Differential lobular induction in rat liver of glutathione S-transferase A1/A2 by phenobarbital

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Selim, Niazy, Gene D. Branum, Xia Liu, Richard Whalen, and Thomas D. Boyer. Differential lobular induction in rat liver of glutathione S-transferase A1/A2 by phenobarbital. Am J Physiol Gastrointest Liver Physiol 278: G542–G550, 2000.—Phenobarbital and other xenobiotics induce drug-metabolizing enzymes, including glutathione S-transferase A1/A2 (rGSTA1/A2). We examined the mechanism of induction of rGSTA1/A2 in rat livers after phenobarbital treatment. The induction of rGSTA1/A2 was not uniform across the hepatic lobule; steady-state transcript levels were threefold higher in perivenous hepatocytes relative to periportal hepatocytes when examined by in situ hybridization 12 h after a single dose of phenobarbital. Administration of a second dose of phenobarbital 12 or 24 h after the first dose did not equalize the induction of rGSTA1/A2 across the lobule. The transcriptional activity of the rGSTA1/A2 gene was increased 3.5- to 5.5-fold in whole liver by phenobarbital, but activities were the same in enriched periportal and perivenous subpopulations of hepatocytes from phenobarbital-treated animals. The half-life of rGSTA1/A2 mRNA in control animals was 3.6 h, whereas it was 10.2 h in phenobarbital-treated animals. We conclude that phenobarbital induces rGSTA1/A2 expression by increasing transcriptional activity across the lobule but induction of rGSTA1/A2 is greater in perivenous hepatocytes due to localized stabilization of mRNA transcripts.

The liver lobule is a three-dimensional structure that is perfused by both portal venous and hepatic arterial blood. The blood moves down the hepatic sinusoid, and hepatocytes near the entry of the vessels (periportal area or zone 1) are exposed to a different environment than are cells located near the exit site for the blood at the terminal hepatic veins (perivenous area or zone 3) (10, 12). Hepatocytes arise from the same precursor cells, and many of their functions, such as the synthesis of albumin, are common to all hepatocytes within the hepatic lobule (6). However, other functions are regionally distributed. For example, carbohydrate and amino metabolism, bile salt transport, and drug metabolism vary across the lobule. These differences appear to reflect differential gene expression in the various hepatocyte subpopulations within the hepatic lobule (10). However, in at least one instance posttranscriptional events account for the perivenous localization of one gene product, the GLUT-1 glucose transporter (3).

Hepatocytes also respond differently to inducing agents depending on their location within the lobule. For example, treatment of rats with phenobarbital leads to an increase, which is greatest in perivenous hepatocytes, in the transcript levels of cytochrome P-450 CYP2B1 and CYP2B2 (7). Differential induction is observed whether the cells are in the liver or transplanted into the spleen, suggesting that the variable response to the inducer is intrinsic to the hepatocytes and is not dependent on their location within the hepatic acinus (17). The differential induction of CYP by phenobarbital is thought to be mediated transcriptionally, with increased rates of transcription in zone 3 and a lack of induction in zone 1 (30).

The glutathione S-transferases (GSTs) are a family of detoxication enzymes found in the cytosol of most cells. Like the CYP enzymes, the GSTs can be induced by a variety of agents, including phenobarbital, 3-methylcholanthrene, and products of oxidant stress (5, 11, 18, 27). The induction appears to be largely transcriptional and is mediated by response elements in the 5′-flanking sequence of the genes (11, 18, 27). Increased expression of rGSTA2 in perivenous hepatocytes is found after treatment of animals with 3-methylcholanthrene (19). In the latter study, an enhancer site in the 5′-flanking sequence of the GST gene mediates the inducible expression. Ischemia-reperfusion injury to the liver is also associated with an increase in rGSTA1/A2 transcripts in the perivenous hepatocytes of the hepatic lobule and is, at least in part, transcriptionally mediated (5). All of these studies suggest that hepatocytes in the perivenous region of the hepatic lobule respond differently to inducing agents compared with periportal hepatocytes. Most investigators have assumed that these differences are transcriptionally mediated; however, transcriptional activity in the different hepatocyte populations has not been determined.

In the current study, we systematically examined the lobular induction of rGSTA1/A2 by phenobarbital. We defined the time course of induction in both periportal and perivenous hepatocytes by in situ hybridization. We then determined whether multiple doses of phenobarbital altered the response of the hepatocytes to the inducing agent. We obtained periportal and perivenous...
enriched subpopulations of hepatocytes and determined the transcriptional activities in the two subpopulations after treatment of the animals with phenobarbital. Finally, we determined the effect of phenobarbital on rGSTA1/A2 mRNA half-life. The results of these studies demonstrate that phenobarbital has effects on rGSTA1/A2 mRNA at both transcriptional and posttranscriptional levels.

METHODS

All chemicals used were of analytical grade and, unless otherwise noted, were purchased from Sigma Chemical (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Restriction enzymes were from Promega (Madison, WI) or New England Biolabs (Beverly, MA), random primer DNA labeling reagents were from Amersham (Arlington Heights, IL), and radiolabeled nucleotides ([³²P]dCTP, [³²P]UTP and [³⁵S]UTP) were from NEN Life Science (Boston, MA). Sodium phenobarbital was from Elkins-Sinn (Cherry Hill, NJ). Adult male (250–350 g), pathogen-free, Sprague-Dawley rats were from Harlan (Prattville, AL), and the experimental protocols received prior approval from the Emory University Institutional Animal Care and Use Committee.

Phenobarbital-treated animals. Phenobarbital (8 mg/100 g body wt) or 0.9% saline for control animals was injected intraperitoneally, and liver samples from the animals were obtained at various times after injection as described below. One group of animals received a second injection of phenobarbital 12 h after the initial injection and was killed 12 h later, whereas a second group received a second injection 24 h after the first and was killed 24 h later.

Tissue collection and mRNA half-life determinations. Livers were collected at laparotomy at prescribed time intervals using pentobarbital anesthesia. At harvest, a small piece of liver was removed and embedded in optimum cutting temperature compound (Miles, Elkhart, IN), frozen immediately at −80°C, and used for in situ hybridization studies. The remaining liver was perfused with saline buffer, and portions were then frozen in liquid nitrogen, pulverized, and homogenized in ice-cold guanidine thiocyanate solution (0.5 g liver/5 ml); total RNA was extracted as described previously (5). Animals were used for determination of mRNA half-life after an intravenous injection of α-amanitin (50 µg/100 g body wt) and intraperitoneal actinomycin D (150 µg/100 g body wt) 6 h after injection of phenobarbital or saline as described previously (14). The animals were killed at various times after injection, and livers were removed and processed as described above.

Preparations enriched in perportal (zone 1) and perivenous (zone 3) hepatocytes were obtained from the same liver by modifications of a previously described method (25). In brief, the portal vein and caudal venous cava were catheterized and livers were perfused with perfusion buffer (per liter: 115 mmol NaCl, 5.6 mmol KCl, 1.2 mmol MgCl₂, 1.2 mmol NaH₂PO₄, 25 mmol NaHCO₃, and 1 g glucose, pH 7.4, at 20°C) for 5 min at a flow rate of 20 ml/min. The buffer was gassed continuously with O₂/CO₂ (19:1). The circulation to different lobes of the liver was sequentially blocked, and the liver was perfused first in a prograde direction and then in a retrograde direction with digitonin (4 mg/ml) until periporal and perivenous blanching were seen, respectively. Digitonin was washed out of the liver by perfusion in the opposite direction with perfusion buffer before each change in perfusion direction. The livers were then perfused with collagenase, and periporal and perivenous enriched hepatocyte preparations were obtained from the same liver. The cells were centrifuged using a Percoll gradient to separate hepatocytes from cellular debris, including nuclei released from lysed cells. Glutamine synthetase and alanine transaminase activities were measured in each preparation to ensure that the populations were enriched (25). Hepatocytes were snap frozen and stored at −80°C for the preparation of nuclei for nuclear runoff assays as described below.

Northern blot analysis. Total RNA from lobes of treated or control animals at each time point were isolated separately. RNA (20 µg) from each sample was denatured in 2.2 M formaldehyde/50% formamide and electrophoresed on 1% (wt/vol) agarose gels containing 2.2 M formaldehyde. The quality of the RNA was judged after staining the gels with ethidium bromide. The RNA was transferred to nylon membranes (NEN Life Science) by capillary blotting in 10× standard saline citrate (SSC; 1× SSC is 0.15 M NaCl, 0.015 M sodium citrate; pH 7.0) overnight. The RNA was linked to the membrane in a UV cross-linker (Stratagene, La Jolla, CA), dried at 80°C for 60 min, and stored at room temperature until used for hybridization.

The cDNAs for rGSTA2 (pGtB 38), rGSTA3 (pGtB 42), and quinone reductase were gifts from Dr. C. Pickett (20, 24). The cDNAs for ribosomal protein S14 (pCS14) and glyceraldehyde-3-phosphate dehydrogenase were from the American Type Culture Collection (ATCC). The cDNA for mouse albumin was from Dr. E. T. Morgan (Emory University, Atlanta, GA). A DNA fragment (300 bp) was purified from a Bgl II/EcoR I digest of the rGSTA2 plasmid that hybridized with rGSTA1 and rGSTA2 transcripts, the predominant alpha class isozymes in rat liver (20). These transcripts run as a single band on formaldehyde-agarose gels, and no other bands were detected. To acknowledge that the probe hybridizes with both rGSTA1 and A2 transcripts, we have used rGSTA1/A2 when referring to transcript levels.

RNA blots were prehybridized for 4 h at 60°C in hybridization solution (7% SDS, 1% BSA, 10% polyethylene glycol, 1.25 M NaCl, 0.15 M NaH₂PO₄, 25 mmol NaHCO₃, and 1 g glucose, pH 7.4). Radiolabeled cDNA probes were added and incubated overnight. Blots were washed three times at 65°C with 1× SSC for 30 min and exposed to film. The resulting autoradiograms were scanned in a densitometer as described previously (15). Blots were stripped in boiling 1% SDS/0.1× SSC for reprobing. Normalizing the GST transcript signal densities to those of pCS14 controlled for intrablot variability in RNA loading.

Nuclear runoff assays. Assays were performed as described previously (15). In brief, nuclei were isolated from whole liver or from periporal or perivenous enriched hepatocyte preparations from control and phenobarbital-treated animals. The nuclei were isolated in a sucrose gradient, resuspended, and snap frozen in a glycerol buffer (75 mM HEPES, pH 7.5, 60 mM KCl, 15 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, and 40% glycerol). Nuclei were used less than 30 days after isolation. After thawing on ice, −10⁷ nuclei (−250 µg DNA) were added to transcription buffer that contained [³²P]UTP. The mixture was incubated at 26°C for 45 min. The reaction was stopped, and labeled RNA was extracted by the addition of TRI Reagent (Molecular Research Center, Cincinnati, OH). Cloned DNA plasmids (5 µg) were linearized, denatured, and transferred onto nitrocellulose filters, which were then baked at 80°C for 2 h. Aliquots of labeled RNA (1 × 10⁷ cpm) were hybridized with the cDNA-containing filters at 60°C for 20 h in ml of hybridization buffer. The filters were washed twice for 20 min each in 2× SSC at 60°C, treated with RNase A (10 µg/ml) for 30 min at 37°C, washed a third time in 1× SSC for 15 min at 37°C, and then exposed to film. The resulting bands on autoradiographs were quantified by densitometry.
35S-labeled RNA probe synthesis for in situ hybridization. Sense (control) and antisense RNA probes labeled with [35S]UTP for in situ hybridization were synthesized on linearized rGSTA2 plasmid templates with riboprobe transcription reagents (Promega) (16). A cDNA fragment (530 bp) from a Pst I digest of the rGSTA2 plasmid was subcloned into the Pst I site of pBluescript II KS+ plasmid (Stratagene), and the orientation of the cDNA was determined by sequencing the resulting construct. To synthesize an antisense RNA probe specific only for rGSTA1 and A2 transcripts, the plasmid was linearized with Bgl II (sequence was same as that used for Northern blots), which, after in vitro transcription, resulted in a 377-bp RNA probe. The sense probe was synthesized after the plasmid was linearized with Eco RI, which resulted in a 615-bp RNA probe. The lengths of the RNA probes were confirmed by autoradiography after PAGE.

Liver sections for in situ hybridization. Liver sections were prepared and hybridizations were performed as described previously (16). Frozen pieces of liver were sectioned (6 µm) in a cryostat, and sections were mounted on slides (Fisher Superfrost/Plus) and immediately placed on dry ice; slides were stored at −80°C over desiccant until used (<3 days). Sections were thawed and then fixed with 4% paraformaldehyde in phosphate buffer (pH 7.4) for 10 min at 4°C. The sections were washed in 0.5× SSC for 5 min and treated with proteinase K (1 µg/ml) in 0.5 M NaCl-0.01 M Tris buffer (pH 8.0) for 5 min at room temperature. The slides were washed in 0.5× SSC and placed in air-tight hybridization boxes containing filter paper saturated with buffer (4× SSC-50% formamide). Prehybridization solution (100 µl of 0.01 M dithiothreitol, 0.3 M NaCl, 0.02 M Tris, 0.05 M EDTA, 1× Denhardt's solution, 10% dextran sulfate, and 50% formamide, pH 8.0) was placed on each section for 3 h at 42°C. Radiolabeled RNA probe (2 µl at 300,000 cpm/µl in 10 mM Tris-1 mM EDTA, pH 8.0) and yeast tRNA (1 µl of 2.5 mg/ml in water) was added to 17 µl of hybridization buffer, placed on each section, and incubated overnight at 55°C. The slides were washed and treated with RNase A (20 µg/ml in 0.5 M NaCl-0.01 M Tris, pH 8.0) and then washed again. Finally, the tissue sections were dehydrated in a graded alcohol series containing 0.3 M sodium acetate and dried under vacuum in a desiccator. The slides were dipped in Kodak Type NTB2 emulsion (diluted 1:1 with water) at 42°C and left to dry in the dark for 2 h. The emulsion-coated slides were sealed in slide boxes with desiccant at 4°C for 4 days of exposure and then were developed. A liver section from a saline-treated animal and a corresponding section from a phenobarbital-treated animal were placed together on each slide, and treatment groups and their controls were all run in the same batch to control for possible variations in thickness of the emulsions between slides and batches. The slides were developed in Kodak D19 developer for 3 min, rinsed in water for 20 s, fixed in Kodak Fixer for 3 min, and washed in water three times for 5 min each. Sections were counterstained with hematoxylin and eosin (28, 29).

Western blots. Cytosols from livers of phenobarbital-treated and saline-treated rats were prepared, and Western blots were made as previously described (16). Protein concentrations were determined by the Bio-Rad protein assay, with BSA as the standard. Cytosolic proteins (5 µg) from individual animals were loaded onto SDS gels for electrophoresis.

Fig. 1. Nuclear runoff assays from nuclei of whole livers from control and phenobarbital-treated animals. Hepatocyte nuclei were isolated from saline-treated animals (A) and from phenobarbital-treated animals after 12 (B), 24 (C), or 48 h (D) to measure transcriptional activity by nuclear runoff assay. Hybridization probes were linearized plasmids containing cloned genes for rat liver glutathione S-transferase A1/A2 (rGSTA1/A2), albumin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and ribosomal protein S14 (latter two as positive controls) plus the parent plasmids lacking genes (negative controls). 1, pBluescript (negative control for rGSTA1/A2); 2, rGSTA1/A2; 3, pBR322 (negative control for GAPDH and albumin); 4, albumin; 5, GAPDH; 6, pGEM4Z (negative control for S14); 7, S14.
Immobilon-P membranes (Millipore, Bedford, MA). The primary antiserum used for GST detection was rabbit anti-rat GSTA1/A2/A3 antiserum (MED 26 YA, 1:3,000; Biotrin, Dublin, Ireland). Bound primary antibodies were detected with enhanced chemiluminescence reagents (Amersham or NEN Life Science). The densities of the resultant bands were quantitated with a densitometer. Although the primary antibody reacts with rGSTA1, A2, and A3, complete separation of rGSTA3 from A1 and A2 was accomplished by electrophoresis (4). Therefore, the densities reported reflect changes in rGSTA1 and A2 protein levels only.

Data analysis. Northern and Western blot analyses were performed on individual animals. Values are means ± SE of at least three animals unless otherwise stated. Samples from control and phenobarbital-treated animals were run on the same blots and compared with each other. In situ hybridization sections were photographed, and fields were digitized and analyzed by computer image analysis software (Matrox Inspector, version 1.7, Matrox, QC, Canada). Briefly, high-power fields (×119) were analyzed by dividing the lobules into equal thirds to represent zone 1 (periportal), zone 2, and zone 3 (perivenous). The total area of exposed silver grains was then measured in a constant-size sample area of each zone and averaged from three different lobules from each of at least three different phenobarbital- or saline-treated animals (nine lobules per time period). Data were analyzed statistically by two-way ANOVA or Student’s t-test where appropriate. Statistical significance was achieved when P ≤ 0.05.

RESULTS

Effect of phenobarbital on mRNA, protein levels, and transcriptional activity of rGSTA1/A2. Previous studies have shown that a single dose of phenobarbital causes an increase in the mRNA and protein levels of rGSTA1/A2 in rat liver (20). In addition, transcrip-

Fig. 2. In situ hybridization using sense and antisense probes. In situ hybridization was performed on liver sections obtained from animals treated for 48 h with saline or phenobarbital. A: saline-treated animal (sense); B: phenobarbital-treated animal (sense); C: saline-treated animal (antisense); D: phenobarbital-treated animal (antisense). Reflective silver grains localize rGSTA1/A2 expression. Sections were 6-µm thick (×119). CV, central vein.
transcriptional activity increases by 2 h and remains elevated for at least 16 h after the administration of phenobarbital (8). We established that a similar series of events occurred in the present study. After a single dose of phenobarbital, liver rGSTA1/A2 mRNA levels increased 2.5 ± 0.24-fold (P < 0.01; n = 3) at 24 h. Protein levels of rGSTA1/A2 also increased 1.2 ± 0.09-fold (P < 0.02; n = 4) at 24 h. Transcriptional activity of rGSTA1/A2 was measured by nuclear runoff assay at 12, 24, and 48 h after phenobarbital treatment and increased 3.7-, 4.6-, and 5.5-fold, respectively, in treated animals compared with control animals (n = 2 at each time point; Fig. 1). Transcriptional activity for albumin was unaffected by treatment with phenobarbital, and differences were 0.49-, 0.99-, and 0.94-fold relative to untreated controls at 12, 24, and 48 h, respectively.

Effect of phenobarbital on lobular distribution of rGSTA1/A2. The induction of rGSTA1/A2 transcripts by 3-methylcholanthrene is greatest in the perivenous region of the hepatic lobule (19). We examined the effect of phenobarbital on lobular induction of rGSTA1/A2 by in situ hybridization. The area covered by silver grains in the lobules of saline-treated animals using the sense probe was three- to fivefold less than the area covered when the antisense probe was used. In addition, with either probe there was no significant intralobular variability (Fig. 2). After treatment with phenobarbital, the silver grain area seen with the sense probe did not change compared with that of saline-treated animals [e.g., saline vs. phenobarbital (48 h): zone 1 = 185 ± 17 vs. 152 ± 9; zone 2 = 143 ± 11 vs. 140 ± 11; zone 3 = 147 ± 8 vs. 136 ± 10; means ± SE; not significant]. In contrast, when the antisense probe was used there was an increase in rGSTA1/A2 transcripts (Fig. 2). The earliest increase in transcript levels was seen in zones 2 and 3.3 h after phenobarbital treatment, and increases were present in all three zones 12 h after phenobarbital treatment. The increase in transcript levels was greatest in zone 3 and least in zone 1 (Figs. 3 and 4). The time course of induction in zones 1 and 3 relative to control animals is shown in Fig. 5. The greatest increase in transcript levels was seen in zone 3 at 12 h (11-fold compared with saline-treated animals), which then decreased to 9-fold at 48 h. The relative induction in zone 1 peaked at a 3.8-fold increase at 24 h.

The slower rate of increase in transcript levels in zone 1 relative to zone 3 suggested that a delay in response or insensitivity to phenobarbital might account for the observed differences. We administered a second dose of phenobarbital 12 or 24 h after the first and then performed in situ hybridization either 12 or 24 h later. Twelve hours after the second dose of phenobarbital, mRNA levels increased 1.7-fold, whereas 24 h after the second dose of rGSTA1/A2 mRNA levels increased 2.6-fold. The increase in transcripts was greatest in zone 3 and least in zone 1. In fact, zone 1 hepatocytes showed relatively less induction following two doses of phenobarbital than they did following one dose (Fig. 6).

Transcriptional activity in periportal and perivenous hepatocytes after treatment with phenobarbital. We questioned whether differences in transcriptional activity accounted for the higher transcript levels of rGSTA1/A2 in perivenous vs. periportal hepatocytes after treatment with phenobarbital. Fractions enriched in either periportal or perivenous hepatocytes were used to perform nuclear runoff assays. Transcriptional activity in the periportal hepatocytes was similar to that in perivenous hepatocyte preparations at 3 h or 12 h after administration of phenobarbital with all cDNA probes used. No increase in transcriptional activity in perivenous hepatocytes relative to periportal hepatocytes for rGSTA1/A2 was observed (Table 1).

Effect of phenobarbital on mRNA half-life of rGSTA1/A2. Since the increase in transcript levels in zone 3 relative to zone 1 after treatment with phenobarbital was not due to a difference in transcriptional activity, we questioned whether phenobarbital might have affected the posttranslational stability of rGSTA1/A2 transcripts. Animals were treated either with phenobarbital or saline and 6 h later given α-amanitin and actinomycin D to block transcription. The half-life of rGSTA1/A2 transcripts in saline-treated animals was 3.6 h, whereas the half-life in phenobarbital-treated animals was 10.2 h (Fig. 7).
DISCUSSION

The induction of detoxication enzymes by xenobiotics has been the focus of extensive investigation for many years. Of particular interest has been the finding that much of the induction was due to regulation of rates of transcription through response elements in the 5′-flanking sequences of the enzyme genes. The rGSTA2 gene has been extensively studied in this regard, and its 5′-flanking sequence contains regulatory elements that are responsive to xenobiotics such as phenobarbital and 3-methylcholanthrene (11, 18). Studies in animals suggest that all of the increase in steady-state levels of GST mRNA after treatment with phenobarbital can be accounted for by an increase in rates of transcription (8, 19, 20). However, studies in cultured cells suggest that the effects of phenobarbital may be more complex with both transcriptional and posttranscriptional events accounting for the increase in rGSTA1/A2 mRNA levels (26).

In the current study, phenobarbital was shown to increase rGSTA1/A2 transcript levels by increasing the transcriptional activity of the gene in rat liver. This increase in transcriptional activity appeared to account for the panlobular induction of rGSTA1/A2; however, it does not account for the greater increase in rGSTA1/A2 transcripts that developed in zones 2 and 3. The overall increase in transcriptional activity was similar to the increase in transcript levels in zone 1. However, the increase in transcript levels in zone 3 was much greater than that in zone 1, despite the fact that rates of transcription in periportal and perivenous hepatocytes were similar. Thus posttranscriptional events rather than an increase in rates of transcription must account for the greater induction of rGSTA1/A2 in perivenous relative to periportal hepatocytes.
Posttranscriptional events have a significant impact on mRNA levels and are important in gene regulation. Changes in mRNA half-lives occur in response to alterations in cell growth, viral infections, and exposure to toxins (2, 22). For example, changes in gene expression during liver regeneration are mediated frequently by changes in mRNA stability and not by alterations in rates of transcription (13). Similarly, when the mechanism behind the increase in collagen synthesis by hepatic stellate cells was examined, an increase in mRNA stability accounted for most of the increase in collagen mRNA (23). In the current investigation, the mRNA half-life of rGSTA1/A2 increased more than threefold in the phenobarbital-treated animals compared with untreated animals. Previous studies in cultured cells also suggest that phenobarbital stabilizes GST message (26). We have not measured rGSTA1/A2 mRNA half-lives in periportal vs. perivenous hepatocytes for technical reasons, but it is reasonable to assume that they are different in the absence of differences in rates of transcription. A three- to fourfold increase in rates of transcription in concert with a threefold increase in mRNA half-life could have led to the 11-fold increase in mRNA levels that was observed in the perivenous hepatocytes.

Changes in mRNA stability are commonly mediated at the 3'-untranslated (3'-UTR) region of the mRNA. Deadenylation, binding of proteins to the poly(A)⁺ tail, or endonucleolytic cleavage within the coding region are mechanisms that may affect mRNA stability (2, 22). The binding of specific proteins to the poly(A)⁺ tail or to regions rich in adenosine or cytosine in the 3'-UTR stabilizes mRNA (2, 22). Treatment of mice with the inducing agents pyrazole or 3-methylcholanthrene leads to induction of specific proteins that bind to the 3'-UTR of CYP2a5 and CYP1a2 mRNA (9, 21). These proteins are thought to stabilize the mRNA and therefore lead to

**Table 1. Relative transcriptional rates in perivenous and periportal hepatocytes**

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Time After Phenobarbital, h</th>
<th>3</th>
<th>12</th>
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<tbody>
<tr>
<td>rGSTA1/A2</td>
<td>1.1</td>
<td>0.8 ± 0.30</td>
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<tr>
<td>rGSTA3</td>
<td>1.7</td>
<td>1.1 ± 0.44</td>
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</tr>
<tr>
<td>Albumin</td>
<td>1.1</td>
<td>0.7 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Quinone reductase</td>
<td>2.5</td>
<td>1.1 ± 0.30</td>
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Zone 1 and zone 3 enriched hepatocytes were prepared at 3 and 12 h after phenobarbital treatment as described in METHODS. Values are transcriptional activities in zone 3 relative to zone 1. Values from the 3-h animals are average of 2 separate runoff experiments, whereas values at 12 h are from 3 separate experiments (means ± SE). rGST, rat liver glutathione S-transferase.
an increase in steady-state transcript levels. Phenobarbital may also induce a protein that binds to the 3′-UTR of rGSTA1/A2 mRNA, decreasing rates of degradation of the message. If perivenous hepatocytes produce more of these proteins in response to phenobarbital and 3-methylcholanthrene than periportal hepatocytes, then transcript levels will be higher in perivenous relative to periportal hepatocytes. Identification of these proteins will be required before this hypothesis can be established with certainty.

In the current study we questioned whether the induction by phenobarbital of rGSTA1/A2 in periportal hepatocytes was delayed relative to the induction in perivenous hepatocytes. We therefore administered a second dose of phenobarbital to determine if a further induction of transcript levels above that induced by a single dose could occur in periportal hepatocytes. However, the mRNA induction in perivenous hepatocytes was always greater than that in periportal cells whether one or two doses of phenobarbital were administered. These results suggest that the difference in rGSTA1/A2 induction between perivenous and periportal hepatocytes is intrinsic to the cells and do not reflect their position within the lobule or how the phenobarbital is administered. This conclusion is consistent with previous work in which increased expression of CYP was greater in perivenous hepatocytes. Transplanting the cells into another location (the spleen) did not affect the induction of the CYP enzyme by phenobarbital, which supports the idea that the difference in periportal and perivenous hepatocytes is intrinsic to the different cell populations (1, 7, 17, 30). However, in contrast to previous investigators, we believe posttranscriptional and not transcriptional events account for the difference.

In conclusion, treatment of rats with phenobarbital causes a nonuniform increase in transcript levels of rGSTA1/A2 in the hepatic lobule. The greatest increase in rGSTA1/A2 transcript levels was found in perivenous hepatocytes and exceeded that which could be accounted for by an increase in the rate of transcription, because transcriptional activity was uniform across the hepatic lobule. Phenobarbital also increased the half-life of the rGSTA1/A2 message. We believe that this increase in message half-life accounts for the higher transcript levels in perivenous relative to periportal hepatocytes. Further studies are required to define exactly how phenobarbital stabilizes rGSTA1/A2 message differentially in perivenous and periportal hepatocytes.

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REFERENCES


