

Developmental regulation of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) gene expression in rat liver

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Abstract. The expression of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) genes has been studied as a function of development in rat liver. The levels of cytochrome P-450 (b+e) mRNAs and their transcription rates are too low for detection in the 19-day old fetal liver before or after phenobarbitone treatment. However, glutathione transferase (Ya+Yc) mRNAs can be detected in the fetal liver as well as their induction after phenobarbitone treatment can be demonstrated. These mRNAs contents as well as their inducibility with phenobarbitone are lower in maternal liver than that of adult non-pregnant female rat liver. Steroid hormone administration to immature rats blocks substantially the phenobarbitone mediated induction of the two mRNA families as well as their transcription. It is suggested that steroid hormones constitute one of the factors responsible for the repression of the cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) genes in fetal liver.

Keywords. Cytochrome P-450; glutathione transferase; transcription; gene expression fetal liver.

Introduction

It is known that the cytochrome P-450 dependent mixed function oxygenase activities are very low in fetal liver and increase rapidly soon after birth. Phenobarbitone fails to induce cytochrome P-450 in fetal liver, although 3-methyl-cholanthrene is capable of a selective but small level of induction. It has also been shown that phenobarbitone readily crosses the placental barrier and accumulates in the fetal liver to almost the same concentration observed in the adult liver. Phenobarbitone is quite effective in inducing cytochrome P-450 soon after birth (Waddel and Mirkin, 1971; Guenther and Mannering, 1977; Cresteil *et al.*, 1979; Nebert *et al.*, 1982).

Glutathione-S-transferase, another important enzyme family-of the drug metabolizing pathway, has been shown to be present at 10–20% of the adult levels in fetal rat liver. The susceptibility of this enzyme family for induction at the fetal stage has not been well studied, except that phenobarbitone induces this enzyme system 2–3-fold after birth and the extent of induction remains constant throughout development (Hales and Neims, 1976; Neims *et al.*, 1976).

The molecular mechanism of regulation of expression of these two gene systems during development has not been well studied, except for a report that phenobarbitone fails to induce cytochrome P-450b or e mRNA in 10- or 19-day old fetal liver but detectable levels of induction are seen on day 22 (Giachelli and Omiecinski, 1986). In the present study, the induction of cytochrome P-450 (b+e)

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and glutathione transferase (Ya+Yc) mRNAs by phenobarbitone in fetal, pup and adult rat livers was examined using cloned cDNA hybridization probes. The run-off transcription rates with isolated nuclei were measured. The effects of circulating steroid hormones on these parameters were also studied and the suggestion is made that these hormones constitute one of the factors responsible for maintaining these genes in the repressed state in fetal liver.

Experimental procedures

Treatment of animals

Pregnant rats (200 g body weight) of the Institute strain were given one, two or three intraperitoneal injections of phenobarbitone (8 mg/100 g body weight) starting on day 17 or 19. The animals were killed 12h after the last injection. Single injections of steroid hormones were given intraperitoneally at a dose of 1 mg/100 g, 15 min before the drug administration.

Quantification of cytochrome P-450(b+e) and glutathione transferase (Ya+Yc) mRNA

Polysomal RNA was isolated from the pooled livers of rats by the magnesium precipitation procedure of Palmiter (1974). Poly (A)-containing RNA was isolated using oligo-dT cellulose chromatography. Specific mRNA concentrations were estimated by dot blot hybridization using specific nick-translated cloned cDNA probes. In these studies 500 ng to 1 μ g of poly (A)-containing RNA or 10-20 μ g of total polysomal RNA was loaded onto nitrocellulose filters. In northern blot experiments 10 μ g of polysomal RNA was used. The dot blot and northern blot hybridizations were carried out as described by Thomas (1980). Agarose (1 %, w/v)-formaldehyde gels were used for RNA analysis (Maniatis *et al.*, 1982). The probes used were inserts from the plasmids pP-450 91 (Ravishankar and Padmanaban, 1985) and pGST 19 (Francis *et al.*, 1986) for quantifying cytochrome P-450(b + e) and glutathione transferase (Ya+Yc) mRNAs respectively. The probes covered more than 2/3 of cytochrome P-450e and glutathione transferase Ya subunit mRNAs respectively from 3'ends. Cytochrome P-450 e and b mRNAs as well as glutathione transferase Yc and Ya subunit mRNAs have extensive regions of homology (Mizukami *et al.*, 1983; Pickett *et al.*, 1984). Albumin mRNA was quantified with the albumin cDNA probe (Sathyabhama *et al.*, 1986). Nick translation was carried out in the presence of [α^{32} -P]dCTP and a labelling of 5×10^7 to 10^8 cpm/ μ g DNA was routinely obtained. After carrying out the hybridization and washing procedures, the blots were exposed to X-ray films and the corresponding dots were punched out from the nitrocellulose blots and radioactivity measured using 0.5% (w/v) PPO in toluene as the scintillation fluid. Linear responses were obtained in the concentration range up to 1.5 μ g for poly (A)-containing RNA and 20 μ g for total polysomal RNA. The experiments were repeated 4 times and the pattern of results was reproducible. However, the absolute radioactivity in the dots varied between experiments depending on the specific activity of the probe used.

Cell-free nuclear transcription

Nuclei were isolated from the livers of rats given the various treatments and cell-free transcription was carried out in the presence of [α^{32} -P] UTP as described by Ravishankar and Padmanaban (1985) based on the reaction conditions given by Guertin *et al.* (1983). The RNA transcribed *in vitro* (10^7 cpm) was hybridized to filters containing pP-450 91 or pGST 19 or pAlb 6 DNA. About 5 μ g of the DNAs were loaded on filters. After hybridization and washing the filters were dried and radioactivity measured. Addition of α -amanitin (1 μ g/ml) to the transcription mixture was found to inhibit total transcription by about 50%, but hybridization to the cloned probes used was found to be inhibited by 96–98%. Non-specific hybridization to pBR 322 DNA was of the order of 10% of specific hybridization and this value was subtracted in the final data presented.

Results

The inducibility of cytochrome P-450 (b + e) and glutathione transferase (Ya+Yc) mRNAs by phenobarbitone in fetal, pup and adult livers were examined by dot blot hybridization and the quantitative values are presented in table 1. The results indicate that detectable levels of cytochrome P-450(b + e) mRNA are not present in control fetal liver. The mRNAs can be detected in the livers of 5-day old pups, maternal and non-pregnant female adult livers. Phenobarbitone administration to the mother fails to induce these mRNAs in fetal liver. This is irrespective of whether the animal has received 1, 2 or 3 injections of the drug. There is about 10-12-fold induction in the 5-day old pup and pregnant mother.

Table 1. Cytochrome P-450 and glutathione transferase (Ya+Yc) mRNA contents in rat liver as a function of development.

Developmental stage	P-450(b + e) mRNA		GST(Ya + Yc) mRNA	
	Control	PB	Control	PB
	(cpm hybridized)			
Fetal	ND	ND	33	55
Pup	18	190	50	85
Maternal	24	256	80	152
Adult (non-pregnant female)	30	626	135	301

Nineteen-day old pregnant rats as well as the other groups received a single injection of phenobarbitone and the animals were killed after 12 h. Poly (A)-containing RNA (1 μ g) isolated from the magnesium-precipitated liver polyribosomes was loaded onto nitrocellulose filters and hybridized with the nick-translated probes. The values presented are those obtained in a typical experiment.

ND, Not detectable.

The induction is nearly 20-fold in the non-pregnant adult female. Unlike cytochrome P-450 (b+e) mRNAs, glutathione transferase (Ya+Yc) mRNAs can be detected in control fetal livers. These mRNA concentrations increase 1.5-, 2.5- and 4-

fold in control pup, pregnant and non-pregnant female livers respectively. Phenobarbitone administration results in a small but consistent pattern of induction of glutathione transferase (Ya+Yc) mRNAs in fetal liver. The induction is around 1.5-fold in pups, 2- and 2.5-fold in the pregnant and non-pregnant adult females respectively 12 h after a single injection of phenobarbitone. The northern blot presented in figure 1 confirms the pattern of data presented in table 1. In particular, it is clear that glutathione transferase (Ya+Yc) mRNAs are present in the fetal liver and are induced by phenobarbitone (figure 1, lanes 3 and 6). It may be pointed out that for northern blot analysis, RNA was isolated from animals which had received 3 injections of the drug and the glutathione transferase (Ya + Yc) mRNA induction obtained is nearly 7–10-fold of the levels observed in control rats. In separate hybrid selection experiments, pGST-19 has been found to pick up mostly Ya mRNA and therefore these results would mostly reflect changes in the concentration of Ya mRNA. On the basis of the mobility of the size markers, it appears that these have a size range of 900 ± 50 nucleotides which is around the value reported earlier (Pickett *et al.*, 1983). Giachelli and Omiecinski (1986) have

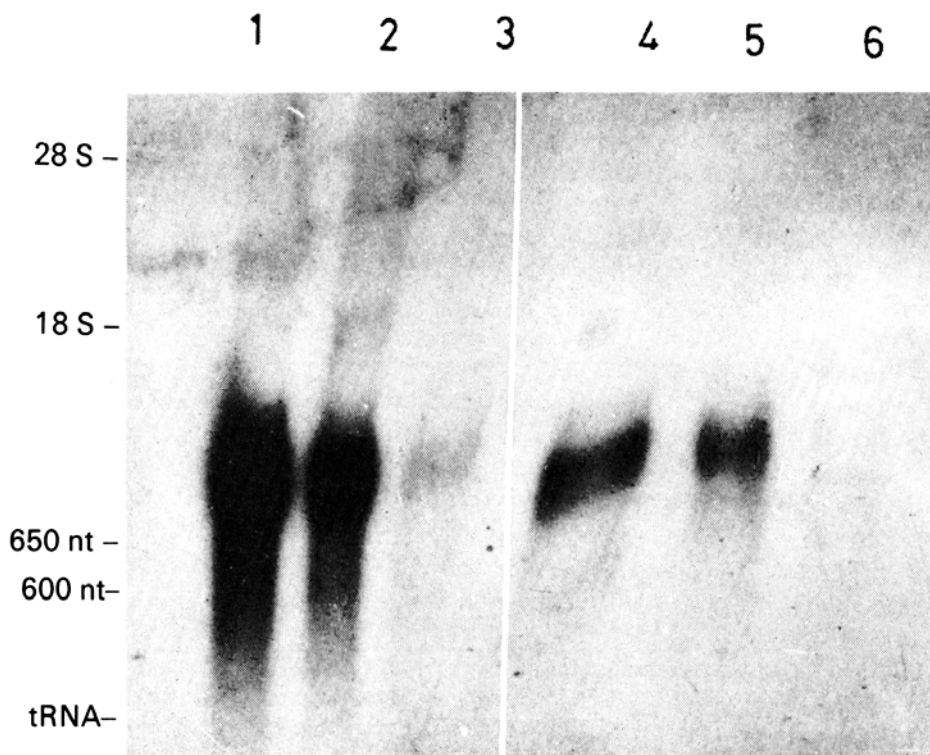


Figure 1. Northern blot analysis of glutathione transferase (Ya+Yc) mRNAs during development and phenobarbitone treatment. The animals received 3 injections of phenobarbitone one each at 24 h intervals. The first injection was given to 17-day old pregnant rats and the maternal and fetal livers were isolated on day 19. Total polysomal RNA (10 μ g) was subjected to northern blot analysis using nick-translated fragment from pGST-19 as probe. 28 S and 18 S ribosomal RNAs, globin mRNA and tRNA were used as size markers. Lanes 1-3, Phenobarbitone treated; lanes 4-6 control; lanes 1 and 4, adult female liver; lanes 2 and 5, maternal liver; lanes 3 and 6, fetal liver.

reported that the size of cytochrome P-450e mRNA remains around 1.8 kb throughout development.

In view of the bindings that phenobarbitone fails to induce cytochrome P-450(b+e) mRNAs and induces glutathione transferase (Ya+Yc) mRNAs to a lower extent in fetal livers than in the adult and that the induction of these mRNA families in the pregnant mother is lower than that of the non-pregnant female, the effects of circulating steroid hormones on the phenobarbitone-mediated induction of these mRNAs were investigated. Figure 2 indicates that estradiol and progesterone administration block significantly the phenobarbitone mediated induction of cytochrome P-450(b+e) mRNAs in the 5-day old pup. Surprisingly, the hormones fail to block the induction in adult male rats. In fact, a certain level of synergism is seen between the hormones and the drug. Detailed quantitative results for cytochrome P-450(b+e) and glutathione transferase (Ya+Yc) mRNAs are presented in table 2. The hormones estradiol, progesterone, testosterone and dexamethasone inhibit the phenobarbitone-mediated induction of both mRNA families by 50-70% in the 5-day old pup liver.

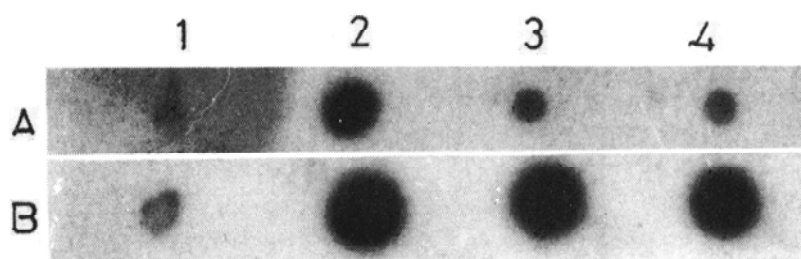


Figure 2. Dot blot analysis of cytochrome P-450(b+e) mRNAs after phenobarbitone and steroid treatments. Five-day old pups and adult male rats received single injections of phenobarbitone along with estradiol and progesterone. Poly (A) containing RNA (1 μ g) was used for the dot blot analysis using nick-translated fragment from pP-450 91 as probe. Lane A (pup): (1), control; (2), phenobarbitone; (3), phenobarbitone + estradiol; (4), phenobarbitone + progesterone. Lane B (adult): (1), control; (2), phenobarbitone + estradiol; (3), phenobarbitone + progesterone; (4), phenobarbitone.

Table 2. Effect of steroid hormones on the induction of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) mRNAs by phenobarbitone.

Treatment	Pup		Adult (male)	
	P-450 (b+e) mRNA	GST (Ya+Yc) mRNA	P-450 (b+e) mRNA	GST (Ya+Yc) mRNA
	(cpm hybridized)			
Control	21	63	32	175
PB	196	126	750	420
PB + estradiol	75	85	855	510
PB + progesterone	96	89	825	435
PB + testosterone	66	79	793	556
PB + dexamethasone	71	75	835	456

The treatment conditions and other experimental details are as described in the text and table 1. The results presented are those obtained in a typical experiment

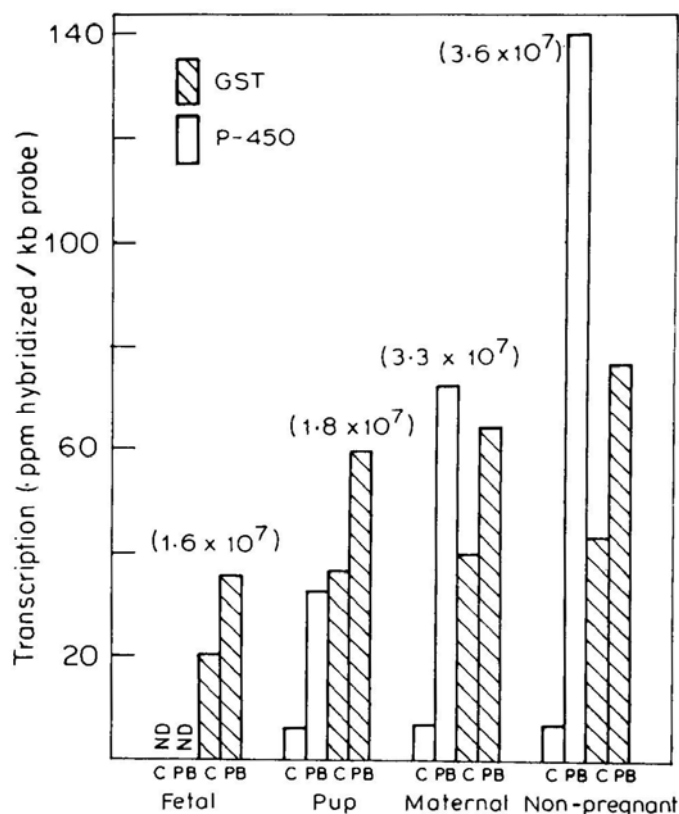


Figure 3. Transcription rates of cytochrome P-450(b+e) and glutathione transferase (Ya +Yc) mRNAs as a function of development. The nuclei isolated from the livers of rats at different developmental stages were incubated for 45 min at 25°C and other components with [α -³²P]UTP and the transcribed RNA was hybridized to the probes. The total RNA transcribed, expressed in cpm in the case of each nuclear preparation, is indicated in parenthesis. The specific transcription rates presented as bars are an average from two independent experiments and are expressed as ppm of RNA hybridized/kb of the probe used. The other details are given in the text, (C), Control; (PB), phenobarbitone, (ND), not detectable.

The results presented in figure 3 indicate that the total RNA transcription rates obtained for fetal and pup livers is consistency around 50% of the adult livers. In the case of cytochrome P-450(b+e) mRNAs, phenobarbitone administration results in about 5-, 10- and 20-fold increase in transcription rates for pup, pregnant and non-pregnant livers. Transcription of these mRNAs cannot be detected in control or phenobarbitone-treated fetal liver nuclei. In the case of glutathione transferase (Ya+Yc) mRNAs, significantly level of transcription is detectable in control fetal liver nuclei. With development, this increases by nearly 2-fold in the adult. Phenobarbitone administration results in a small but consistency detectable increase in the transcription rates in the fetal liver nuclei. The increase obtained with the pup, pregnant and non-pregnant liver nuclei is less than 2-fold of the values obtained with control animals, 12 h after a single injection of the drug.

Since estradiol and progesterone significantly inhibited the induction of cytochrome P-450(b+e) mRNAs and glutathione transferase (Ya+Yc) mRNAs in

5-day old pup livers, the run-off transcription rates were also measured under these conditions. The results presented in figure 4 indicate that the two hormones tested inhibit the phenobarbitone-mediated increase in the transcription rates of the two mRNA families by 50-60%. It is of interest to point out that albumin mRNA transcription rates measured under these conditions also show significant changes. First of all, phenobarbitone administration *per se* decreases albumin transcription rate in the pup. Estradiol as well as testosterone treatments, further decrease the albumin mRNA transcription rates. The albumin mRNA transcription rate observed in the normal pup is around 40% of that seen with the adult (data not presented). It should, however, be pointed out that none of the treatments affects total RNA transcription rates to any significant extent.

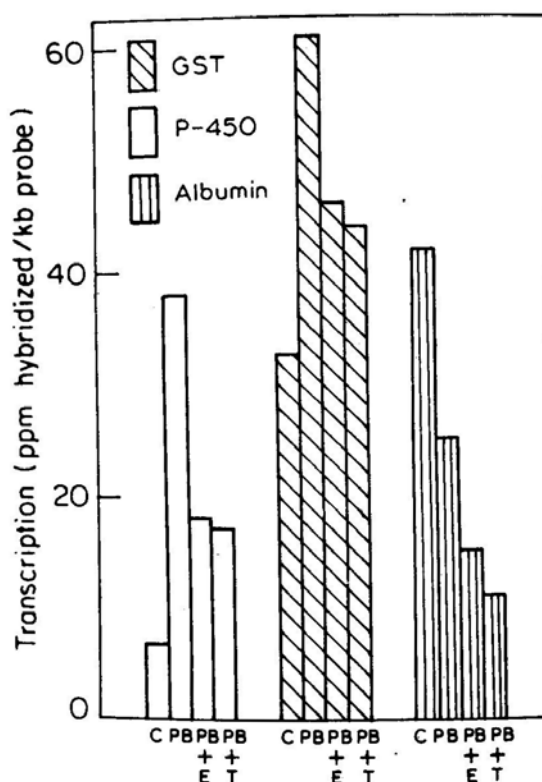


Figure 4. Effect of steroid hormones on the phenobarbitone-mediated increase in transcription rates of cytochrome P-450(b+e), glutathione transferase (Ya+Yc) and albumin mRNAs in pup liver. Nuclei were isolated from the livers of pups given the different treatments. The experimental details are given in figure 3 and text. The values depicted are an average obtained from two independent experiments. (C), Control, (PB), phenobarbitone; (E), estrogen; (T), testosterone.

Discussion

Several interesting features of developmental regulation of the hepatic cytochrome P-450(b+e) and glutathione transferase (Ya+Yc) gene systems are apparent from this study. First of all, detectable levels of expression of the cytochrome P-450(b + e)

mRNAs in control or phenobarbitone-treated 19-20-day old fetal livers are not manifest, confirming the report of Giachelli and Omiecinski (1986). However, glutathione transferase (Ya+Yc) mRNAs are expressed as well as induced in fetal livers.

Steroid hormones appear to exert a negative control in regulating the expression of both cytochrome P-450(b+e) and glutathione transferase (Ya+Yc) mRNAs in fetal liver. The poor or non-expression of the former and the lower level of expression of the latter gene systems in fetal liver in the presence or absence of the inducer, the inhibitory effects of the steroid hormones on phenobarbitone mediated induction of the two mRNA families in the immature animal and the lower levels of these mRNAs in the drug-treated pregnant mother compared to the non-pregnant adult female, all point to the role of circulating steroid hormones in exerting a negative control on the expression of these two gene systems in fetal and pregnant rat livers. It has not been possible to examine the effect of steroid hormones on cytochrome P-450(b+c) gene transcription directly in fetal liver in view of poor expression. The possibility of factors in the fetomaternal environment suppressing the phenobarbitone-mediated induction of the drug-metabolizing enzymes in fetal liver has been suggested by Guenther and Mannering (1977). Further studies are needed to examine the hormone-dependent changes in the expression of these genes during different phases of the estrous cycle in non-pregnant female adult rats.

It is also clear that the responsiveness of the gene involved for interaction with the environment also changes during development. For example, in the adult male the steroid hormones fail to repress the phenobarbitone-mediated induction of these mRNAs. We have recently shown that dexamethasone inhibits phenobarbitone-mediated increase in the transcription of cytochrome P-450(b+e) genes both in adult and pup livers. However, dexamethasone inhibits the drug mediated induction of cytochrome P-450(b + e) mRNAs only in pup livers, but actually shows synergism with the drug in adult livers (table 2; Venkateswara Rao *et al.*, 1990). Thus, development dependent post-transcriptional mechanisms are also involved in determining the ultimate levels of the functional mRNA and perhaps of the protein species as well. This is further indicated by the lack of a strict correlation between changes in transcription rates and mRNA contents under different developmental and physiological states.

Two other features of this study are of interest. One is that all the 4 steroid hormones examined share the ability to inhibit phenobarbitone-mediated increase in the transcription of cytochrome P-450(b+e) and glutathione transferase (Ya+Yc) genes. Steroid receptors belong to a super family and are related structurally. The DNA consensus elements, involved in receptor binding are also similar, but distinct identity is maintained in mediating transcriptional activation by the individual steroids (Evans, 1988; Green and Chambon, 1988). However, the DNA consensus elements involved in mediating the negative modulatory effects of steroids have not been well characterized. The negative glucocorticoid receptor element does not appear to share much of homology with the positive element or with the negative elements detected in different gene systems (Sakai *et al.*, 1988). It is possible that the negative steroid elements are degenerate and recognise more than one steroid receptor *in vitro*. The second feature is that the steroids used repress the expression of all the 3 gene systems, namely cytochrome P-450(b+ e), glutathione transferase (Ya+Yc) and albumin in pups. All these 3 genes are poorly

expressed in the fetal liver. Thus, the role of steroids as negative modulators of the expression of major liver proteins in fetal liver is indicated and would need further investigation.

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