of the Ah (aryl hydrocarbon) battery group of genes activated in the liver by polycyclic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The physiological role of the Ah receptor (AHR), its gene-activating mechanism and its endogeneous ligands are still poorly clarified. We had previously observed that 3-methylcholanthrene (3MC) and β-naphthoflavone (βNF) induced the AHR-associated CYP1A1/1A2 pair in different liver regions, an effect not explained by the acinar distribution of the AHR protein. Here, we investigated AHR-associated regional induction by comparing the expression patterns of ALDH3 and GSTYa. Analysis of samples from periportal and perivenous cell lysates from 3MC-treated animals revealed that ALDH3 mRNA, protein and benzaldehyde-NADP associated activity were all confined to the perivenous region. In contrast, such regio-specific induction was not seen after β-NF induction. Immunohistochemically, a peculiar mono- or oligocellular induction pattern of ALDH3 was seen, consistently surrounding terminal hepatic veins after 3MC but mainly in the midzonal region after βNF. A ligand-specific difference in regional induction of GSTYa1 mRNA was also observed: The constitutive perivenous dominance was preserved after 3MC while induction by βNF was mainly periportal. A 3MC–βNF difference was also seen by immunohistochemistry and at the GSTYa protein level, in contrast to that of the AHR-unassociated GSTYb protein. However, experiments with hepatocytes isolated from the periportal or perivenous region to replicate these inducer-specific induction responses *in vitro* were unsuccessful. These data demonstrate that the different acinar induction patterns by 3MC and βNF previously observed for CYP1A1 and CYP1A2 are seen also for two other Ah battery genes, GSTYa1 and ALDH3, but in a modified, gene-specific form. We hypothesize that unknown protein(s) operating *in vivo* and modifying the Ah-mediated response at the common XRE element located upstream of these genes is affected zonespecifically by 3MC and βNF. BIOCHEM PHARMACOL 55;4: 413–421, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. liver gene expression; perivenous hepatocytes; Ah receptor; glutathione transferase; aldehyde dehydrogenase; 3-methylcholanthrene

The class 3 aldehyde dehydrogenase (ALDH3) isoform is not normally expressed in rodent liver, but is found in other organs including stomach and cornea [1]. The enzyme is described as a tumor-associated ALDH, since exposure to carcinogens activates the *ALDH3* gene in the liver and the gene product is abundant in preneoplastic cells [1]. The ALDH3 isozyme is also induced by polycyclic aromatic hydrocarbons, not all of which are classified as carcinogens, with this induction thought to be regulated differently. Several glutathione transferase (GST) isozymes are also induced in the liver upon exposure to polycyclic hydrocarbons. Among members of the four cytosolic GST families (α, μ, π and τ), at least GSTYa and GSTYc (belonging to the α family) and GSTYb (belonging to the μ family) are induced [2, 3], in spite of their low degree of homology and large differences in their upstream DNA sequences [4]. Among these, the GSTYa form exhibits the most prominent induction [3]. Both the genes encoding this enzyme, *GSTYa1* and *GSTYa2*, as well as *ALDH3* are considered members of the Ah battery of genes [5], which are strongly induced by TCDD and other polycyclic hydrocarbons [6, 7].

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§ *Abbreviations:* Ah, aryl hydrocarbon; AHR, aryl hydrocarbon receptor; ALAT, alanine aminotransferase; ALDH3, class 3 aldehyde dehydrogenase; βNF, β-naphthoflavone; CDNB, 1-chloro-2,4-dinitro-benzene; CYP, cytochrome P450; GST, glutathione-S-transferase; RT-PCR, reverse transcriptase-coupled polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 3MC, 3-methylcholanthrene; XRE, xenobiotic response element.

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The induction is mediated by a cytosolic aryl hydrocarbon (Ah) receptor (AHR), which belongs to a family of basic helix-loop-helix (bHLH) DNA binding proteins [7, 8]. The Ah receptor also mediates the transcriptional activation of several other genes associated with drug metabolism, including the *CYP1A1* and *CYP1A2*, *UGT1*06* and NQO_1 [6]. Although the exact mechanisms leading to transcriptional activation of these genes is still only partly known, it is generally accepted that upon ligand binding AHR dissociates from a 90-kDa heat shock protein (hsp90) and then binds to the nuclear transcription factor ARNT, which has a basic helix-loop-helix (bHLH) element similar to that of AHR [9, 10]. Via the bHLH element, the AHR-ARNT heterodimer complex binds to common xenobiotic responsive elements (XREs) located upstream of these genes and initiates transcriptional activation [11].

We have been investigating the regio-specific expression of drug metabolizing proteins in liver and how this relates to development of local damage [12]. Most of the phase I enzymes belonging to the cytochrome P450 superfamily and coded by *CYP* genes are constitutively expressed and also induced by xenobiotics mainly in the perivenous region [13, 14]. The factors regulating this positional expression pattern are not characterized. We and others have observed that while pretreatment with 3-methylcholanthrene caused an expected induction of the two Ah battery gene members *CYP1A1* and *CYP1A2* mainly in the perivenous liver region, β-naphthoflavone induced these genes rather in the opposite, periportal liver region [15, 16], a phenomenon that was found to be a pretranslational event [16]. In order to understand the regulation of this regional induction, we investigated the acinar distribution of the AHR. In the normal rat liver, expression of AHR protein was found almost exclusively in the perivenous region [17]. However, since little change in the zonation occurred after pretreatment with either 3MC or βNF, the inducer-specific expression pattern remained unexplained. Revealing the molecular mechanism for this inducer-specific effect might help to understand how AHR-mediated responses and regional gene expression are regulated. It may also throw additional light on the physiology of the receptor, which according to recent experiments with AHR deficient mice may play an important role during development [18]. Consequently, we investigated the induction pattern of ALDH3 and GSTYa to explore whether there are common factors that regulate the regional expression and induction of Ah battery genes.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 170 to 370 g and fed standard R3 diet (Ewos AB) were used. Rats received 100 mg/kg β-naphthoflavone (Aldrich Chemical Co. Inc.) or 25 mg/kg 3-methylcholanthrene (Sigma Chemical Co.) 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

For immunohistochemical localization of ALDH3 and GSTYa, paraffin embedded liver sections were treated with polyclonal rabbit antibodies against purified rat liver ALDH3 or GSTYa (Biotrin) and stained as described previously [14].

Isolation and Characterisation of ALDH3 Antibody

ALDH3 (tumor-associated, class 3) protein was purified from livers of rats treated with 3-methylcholanthrene, using AMP-Sepharose affinity chromatography combined with chromatofocusing [19]. A crude IgG fraction from polyclonal antibodies raised in rabbits was prepared by ammonium sulfate (40%) precipitation and used in double immunodiffusion tests with purified ALDH3 from stomach and lung and from livers from 3-methylcholanthrene- or βNF-treated animals. All samples performed identically. No precipitin band was formed with cytoplasm from normal rat liver [20], and immunotitration experiments demonstrated that the antibodies inhibited ALDH3 activity. Residual active soluble immunocomplexes were removed by centrifugation. Protein-A Sepharose treatment also completely removed ALDH3 activity from the antibody-enzyme mixtures. NAD, when added prior to antibodies, was found to offer partial protection against immunoinhibition. Addition of antibodies completely removed the ALDH3 bands at pH 6.5–7.0 but had no effect on the normal cytoplasmic ALDH bands in isoelectric focussing.

Collection of Periportal and Perivenous Cell Lysates

Digitonin cell lysates from the periportal and perivenous region were obtained by dual pulse technique [21] as modified in [22] and samples for analysis of protein and mRNA treated as recently described [23]. Briefly, periportal cells were lysed by infusion of 6.7 mL/kg body wt. of 3.5 mM digitonin (ICN Chemicals) via the portal vein and the lysate collected by immediate retrograde flushing. Perivenous cell lysates were obtained by infusing 10 mL/kg digitonin solution via the upper vena cava followed by antegrade flushing. The terms periportal and perivenous are arbitrarily used and refer to this model. The zone-specificity of the lysates was verified by assay of alanine aminotransferase as in [16].

Isolation and Culture of Periportal and Perivenous Hepatocytes

Intact hepatocytes were isolated from either the periportal or the perivenous region of the liver acinus after zone-specific destruction with digitonin followed by conventional collagenase perfusion as described in detail previ-

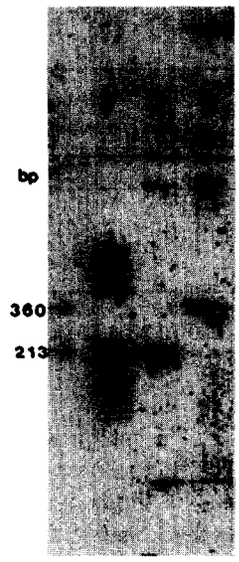

FIG. 1. Identification of GST Ya1 and Ya2 PCR fragments. Silver staining from a polyacrylamide electrophoresis (Pharmacia's Phast™ System) with 20% gels and native buffer strips is shown. On lane 1 a Boehringer V molecular weight standard (Hae II digest of pBR 322) was run and on lanes 2 and 3 the GSTYa1 and GSTYa2 products, respectively. The position of the size of the expected products is indicated by arrows.

ously [24]. The hepatocyte fractions were plated at a density of 1.4 times 10^5 cells/cm^2 on plastic dishes (Nunc) using MEM medium (Gibco) supplemented as described previously [16]. Phenobarbital (2 mM), 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 with fresh medium at 4 hr after plating and cells removed after 48 hr of culture.

Immunoblotting

For analysis of ALDH3, GST Ya and GST Yb apoprotein in periportal and perivenous cell samples, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was run [25] in 8.7% or 12% gels. Proteins were electroblotted on nitrocellulose filters, blocked in 5% milk and probed with rabbit anti-rat ALDH3, GST Ya (Biotrin) or GST Yb antiseras (kindly donated by Dr. Andrew Dannenberg, New York). The filters were stained with the Proto-Blot AP (alkaline phosphatase) system (Promega) and staining quantified with videodensitometer (Ultraviolet Products).

RT-PCR

The relative amount of ALDH3, GST Ya1, and GST Ya2 mRNA in cell lysates was analysed by semi-quantitative RT-PCR as described [23, 26]. Total RNA was isolated [27], the concentration and purity were determined spectrophotometrically (A_{260} and nucleic acid/protein ratio A_{260}/A_{280}) and the integrity ascertained by electrophoresis in formaldehyde denatured agarose gels. One μg of total RNA was reverse transcribed using Promega's Reverse Transcription system and random hexanucleotide primers. 0.5–5 μL of cDNA were amplified in a 100 μL reaction volume containing 50 or 25 (GST Ya2) pmol of both primers, 2 or 1.25 (GST Ya2) U of Taq DNA polymerase, 1*PCR reaction buffer (both from Boehringer Mannheim GmbH), 3.5 (ALDH3), 4 (GST Ya1), or 1.5 (GST Ya2) mM $MgCl_2$ and 0.2 or 0.025 (GST Ya2) mM of each deoxynucleotide triphosphate (Promega). Finally, 100 μL of mineral oil was added. After heating the reaction mixture for 4 min at 94°, 25–26 cycles, each consisting of 30 sec 94°, 1 min 63 (ALDH3), 53 (GST Ya1), or 58 (GST Ya2)°, and 1 min 72°, were performed. The last extension step was continued for 5 min. The following primers were used to amplify a product. The calculated size is in parenthesis.

ALDH3 (248 bp):
5′-ACTGGAGGAGCTTGATACCACAA-3′, forward
5′-GCAGGTCTGCCATGTGCCCG-3′, reverse
GST Ya1 (213 bp):
5′-ATGAGAAGTTTATACAAAGTCC-3′, forward
5′-GATCTAAAATGCCTTCGGTG-3′, reverse
GST Ya2 (360 bp):
5′-GATTGACATGTATTCAGAGGGT-3′, forward
5′-TTTGCATCCATGGCTGGCTT-3′, reverse

The size of the PCR fragments was determined by PAGE with Pharmacia's Phast™ System (Pharmacia LKB Biotechnology) using 20% gels and native buffer strips (Fig. 1). The run was 90 and 250 Vh at 15° and gels were stained with Phast Gel Silver Kit (Pharmacia). The linearity of the PCR amplification was ensured by varying the number of cycles and the amount of cDNA in the reaction. The amplification products were quantified by anion exchange HPLC [28] as modified in [26]. Since the coding regions of GST *Ya1* and *Ya2* genes are almost identical [29], the primers were chosen from regions with maximal deviation between the *Ya1* and *Ya2* genes. For this purpose, the GCG 7.0 Stringsearch sequence analysis program (Genetic Computer Group) coupled with the GCG Bestfit program was used. The identity of the amplified fragments was verified by sequencing the PCR products and using restriction endonucleases which differentiate between the products, *HindIII*, *AluI* (both from Boehringer Mannheim), and *MnlI* (New England BioLabs, Inc.) This was followed by size analysis of the digestion fragments on 20% native Phast™ gels as described above. Sequencing reactions were performed with a TaqDyeDeoxy™ Terminator Cycle Sequencing kit (Applied Biosystems) and the primers described above, and analysed with a ABI 373A DNA Sequenator (Applied Biosystems).

Data Analysis

To reduce interseries variation in quantification of PCR products, data from HPLC quantitations of RT-PCR products from the interdependent parameters, GST Ya1, GST Ya2, and ALDH3 as well as AHR and ARNT [17] were

TABLE 1. Acinar distribution of aldehyde dehydrogenase 3 and glutathione S-transferase activity after induction by 3-methylcholanthrene or β-naphthoflavone

	ALDH3 (benzaldehyde + NADP, nmol/min per mg prot)			GST (CDNB, μmol/min per mg prot)		
	C	3MC	βNF	C	3MC	βNF
pp	0.3 ± 0.5	2.2 ± 2.7	14.8 ± 5.5	0.78 ± 0.11	1.14 ± 0.25	1.99 ± 0.56
pv	0.9 ± 0.7	242 ± 78	103 ± 35	1.67 ± 0.17	2.81 ± 0.45	2.71 ± 0.45
pv/pp ratio	2.0 ± 3.0	307 ± 230	7.4 ± 2.6*	2.13 ± 0.23	2.61 ± 0.40	1.38 ± 0.26*

Rats received 100 mg/kg β-naphthoflavone (βNF) or 25 mg/kg 3-methylcholanthrene (3MC) or vehicle only (corn oil, 5 mL/kg), by i.p. injection once a day for three or four consecutive days. Periportal (pp) and perivenous (pv) cell lysates were extracted and the activity of aldehyde dehydrogenase (ALDH3) and glutathione S-transferase (GST) was assayed as described in "Materials and Methods." Means ± SD of 5–6 experiments is given. *$P < 0.01$ for difference between 3MC and βNF treatment.

treated as follows. Within each treatment and sample type (periportal or perivenous) group and for each PCR run, the relative ratio of a sample to the mean of the group was calculated. Then the mean of the ratios obtained with five pairs of primers was calculated for each sample, describing the coefficient for the relative deviation of the sample from the mean of the group. Normalisation was achieved by dividing sample values by the coefficient. Significance of differences between means of periportal and perivenous samples within treatment groups was determined with Student's *t*-test.

RESULTS

Alanine Aminotransferase (ALAT) Activity in Periportal and Perivenous Cell Lysates

The zonal origin of digitonin cell lysates was controlled by analysis of ALAT (a periportal marker) in periportal and perivenous cell lysates. In this study in untreated animals, the activity of ALAT in periportal samples was 17.2 ± 8.6 (mean ± SD, $n = 6$) times higher than in perivenous samples. The corresponding periportal to perivenous ratios for ALAT in livers from 3MC- and βNF-treated rats were 22.1 ± 14.6 and 16.4 ± 10.3, respectively, indicating complete zone selectivity and full agreement with previous investigations [16, 23].

Zonal Distribution of ALDH3 Induction

The distribution of ALDH3 after induction by 3MC or βNF was studied by measuring enzyme activity, by immunoblotting and by RT-PCR analysis of mRNA from periportal and perivenous cell lysates. As expected, practically no ALDH3 expression was observed in liver cell lysates from control animals. After treatment with 3MC, high ALDH activity, as assayed with benzaldehyde and NADP, was observed, but almost exclusively in perivenous samples (Table 1). The mean activity was *ca.* 300 times higher than in periportal samples. In contrast, after treatment with βNF, high ALDH activity was observed in periportal samples as well. The activity in perivenous samples still exceeded that in periportal samples, but the mean activity was only ca. 7 times higher than in periportal samples.

Using polyclonal antibodies raised in rabbits against purified liver ALDH3, a similar difference in the zonal induction pattern was discerned from Western blot analysis of ALDH3 protein. No immunoreactive protein was detected in cell lysates from control livers (Fig. 2). Induction by 3MC was 6.8 times higher in perivenous samples as compared to the periportal ones. In comparison, treatment with βNF resulted in a less zonated induction, the pv/pp ratio being reduced to 2.0. The polyclonal antibodies were also applied for immunohistochemical analysis. No staining was observed in livers from untreated animals, in contrast to the marked staining seen in induced livers. However, the patterns of staining differed from those normally exhibited by phase 1 or phase 2 gene products. While a zonal distribution pattern, both constitutively and after induction, is a common observation for these proteins, this pattern usually appears as rather uniform, indicating that all

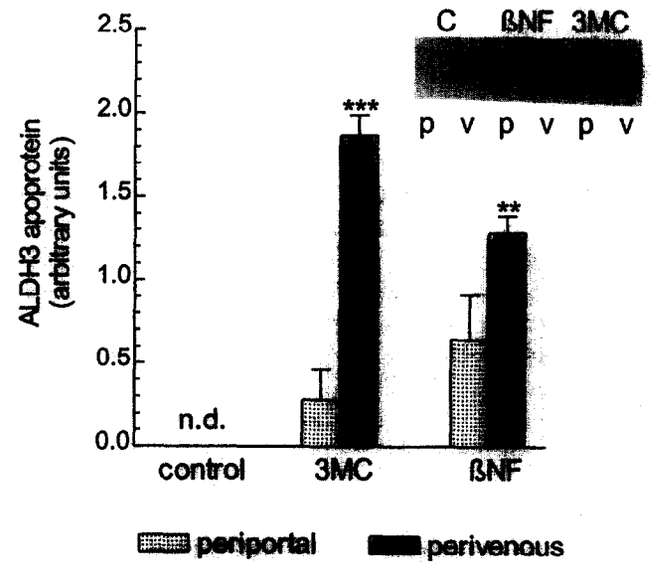

FIG. 2. Acinar distribution of ALDH3 apoprotein after induction by 3MC or βNF. Rats were treated and samples taken as described in the legend to Table 1. Western blots, using polyclonal antiserum against ALDH3, were performed as described in the "Materials and Methods." The bars depict means ± SD of videodensitometric quantitations of samples obtained from 6 animals. **$P < 0.01$ and ***$P < 0.001$ for difference in the amount of ALDH3 protein between periportal and perivenous samples. ND = not detected. A series of samples stained with ALDH3 antibody is shown in the insert.

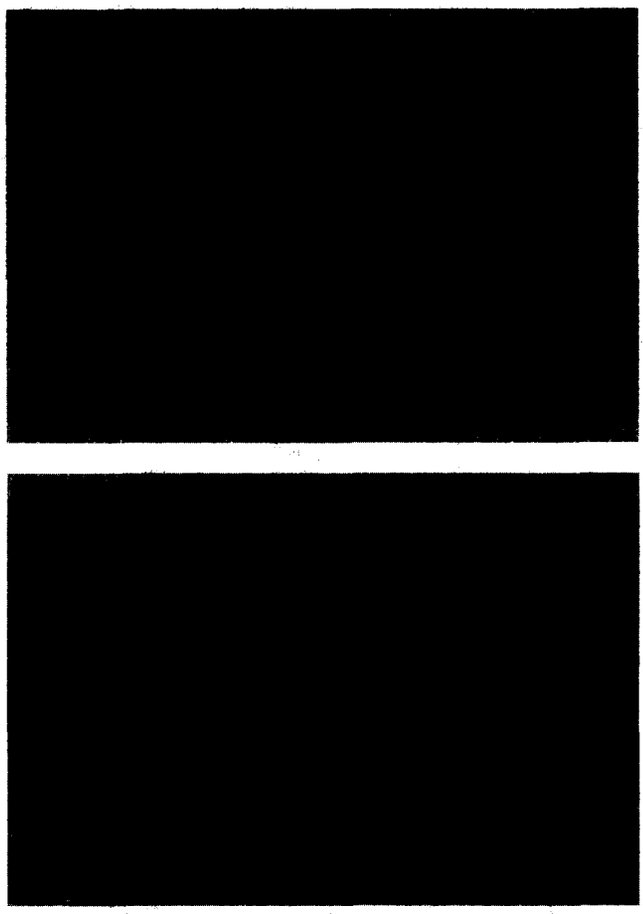

FIG. 3. Localization of immunoreactive ALDH3 apoprotein in liver sections after treatment with 3MC or βNF. Rats were treated with 3MC (A) or βNF (B) and sections stained as described in the legend to Table 1. Terminal central venules (cv) and terminal portal venules (pv) are marked on the photographs. Note the scattered cell-specific staining pattern and the differently localized ALDH3 positive staining seen after 3MC and βNF.

cells in the affected area are immunoreactive. A deviant pattern was seen after induction of ALDH3. Immunostaining appeared to be limited to single isolated cells or small clusters of cells located in the inducible area, while the majority of adjacent cells in the same region were immunonegative (Fig. 3). This spotty pattern was seen both after 3MC and βNF induction, but different zones were stained. In livers of 3MC-treated rats, strongly immunopositive cells were located very close to the terminal hepatic venules, while after βNF treatment induced cells appeared in a broad region extending throughout the acinus but being strongest in the midzonal region (Fig. 3).

To establish whether corresponding zonal induction patterns are seen at the pretranslational level, lysates were analysed for their content of ALDH3 mRNA, using RT-PCR followed by quantitation of the PCR products by HPLC. This method indicated that 3MC caused appearance of ALDH3 mRNA almost exclusively in perivenous cell lysates and that only traces (1–2%) were found in periportal lysates (Fig. 4). In contrast, after βNF induction,

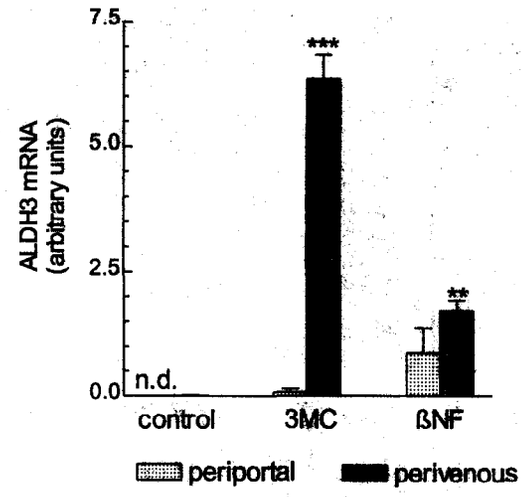

FIG. 4. Effect of 3MC or βNF induction on ALDH3 mRNA in periportal and perivenous cell lysates. The bars represent quantitation by PLC of RT-PCR products based on analysis of cell lysates from the periportal or perivenous region. Animals were treated with 3MC or βNF as described above. *$P < 0.01$ and ***$P < 0.001$ for statistical significance of pp-pv difference in each treatment group ($n = 4–6$). ND = not detected.

much ALDH3 mRNA was also found in periportal samples, although approximately twice as much was still detected in perivenous lysates.

Zonal Distribution of GSTYa Induction

The zonation of GST protein induction was studied using polyclonal antibodies against both the GSTYa and the GSTYb form. The genes encoding the GSTYa form are considered members of the Ah battery family, as opposed to the GSTYb form [4, 30]. Although under the experimental conditions used, the increase in immunoreactive GSTYa protein observed after 3MC or βNF treatment was modest, i.e. 1.5- to 2-fold, a clear difference between the compounds in their zonal effects was seen. Immunohistochemically, GSTYa staining was restricted to a 6–8 cell thick layer of cells surrounding the terminal hepatic veins, both constitutionally and after 3MC treatment (Fig. 5). After βNF treatment, GSTYa positive staining extended further into the periportal region, but was still strong in the perivenous region. Immunoblot analysis of periportal and perivenous cell lysates showed that treatment with 3MC increased the amount of immunoreactive GSTYa protein in both regions (Fig. 6). However, the relative increase was greater in the perivenous region, so that the perivenous/periportal ratio increased from 1.6 in controls to 2.0. In contrast, treatment with βNF caused a relatively stronger response in the periportal region, and the corresponding ratio fell to 0.9. The response of GSTYb was clearly different. There was no effect of either 3MC or βNF on the amount of GSTYb protein and the perivenous dominance observed in control livers prevailed.

The different zonal response of GSTYa to 3MC and βNF was reflected at the mRNA level, demonstrating regulation

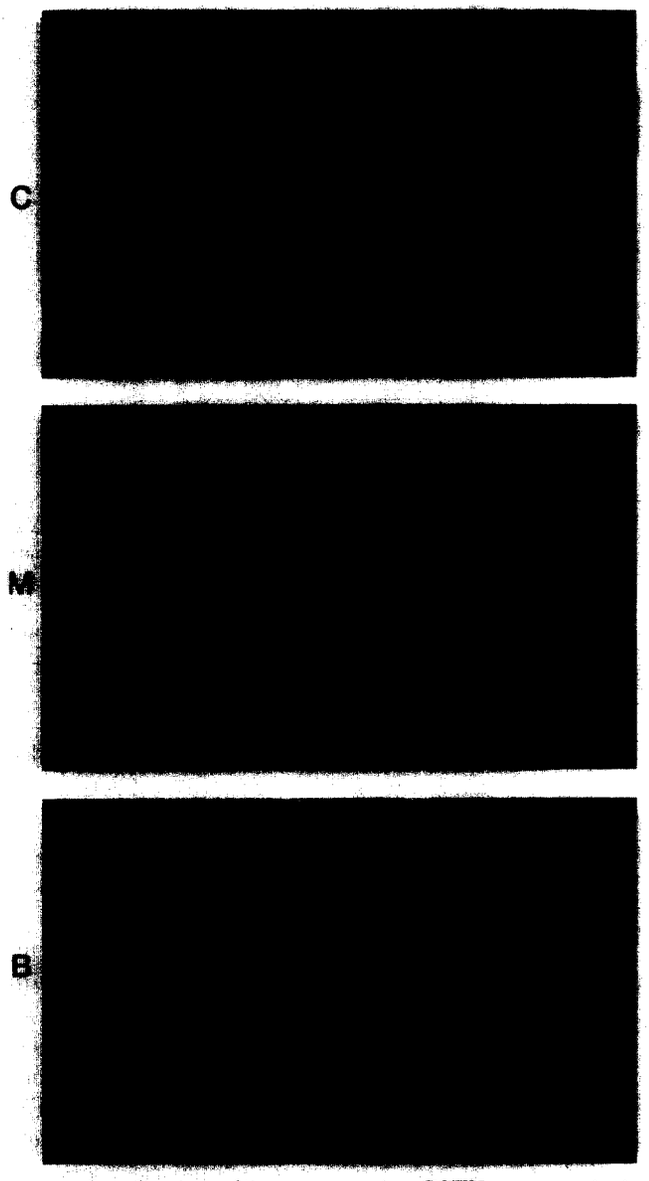

FIG. 5. Localization of immunoreactive GSTYa apoprotein in liver sections after treatment with 3MC (M) or βNF (B) as described in the legend to Table 1. Terminal central venules (cv) and terminal portal venules (pv) are marked on the photographs. Note that in livers from βNF-treated rats, GSTYa positive staining extends further into the periportal regions, in contrast to the restricted staining around the terminal central veins seen in samples from untreated (C) or 3MC-treated rats.

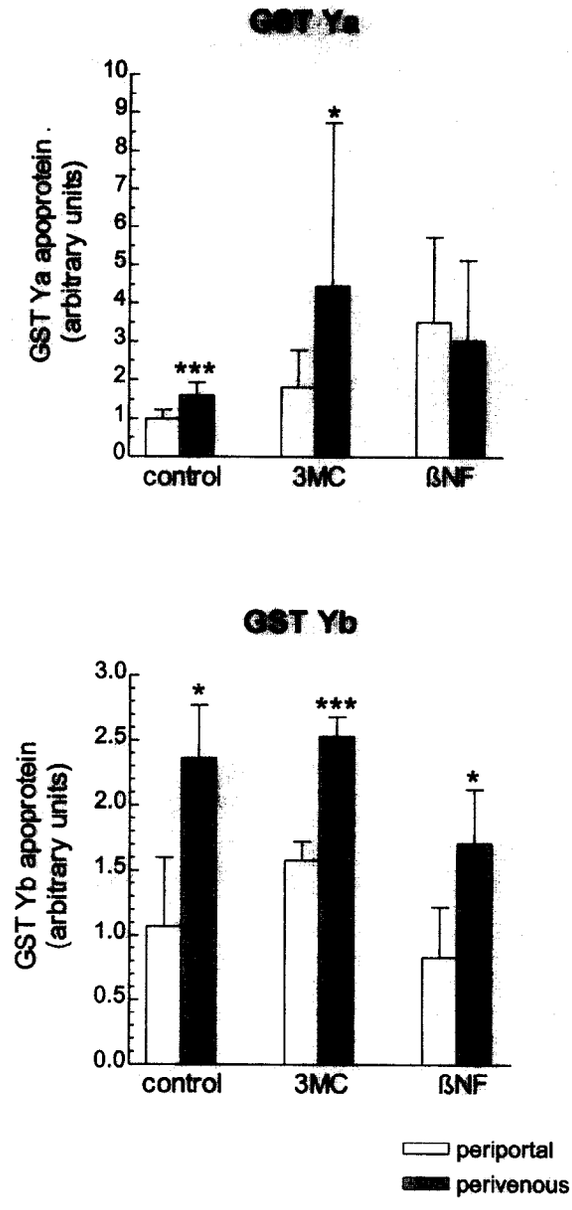

FIG. 6. Acinar distribution of GST Ya and Yb apoprotein after induction by 3MC or βNF. Rats were treated and samples taken as described in the legend to Table 1. Western blots, using polyclonal antiserum against GST Ya and Yb, respectively, were performed and the stained bands subjected to videodensitometric quantitation as described in "Materials and Methods". Means ± SD of 4–6 experiments are shown. *P < 0.05 and ***P < 0.001 for pp-pv difference in each treatment group.

at the pretranslational level. Both GSTYa1 and GSTYa2 mRNA species were analysed. These genes being highly homologous [28], the GSTYa antibody will detect both gene products. In control livers, somewhat higher amounts of Ya1 and Ya2 mRNA were seen in the perivenous cell lysates (Fig. 7). Both 3MC and βNF moderately increased these mRNA species, but the zonal effects appeared to differ. Thus, 3MC appeared to increase GSTYa1 mRNA more in perivenous cell lysates, in contrast to Ya2. Treatment with βNF increased the concentration of both Ya1 and Ya2 mRNA mainly in the pp region. For GSTYa1, this resulted in a significantly higher ($P < 0.05$) mRNA concentration in the periportal region.

In Vitro Induction of GSTYa

Induction of GST by various chemicals, including phenobarbital, is observed in isolated hepatocytes during primary culture [31]. We previously demonstrated that hepatocytes isolated from the perivenous region retain their greater *in vivo* inducibility of CYP2B1/2 by phenobarbital during culture [32]. Attempts were therefore made to elucidate factors responsible for the zone-specific induction effects of 3MC and βNF in hepatocyte fractions isolated from the periportal or perivenous region [24]. When cells from the

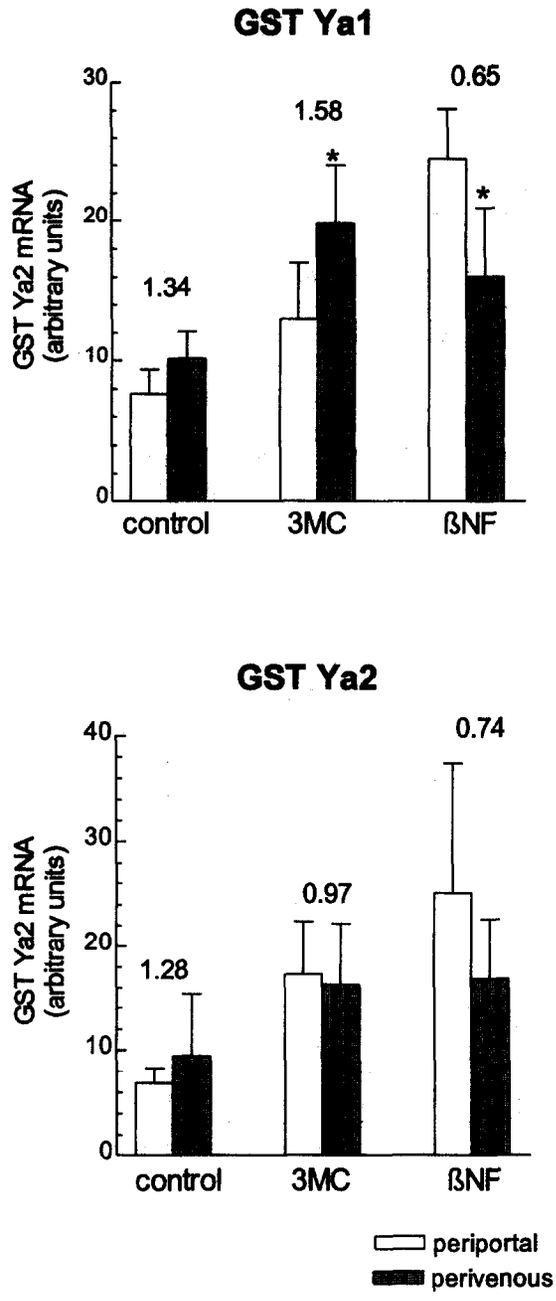

FIG. 7. Acinar distribution of GST Ya1 and GST Ya2 mRNA after induction by 3MC or βNF. RT-PCR products of GST Ya1 mRNA or GST Ya2 mRNA from periportal and perivenous liver eluates obtained from rats pretreated with 3MC, βNF or vehicle only (control) were quantified by HPLC as described under "Materials and Methods". The bars are means ± SD of peak areas (n = 3–6). The mean of the perivenous/periportal ratio from each pair is given above the bars. *$P < 0.05$ for pp-pv difference within the treatment group.

perivenous region were cultured on plastic dishes for 48 hr [32], addition of 2 mM phenobarbital induced GST Ya and Yb protein somewhat more (+40%) as compared to periportal cells (+30%, means from 5 pairs of cell populations), but statistically significant differences were not achieved. Neither was any significant difference between the induction response of the cell populations observed by addition of 3MC or βNF to the culture medium. Other experimental conditions, resulting in stronger induction, may have revealed periportal-perivenous *in vitro* GST inducibility differences similar to those seen for CYP2B1/2, but our present data were inconclusive.

DISCUSSION

The present study demonstrates distinct similarities in the regional induction response of two gene products, ALDH3 and GSTYa, after exposure to two different chemicals. Treatment with 3MC activates these genes exclusively in the perivenous liver region while βNF exposure, in addition to activating cells in the perivenous region, also causes a marked response in periportally located cells. We previously reported an even more dramatic zonal difference in the activation of the *CYP1A1* and *CYP1A2* genes by these inducers [16]. A moderate dose of βNF almost selectively activated the *1A1* and *1A2* genes in periportally located cells, in sharp contrast to the distinct perivenous induction seen after 3MC. Corresponding changes in the mRNAs of these genes indicated pretranslational regulation, suggesting the involvement of common regionally operating gene-activating or derepressing factors in their upstream region. However, as indicated in the schematic summary of these actions in Fig. 8, the zonal induction effects of these genes are not identical. As members of the Ah battery gene family [7, 8], these genes are activated by xenobiotic ligands to the Ah receptor. A heterodimer is formed with the ARNT (Ah receptor nuclear translocator) protein, and this binds in the nucleus to a common XRE (xenobiotic response element) located in the upstream region of these genes [7]. Since the AHR-mediated induction process consists of several steps recruiting different proteins, some of which are probably still undetected, a number of ligand- and zone-specific effects are feasible. Either the Ah receptor or the ARNT protein could be subject to a ligand-specific acinar redistribution. The AHR was indeed found to be expressed selectively in the perivenous region [17]. This distribution pattern could relate to the higher expression of all AHR-associated genes in the perivenous region and is also compatible with the selective perivenous induction of these genes elicited by 3MC. Thus, a functional Ah receptor would be required for normal constitutive expression and induction of the members of the Ah battery genes. The observation that in transgenic AHR-deficient mice, the expression of the XRE-possessing genes, *CYP1A1* and *UDPGT*, was absent or extremely low [18] supported this notion. Consequently, the very low level of AHR expression in the periportal region may explain the limited expression of the Ah battery genes in this region. However, after βNF treatment the perivenous expression of the Ah receptor persisted [17]. We have at the moment no valid explanation for the deviant induction pattern by βNF. There may exist an additional periportally distributed Ah receptor-like protein with high affinity for βNF or its metabolites, but with low affinity for 3MC. Alternatively,

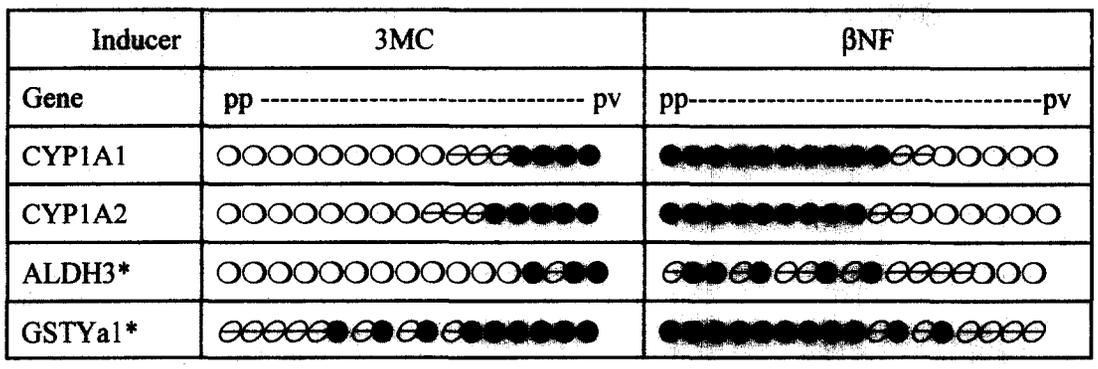

FIG. 8. Schematic comparison of the acinar mRNA distribution of four Ah battery genes after induction by 3MC or βNF. The figures indicate the relative distribution of four Ah battery gene transcripts along the sinusoids, from the periportally most proximal cells (pp) to the most distal ones surrounding the terminal hepatic vein (pv). The filled (●), hatched (⊖) and open (○) circles depict, respectively, high, intermediate or low/undetectable mRNA as analysed from periportal and perivenous cell lysates. The figure is compiled from data presented here and in our previous study [16].

common transcription factors interacting with proposed Negative Response Elements may be removed by the action of βNF, selectively in the periportal region. Transfection experiments, using deletion constructs of the *GSTYa* gene, have identified different regulatory elements, including HNF1, HNF4, a glucocorticoid responsive element and an Antioxidative Response Element (ARE), in the 5′ flanking region of the gene [33, 34]. It is possible that an inducer-specific signal could be mediated via one of these elements.

Other factors, unrelated to regional expression and probably permissive, affect selective genes. For instance, while *GSTYa1*, *Ya2* and *CYP1A2* are constitutively expressed in liver, the *CYP1A1* and *ALDH3* genes are dormant. However, both these dormant genes are dramatically activated, and exclusively in the perivenous region, by 3MC. This would suggest that they share responsive elements in the upstream region that are activated by the same perivenously located protein. However, while CYP1A induction occurs relatively evenly among cells in the affected region [15], the immunohistochemical studies revealed that ALDH3 induction was intense in isolated cells or cell clusters, while adjacent cells in the same region appeared nonresponsive. A similar spotty pattern has previously been reported after histochemical staining of liver sections from ethionine-treated rats [35]. This spotty induction pattern was also observed after βNF treatment, albeit in different areas. In our previous study, we discussed the possibility that the unique periportal induction pattern by βNF would be due to its exclusive uptake in that region. Given that after a large dose of βNF panacinar CYP1A induction has been observed, this explanation was considered unlikely. This view is strengthened by a closer examination of the immunohistochemical staining pattern of ALDH3. It is obvious that more staining is seen in the distal than in the proximal periportal region where, according to the uptake theory, βNF action should be strongest.

In conclusion, our findings demonstrate that there are common inducer-specific regionally acting factors that coregulate the acinar pattern of induction of five different genes belonging to the Ah battery gene family. Plausible targets for these ligand- and region-specific effects are the XREs located in the promotor region of these genes. Elucidation of the protein(s) involved should clarify the mechanism of activation of these genes and the mediatory action of the Ah receptor in this process constitutes an interesting challenge for future research.

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