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Hemin Administration to Rats Reduces Levels of Hepatic mRNAs for Phenobarbitone-Inducible Enzymes

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SUMMARY

The levels of hepatic mRNAs for several enzymes involved in drug metabolism were measured following administration to rats of either phenobarbitone or 2-allyl-2-isopropylacetamide. There was a substantial elevation in the mRNA levels for cytochromes P450 IIB1, IIB2, and IIIA1, epoxide hydrolase, glutathione-*S*-transferase Y_a/Y_c subunit, UDP-glucuronosyltransferase isoenzyme ($UDPGT_r$-2), NADPH-cytochrome P450 oxidoreductase, and 5-aminolevulinate synthase. When rats were treated with hemin, together with inducing drug, there was a marked reduction in the induced levels of these mRNAs, with decreases in the range of 55–95%. Basal levels of these mRNAs in the noninduced rat liver were also lowered by hemin administration. Nuclear run-on transcriptional experiments showed that hemin administration substantially lowered both the basal and drug-induced transcriptional activities of the genes for cytochrome P450IIB1/IIB2 and 5-aminolevulinate synthase. In contrast, the mRNA for heme oxygenase was elevated by hemin treatment, whereas the mRNA levels of β-actin, albumin, and ornithine transcarbamylase, used as controls, were not affected. Treatment of rats with clofibrate resulted in increased levels of mRNA for cytochrome IVA1 and, in addition, those for cytochromes P450IIB1 and P450IIB2. Hemin administration repressed the induction of mRNA levels for cytochromes P450IIB1 and IIB2 but not that for cytochrome P450 IVA1. Additionally, the induction of P450IA1 by β-naphthoflavone was not affected by hemin. The results suggest that heme may negatively control the induction of cytochromes P450IIB1 and IIB2 and other hepatic enzymes by phenobarbitone and phenobarbitone-like drugs and perhaps play a role in regulating drug metabolism. There is, however, no evidence at present as to whether heme has a direct role in such a mechanism or whether injected hemin promotes a secondary response.

The P450-dependent monooxygenase system located in the liver endoplasmic reticulum is responsible for the oxidative metabolism of a large number of endogenous and foreign hydrophobic compounds (1, 2). This system is composed of a single enzyme species of NADPH-cytochrome P450 oxidoreductase and multiple species of P450s (1, 2). The latter are encoded by a superfamily of related genes divided into families and subfamilies on the basis of amino acid sequence data. Many hepatic P450s are selectively induced by different foreign chemicals. Of particular interest in the present study is the sedative phenobarbitone. When administered to rats, phenobarbitone dramatically increases the levels of the hepatic P450s, chiefly P450IIB1 and P450IIB2 (3), in addition to NADPH-cytochrome P450 oxidoreductase (4).

Other enzymes are required for the final conversion of oxygenated foreign compounds to water-soluble metabolites that can be more easily excreted from the body. These include the microsomal epoxide hydrolases and UDP-glucuronosyltransferases and cytosolic enzymes such as glutathione *S*-transferases. UDP-glucuronosyltransferases and glutathione *S*-transferases each constitute a family of proteins and, in rat liver, phenobarbitone induces the UDP-glucuronosyltransferase isozyme $UDPGT_r$-2 (5); the Y_a, Y_b, and Y_c subunits that may comprise the dimeric glutathione *S*-transferase isozymes are also induced by this drug (6, 7). The rate-limiting mitochondrial enzyme of the hepatic heme biosynthetic pathway, 5-aminolevulinate synthase, can also be induced by phenobarbitone (8) and we have demonstrated, in rats, that this control is exerted at the level of transcription of the gene (9). The level of 5-aminolevulinate synthase is subject to a negative end-product control mechanism, with heme acting to inhibit gene transcription (9).

In the present work, the effect of administered hemin on the synthesis of several drug-inducible mRNAs in rat liver has been examined. We have made the unexpected observation that hemin injected into rats reduces the levels of mRNAs for a number of genes associated with drug metabolism, and the findings may be relevant to the mechanism by which phenobarbitone induces hepatic mRNAs.

This research was supported by grants from the National Health and Medical Research Council of Australia, the University of Hong Kong, and the Croucher Foundation.

ABBREVIATIONS: P450, cytochrome P450; NaCl/P_i/EDTA, 0.9 M NaCl, 50 mM sodium phosphate buffer, pH 7.0, 5 mM EDTA; kb, kilobases; SDS, sodium dodecyl sulfate.

Materials and Methods

Treatment of animals. Male albino Wistar rats (about 100–200 g of body weight), fasted overnight, received injections of drugs at time zero and at 12 hr before slaughter at 18 hr. 2-Allyl-2-isopropylacetamide was administered subcutaneously (400 mg/kg of body weight, in 0.9% saline), phenobarbitone intraperitoneally (80 mg/kg of body weight, in 0.9% saline), clofibrate intraperitoneally (400 mg/kg of body weight, in peanut oil), and β-naphthoflavone intraperitoneally (50 mg/kg of body weight, in corn oil).

Hemin was administered to rats as heme arginate, prepared as described by Tokola (10). Heme arginate was diluted 3-fold with 0.9% saline immediately before injection intraperitoneally at 40 mg/kg of body weight; injections were given at the same time as drugs.

Analysis of RNA. Total RNA was isolated from 2.5 g of liver, using the guanidine-hydrochloride procedure described previously (11). Livers from two rats were pooled for each treatment and each experiment was repeated three times. Similar results were obtained for each experiment and representative results are shown. For Northern blot analysis, total RNA (20 μg/lane) was electrophoresed in 1.0% agarose gels containing 1.1 M formaldehyde (11) and was transferred to nitrocellulose filters (BA85 from Schleicher & Schuell). Filters were hybridized with radiolabeled cDNA or oligomer probes. The cDNA clones employed as probes were radiolabeled by nick translation with both [α-^{32}P]dATP and [α-^{32}P]dcTP, to give specific activities of 1–5 × 10^8 cpm/μg. The cDNA probes used were rat liver 5-aminolevulinate synthase cDNA clone p101B1 in pBR322 (2.3-kb insert) (9), rat P450IIB1 cDNA clone pB7 (1.9-kb insert), which hybridizes to P450IIB1 and P450IIB2 mRNAs (12) and which was generously provided by A. Anderson, Université Laval, Québec, Canada, a chicken β-actin cDNA clone in pBR322 (1.8-kb insert), a rat albumin cDNA clone in pBR325 (13), and a rat ornithinine transcarbamylase cDNA clone in pUC9 (14). Specific DNA oligomeric probes complementary to mRNAs were synthesized by Bresatec (Australia). Oligomers were derived from the following published cDNA sequences: nucleotides 1032–1049 of the rat P450s IIB1 [3′-d(AGTGTGGCCGATGGTTGG)-5′] and IIB2 [3′-d(AGAGTGTCCGGTGGTAGG)-5′] (3), nucleotides 1594–1623 of rat P450IIIA1 [3′-d(CTCAAGACGACTTAAGCAGTCTTTACACCA)-5′] (15), nucleotides 209–238 of rat NADPH-cytochrome P450 oxidoreductase [3′-d(AAGCACCTTTTCTACTTCTTTTGCCCTTCC)-5′] (16), nucleotides 1169–1198 of rat epoxide hydrolase [3′-d(CTCCTACCTCCGGACCTCTCCTTCAAGAGG)-5′] (17), nucleotides 305–334 of rat UDP-glucuronosyltransferase (UDPGT$_r$-2) [3′-d(CTTTGTGAGAGCTAAACCTGTATAATAAGT)-5′] (5), nucleotides −30 to −1 of rat heme oxygenase [3′-d(CCTCGGTCGGACTTGATCGGGTCAGGGCGC)-5′] (18), nucleotides 598–627 of rat glutathione *S*-transferase Y_a/Y_c subunit [3′-d(GGAGACGACTTCCGGAAGTTCTCGTCTTAG)-5′] (7), nucleotides 988–1017 of the rat P450IVA1 coding sequence [3′-d(CGGTCACCTCAGAGGACCTAGAAGATACGA)-5′] (19), and nucleotides 1645–1674 of the rat P450IA1 coding sequence [3′-d(GCCAGAAGACCAGGAGTCGTAGAGGTCCGA)-5′] (20). The oligomeric probes were 5′-labeled with [γ-^{32}P]ATP, using T_4 polynucleotide kinase. For the nick-translated cDNA probes, filters were hybridized in a solution containing α-^{32}P-labeled cDNA (5 ng/ml), 50% formamide, 5× NaCl/P_i/EDTA, 5× Denhardt's (0.1% Ficoll, 0.1% polyvinyl pyrrolidone, and 0.1% bovine serum albumin), 10% dextran sulfate, 0.1% SDS, 0.05% sodium pyrophosphate, and 200 μg/ml sheared denatured salmon sperm DNA, at 42° for 20 hr. Filters were washed in a solution containing 0.5× NaCl/P_i/EDTA, 0.1% SDS, and 0.05% sodium pyrophosphate, at room temperature for 20 min and subsequently at 60° for 40 min. For the oligomeric probes, filters were hybridized in the above solution without formamide, at the same probe concentration, for 20 hr at 52°. Filters were washed in 5× NaCl/P_i/EDTA, 0.1% SDS, 0.05% sodium pyrophosphate, at room temperature for 20 min and subsequently at 52° for 20 min, and were autoradiographed.

***In vitro* transcription of isolated nuclei.** Rats were treated with drug and/or hemin and nuclei were prepared from the livers, as described by Schibler *et al.* (21). Nuclei were prepared from the livers of two rats, which were pooled for each treatment.

The transcription reactions contained 100 mM Tris·HCl, pH 7.9, 50 mM NaCl, 5 mM $MgCl_2$, 1.5 mM $MnCl_2$, 0.4 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1.2 mM dithiothreitol, 30% glycerol, 2 μM UTP, 1 mM concentrations each of ATP, CTP, and GTP, 100 μCi of [α-^{32}P]UTP (1500 Ci/mmol) from Bresatec (Australia), and 1.5 × 10^7 nuclei, in a final volume of 150 μl. The reactions were incubated at 26° for 15 min and ^{32}P-labeled RNA was extracted as described (9).

For quantitation of specific transcripts, nitrocellulose filters, containing 5 μg of appropriate plasmid applied in duplicate using a slot-blot apparatus, were prehybridized in 50% formamide, 5× NaCl/P_i/EDTA, 10 mM Tris·HCl, pH 7.6, 1 mM EDTA, 0.1% sodium pyrophosphate, 0.1% SDS, 100 μg/ml *Escherichia coli* tRNA, 10× Denhardt's (0.2% Ficoll, 0.2% polyvinyl pyrrolidone, and 0.2% bovine serum albumin), for 12 hr at 52°. Hybridization was performed in the same solution at 52° for 72 hr with 5 × 10^6 cpm of ^{32}P-labeled RNA. Filters were washed twice at room temperature for 30 min in 2× NaCl/P_i/EDTA, 0.1% SDS, 0.05% sodium pyrophosphate, and then twice in 0.5× NaCl/P_i/EDTA, 0.1% SDS, 0.1% sodium pyrophosphate, at 65° for 60 min. This was followed by treatment with RNase A (10 μg/ml) in 1× NaCl/P_i/EDTA at 37° for 30 min.

Filter-bound plasmid DNAs (5 μg) were in excess and the hybridizations were shown to be linear with increasing amounts of ^{32}P-labeled RNA. Incorporation by nuclei of [α-^{32}P]UTP was linear for at least 30 min, and the extent of incorporation was similar in nuclei from untreated rats and rats treated with drugs, hemin, or both. Background hybridization was assessed using pBR322. α-Amanitin added to the reaction at 2 μg/ml inhibited the transcription signal for all genes tested by about 75%, whereas the addition to the transcription reaction of hemin at concentrations up to 10 μM did not affect run-on transcription activities of the genes examined.

Densitometric quantitation of autoradiographs. All nitrocellulose filters were exposed to Kodak X-Omat AR film between two intensifying screens at −80°. Signals were quantitated by densitometric scanning of the autoradiograms, using a Zeineh soft laser densitometer. The exposure times employed resulted in hybridization signals that were in the linear response range of the film.

Results

The mRNA levels for several hepatic enzymes were measured following administration to rats of the drugs phenobarbitone or 2-allyl-2-isopropylacetamide. Levels of the major phenobarbitone-inducible P450 mRNAs, P450IIB1 and P450IIB2, were first examined. The cDNA probe employed hybridized to both of these mRNAs and, because these are of the same size (2.1 kb), the two mRNAs were, therefore, indistinguishable in our Northern blots. As seen in Fig. 1a, both phenobarbitone and 2-allyl-2-isopropylacetamide substantially elevated levels of the 2.1-kb mRNA species, with approximate 15-fold and 12-fold increases, respectively, in mRNA amounts, compared with basal levels. Specific oligomer probes that could distinguish between these two mRNAs (3) confirmed that both mRNAs are induced by these drugs (Fig. 1, b and c). Levels of mRNA for P450IIIA1 were also examined. The best known inducer of this mRNA in rat liver is dexamethasone, but this mRNA is also known to be induced to a lesser extent by phenobarbitone (22). The P450IIIA1 mRNA of 2.2-kb size was elevated by either phenobarbitone or 2-allyl-2-isopropylacetamide (Fig. 1d). The DNA oligomer probe employed also hybridized to a second mRNA of 2.0-kb size, which was drug induced and probably represents another member of the P450III gene family (22).

The effect of hemin injections on the basal and drug-induced

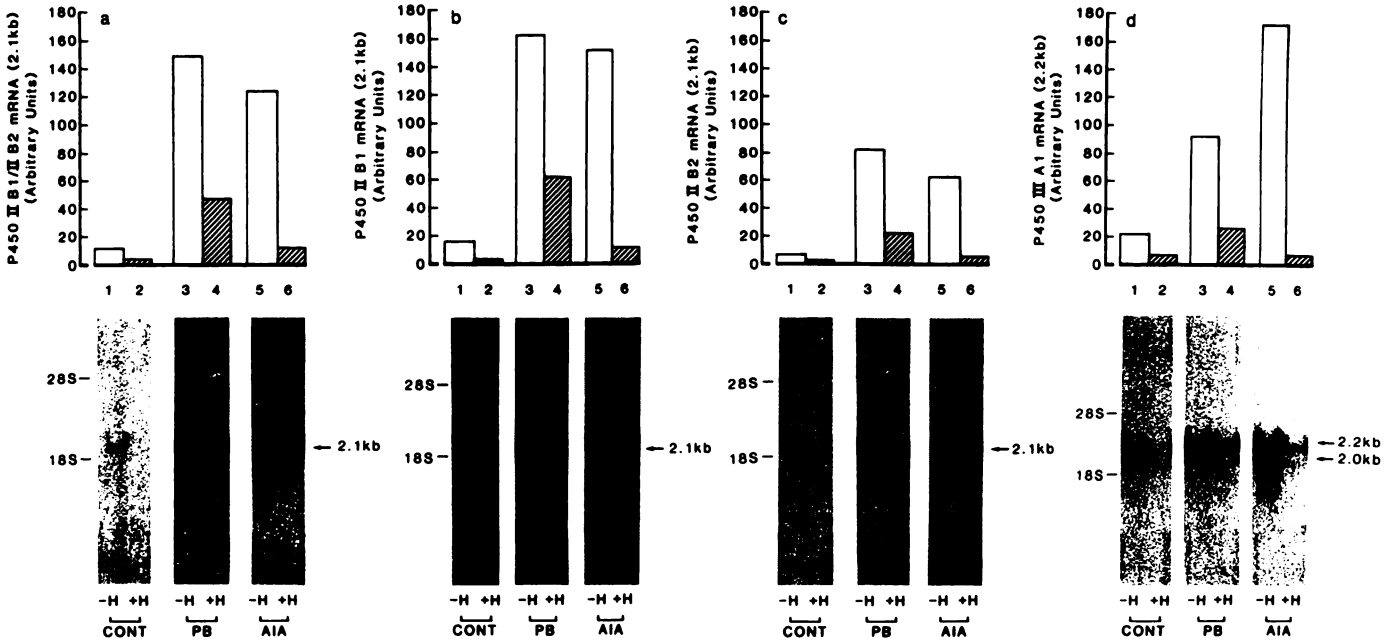

Fig. 1. Effect of hemin on basal and drug-induced levels of P450 mRNAs. Rats were administered two injections of either hemin alone, drug alone, or drug plus hemin at 18 and 6 hr before isolation of total hepatic RNA. Total RNA (20 μg/lane) was electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose filters, and hybridized with a nick-translated 450-base pair *Hind*III-*Nco*I fragment of pB7 (a) or with end-labeled specific γ ^{32}P-oligomers for P450IIB1 (b), P450IIB2 (c), or P450IIIA1 (d). Treatments: no drug (*CONT*), phenobarbitone (*PB*), 2-allyl-2-isopropylacetamide (*AIA*), no hemin (*−H*), hemin (*+H*). Autoradiographic signals were quantitated by laser densitometry and are presented as *bar graphs* (*1–6*) directly above each respective track. □, No hemin; ▨ hemin administration. The positions of 18 S and 28 S rRNA are indicated.

mRNA levels for these P450s was investigated. Rats were injected with hemin, as heme arginate (10), at the same times as either phenobarbitone or 2-allyl-2-isopropylacetamide. Hemin markedly repressed both the basal and drug-induced levels of the 2.1-kb mRNA species for P450IIB1/IIB2 (Fig. 1a). The phenobarbitone-induced and 2-allyl-2-isopropylacetamide-induced mRNA levels were reduced after hemin treatment by about 70 and 90%, respectively. This reduction was observed consistently following hemin treatment. Moreover, both P450IIB1 and P450IIB2 mRNA levels were reduced, as shown by use of the specific oligomeric probes (Figs. 1, b and c). Hemin administration also markedly reduced the basal and drug-induced levels of both the P450IIIA1 mRNA and the P450IIIA1-like mRNA (Fig. 1d).

The levels of mRNA for several other known phenobarbitone-inducible proteins involved in drug metabolism were similarly determined. Following either phenobarbitone or 2-allyl-2-isopropylacetamide treatment of rats, hepatic levels of mRNA were induced for the NADPH-cytochrome P450 oxidoreductase mRNA of 2.4-kb size (Fig. 2a), for epoxide hydrolase mRNA of 1.8-kb size (Fig. 2b), for the glutathione *S*-transferase Y_a/Y_c subunit mRNA of 1.0-kb size (Fig. 2c), and for $UDPGT_r$-2 mRNA of 2.3-kb size (Fig. 2d). These drugs gave similar induced levels of the various mRNAs, except for the Y_a/Y_c subunit mRNAs, where the 2-allyl-2-isopropylacetamide induction was 50% of that observed with phenobarbitone. As seen in Fig. 2, when rats were treated with hemin together with the inducing drug, there was a substantial reduction in the levels of mRNA for these four drug-metabolizing enzymes, with decreases in the range of 55–95%. In the same figure, basal levels of mRNA for these enzymes were also reduced by treatment of rats with hemin.

Levels of mRNA were also determined for hepatic 5-aminolevulinate synthase, which is the rate-limiting enzyme of the heme biosynthetic pathway (8), and for hepatic heme oxygenase, which catalyzes the rate-limiting step of heme degradation (18). The level of the 5-aminolevulinate synthase mRNA (2.3 kb) was induced about 12-fold by either 2-allyl-2-isopropylacetamide or phenobarbitone, and this induction was prevented by hemin administration (Fig. 3a). These results are in agreement with our previous findings (9) and indicate that 5-aminolevulinate synthase mRNA levels are regulated through an end-product repression mechanism. In contrast, hemin treatment markedly elevated the basal level of the mRNA for heme oxygenase (Fig. 3b). This finding has been well established (18) and reflects an increased capacity of the liver to metabolize additional hemin. Interestingly, in the presence of drugs alone, a small increase in the basal level of the heme oxygenase mRNA was observed. This probably reflects an increased amount of heme following drug induction of 5-aminolevulinate synthase; a similar response has been observed in chick embryo liver (23).

In all experiments described, the amount of β-actin mRNA of 1.9-kb size (measured as a control) was found to be essentially unchanged by drug or hemin treatment of rats (Fig. 3c). Similarly, mRNA levels for hepatic albumin and ornithine transcarbamylase were not affected by administration of drugs or hemin (results not shown). Although in all experiments hemin was administered as heme arginate (10), hemin was responsible for the effects observed, because injection of "heme arginate" lacking the hemin component had no effect on levels of any of the mRNAs investigated (results not shown). These findings together indicate that administered drugs and hemin modulate mRNA levels for drug-metabolizing enzymes without affecting general cellular events.

To investigate the level at which administered hemin may

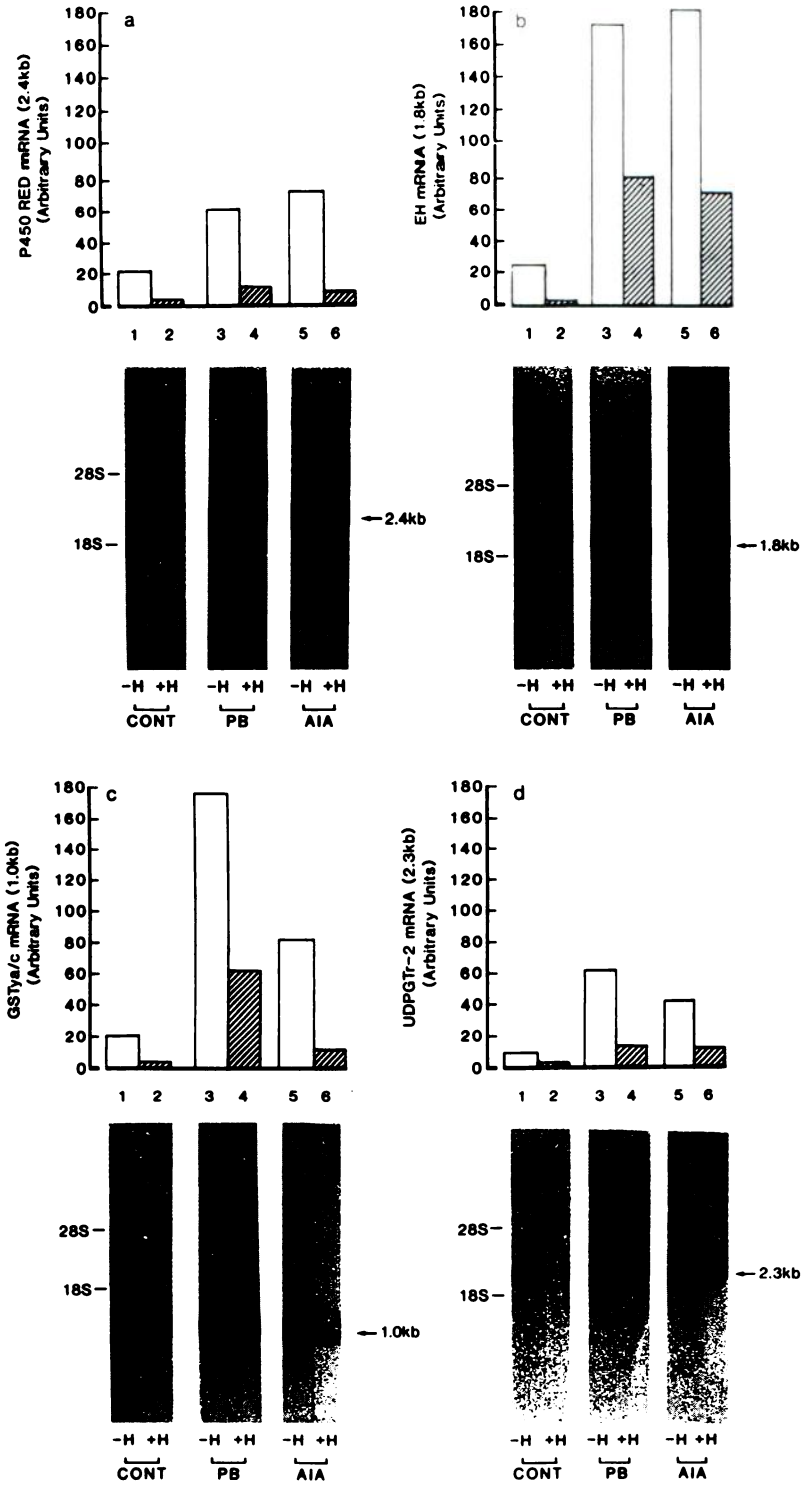

Fig. 2. Effect of hemin on basal and drug-induced mRNA levels for NADPH P450 oxidoreductase, epoxide hydrolase, glutathione *S*-transferase, and UDPGT$_r$-2. Rats were administered drugs and hemin, as in the legend to Fig. 1. Total RNA (20 μg/lane) was electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose filters, and hybridized with end-labeled [γ-^{32}P]DNA oligomers for NADPH P450-oxidoreductase (a), epoxide hydrolase (*EH*) (b), glutathione S-transferase (c), or UDPGT$_r$-2 (d). Treatments: no drug (*CONT*), phenobarbitone (*PB*), 2-allyl-2-isopropylacetamide (*AIA*), no hemin (*–H*), hemin (*+H*). Autoradiographic signals were quantitated by laser densitometry and are presented as *bar graphs* (*1–6*) directly above each respective track. □, No hemin; ▨ hemin administration. The positions of 18 S and 28 S rRNA are indicated.

exert its control, nuclear run-on transcriptional activities of the genes for P450IIB1/IIB2 and 5-aminolevulinate synthase were quantitated using nuclei isolated from livers of rats. Treatment of rats with phenobarbitone alone increased the rate of P450IIB1/IIB2 gene transcription 15-fold and that of the 5-aminolevulinate synthase gene 10-fold, relative to basal transcription rates (Fig. 4, a and b). This was accompanied by comparable increases in mRNA levels for P450IIB1/IIB2 and 5-aminolevulinate synthase measured at this time (Figs. 1 and 3). Similar results were obtained with 2-allyl-2-isopropylacetamide (Fig. 4, a and b), although the run-on transcriptional activities were somewhat less. Administration of hemin to untreated rats reduced the basal transcription rates of the genes for P450IIB1/IIB2 and 5-aminolevulinate synthase, whereas administration of hemin to phenobarbitone-treated rats lowered the drug-induced increase in the transcriptional activities of these genes by about 60% (Fig. 4, a and b). These decreases were accompanied by an observed reduction in mRNA levels for P450IIB1/IIB2 and 5-aminolevulinate synthase of about 75% and 90% respectively (Figs. 1 and 3). A reduction of both transcriptional activities and mRNA levels for these genes was also observed following hemin treatment of rats induced with

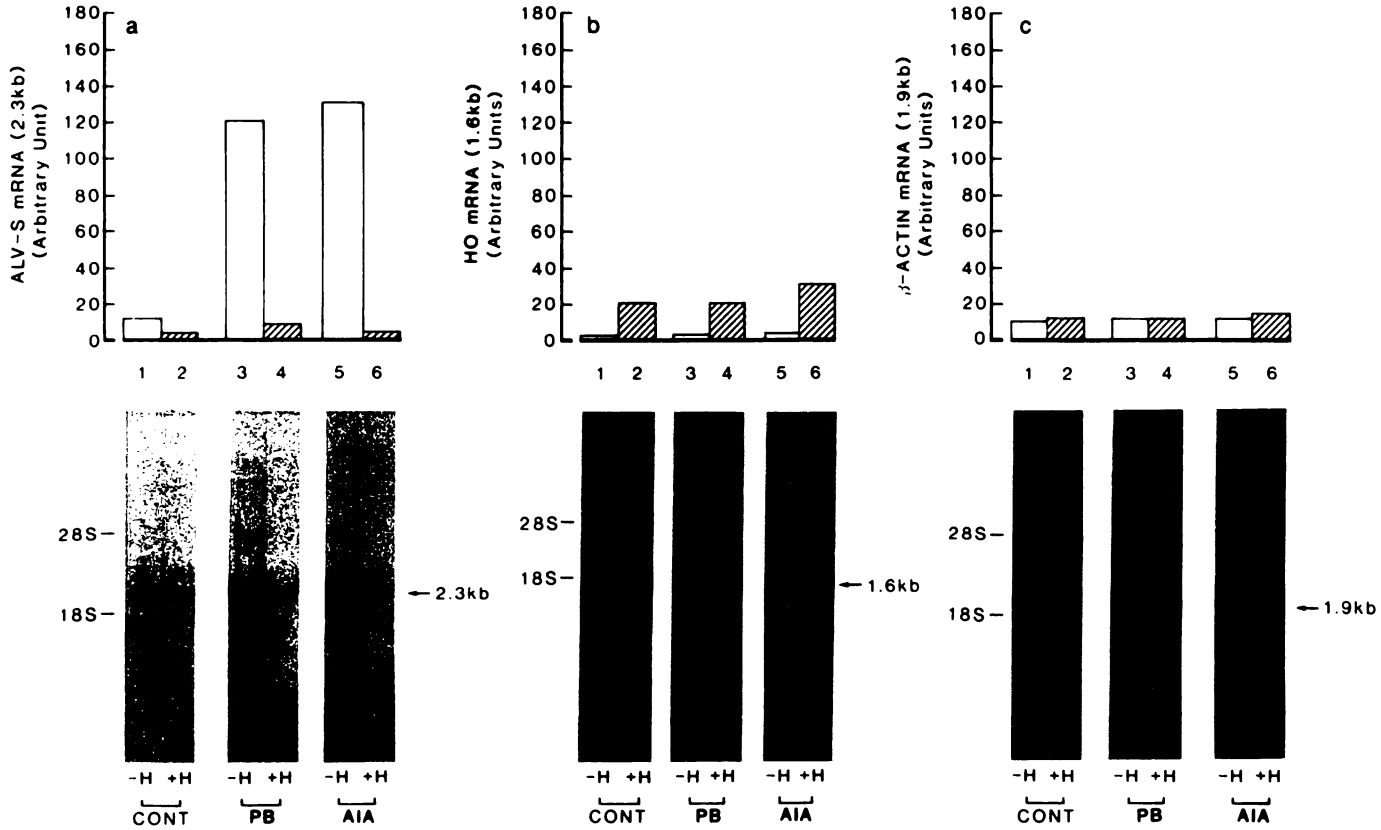

Fig. 3. Effect of hemin on 5-aminolevulinate synthase, heme oxygenase, and β-actin mRNA levels. Rats were administered drugs and hemin, as stated in the legend to Fig. 1. Total RNA (20 μg/lane) was electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose filters, and hybridized with nick-translated cDNA clones for 5-aminolevulinate synthase (*ALV-S*) (a) or β-actin (c) or, alternatively, filters were probed with an end-labeled [γ^{32}P]DNA oligomer specific for heme oxygenase (*HO*) (b). Treatments: no drug (*CONT*), phenobarbitone (*PB*), 2-allyl-2-isopropylacetamide (*AIA*), no hemin (*–H*), hemin (*+H*). Autoradiographic signals were quantitated by laser densitometry and are presented as *bar graphs* (*1–6*) directly above each respective track. □, No hemin; ■ hemin administration. The positions of 18 S and 28 S rRNA are indicated.

2-allyl-2-isopropyl-acetamide (Figs. 1 and 3). The fact that the transcription rates and mRNA levels measured at this time do not correlate precisely probably reflects a delay in mRNA synthesis following recovery of transcription rates, and measurements at other times must be made to investigate this. Nevertheless, it is clear that hemin administration substantially affected the transcription rates of these genes. In contrast, transcription of the control β-actin gene was not affected by these treatments (Fig. 4c).

In the next phase of the work, the effect of hemin administration on the amounts of P450 mRNAs elevated by either clofibrate or β-naphthoflavone was investigated. Clofibrate, a peroxisome-proliferating agent, induces both hepatic P450IVA1 mRNA (19) and a P450IIB1-like mRNA species (24). Using specific oligomeric probes, we confirmed that the P450IVA1 mRNA of 2.2-kb size was induced and we established that both P450IIB1 and P450IIB2 mRNAs of 2.1-kb size were also elevated (Fig. 5). When rats were administered hemin, the basal and drug-induced levels of mRNAs for P450IVA1 were not significantly affected. However, following hemin treatment, the P450IIB1 and P450IIB2 mRNA levels were dramatically reduced to levels below the control and were not detectable.

It was also of interest to examine the effect of hemin administration on the induction of the P450IA1 mRNA. Using a specific oligomeric probe for this mRNA, we established that mRNA of 2.7-kb size is strongly induced in rat liver by β-naphthoflavone (Fig. 6). However, hemin administration did not affect this induction. In this experiment, basal levels of the P450IA1 mRNA were not detected.

Discussion

The results show that hemin administration to rats prevents the induction by phenobarbitone of several liver mRNAs, in particular P450IIB1 and P450IIB2 mRNAs. Induction of these latter mRNAs by clofibrate is also inhibited by hemin. In these studies, hemin cannot be eliciting a general cellular toxic effect, because the induction of P450IVA1 and P450IAI mRNAs by clofibrate and β-naphthoflavone, respectively, is not affected. In addition, the mRNA levels of β-actin, albumin, and ornithine transcarbamylase were not affected by hemin treatment. The present findings imply that the induction of P450IIB1 and P450IIB2 mRNAs by phenobarbitone, 2-allyl-2-isopropylacetamide, and clofibrate occurs through a common mechanism, which involves a heme-sensitive step, with induction of P450IVA1 mRNA by clofibrate occurring through a separate mechanism. Whether the phenobarbitone induction of all mRNAs is repressed by heme remains to be established. Interestingly, preliminary data indicate that P450IIB1 and P450IIB2 mRNAs, when induced by dexamethasone, are repressed in rat liver by hemin administration but the P450IIIA1 mRNA induced by dexamethasone is not repressed.[1]

[1] Unpublished observations.

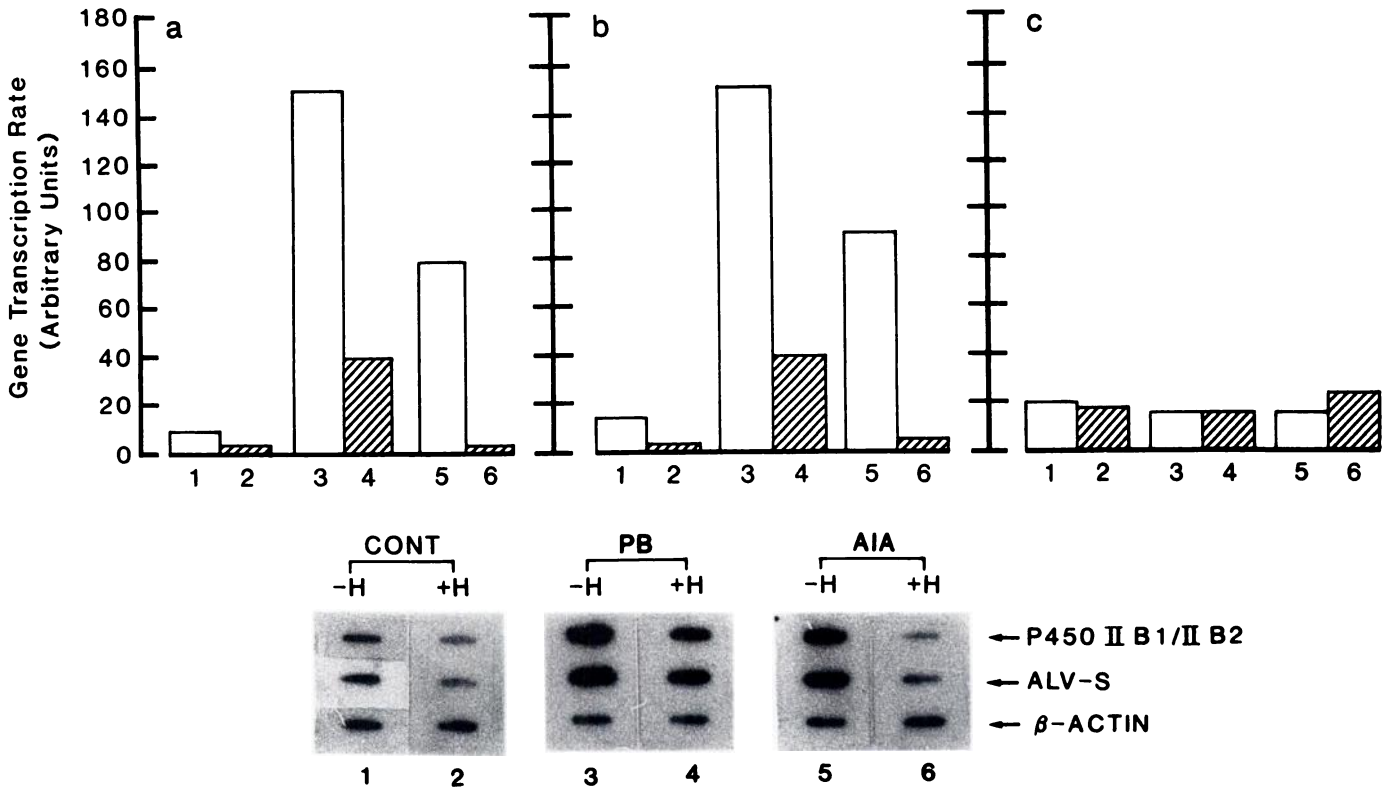

Fig. 4. Effect of hemin on P450IIB1/IIB2 and 5-aminolevulinate synthase gene transcription rates. Rats were treated with drugs and hemin, as described in the legend to Fig. 1. [^{32}P]RNA was isolated from rat liver nuclei and hybridized to nitrocellulose filter-bound cloned DNA. Transcription rates were quantitated from the slot blots by densitometric scanning (following 48-hr exposure with two intensifying screens) and gene transcription rates corrected for vector background are shown as *bar graphs*. □ No hemin; ▨ hemin administration. Filter-bound cDNA clones employed were P450IIB1/IIB2 (a), 5-aminolevulinate synthase (b), and β-actin (c). Treatments: control (*1*), hemin alone (*2*), phenobarbitone alone (*3*), phenobarbitone plus hemin (*4*), 2-allyl-2-isopropylacetamide alone (*5*), 2-allyl-2-isopropylacetamide plus hemin (*6*).

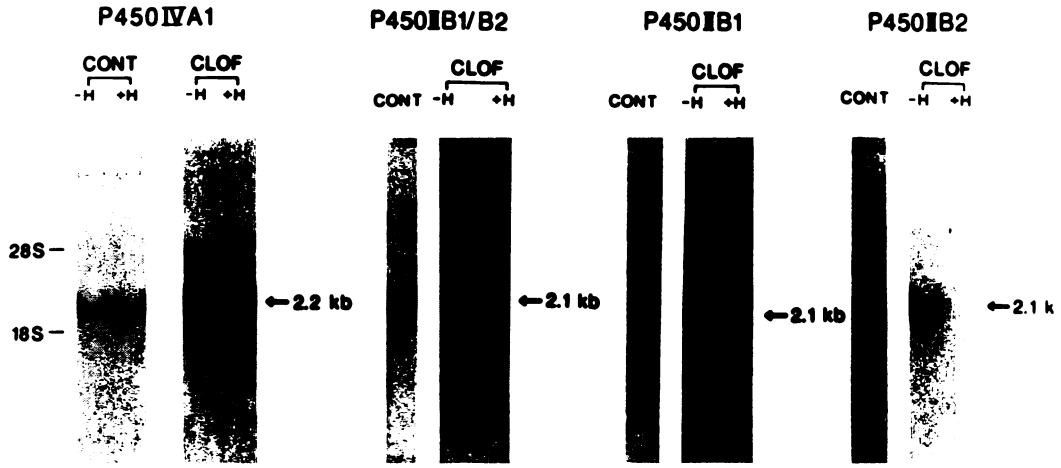

Fig. 5. Effect of hemin on clofibrate-induced P450 mRNA levels. Rats were administered clofibrate and hemin according to the schedule stated in the legend to Fig. 1. Total RNA (20 μg/lane) was electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose filters, and hybridized with a nick-translated 450-base pair *Hind*III-*Nco*I fragment of pB7 (P450IIB1/B2) or with end-labeled [γ-^{32}P]DNA oligomers specific for P450IVA1 and P450IIB1 and P450IIB2. Treatments: no drug (*CONT*), clofibrate (*CLOF*), no hemin (*–H*), hemin (*+H*). The positions of 18 S and 28 S rRNA are indicated.

Although the induction of hepatic heme oxygenase and the repression of 5-aminolevulinate synthase mRNA levels following hemin administration are consistent with the proposed role of these enzymes, the physiological significance of heme repressing mRNA levels of P450IIB1 and P450IIB2 and other hepatic enzymes is not clear. Moreover, there is the possibility that injected hemin may initiate an indirect response, and this requires further investigation. In this regard, it is perhaps of note that the induction of P450IIB1 and P450IIB2 mRNAs in rat hepatocytes is repressed by growth hormone (25).

Padmanaban and co-workers (26–29) have reported that heme at low concentrations is required for the drug-induced transcription of P450 genes in rats that were made heme deficient by treatment with either $CoCl_2$, 3-amino-1,2,4-triazole, or thioacetamide, although studies using succinylacetone as a specific inhibitor of heme biosynthesis have resulted in the

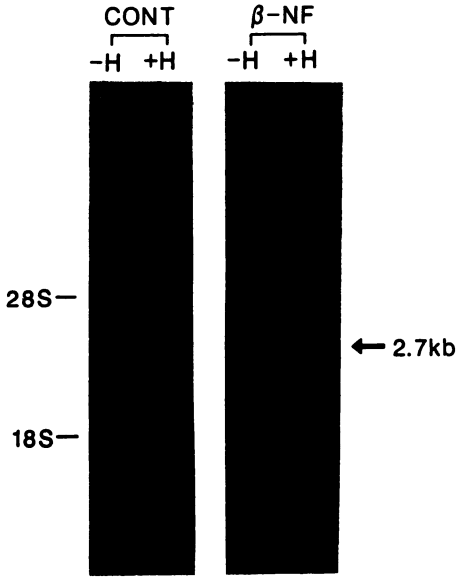

Fig. 6. Effect of hemin on β-naphthoflavone-induced P450IAI mRNA levels. Rats were administered β-naphthoflavone and hemin according to the schedule outlined in the legend to Fig. 1. Total RNA (20 μg/lane) was electrophoresed on formaldehyde/agarose gels, transferred to nitrocellulose filters, and hybridized with an end-labeled [γ-^{32}P]DNA oligomer specific for P450IAI. Treatments; no drug (*CONT*), β-naphthoflavone (*β-NF*), no hemin (*–H*), hemin (*+H*). The positions of 18 S and 28 S rRNA are indicated.

questioning of this proposal (24). Moreover, in their study on nuclear run-on experiments, addition of hemin (10^{-5} to 10^{-6} M) *in vitro* to nuclei isolated from livers of heme-depleted rats was shown to restore P450 gene transcription (28, 29), suggesting that a heme requirement is predominantly at the elongation step of gene transcription. The present results are compatible with the proposal of Padmanaban and co-workers (26–29), because it is conceivable that administered hemin at low levels (25 μg/100 g of rat) is required permissively for the elongation of P450 gene transcripts, whereas hemin at higher levels (4 mg/100 g of rat) elicits an inhibitory response on the initiation of P450 gene transcription.

It is interesting to note that hemin (8) and more recently heme arginate (10) are used as therapy for patients suffering attacks of acute intermittent porphyria. Symptoms in this disease correlate with abnormally elevated levels of hepatic 5-aminolevulinate synthase. From the work described here, it might be predicted that, as well as repressing levels of 5-aminolevulinate synthase, hemin therapy may also repress levels of other drug-metabolizing proteins. In this regard, it has been reported that hemin administration to rats (30) and monkeys (31) and heme arginate administration to rats and dogs (10) caused a decrease in the activities of enzymes associated with drug metabolism. It has been assumed that this results from increased microsomal membrane lipid peroxidation catalyzed by free iron released from heme (32). The work here indicates that administration of hemin can affect levels of phenobarbitone-inducible enzymes by lowering mRNA amounts. The molecular mechanism of activation of phenobarbitone-inducible genes is unknown (2, 33) and no drug-receptor protein has been isolated thus far; the current findings raise the possibility that a heme-responsive step is involved in this mechanism.

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