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Dexamethasone Negatively Regulates Phenobarbitone-activated Transcription but Synergistically Enhances Cytoplasmic Levels of Cytochrome P-450*b*/*e* Messenger RNA*

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Dexamethasone has a potentiating effect on phenobarbitone mediated induction of cytochrome P-450*b* + *e* mRNAs in adult rat liver. However, the glucocorticoid inhibits phenobarbitone-activated transcription of cytochrome P-450*b* + *e* mRNAs by 60–70%. This inhibitory effect is evident in run-off transcription of the endogenous genes as well as in the transcription of an added cloned gene fragment. Dexamethasone inhibits the phenobarbitone-mediated increase in the binding of a transcription factor(s) to the upstream region of the gene as evidenced by gel retardation and Southwestern blot analysis. The glucocorticoid does not stabilize the phenobarbitone-induced polyribosomal cytochrome P-450*b* + *e* mRNAs but appears to stabilize the nuclear transcripts. It is proposed that a negative element may mediate the action of dexamethasone at the level of nuclear transcription and stabilization of the nuclear transcript may account for the potentiating effect of the glucocorticoid on phenobarbitone-mediated increase in cytochrome P-450*b* + *e* mRNAs in the cytoplasm of the adult rat liver. However, the cytochrome P-450*b* protein levels are slightly lower in phenobarbitone+dexamethasone treatment than in phenobarbitone-treated liver microsomes.

A sufficient amount of sequence information at the gene level is now available for the cytochrome P-450 supergene family to permit investigators to embark on examination of the features of regulation of gene expression. In particular, cis and trans responsive elements have been identified in the 5′ upstream region of the 3-methylcholanthrene inducible cytochrome P-450IA gene family (1–4). The mechanism of induction of the phenobarbitone (PB)[1]-inducible cytochrome P450IIB gene family (cytochrome P-450*b* and *e* in rat liver) has not been well studied, except for the finding that the drug acts primarily by activating transcription (5, 6). DNA sequence information of the 5′ upstream region of the cytochrome P-450*b*/*e* gene reveals several interesting regulatory motifs such as direct and indirect repeats, an alternating pyrimidine-purine stretch and potential glucocorticoid responsive core sequences, TGTCCT (7, 8). Recently, studies in this laboratory have led to the identification of a PB- and heme-modulated transcription factor in rat liver (9). Simmons *et al.* (10) have shown that dexamethasone (Dex, 80 mg/kg body weight) increases cytochrome P-450*b*/*e* mRNA in rat liver by about 12-fold without any significant effect on transcription and mRNA stabilization has been suggested to be the likely reason for this effect. In the present study, we report the novel finding that Dex negatively interferes with the PB-mediated activation of cytochrome P-450*b*/*e* gene transcription but shows a potentiating effect in enhancing the corresponding mRNA levels. The possible mechanisms of Dex action are discussed.

MATERIALS AND METHODS

Treatment of Animals—Male rats (80–85 g) of the Institute strain were injected with PB (8 mg/100 g body weight), Dex (5 mg/100 g) and actinomycin D (100 μg/100 g), intraperitoneally. $CoCl_2 \cdot 6H_2O$ (6 mg/100 g) was injected subcutaneously. In all the experiments, the animals received the various treatments for a period of 6 h, except in experiments involving the measurement of cytochrome P-450*b* protein content, where the treatment period was 10 h.

Cytochrome P-450(b+e) mRNA Quantification—Total RNA was isolated from magnesium precipitated polyribosomes of rat liver by phenol/chloroform extraction method (11). Dot blot analysis with 10 and 20 μg of total polyribosomal RNA was carried out (12) using pP-450-91, a cloned cDNA probe isolated in this laboratory (6). In some experiments, cytochrome P-450*b* and *e* mRNAs were quantified separately using oligonucleotide probes as described by Omiecinski *et al.* (13).

Nuclear Run-off Transcription—This was carried out by the procedure of Guertin *et al.* (14) and has been described previously (6, 15). Briefly, nuclei were incubated with 200 μCi of [α-^{32}P]UTP and other components including human placental RNase inhibitor in a total volume of 200 μl for 45 min at 25 °C. The labeled RNA transcribed was isolated by phenol/chloroform extraction after DNase I and proteinase K treatments. pP-450-9l DNA was linearized and loaded onto nitrocellulose filters and hybridization carried out with the labeled RNA transcripts (10^7 cpm). The hybridized radioactivity was measured after washing and pancreatic DNase I and RNase T1 treatments. α-Amanatin (1 μg/ml) was added to establish the specificity of mRNA transcription. Filters containing pUC19 DNA served as controls for nonspecific hybridization.

Transcription Analysis of Cloned DNA Fragment by S1 Nuclease Protection Assay—The plasmid pP-450-e4 containing a 360-bp fragment of the cytochrome P-450*e* gene (identical with cytochrome P-450*b* gene except for four base substitutions) cloned into the *Sma*I site of pUC19 was used. The fragment can be released from the plasmid with *Bam*HI and *Eco*RI (polylinker sites) and contains −179 nucleotides of the 5′-flanking region and +181 nucleotides of the first exon. The fragment has a unique *Nco*I site at +29 and a *Sph*I site at −1 (9). The plasmid DNA (3 μg) was added to freeze-thawed rat liver nuclei and transcription carried out as already described except that cold UTP replaced the radioactive precursor. The RNA transcripts were isolated and hybridized to the *Nco*I-*Eco*RI fragment of pP-450-*e*4 which had been labeled at the *Nco*I end using Klenow polymerase (50,000 cpm). S_1 nuclease protection analysis was carried out as

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[1] The abbreviations used are: PB, phenobarbitone; Dex, dexamethasone; bp, base pairs; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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described by Favaloro *et al.* (16) and the protected fragment was analyzed on urea-polyacrylamide gel electrophoresis (8% acrylamide) and subjected to autoradiography.

Gel Retardation Assay—Nuclei were prepared using citric acid buffer homogenization (17) and nuclear extracts were prepared using 0.45 M NaCl (in final concentration) and dialysis (9). The nuclear extract (1–5 μg of protein) was incubated with 1 μg of poly(dI)·poly(dC) (double-stranded, average length 262 bp) in a volume of 20 μl containing 25 mM HEPES-NaOH, pH 7.5, 5 mM $MgCl_2$, 2 mM dithiothreitol and 50 mM NaCl at 25 °C for 10 min. *Nco*I-*Bam*HI fragment (10,000 cpm) was then added and incubation continued for another 30 min. After incubation, 2 μl of loading buffer (0.05% bromphenol blue in 50% glycerol) was added, and the samples were analyzed by electrophoresis on 6% polyacrylamide gel (acrylamide to bis ratio, 30:1) in a low ionic strength buffer (6.7 mM Tris-HCl, pH 7.5, 3.3 mM sodium acetate and 1 mM EDTA) at 4 °C for 3 h (10 volts/cm). The gel was then soaked in 10% glycerol, dried, and subjected to autoradiography.

Southwestern Blot Analysis—Nuclear extracts (100 μg of protein) were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8% polyacrylamide), and the proteins were electrophoretically transferred to a nitrocellulose membrane in a buffer containing 25 mM Trizma base and 190 mM glycine for 16 h at 100 mA. The filters were first incubated with blocking buffer (2% nonfat dry milk, 1% bovine serum albumin, 10 mM HEPES-NaOH, pH 7.5, 0.1 mM EDTA, 60 mM NaCl, 100 mM $MgCl_2$, and 16 μg/ml sonicated salmon sperm DNA) for 2 h at room temperature. The filters were then incubated in binding buffer (same as blocking buffer but containing only 0.2% nonfat dry milk) containing nick-translated *Nco*I-*Bam*HI upstream fragment (10^6 cpm/ml) for 2 h, washed, and subjected to autoradiography (9).

Cytochrome P-450b Protein Content—This analysis was carried out by radial immunodiffusion using cytochrome P-450*b*-specific antibody as described by Thomas *et al.* (18). Purified cytochrome P-450*b* was used as standard.

RESULTS

The effect of different concentrations of Dex on PB-mediated induction of cytochrome P-450*b*+*e* mRNA was assessed by dot blot analysis using pP-450-9l probe. The results presented in Fig. 1 indicate a potentiating effect of Dex at a concentration of 50 mg/kg body weight on the changes elicited by PB. The effects are less pronounced at Dex concentrations of 10 and 20 mg/kg (data not presented). Quantitative analysis of the dots presented in Fig. 1 indicate that Dex, PB, and PB+Dex enhanced cytochrome P-450*b*+*e* mRNA levels by 2.5-, 8-, and 14-fold, respectively. Even at a Dex concentration of 80 mg/kg, the enhancement did not change significantly. The results obtained on the concentrations of cytochrome P-450*b* and *e* mRNAs separately under these conditions, using specific oligonucleotide probes are presented in Table I. The potentiating effects of PB+Dex are quite evident, although the effect on cytochrome P-450*b* mRNA concentration is more marked than that on the *e* species. The results presented in Fig. 2 indicate that in 5-day-old pups, PB+Dex treatment fails to show any potentiating effect on cytochrome P-450*b*+*e* mRNA concentration. On the contrary, Dex inhibits the PB-mediated increase.

Run-off transcription of cytochrome P-450*b*+*e* mRNAs was then carried out with nuclei isolated from PB- and Dex-treated adult and pup rat livers. The results presented in Table II reveal some interesting features. Quite unexpectedly, Dex inhibits the PB-mediated increase in cytochrome P-450*b*+*e* mRNA transcription by 60–70% in both adult and pup rat liver nuclei. Dex injection to saline-treated controls has no significant effect. Thus, while Dex inhibits the PB-mediated increase in cytochrome P-450*b*+*e* mRNA levels as well as run-off transcription in pups, it has the unusual effect of increasing the mRNA levels, while inhibiting transcription in the adult rat liver.

To further elucidate the mechanism of action of Dex in adult rats, attempts were made to examine the transcription

TABLE I

Quantification of cytochrome P-450b and e mRNAs using specific oligonucleotide probes

The specific oligonucleotide probes described by Omiecinski *et al.* (13) were synthesized and end labeled with [γ-^{32}P]ATP. Dot blot analysis was carried out with 20 μg of total polysomal RNA isolated from the livers of adult rats given the different treatments for a period of 6 h. The specificity of the probe for cytochrome P-450*b* or *e* species was established by the ability of the corresponding excess cold oligonucleotide to chase the radioactive signal obtained with the radioactive probe. The spots were punched out from the nitrocellulose filter and radioactivity measured.

Treatment	mRNA content	
	Cytochrome P-450*b*	Cytochrome P-450*e*
	cpm	
Control	35	28
PB	223	143
Dex	46	33
PB + Dex	436	225

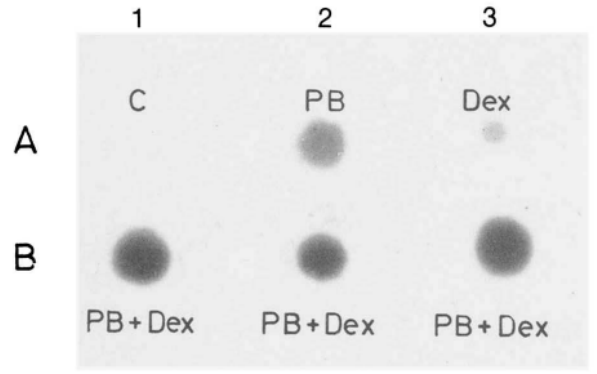

FIG. 1. **Dot blot analysis of cytochrome P-450*b*+*e* mRNA concentration in polyribosomes of adult rat livers treated with phenobarbitone and dexamethasone (50 mg/kg), for a period of 6 h.** Polyribosomal RNA (10 μg) was loaded onto nitrocellulose filters and hybridized with nick-translated pP-450e-91 DNA. *Lane A:* 1, control (35); 2, PB (305); 3, Dex (69). *Lane B* represents three different groups treated with PB+Dex, each group consisting of two rats, 1 (510); 2 (423); 3 (535). The values given in parentheses represent the radioactivity (*cpm*) measured after punching out the dots from the nitrocellulose sheets.

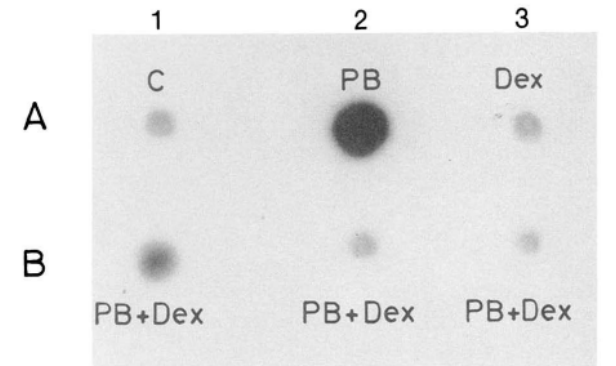

FIG. 2. **Dot blot analysis of cytochrome P-450*b*+*e* mRNA concentration in polyribosomes of 5-day-old rat livers treated with phenobarbitone and dexamethasone (50 mg/kg), for a period of 6 h.** The experimental details are as given in text and Fig. 2. 20 μg of polyribosomal RNA was used for analysis. *Lane A*, 1, control (55); 2, PB (620); 3, Dex (83). *Lane B* represents three different groups treated with PB+Dex, each group consisting of four rats: 1 (115); 2 (43); 3 (51). The values given in parentheses represent the radioactivity (*cpm*) measured after punching out the dots from the nitrocellulose sheets.

of an added cloned gene fragment under conditions of PB and Dex treatment. We have recently shown that pP-450-e4 DNA containing −179 bp of 5′-flanking region and a portion of the first exon can be faithfully transcribed in freeze-thawed rat liver nuclei. The transcripts analyzed by S_1 nuclease mapping using the end-labeled *Nco*I-*Eco*RI fragment reveal protected fragments of size 170 and 152 bp generated by exogenous and endogenous gene transcripts, respectively. The difference in the size of the protected fragments in the two cases is due to the contribution by transcription and protection of the polylinker region in the case of added gene fragment and the absence of such a polylinker region in the endogenous gene (9). The results presented in Fig. 3 indicate interesting features. In the absence of added cloned gene fragment, PB treatment shows a striking increase in the S_1-protected band due to the endogenous P-450*b*+*e* gene transcripts (152 bp). Dex has a marginal effect, and, surprisingly, PB+Dex treatment has an effect almost similar to that of PB. When the cloned gene fragment is added, once again PB treatment shows an increase in the protected band resulting from the exogenous DNA transcript (170 bp), but Dex inhibits this increase very significantly. It may be pointed out that addition of exogenous DNA decreases the S_1-protected lower band arising due to endogenous gene transcription.

We have recently identified a transcription factor(s) interacting with the 5′-flanking region of the cytochrome P-450*b*+*e* gene by gel retardation assay and Southwestern blot analysis. The binding of this factor(s) correlates very well with the transcription status of the gene (9). It was, therefore, of interest to examine the effect of Dex treatment on the PB-mediated increase in the binding of the transcription factor(s) to the 5′-flanking region. Gel retardation experiments were performed with the *Nco*I-*Bam*HI fragment labeled at the *Nco*I end and nuclear extracts prepared from rats given different treatments. The results presented in Fig. 4, *A* and *B* indicate the identification of at least two retarded complexes generated by the upstream fragment. The pattern generated depends upon the protein concentration of the nuclear extract and the nature and concentration of the competitor DNA used. It is, however, clear that PB treatment leads to a striking increase in the intensity of the lower band compared with control. At the higher protein concentration (3 μg of protein), the upper band shows a striking increase in intensity in the control, whereas the lower band shows a striking increase in intensity in PB treatment. Dex treatment counteracts the effects of PB treatment and leads to a decrease in the intensity of the lower band with a concomitant increase in the intensity of the upper band. Dex treatment *per se* gives a pattern, which is somewhat intermediate between control and PB treatment. The significance of these changes is as yet unclear, although there appears to be an inverse relationship between the formation of the upper and lower complexes. It is clear, however, that PB treatment gives rise to a characteristic gel retardation pattern and Dex treatment counteracts this effect.

The nuclear proteins were then analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose and then probed with labeled *Nco*I-*Bam*HI upstream fragment. The results presented in Fig. 5 indicate a striking increase in a set of protein bands after PB treatment, the maximum response being with the band of M_r 90,000 ± 5,000. Dex treatment clearly inhibits this PB-mediated increase. We had earlier assessed the molecular weight of this species responding to PB treatment as 85,000 (9) and a revision is now made on the basis of mobility studies in several gels run with standard markers.

Finally, attempts were made to examine the effects of Dex on the stability of cytochrome P-450*b*+*e* mRNAs induced by PB. For this purpose, the animals received a single injection of PB after 6 h, fresh transcription was blocked with $CoCl_2$ or actinomycin D. $CoCl_2$ was used as a specific inhibitor of cytochrome P-450*b*+*e* mRNA transcription (6, 15) and actinomycin D as a general inhibitor of transcription. Cytochrome P-450*b*+*e* mRNA was quantified in polyribosomes by dot blot

TABLE II

Effect of phenobarbitone and dexamethasone treatments on cytochrome P-450b+e mRNA run-off transcription

The experimental details are given in the text. The animals were killed 6 h after drug treatment, and run-off transcription was carried out with isolated nuclei. The RNA transcripts (10^7 cpm) were hybridized to 5 μg of pP-45091 DNA loaded onto nitrocellulose filters. The hybridization efficiency was around 50%. The results presented represent an average obtained from two independent experiments.

Treatment	Cytochrome P-450*b*+*e* mRNA transcription	
	Adults	Pups
	cpm	
Control	38	32
PB	674	423
Dex	47	43
PB+Dex	183	93

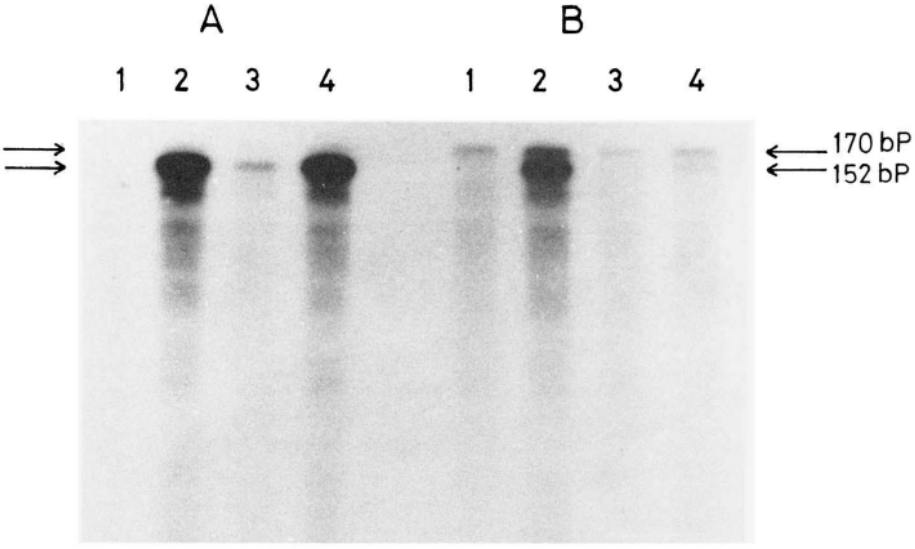

FIG. 3. **Effect of phenobarbitone and dexamethasone treatment on the transcription of the endogenous cytochrome P-450*b*+*e* genes and the added pP-450e4 DNA.** Freeze-thawed nuclei were incubated with or without added DNA as described in the text. The RNA transcripts were analyzed by S_1 nuclease protection assay. The top (170 bp) and bottom (152 bp) bands are protected DNA fragments arising from RNA transcripts derived from exogenous and endogenous gene fragments respectively. *Panel A* (endogenous run-off transcription): *lane 1*, control; *lane 2*, PB; *lane 3*, Dex; *lane 4*, PB+Dex. *Panel B* (pP-450e4 DNA transcription): *lane 1*, control; *lane 2*, PB; *lane 3*, Dex; *lane 4*, PB+Dex.

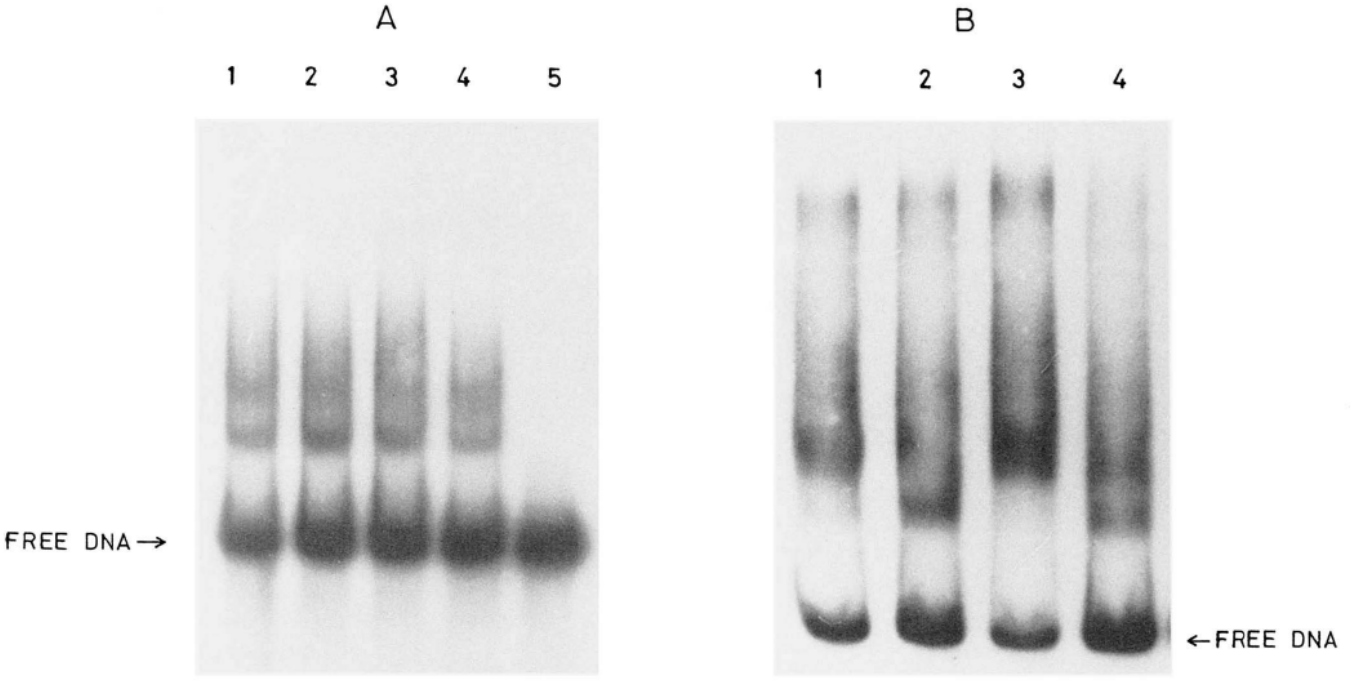

FIG. 4. **Gel retardation analysis of the interaction between nuclear extract and ^{32}P-end labeled upstream region (*Nco*I-*Bam*HI fragment).** The experimental details are given in text and 1.5 μg (*A*) and 3 μg (*B*) of protein were used. *Lane 1*, control; *lane 2*, PB; *lane 3*, Dex; *lane 4*, PB+Dex; *lane 5*, free DNA.

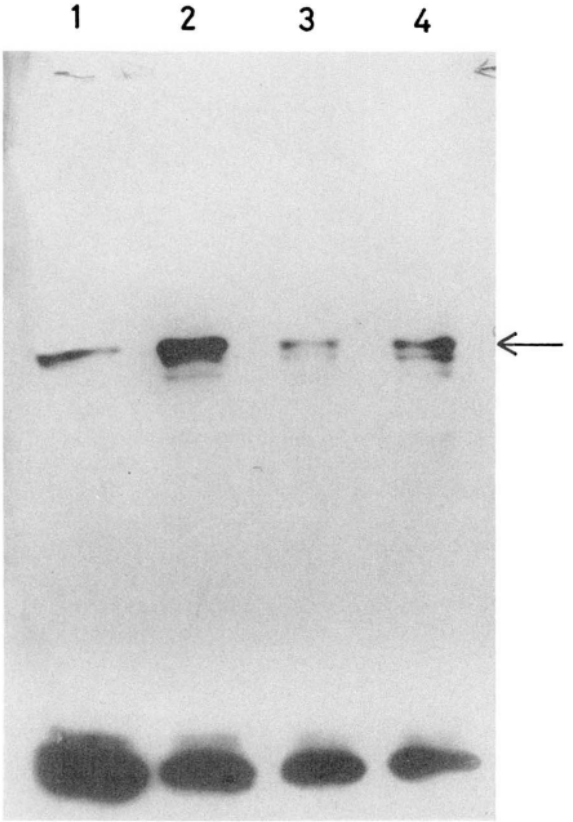

FIG. 5. **Southwestern blot analysis of nuclear extracts.** *Nco*I-*Bam*HI fragment was used as a probe. *Lane 1*, control; *lane 2*, PB; *lane 3*, Dex; *lane 4*, PB + Dex. The *arrow* corresponds to the protein band of M_r 90,000 ± 5,000. The intense bands seen at the bottom are due to histones. Cold marker proteins were run for molecular weight calibration.

analysis at different time intervals after transcription arrest. The results presented in Fig. 6 indicate that the half-life of PB-induced cytochrome P-450*b+e* mRNA is not significantly changed after Dex treatment, even though the content of these mRNAs is higher in PB+Dex treatment than in PB treatment. Similar results were obtained with $CoCl_2$ or actinomycin D treatments.

We have quantified the levels of cytochrome P-450*b* protein under the different treatment conditions using radial-immunodiffusion. The results reveal that there is a small increase in cytochrome P-450*b* protein content after Dex treatment compared with controls. PB treatment results in a significant increase, whereas PB+Dex treatment actually shows a slight decrease compared with the former (cytochrome P-450*b* protein content in nmol/mg protein: control, not detectable; PB, 1.01; Dex, 0.16; PB+Dex, 0.81). As already pointed out, Simmons *et al.* (10) have shown that Dex treatment *per se* increases cytochrome P-450*b* mRNA levels by nearly 12-fold, but there is no concomitant increase in the protein levels.

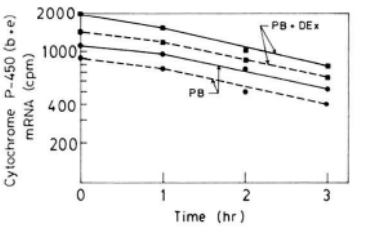

FIG. 6. **Effect of dexamethasone treatment on the stability of cytochrome P-450*b+e* mRNAs induced by phenobarbitone.** The experimental details are given in text. $CoCl_2$ or actinomycin D was given 6 h after phenobarbitone injection to block fresh cytochrome P-450*b+e* mRNA transcription. The specific mRNAs were quantified by dot blot analysis of polyribosomal RNA (20 μg), hybridization, and measurement of radioactivity of the dots. The data obtained at 6 h are taken as the zero time values. The results presented are an average obtained from two independent experiments. ——, $CoCl_2$ treatment; – – – actinomycin D treatment.

They have considered various possibilities such as induction of a species in the cytochrome P-450*b* mRNA family hybridizing to the probe but the protein species weakly cross-reacting with the *b* antibody, selective degradation of the protein species and a Dex-inducible translation inhibitor. These possibilities need to be examined for the results obtained with PB and PB+Dex treatments in the present investigation. However, in the present study, Dex treatment alone shows a small increase in cytochrome P-450*b* protein content compared with controls.

DISCUSSION

Dex is well known to act as a positive modulator of certain genes and exerts its effects through the glucocorticoid receptor interacting with glucocorticoid responsive elements, GRE (19–21). Negative regulation of transcription of certain eukaryotic genes is also clearly documented (22–25), although the mechanistic relationships between positive and negative effects are not very clear.

In the present study, Dex has been shown to negatively modulate the PB-mediated activation of cytochrome P-450*b*+*e* gene transcription. Two significant points emanate out of this study. One is that Dex shows a potentiating effect with PB on cytochrome P-450*b*+*e* mRNA levels, although it negatively modulates their transcription. Simmons *et al.* (10) have shown that Dex (80 mg/kg) by itself enhances cytochrome P-450*b*+*e* mRNA levels in rat liver by 12-fold without significantly influencing their transcription rates. They have suggested the possibility of mRNA stabilization. We have not been able to obtain 12-fold induction of cytochrome P-450*b*+*e* mRNA by Dex even under conditions described by Simmons *et al.* (10), perhaps due to strain variation. Under the conditions of our experimentation, Dex induces cytochrome P-450*b*+*e* mRNA levels by 2.5-fold without having a significant effect on basal transcription. However, Dex shows a potentiating effect with PB on the induction of cytochrome P-450*b*+*e* mRNA levels but does not manifest any stabilization effect at the cytoplasmic level. However, it appears that Dex may be stabilizing the cytochrome P-450*b*+*e* mRNA transcripts in the nuclei. This is evident from the S_1 nuclease protection analysis of endogenous P-450*b*+*e* gene nuclear transcripts (Fig. 3*A*). The RNA isolated from the nuclei under these conditions consists of steady state cytochrome P-450*b*+*e* mRNA transcripts in addition to a small amount of fresh transcripts. If PB+Dex treatment has stabilized the nuclear transcripts derived from the endogenous cytochrome P-450*b*+*e* gene during the period of treatment, even though fresh transcription is inhibited by 60–70%, S_1 nuclease protection analysis would not reveal a significant decrease compared with nuclei derived from PB-treated livers. An attempt was also made to measure the half-life of nuclear cytochrome P-450*b*+*e* mRNA transcripts under conditions described in Fig. 6 for the measurement of half-life of cytoplasmic cytochrome P-450*b*+*e* mRNA. The results did indicate a longer half-life for the cytochrome P-450*b*+*e* nuclear RNA transcripts under conditions of PB+Dex treatment than in PB treatment (data not presented). However, the differences obtained were small, and therefore the conclusions based on this experiment can only be considered tentative but support data obtained with S_1 nuclease protection analysis. At the same time, it is clear that Dex inhibits the synthesis of fresh transcripts induced by PB as evidenced by run off transcription experiments using [α-^{32}P]UTP or S_1 nuclease protection analysis of fresh transcripts synthesized in response to the addition of cloned gene fragment to nuclei.

As already mentioned, transcription of added cloned gene fragment is accompanied by a suppression of the S_1-protected band due to endogenous cytochrome P-450*b*+*e* gene transcript, both in PB as well as PB+Dex treatments (Fig. 4*B*). In this particular experiment, the suppression seen is about 50% in the case of nuclei isolated from PB-treated rats and is nearly 95% in the case of nuclei isolated from PB+Dex-treated rats (compare S_1 nuclease-protected band in Fig. 4*A* with the lower band in Fig. 4*B*). It may, however, be pointed out that the suppression level seen in endogenous transcripts due to the addition of exogenous gene is variable and a high level of suppression has been obtained in nuclei from PB-treated animals as well in other experiments. It appears possible that the entire nuclear mRNA transcript is template bound and addition of excess of cloned gene fragment may bind all the available transcription factors. This can lead to an arrest of the endogenous gene transcription accompanied by degradation of the template bound transcripts, resulting in a striking decrease in the generation of S_1-protected band. In 5-day-old pups, Dex inhibits PB-mediated increase in transcription of cytochrome P-450*b*+*e* mRNA as well as their levels in the cytoplasm. Thus, the stabilization effect of Dex is seen only in adults and therefore is developmentally programmed.

The second significant point is on the mechanism by which Dex negatively modulates PB-mediated activation of cytochrome P-450*b*+*e* mRNA transcription. Three different types of observations have been made in attempts to explain negative modulation of transcription by glucocorticoids. Sakai *et al.* (25), in a study with bovine prolactin gene, have proposed that the glucocorticoid receptor can bind both positive and negative GREs, which are distinct elements, giving rise to alternate conformations resulting in positive or negative effects. Additionally, they have shown that in the absence of glucocorticoids or the receptor, the negative GRE acts as a constitutive enhancer-like element activating transcription, perhaps due to the binding of an activator protein. Receptor binding at the negative GRE is visualized to reverse the constitutive enhancer action presumably by removing or otherwise neutralizing the actions of the enhancer activating protein bound at an overlapping site. In another study, Langer and Ostrowski (26), have examined the effect of negative GRE from mouse mammary tumor virus gene in cell-free assay. According to these workers, negative GRE binds a repressor protein distinct from the glucocorticoid receptor and decreases the basal level of transcription from some glucocorticoid-regulated promoters by preventing the assembly of active transcription complexes at promoter elements such as CCAAT box and TATA box. Oro *et al.* (28) have shown that trans-repression by human glucocorticoid receptor may involve a steric hindrance mechanism wherein the carboxyl-terminal domain of the receptor inhibits activation by preventing other factors from binding to the promoter or interacting with the transcription machinery. Despite the differences in the experimental systems used and the consequence of results, the common feature is that functional manifestation of negative GRE involves interference with the assembly of an activation complex at the enhancer or promoter site. In that respect, the present study also indicates that Dex administration counteracts the binding of the PB-activated transcription factor(s) to the 5′-flanking region of the cytochrome P-450*b*/*e* gene. As already indicated, this binding correlates very well with the transcription status of the gene, as evidenced by the results obtained in the present study and elsewhere (9).

A tentative model can be proposed as follows. The cytochrome P-450*b*+*e* gene transcription is in the repressed state

in the normal animal. This may be due to a repressor protein functioning independent of Dex. Dex may not have any additional repressive effect, since the gene is already in a repressed state. In fact, Dex treatment shows a small increase in mRNA and protein contents. Administration of PB results in the augmented synthesis of a positive transcription factor(s), that can reverse the effect of the repressor. Administration of Dex can recruit the receptor or some other Dex-modulated protein to bind at the negative GRE that may once again reverse the effects of the positive transcription factor(s) induced by PB. At this stage it is not clear whether Dex exerts its negative effects at the level of transcription through its receptor or some other Dex-modulated protein. The present study has shown that Dex has a negative effect in 5-day-old pups as well, where the glucocorticoid receptor levels are low. Thus, the involvement of some other Dex-modulated protein cannot be ruled out. A similar possibility has been suggested to explain the positive modulation effects of Dex on the transcription of cytochrome P-450*pcn* gene (27). The negative GRE may include or be very close to the sites for the binding of the repressor as well as the receptor/Dex-modulated protein as is the case in other systems where the negative GRE has sites for binding an activator or repressor (25, 26). The negative modulating effects of Dex at the level of transcription are clearly evident from its efficacy to counteract the effects of PB on cytochrome P-450*b+e* gene transcription as well as the binding of specific nuclear proteins to the 5′-flanking region of the gene as demonstrated by gel retardation and Southwestern blot analysis. We have identified a footprint for the positive transcription factor(s) induced by PB in the region −56 to −88 that includes a consensus nuclear factor I-binding site and a 13-bp pseudopalindrome (9). The region −122 to −150 includes the two TGTCCT repeats, and it is known that the different negative GREs identified do not closely conform to each other or to the positive GRE in terms of consensus sequence (25). It would be of interest to identify the negative GRE of the cytochrome P-450*b+e* genes and further examine the modalities of this negative regulation.

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REFERENCES

1. Jones, P. B. C., Durrin, L. K., Fisher, J. M., and Whitlock, J. P., Jr. (1986) *J. Biol. Chem.* **261,** 6647–6650
2. Gonzalez, F. J., and Nebert, D. W. (1985) *Nucleic Acids Res.* **14,** 1465–1477
3. Fujisawa-sehara, A., Sogawa, K., Yamane, M., and Fujii-kuriyama, Y. (1987) *Nucleic Acids Res.* **15,** 4179–4191
4. Fujisawa-sehara, A., Yamane, M., and Fujii-kuriyama, Y. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 5859–5863
5. Hardwick, J. P., Gonzalez, F. J., and Kasper, C. B. (1983) *J. Biol. Chem.* **258,** 8081–8085
6. Ravishankar, H., and Padmanaban, G. (1985) *J. Biol. Chem.* **260,** 1588–1592
7. Suwa, Y., Mizukami, Y., Sogawa, K., and Fujii-Kuriyama, Y. (1985) *J. Biol. Chem.* **260,** 7980–7984
8. Rangarajan, P. N., Ravishankar, H., and Padmanaban, G. (1987) *Biochem. Biophys. Res. Commun.* **144,** 258–263
9. Rangarajan, P. N., and Padmanaban, G. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 3963–3967
10. Simmons, D. L., McQuiddy, P., and Kasper, C. B. (1987) *J. Biol. Chem.* **262,** 326–332
11. Palmiter, R. D. (1974) *Biochemistry* **13,** 3601–3605
12. Thomas, P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 5201–5205
13. Omiecinski, C. J., Walz, F. G., Jr. and Vlasuk, G. P. (1985) *J. Biol. Chem.* **260,** 3247–3250
14. Guertin, M., Baril, P., Bartkowiak, J., Anderson, A., and Belanger, L. (1983) *Biochemistry* **22,** 4296–4302
15. Dwarki, V. J., Francis, V. S. N. K., Bhat, G. J., and Padmanaban, G. (1987) *J. Biol. Chem.* **262,** 16958–16962
16. Favaloro, J., Treisman, R., and Kamen, R. (1980) *Methods Enzymol.* **65,** 718–749
17. Taylor, C. W., Yeoman, L. C., Dashal, I., and Bush, H. (1973) *Exp. Cell. Res.* **82,** 215–226
18. Thomas, P. E., Korzeniowski, D., Ryan, D., and Levin, W. (1979) *Arch. Biochem. Biophys.* **192,** 524–532
19. Chandler, V. L., Mahler, B. A., and Yamamoto, K. R. (1983) *Cell* **33,** 489–499
20. Miksicek, R., Heber, A., Schmid, W., Danesch, U., Possekert, B., Beato, M., and Schutz, G. (1986) *Cell* **46,** 283–290
21. DeFranco, D., and Yamamoto, K. R. (1986) *Mol. Cell. Biol.* **6,** 993–1001
22. Camper, S. A., Yao, Y. A. S, and Rottman, F. M. (1985) *J. Biol. Chem.* **260,** 12246–12251
23. Charron, J., and Drouin, J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 8903–8907
24. Weiner, F. R., Czaja, M. J., Jefferson, D. M., Giamborne, M., Tur-Kaspa, R., Reid, L. M., and Zern, M. A. (1987) *J. Biol. Chem.* **262,** 6955–6958
25. Sakai, D. D., Helms, S., Carlstedt-Duke, J., Gustaffson, J-A., Rottman, F. M., and Yamamoto, K. R. (1988) *Genes & Dev.* **2,** 1144–1154
26. Langer, S. J., and Ostrowski, M. C. (1988) *Mol. Cell. Biol.* **8,** 3872–3881
27. Schuetz, E. G., and Guzelian, P. S. (1984) *J. Biol. Chem.* **259,** 2007–2012
28. Oro, A. E., Hollenberg, S. M., and Evans, R. M. (1988) *Cell* **55,** 1109–1114