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Regulation of Cytochrome P-450 Messenger RNA and Apoprotein Levels by Heme*

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2-Allylisopropylacetamide, a porphyrinogen which decreases the microsomal and cytosolic heme pools, is a phenobarbitone-like inducer of cytochrome P-450(b + e) messenger RNAs in rat liver. The porphyrinogen, however, does not affect the nuclear heme pool and enhances the transcription of cytochrome P-450(b + e) messenger RNAs strikingly. Inhibitors of heme biosynthesis, such as $CoCl_2$ and 3-amino-1,2,4-triazole, which decrease the total heme levels including that of the nuclear heme pool, block the 2-allylisopropylacetamide- or phenobarbitone-mediated increase in the transcription of cytochrome P-450(b + e) messenger RNAs. Administration of exogenous heme at a very low concentration (25 μg/100 g) is able to counteract the inhibitory effects of the heme biosynthetic inhibitors. Addition of heme *in vitro* to heme-depleted nuclei leads to a significant increase in the transcription rates for cytochrome P-450(b + e) messenger RNAs. 2-Allylisopropylacetamide, unlike phenobarbitone, fails to increase the levels of cytochrome P-450b protein at 12 h after the drug administration, although there is a striking increase in the messenger RNA levels. Under conditions of 2-allylisopropylacetamide treatment, the cytochrome P-450 messenger RNA is translated, but the newly synthesized apoprotein undergoes rapid degradation. It is concluded that heme is a positive modulator of cytochrome P-450 gene transcription and is also required to stabilize the freshly synthesized apoprotein.

The hepatic cytochrome P-450 gene system constitutes a multigene family, and the structure of prototype subfamilies is under intense investigation (1, 2). While the different subfamilies are activated by prototype chemicals such as phenobarbitone (PB),[1] 3-methylcholanthrene, and pregnenolone 16α-carbonitrile in the liver, it is also clear that a variety of other factors, small and large, are involved in the regulation of expression of these genes (3–7). One such factor implicated, arising out of studies in this laboratory, is heme (8–10), the prosthetic group of cytochrome P-450. It has been shown that inhibitors of heme biosynthesis such as $CoCl_2$, 3-amino-1,2,4-triazole (AT), and thioacetamide block the transcription of PB-inducible cytochrome P-450(b + e) messenger RNAs in rat liver and consequently decrease the levels of the protein species. Based on this indirect evidence, it has been suggested that heme is perhaps a positive modulator of cytochrome P-450 gene expression.

Heme is a versatile regulatory molecule and is known to regulate a variety of cellular processes such as differentiation, transcription, and post-translational processing in different systems (11–14). It is thus of interest to examine the site(s) of action of heme and provide direct evidence for its involvement in the regulation of cytochrome P-450(b + e) gene expression. For this purpose, first of all, the effects of 2-allylisopropylacetamide (AIA) on cytochrome P-450(b + e) messenger RNA and protein synthesis have been compared with those of $CoCl_2$ and AT in the present study. AIA is a porphyrinogen and is known to selectively degrade cytochrome P-450-heme and deplete the cytoplasmic heme pool as a result of the hemeprotein acting as a sink (15, 16). $CoCl_2$ depletes the total heme pool by inhibiting its synthesis and promoting its degradation. The effects of supplementing exogenous heme under these conditions of heme depletion on cytochrome P-450(b + e) mRNA contents and their transcription as well as on the apoprotein levels have been examined.

MATERIALS AND METHODS

Treatment of Animals—Male rats (80–90 g) of the Institute strain were injected with PB (8 mg/100 g body weight, intraperitoneally), $CoCl_2 \cdot 6H_2O$ (6 mg/100 g, subcutaneously), AIA (30 mg/100 g, subcutaneously), AT (300 mg/100 g intraperitoneally), and hemin (dissolved in alkali and neutralized with Tris buffer, pH 8.0, 25 μg–2 mg/100 g, intraperitoneally) wherever indicated. In some experiments, the dose of PB was decreased to 2 mg/100 g.

Labeling of Microsomal and Nuclear Heme Pools—To study the effect of AIA on the degradation of these heme pools, the animals first of all received 5 μCi of [4-^{14}C]δ-aminolevulinate as the heme precursor. After 6 h, the animals received AIA or PB, and, after another 2 h, the animals were killed. In another set of experiments, the effects of $CoCl_2$ and AT on heme synthesis were examined by injecting the labeled precursor simultaneously with these compounds. The animals were killed after 4 h, and the livers were processed. The microsomal and purified nuclear fractions were obtained by standard procedures as described earlier (8–10). The nuclei were additionally washed with buffered 0.25 M sucrose containing 1% (w/v) Triton X-100 to remove the outer membrane along with the endoplasmic reticulum contaminants. The heme fraction was isolated by the ethyl acetate/acetic acid extraction procedure (16). The ethyl acetate layer was extracted with 1.5 N HCl to remove the porphyrins, thoroughly washed with water, and then concentrated for radioactivity measurements. Separate chromatographic analysis revealed that the entire radioactivity in the final ethyl acetate layer is only associated with heme.

Cytochrome P-450(b + e) mRNA Quantification—Poly(A)-containing RNA was isolated from magnesium-precipitated polyribosomes using oligo(dT)-cellulose chromatography (17). Cytochrome P-450(b + e) mRNA was quantified by dot-blot and Northern blot hybridizations (18) using nick-translated pP-450-91 DNA, a cloned probe isolated in this laboratory (8). Quantification of nuclear premessenger

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[1] The abbreviations used are: PB, phenobarbitone; AT, 3-amino-1,2,4-triazole; AIA, 2-allylisopropylacetamide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

RNA for cytochrome P-450(b + e) was also carried out as described by Ravishankar and Padmanaban (8).

Nuclear Run-off Transcription—This was carried out essentially by the procedure of Guertin *et al.* (19) and has been described (8). Briefly, nuclei were isolated from control and drug-treated animal livers and incubated with 200 μCi of [α-^{32}P]UTP and other components including placental RNase inhibitor in a total volume of 200 μl. Incubation was carried out for 45 min at 25 °C. The labeled RNA transcribed was isolated by phenol/chloroform extraction after proteinase K and DNase I treatments. pP-450-91 DNA was linearized and loaded onto nitrocellulose filters, and hybridization was carried out with the labeled RNA transcripts. The hybridized radioactivity was measured after washing and pancreatic and T_1 RNase treatments. α-Amanitin (1 μg/ml) was added to check for the specificity of mRNA hybridization with the cloned probe. Filters containing pBR322 DNA served as blank controls.

Cytochrome P-450(b + e) Protein Synthesis in Cell-free Systems—Cell-free protein synthesis was carried out in the reticulocyte system as well as in a homologous system using microsomes and the S100 fraction. The procedures have been described earlier (8–10, 20). The incubation mixture was solubilized and immunoprecipitated with the anti-cytochrome P-450b antibody, and the immunoprecipitate was analyzed by SDS-PAGE (8% polyacrylamide) and autoradiography. The cytochrome P-450 band was cut out of the gel and digested with H_2O_2, and radioactivity was measured.

Effect of Drug Treatments on the Stability of Freshly Synthesized Cytochrome P-450(b + e) Protein—The animals received AIA or PB, and, after 4 h, 100 μCi of [^{35}S]methionine was injected intraperitoneally. After 30 min, cycloheximide (1 mg/100 g) was injected to block further protein synthesis. The animals were killed 30 min and 2 h after cycloheximide administration, cytochrome P-450(b + e) protein was immunoprecipitated from solubilized microsomes, and radioactivity was measured. Cycloheximide was found to inhibit cytosolic protein synthesis by more than 95% at the concentration and time intervals used in the study.

Distribution of [^{14}C]PB in Vivo in the Presence and Absence of AIA—Pheno[2-^{14}C]barbitone (25 μCi) was injected into two sets of rats (50 g) intraperitoneally. One set received saline and another AIA, 15 min before the administration of the radioactive drug. The animals were killed after 4 h, and microsomal, cytosolic, and nuclear fractions were isolated from the liver. The purified nuclear fraction was further fractionated into chromatin extract (0.3 M NaCl) and insoluble chromatin pellet. The distribution of radioactivity in all the fractions was measured.

Other Procedures—Antibodies to purified cytochrome P-450b were raised in rabbits (8–10). Total cytochrome P-450 was estimated in microsomal suspensions by the procedure of Omura and Sato (21). Cytochrome P-450b protein was quantified by the radial immunodiffusion procedure as described by Thomas *et al.* (22).

RESULTS AND DISCUSSION

First of all, it was of interest to compare the effects of AIA, $CoCl_2$, and AT on the nuclear heme pool, since the porphyrinogen is known to deplete microsomal and cytosolic heme, and the latter two chemicals are known inhibitors of heme biosynthesis. This was carried out as per the protocol described under "Materials and Methods" and Table I. Triton X-100 was used to remove the outer membrane from purified nuclei and the endoplasmic reticulum contamination of the nuclear preparation, based on cytochrome P-450 and cytochrome b_5 estimations, could not have been more than 1%. The results presented in Table I indicate that AIA administration decreases prelabeled microsomal heme by nearly 50% within 2 h. The labeled nuclear heme pool accounts for nearly 5% of the microsomal heme pool on protein basis, but is not significantly affected by the porphyrinogen administration. PB administration does not affect the labeling of microsomal or nuclear heme pool under the experimental conditions employed. The effects of $CoCl_2$ and AT on microsomal and nuclear heme were studied in separate experiments, where the radioactive precursor was injected along with the inhibitors. The results presented in Table I indicate that $CoCl_2$ and AT inhibit by 80% and 60%, respectively, the microsomal and nuclear heme labeling.

TABLE I

Effect of AIA, $CoCl_2$, and AT administration on the labeling of microsomal and nuclear heme pools

The experimental details are given in text. The data given for AIA and PB pertain to their effects on prelabeled heme. The data given for $CoCl_2$ and AT pertain to their effects on fresh heme synthesis and represent the mean obtained from two experiments.

Treatment	Radioactivity in heme fraction	
	Nuclei	Microsome
	cpm/10 mg protein	
Experiment I		
Control	610	18,095
PB	660	16,178
AIA	590	8,755
Experiment II		
Control	512	11,082
$CoCl_2$	106	2,131
AT	186	5,078

TABLE II

Effect of saturating and suboptimal doses of PB and AIA on the induction of cytochrome P-450(b + e) mRNAs

PB (8 mg or 2 mg/100 g, intraperitoneally) and AIA (30 mg or 5 mg/100 g, subcutaneously) were given independently or together for 12 h, and the cytochrome P-450(b + e) mRNA concentration was estimated by dot-blot hybridization. Poly(A)-containing RNA (1 μg) was loaded on the filters. The data represent the mean ± S.D. obtained from four experiments.

Treatment	Cytochrome P-450(b + e) mRNA
	cpm hybridized
Saturating dose	
Control	63 ± 12
PB	1050 ± 72
AIA	972 ± 51
PB + AIA	1123 ± 96
Suboptimal dose	
Control	71 ± 14
PB	483 ± 27
AIA	362 ± 21
PB + AIA	910 ± 43

The effects of AIA, $CoCl_2$, and AT on PB-mediated induction of cytochrome P-450(b + e) mRNAs were then studied. A quantitative evaluation of the dot-blot hybridization data presented in Table II indicates that AIA does not affect the PB-mediated induction of cytochrome P-450(b + e) mRNA, but is actually a good inducer of these messenger RNAs by itself. Although saturating doses of PB and AIA given together do not show inhibitory or additive effects, suboptimal doses when given together show an additive effect. Earlier, Brooker *et al.* (23) have shown that AIA behaves as a PB-like inducer in the chick embryo liver system. The Northern blot (Fig. 1*a*) indicates that PB and AIA induce cytochrome P-450(b + e) messenger RNA species with similar electrophoretic mobilities, and the size range works out to around 1800 ± 100 nucleotides. In addition, the blot also depicts the additive effects of PB and AIA when given at suboptimal doses. Administration of $CoCl_2$ and AT inhibits the PB- or AIA-mediated induction process (Fig. 1*b*). These inhibitors block the cytochrome P-450(b + e) nuclear premessenger RNA induction by AIA (Fig. 1*c*). Similar results with PB have been reported earlier (8).

The nuclear run-off transcription experiments reveal that AIA, like PB, enhances the transcription of cytochrome P-

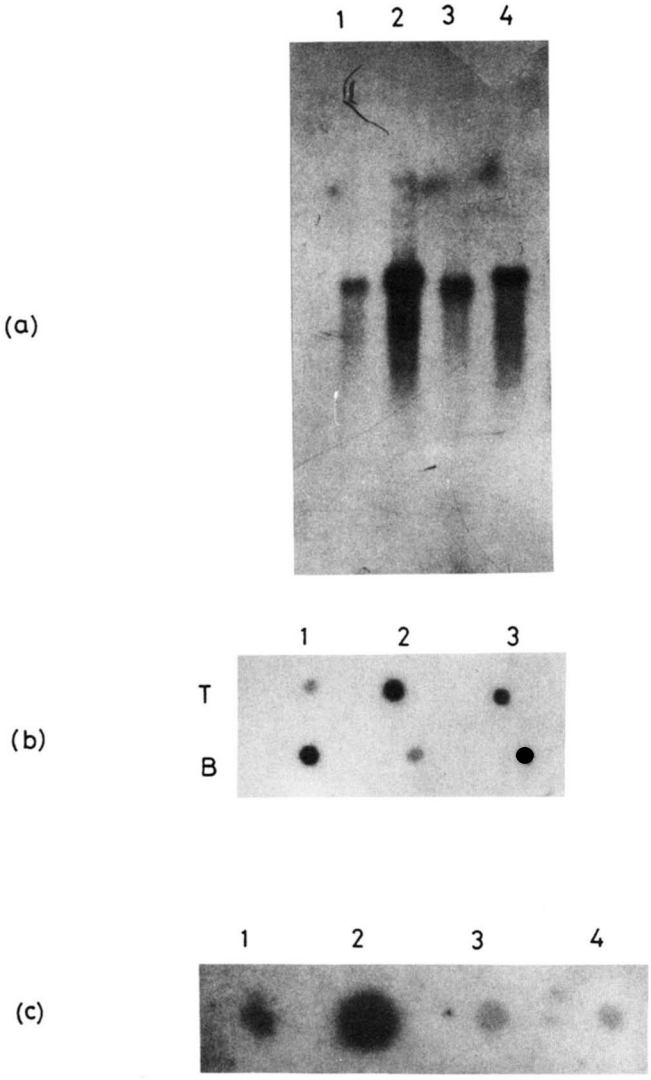

FIG. 1. **Effect of PB, AIA, and inhibitors of heme biosynthesis on the induction of cytochrome P-450(b + e) mRNAs.** *a*, Northern blot analysis of poly(A)-containing RNA isolated 12 h after PB or AIA treatments. Poly(A)-containing RNA (20 μg) was run on agarose/formaldehyde gels. *1*, control; *2*, (PB + AIA)-treated; *3*, PB-treated; *4*, AIA-treated. All the drugs were given at suboptimal doses. *b*, dot-blot analysis of poly(A)-containing RNAs. The animals were given drug treatments for 6 h, and 1 μg of RNA was loaded on nitrocellulose filters. *T*: *1*, control (42); *2*, PB (655); *3*, PB + $CoCl_2$ (157); *B*: *1*, AIA (553); *2*, AIA + $CoCl_2$ (55); *3*, AIA + AT (95). The radioactivity (cpm) in the *dots* is given in parentheses. *c*, dot-blot analysis of nuclear RNA. Total nuclear RNA (10 μg) was loaded on the filters. *1*, control; *2*, AIA; *3*, AIA + $CoCl_2$; *4*, AIA + AT.

450(b + e) mRNAs and $CoCl_2$ or AT administration blocks this effect (Table III). It is clear that PB and AIA induce the same or very closely related cytochrome P-450 mRNA species and both drugs act at the level of transcription. In view of these results and the data that at subsaturating levels the two drugs show an additive effect on cytochrome P-450(b + e) mRNA induction (Table II, Fig. 1*a*), the possibility of the presence of common or identical intracellular binding sites for the two drugs was examined. The results presented in Table IV indicate that, in presence of cold AIA, there is a significant decrease in the localization of pheno[2-^{14}C]barbitone in the microsomal fraction and the 0.3 M NaCl extract of the chromatin. Under these conditions, the total uptake of the labeled drug into the liver is not affected and there is an increase in the cytosolic radioactivity. The competition between the two drugs at the microsomal level is perhaps due

TABLE III

Effect of PB, AIA, $CoCl_2$, and AT on the transcription of cytochrome P-450(b + e) mRNA with isolated nuclei

Nuclei were isolated from animals given 12 h of drug treatment. The total RNA transcripts used for hybridization were adjusted to provide 10^7 cpm of radioactivity. There was no significant difference in total RNA transcription in any of the treatment conditions. Inclusion of α-amanitin (1 μg/ml) in the incubation mixture inhibited total RNA transcription by about 50%. Cytochrome P-450(b + e) mRNA was inhibited by about 96%. pBR322 DNA-loaded filters served as blank controls. The data obtained from two independent experiments are given and are expressed in terms of parts per million of RNA transcripts hybridized.

Treatment	Cytochrome P-450 (b + e) mRNA transcription	
	I	II
	ppm/kilobase pairs cloned probe	
Control	6	8
PB	145	167
AIA	152	146
PB + $CoCl_2$	33	25
PB + AT	46	60
AIA + $CoCl_2$	25	21
AIA + AT	52	46

TABLE IV

Effect of AIA on [^{14}C]PB distribution in different cellular fractions

Pheno[2-^{14}C]barbitone (25 μCi) was injected into rats (50–60 g) that had received saline or AIA, 15 min before the administration of the label. The animals were killed after 4 h, livers from two animals were pooled, and the radioactivity distribution in the different cellular fractions was examined. The data obtained from two independent experiments are given.

Fraction	Radioactivity			
	−AIA		+AIA	
	I	II	I	II
	cpm/mg protein			
Homogenate	2493	2635	2669	2915
Cytosol	3827	4007	5045	5401
Microsomes	1692	1758	915	1005
Chromatin extract (0.3 M NaCl)	719	773	315	381
Chromatin pellet	33	39	30	40

to competition for binding to the same cytochrome P-450 species. The competition in the 0.3 M NaCl extract of the chromatin may be indicative of a common receptor for the two drugs. This aspect needs further study, since the identification of a receptor for PB has remained elusive (24, 25).

These results clearly establish that AIA, which competes for PB binding sites in the microsome and nucleus, is a typical PB-like inducer of cytochrome P-450 mRNAs. However, while PB does not affect the heme pools at least at early periods of drug action, AIA depletes the microsomal and cytosolic heme pool without significantly affecting the nuclear heme pool. In order to provide direct proof that heme regulates cytochrome P-450(b + e) mRNA transcription and that the nuclear heme pool may influence these transcription rates, the following experiments were carried out. First of all, the ability of exogenously administered heme (hematin) to counteract the inhibitory effects of $CoCl_2$ on PB-mediated induction of cytochrome P-450(b + e) mRNAs was examined. In preliminary experiments, it was found that heme in doses ranging from 25 μg/100 g to 2 mg/100 g was not able to counteract the effects of $CoCl_2$ in this regard (data not presented). However, in parallel expriments, it was found that heme at as low a concentration as 50 μg/100 g was able to counteract the

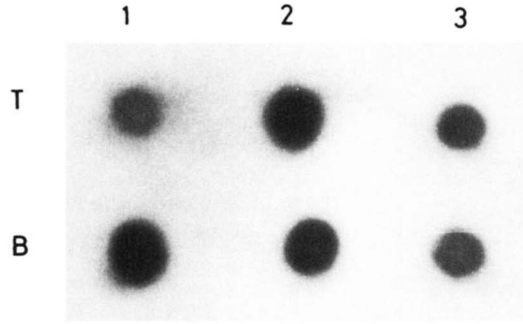

FIG. 2. **Effect of exogenous hemin on $CoCl_2$-mediated inhibition of cytochrome P-450(b + e) mRNA induction by suboptimal doses of phenobarbitone.** The animals were killed 12 h after the drug treatments and 20 μg of total polyribosomal RNA was loaded on the filters. *T*: *1*, control (134); *2*, PB (712); *3*, PB + $CoCl_2$ (176); *B*: *1*, PB + $CoCl_2$ + hemin, 25 μg (650); *2*, PB + $CoCl_2$ + hemin, 50 μg (353); *3*, PB + $CoCl_2$ + hemin, 100 μg (186). The radioactivity (cpm) in the *dots* is given in parentheses.

inhibitory effects of $CoCl_2$ on 3-methylcholanthrene-mediated induction of cytochrome P-450(c + d) mRNAs.[2] Therefore, it was felt that the failure of exogenous heme to counteract the effects of $CoCl_2$ in the case of PB-mediated induction of cytochrome P-450(b + e) mRNAs could perhaps be due to PB inhibiting heme uptake into the regulatory heme pools. Such a competition at the intracellular level for microsomal localization was noticed by Marver (26). Therefore, in the present study, the heme reversal experiments were carried out at a lower PB concentration of 2 mg/100 g body weight. The results presented in Fig. 2 indicate that, at this concentration, phenobarbitone induces cytochrome P-450(b + e) mRNAs by 5-fold. The interesting result is that heme at as low a concentration as 25 μg/100 g body weight is now able to counteract the inhibitory effects of $CoCl_2$. Surprisingly, when the heme concentration is increased to 100 μg/100 g body weight, the counteracting potential is lost. The reasons for this intriguing concentration-dependent action of heme are not clear. It may be that the size of the regulatory heme pool is strictly modulated and the presence of $CoCl_2$ together with a slight excess of exogenous heme perhaps promotes degradation as well as inhibition of synthesis of the regulatory heme pool. In any case, the results clearly establish that heme is a positive modulator of cytochrome P-450(b + e) mRNA transcription.

In order to examine whether the nuclear heme pool regulates cytochrome P-450(b + e) mRNA transcription, run-off transcription experiments were carried out in presence and heme added *in vitro* to heme-depleted nuclei. The results presented in Table V indicate that heme, when added at a concentration of 10^{-6} M to nuclei isolated from (PB + $CoCl_2$)-treated animals, is able to increase cytochrome P-450(b + e) mRNA transcription rates to as much as 60% of the maximal transcription rates obtained with nuclei from PB-treated animals. Heme, when added at concentrations of 10^{-5} M or 10^{-7} M, is only marginally effective. Further studies are needed to study the effects of a closer range of heme concentrations. While these results lend evidence to the suggestion that the nuclear heme pool does regulate cytochrome P-450(b + e) mRNA transcription, they also raise an intriguing point for consideration. In these run-off nuclear transcription experiments, there is very little fresh initiation. The fact that *in vitro* addition of heme is able to enhance significantly cytochrome P-450(b + e) mRNA transcription rates when added to heme-depleted nuclei would indicate that heme may (also) be acting at downstream sites after initiation, as well as influencing RNA processing and stabilization in the nucleus.

[2] G. J. Bhat and G. Padmanaban, unpublished data.

TABLE V

Effect hemin addition in vitro to heme-depleted nuclei on run-off transcription of cytochrome P-450(b + e) mRNAs

The experimental details are given in text. The results represent the mean ± S.D. obtained from three independent experiments.

Nuclei	Cytochrome P-450(b + e) mRNA transcription
	ppm/kilobase pairs cloned probe
Control	8 ± 2
PB (6 h)	135 ± 10
PB + $CoCl_2$ (6 h)	31 ± 4
PB + $CoCl_2$ + hemin (*in vitro*) (10^{-7} M)	44 ± 5
PB + $CoCl_2$ + hemin (*in vitro*) (10^{-6} M)	88 ± 6
PB + $CoCl_2$ + hemin (*in vitro*) (10^{-5} M)	53 ± 9
PB + hemin (*in vitro*) (10^{-6} M)	128 ± 11
PB + hemin (*in vitro*) (10^{-4} M)	109 ± 8

TABLE IV

Effect of exogenous hemin administration on the effects of $CoCl_2$ and AIA on cytochrome P-450b protein levels

Cytochrome P-450b was quantified by radial immunodiffusion. The animals were killed 12 h after the drug treatments.

Treatment	Cytochrome P-450b
	nmol/mg protein
Control	0.03
PB	0.59
PB + $CoCl_2$	0.09
PB + $CoCl_2$ + hemin (25 μg)	0.49
AIA	0.09
AIA + hemin (25 μg)	0.11

The ability of AIA to selectively degrade cytochrome P-450 heme and to induce cytochrome P-450(b + e) mRNA has given us an opportunity to examine the role of heme in cytochrome P-450 protein synthesis. The results on the radial immunodiffusion assay to quantify the cytochrome P-450b protein are presented in Table VI. It is interesting to note that PB enhances the cytochrome P-450b protein content significantly in 12 h. However, AIA which, like phenobarbitone, induces cytochrome P-450(b + e) mRNA, fails to increase cytochrome P-450b protein levels substantially. Therefore, the possible role of heme in the translation of cytochrome P-450(b + e) mRNA was examined. Poly(A)-containing RNA isolated from the drug-treated rats was translated in the reticulocyte lysate cell-free system, and the cytochrome P-450b synthesized was quantified by immunoprecipitation and estimation of radioactivity in the protein band after electrophoresis. The same experiment was also carried out in homologous cell-free systems, where protein synthesis was carried out with microsomes and S100 fraction isolated from the drug-treated rats. The results presented in Table VII indicate that both in the homologous as well as heterologous cell-free systems, cytochrome P-450b was synthesized in equivalent quantities under conditions of PB and AIA treatments. These results indicate that the cytochrome P-450b mRNA induced by PB or AIA is translatable, and lack of heme or any tissue-specific factor is not responsible for the failure of the latter drug to induce cytochrome P-450b protein at the time period examined. Therefore, the possibility that the freshly synthesized cytochrome P-450b apoprotein may undergo degradation *in vivo* under conditions of AIA treatment due to lack of heme was examined. The results presented in Fig. 3 indicate that, in the case of PB treatment, freshly labeled cytochrome P-

TABLE VII

Effect of PB and AIA on cytochrome P-450b protein synthesis in heterologous and homologous cell-free translation systems

Poly(A)-containing RNA (8 μg) from PB- or AIA-treated (12 h) rats was translated in the reticulocyte lysate cell-free system in the presence of [^{35}S]methionine (50 μCi) in a total volume of 100 μl. In the homologous cell-free system, microsomes from PB- or AIA-treated (12 h) rats were incubated with the respective S100 fraction in the presence of 50 μci ob [^{35}S]methionine and other components in a total volume of 250 μl. After incubation, cytochrome P-450b was immunoprecipitated, and the precipitate was analyzed on SDS-PAGE (8% gels). The cytochrome P-450 band was cut and digested with H_2O_2, and radioactivity was measured.

Treatment	Total protein synthesized	Cytochrome P-450b synthesized
	cpm	*% total*
Reticulocyte lysate		
Control	8.8×10^5	0.04
PB	9.0×10^5	0.71
AIA	8.8×10^5	0.82
Microsomal system		
Control	2.0×10^6	0.07
PB	1.8×10^6	1.20
AIA	2.1×10^6	1.13

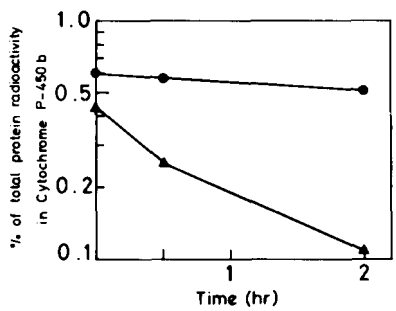

FIG. 3. **Effect of PB and AIA on the degradation of freshly synthesized cytochrome P-450b protein.** The experimental details are given in text. The immunoprecipitable radioactivity due to prelabeled cytochrome P-450b was measured after blocking fresh protein synthesis. ●, PB; ▲, AIA.

450b is quite stable and there is about 10% loss of radioactivity in about 2 h, when continued protein synthesis is blocked by cycloheximide. However, under conditions of AIA treatment, there is massive degradation of freshly synthesized cytochrome P-450b protein.

Exogenous administration of heme fails to counteract the inability of AIA to enhance cytochrome P-450b protein levels. At the same time, exogenous heme is able to counteract the inhibitory effects of $CoCl_2$ on PB-mediated induction of cytochrome P-450b protein (Table VI). It is possible that exogenously administered heme also undergoes degradation during AIA treatment by getting incorporated into cytochrome P-450, just as is the case with cytosolic heme (16). Thus, while depletion of heme brought by AIA is probably responsible for the degradation of the freshly synthesized apocytochrome P-450b, the possibility of the drug or its metabolite having a direct effect on the degradation of this protein cannot be ruled out.

In summary, the results of the present investigation clearly establish that heme is a positive modulator of cytochrome P-450(b + e) mRNA transcription and/or the immediate events following transcription initiation. The nuclear heme pool appears to influence this rate of transcription. Heme also appears necessary to stabilize the freshly synthesized cytochrome P-450 protein in response to PB.

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REFERENCES

1. Atchison, M., and Adesnik, M. (1983) *J. Biol. Chem.* **258,** 11285–11295
2. Sogawa, K., Gotoh, O., Kawajiri, K., and Fujii-Kuriyama, Y. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 5066–5070
3. Gonzalez, F. J., and Kasper, C. B. (1982) *J. Biol. Chem.* **257,** 5962–5968
4. Gonzalez, F. J., Tukey, R. H., and Nebert, D. W. (1984) *Mol. Pharmacol.* **26,** 117–121
5. Hardwick, J. P., Gonzalez, F. J., and Kasper, C. B. (1983) *J. Biol. Chem.* **258,** 10182–10186
6. Jones, P. B., Galleazzi, D. R., Fisher, J. M., and Whitlock, J. P., Jr. (1985) *Science* **227,** 1499–1502
7. Gonzalez, F. J., and Nebert, D. W. (1985) *Nucleic Acids Res.* **13,** 7269–7288
8. Ravishankar, H., and Padmanaban, G. (1985) *J. Biol. Chem.* **260,** 1588–1592
9. Ravishankar, H., and Padmanaban, G. (1983) *Arch. Biochem. Biophys.* **225,** 16–24
10. Sathyabhama, S., and Padmanaban, G. (1984) *Biochem. J.* **218,** 371–377
11. Chen, J. J., and London, I. M. (1981) *Cell* **26,** 117–122
12. Marks, P. A., Rafkind, R. A., Gambari, R., Epner, E., Chen, Z.-X., and Banks, J. (1982) *Curr. Top. Cell. Regul.* **21,** 189–203
13. Gasser, S. M., Ohashi, A., Daum, G., Bohni, P. C., Gibson, J., Reid, G. A., Yonetani, T., and Schatz, G. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 267–271
14. Ochoa, S. (1983) *Arch. Biochem. Biophys.* **223,** 325–349
15. Maines, M. D., and Kappas, A. (1975) *J. Biol. Chem.* **250,** 4171–4177
16. Levin, W., Jacobson, M., Sernatinger, E., and Kuntzman, R. (1973) *Drub Metab. Dispos.* **1,** 267–274
17. Palmiter, R. D. (1974) *Biochemistry* **13,** 3601–3605
18. Thomas, P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 5201–5205
19. Guertin, M., Baril, P., Bartkowiak, J., Anderson, A., and Belanger, L. (1983) *Biochemistry* **22,** 4296–4302
20. Bhat, K. S., and Padmanaban, G. (1979) *Arch. Biochem. Biophys.* **198,** 110–116
21. Omura, T., and Sato, R. (1964) *J. Biol. Chem.* **239,** 2379–2385
22. Thomas, P. E., Korzenioswki, D., Ryan, D., and Levin, W. (1979) *Arch. Biochem. Biophys.* **192,** 524–532
23. Brooker, J. D., Srivastava, G., Borthwick, I. A., May, B. K., and Elliot, W. H. (1983) *Eur. J. Biochem.* **136,** 327–332
24. Poland, A., Glover, E., Boatman, R. J., Ebetino, F. H., and Kende, A. S. (1980) *Mol. Pharmacol.* **18,** 571–580
25. Poland, A., Mak, I., and Glover, E. (1981) *Mol. Pharmacol.* **20,** 442–450
26. Marver, H. S. (1969) in *Microsomes and Drug Oxidations* (Gillette, J. R., Conney, A. H., Cosmides, G. J., Estabrook, R. W., Fouts, J. R., Mannering, G. J., eds) pp. 495–515, Academic Press, Orlando, FL