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EXPRESSION OF THE CYTOCHROME P4502E AND 2B GENE FAMILIES IN THE LUNGS AND LIVERS OF NONPREGNANT, PREGNANT, AND FETAL HAMSTERS

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Abstract—Members of the cytochrome P4502E and 2B gene families have been implicated in the activation of nitrosamines to reactive species capable of binding to cellular macromolecules and initiating tumor formation in various rodent species. This study was initiated to determine the relative prevalence of these isozymes and their response to ethanol during pregnancy and late gestation. Nonpregnant and pregnant hamsters were given a 10% ethanol solution in their drinking water for 10 days (gestation days 5–15) prior to being killed. RNA blot analysis of liver and lung tissue from nonpregnant, pregnant, and fetal hamsters demonstrated tissue-specific expression of CYP2E and 2B in adult and fetal animals. The levels of RNA expression of both P450s in fetal hamsters were <30% of nonpregnant adult values. In pregnant hamsters, the hepatic levels of CYP2E and 2B RNAs were decreased compared to nonpregnant animals. In contrast, the pulmonary levels of CYP2B RNA were increased in pregnant versus nonpregnant hamsters, while no effect of pregnancy on the levels of CYP2E RNA was seen. Although rats contain a single CYP2E1 gene transcript, Northern analysis demonstrated the presence of 1.8 and 2.8 kb bands in both liver and lung tissue of the hamster. Pretreatment with ethanol had little effect on the levels of either P450 RNA species in the lungs or livers of nonpregnant, pregnant, and fetal hamsters. These results demonstrate differences in the levels of expression of members of the CYP2E and 2B gene families during pregnancy and late gestation compared to nonpregnant adult hamsters. Fetal animals, like the adults, apparently respond to ethanol treatment by altering the levels of these P450 isozymes at the post-transcriptional level.

The cytochrome P450 monooxygenase system consists of a superfamily of hemoproteins responsible for the metabolism of a wide variety of endogenous and exogenous substrates, including steroids, fatty acids, drugs, and environmental toxicants such as chemical carcinogens. In addition to the detoxication and elimination of these compounds from the body, several isozymes of cytochrome P450 have been implicated in the activation of parent compounds to highly reactive, carcinogenic metabolites [1, 2].

Recent studies have focused on the importance of tobacco-specific nitrosamines as causative agents in the etiology of smoking-induced lung cancers [3, 4]. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK§) has been shown to be a particularly potent lung carcinogen. Administration of NNK to rats [5], mice [6], or hamsters [7] results primarily in the formation of lung tumors, with incidences of 70–100% depending on the species studied. In addition, NNK also has been shown to be an active transplacental carcinogen in both Syrian Golden hamsters [8] and mice [9]. Several studies have shown that NNK is metabolized by cytochrome P450 monooxygenases and is apparently activated by α-hydroxylation to both α-methylene and α-methyl hydroxylated carbons, which can produce reactive intermediates that form methylated or pyridyloxobutylated adducts, respectively, with both DNA and proteins [6, 10–14]. Belinsky *et al.* [6] have provided strong evidence suggesting that methylation of the O^6 residue of guanine may be the most important lesion for the induction of lung tumors in A/J mice. Approximately 90% of NNK- and dimethylnitrosamine-induced lung tumors contained an activated Ki-*ras* oncogene, primarily with a GC to AT transition in codon 12, a mutation consistent with methylation of guanine at the O^6 position. This hypothesis has been confirmed by recent studies [13] demonstrating the higher tumorigenicity of precursors that can only be metabolized to methylating species compared to derivatives that produce pyridyloxobutylating metabolites.

Results obtained in rats and mice [10, 13] using specific P450 antibodies to inhibit NNK metabolism, and more recent studies in hamsters [15], have suggested that the metabolism of NNK is mediated by at least two isozymic forms of cytochrome P450, and members of both the CYP2E and CYP2B gene families have been implicated as playing important roles in NNK metabolism. Guo *et al.* [16] have recently demonstrated the metabolism of NNK in a reconstituted enzyme system utilizing purified CYP2B1. While these two forms of P450 have been

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§ Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; SDS, sodium dodecyl sulfate; and 1 × SSC, 0.15 M NaCl/0.015 M sodium citrate.

studied extensively in adult rodent species, little information is available on their role in drug metabolism during the fetal period. Studies in fetal hamsters have shown that NNK crosses the placenta into the fetal compartment when administered to pregnant hamsters and is metabolized by fetal tissues to reactive intermediates capable of causing DNA damage [17, 18]. Data obtained from our laboratories [15] have demonstrated, using HPLC analysis, the metabolism of NNK by microsomal fractions from nonpregnant, pregnant, and fetal hamster lung and liver tissue. The catalytic activity of the microsomes for different NNK metabolites varied depending on age, pregnancy status, or tissue being assayed. Fetal tissues demonstrated similar profiles of NNK metabolites as adult tissues, although at much lower levels. Interestingly, similar to adult animals, fetal hamsters responded to ethanol pretreatment with enhanced levels of NNK metabolism. Western blot analysis of CYP2E and 2B* protein has documented tissue-, pregnancy-, and age-specific changes in the levels of expression of members of these two P450 families following administration of ethanol to hamsters [15]. The studies reported here were initiated to determine the molecular mechanisms responsible for the regulation of CYP2E and CYP2B isozymes in hamsters during pregnancy and late gestation.

METHODS

Chemicals. Bovine serum albumin (fraction V), salmon sperm DNA, Ficoll, and polyvinylpyrrolidone were purchased from the Sigma Chemical Co., St. Louis, MO; guanidine isothiocyanate was obtained from Bethesda Research Laboratories, Gaithersburg, MD; formamide was purchased from the Fluka Chemical Corp., Hauppauge, NY; glyoxal was obtained from the Fisher Scientific Co., Springfield, NJ; dextran sulfate was purchased from 5 Prime→3 Prime, Inc., Paoli, PA; and [α-^{32}P]dCTP (~3000 Ci/mmol) was obtained from ICN Biomedicals, Inc., Costa Mesa, CA.

Animals and treatment protocols. Outbred Syrian Golden hamsters (*Mesocricetus auratus*) were purchased from Charles River Inc. (St. Constant, Québec). The animals were housed in plastic cages on wood chip bedding and were fed Rodent Laboratory Chow 5001 (Purina, Québec). The animals were mated when 2 months old as described previously [17]. The day of mating was considered day 0 of gestation.

Both nonpregnant and pregnant hamsters were supplied with a 10% ethanol solution in their drinking water for 10 days, from gestation days 5–15, while control hamsters were allowed free access to tap water alone. Both control and pregnant hamsters (day 15 of gestation) were euthanized by decapitation between 10:00 and 11:00 a.m. Liver and lung tissues from both adult and fetal animals were immediately removed and frozen in liquid nitrogen. Fetal livers and lungs from the same litter were pooled. The tissue was stored at −80° for purification of RNA.

Purification of total cellular RNA. RNA was purified as described previously [19] by the method of MacDonald *et al.* [20]. Frozen liver and lung tissues were placed in 8 mL of a cold 4 M guanidine isothiocyanate/0.1 M Tris–HCl (pH 7.5)/1% 2-mercaptoethanol solution. The tissues were homogenized by a 60-sec burst with a Polytron homogenizer and then passed six times through a 22G syringe needle to shear DNA and reduce the viscosity of the solution. Sodium sarkosyl (*N*-lauroylsarcosine, sodium salt) was added to a final concentration of 0.5% and the homogenates were layered over a 6.1 M CsCl/25 mM NaAc (pH 5.2)/10 mM EDTA cushion. RNA was collected by centrifugation for approximately 20 hr at 20° in a Beckman SW41 rotor at 110,000 *g*. The RNA pellets were redissolved in 0.5 mL of autoclaved, glass-distilled, diethylpyrocarbonate-treated water and precipitated by centrifugation from a 70% ethanol/0.3 M NaAc (pH 5.2) solution. The pellets were rinsed once with 70% ethanol and dried in a Jouan vacuum desiccator. RNA was then dissolved in 10 mM sodium phosphate buffer (pH 7.0) and the RNA concentration determined by measurement of absorbance at 260 nm.

RNA blot analysis. Purified RNA was denatured by treatment with glyoxal and either blotted directly onto a nylon membrane filter using a Schleicher & Schuell Minifold II apparatus or first fractionated by electrophoresis in a 1% agarose gel containing 1 μg/mL of ethidium bromide with constant recirculation of the 10 mM sodium phosphate (pH 7.0) buffer [21]. Prior to Northern blot transfer, the agarose gel was photographed under a UV light to determine the integrity of rRNA in each sample. RNAs were cross-linked to the nylon membranes with a Hoefer UVC 1000 Crosslinker, and the membranes were incubated for 15 min at 95° in 20 mM Tris–HCl (pH 8.0) to remove the glyoxal.

The blots were prehybridized and hybridized as described [21]. The rat pP450j cDNA probe to CYP2E1 was provided by Dr. Frank Gonzalez [22]. The pSP450 oligo plasmid containing the cDNA sequence to rat CYP2B1 was a gift from Dr. Milton Adesnik [referred to as pSR-P450 in Ref. 23]. Both of these CYP plasmids are full-length cDNA probes that will probably hybridize to all members of their respective gene families. The pαT14 genomic clone of the rat α-tubulin gene was obtained from Dr. Ihor Lemischka [24]. The DNA probes were labeled by the random primer labeling technique [25] using a kit purchased from Bethesda Research Laboratories. Following hybridization, the blots were washed two

* Although the hamster cytochrome P450 RNAs and proteins cross-hybridize to rat DNA probes and antibodies, it has not as yet been established that the hamster actually contains genes that are orthologous to the rat forms of CYP2E1 and 2B1/2. We have therefore referred to the hamster RNA transcripts as being members of the CYP2E and 2B gene families. In discussing the results of the Western blot analysis, we have referred to the hamster CYP2B proteins as B1 and B2 based on their mobilities in polyacrylamide gels, which were comparable to the rat 2B1 and 2B2 forms. At this time, there is no evidence to indicate that these hamster forms have the same catalytic specificity as the rat isozymes. It should also be noted that we have no evidence to indicate whether the forms found in the hamster lung and liver are necessarily the same isozymic forms.

Table 1. Immunoblot analysis of CYP2E and 2B protein expression

Treatment group	Liver			Lung
	CYP2E	CYP2B1	CYP2B2	CYP2B1
Nonpregnant control	0.52	2.90	1.35	3.20
Nonpregnant ethanol	1.62	2.77	2.03	3.00
Pregnant control	0.42	0.10	1.48	3.97
Pregnant ethanol	0.60	0.10	1.41	3.96
Fetal control	0.19	0.20	0.10	0.17
Fetal ethanol	0.44	0.30	0.10	0.17

Adult microsomes (30 μg) and fetal microsomes (100 μg) were analyzed by Western blot with anti-P450 antibodies to the specific isozymic forms of the rat. Results shown are scanned data from a previously published figure (see Fig. 2 in Ref. 15). CYP2E was undetectable in the lung by immunoblot analysis. The hamster forms of CYP2B are referred to as B1 and B2 based on their similar electrophoretic mobilities to the rat 2B1 and 2B2 forms. CYP2E values were determined for the major band detected in hamsters that cross-hybridizes to the rat CYP2E1 antibody.

times with 2 × SSC/0.1% SDS at room temperature for 5–10 min followed by three washes with 0.1 × SSC/0.1% SDS at 50° for 15 min each. The blots were wrapped in Saran-Wrap and autoradiographed in the presence of an intensifying screen (Cronex Lightning Plus, DuPont, Wilmington, DE) with preflashed Kodak X-Omat XAR-5 film at −80°. RNA levels were quantitated from the slot blots by densitometric scanning of the autoradiographs using an LKB laser densitometer. Multiple exposures of each blot were scanned to ensure quantitation over the linear range of the film.

RESULTS

Previous studies from our laboratories have shown that treatment of nonpregnant and pregnant hamsters with ethanol results in an increase in the metabolism of specific pathways of NNK metabolism in nonpregnant, pregnant, and fetal microsomal preparations in an age-, pregnancy-, and tissue-dependent manner [15]. These results correlated with alterations in the levels of specific CYP isozymes when the hamster microsomal preparations were subjected to immunoblot analysis using isozyme-specific antibodies for the rat CYP2E1 and 2B1/2 forms. Quantitative results of the Western blot data from these experiments are shown in Table 1 for comparison to the RNA data described below. CYP2E levels were not detectable in lung preparations from hamsters by Western blot analysis.

In the liver, pregnancy appeared to cause a slight decrease in the levels of immunodetectable CYP2E, while fetal microsomes showed markedly lower levels of this isozyme. Indeed, the levels of the P450 isozymes were not detected in fetal microsomal preparations unless a 3-fold greater amount of protein was used in the immunoblot analysis (Table 1). Determination of the RNA levels (Fig. 1A, Table 2) demonstrated that hepatic CYP2E RNA levels in pregnant hamsters are decreased to 27% of the value seen in nonpregnant controls, while fetal RNA levels were only 5% of nonpregnant control values. In contrast, the pulmonary levels of CYP2E RNA were not affected by pregnancy (Fig. 2A, Table 2). As seen in the liver, levels of lung CYP2E RNA were much lower in fetal than in adult animals (1% of nonpregnant adult values).

Treatment with ethanol caused a 2- and 3-fold induction of CYP2E protein in fetal and nonpregnant hamster livers, respectively (Table 1). There were no apparent effects of ethanol treatment on the

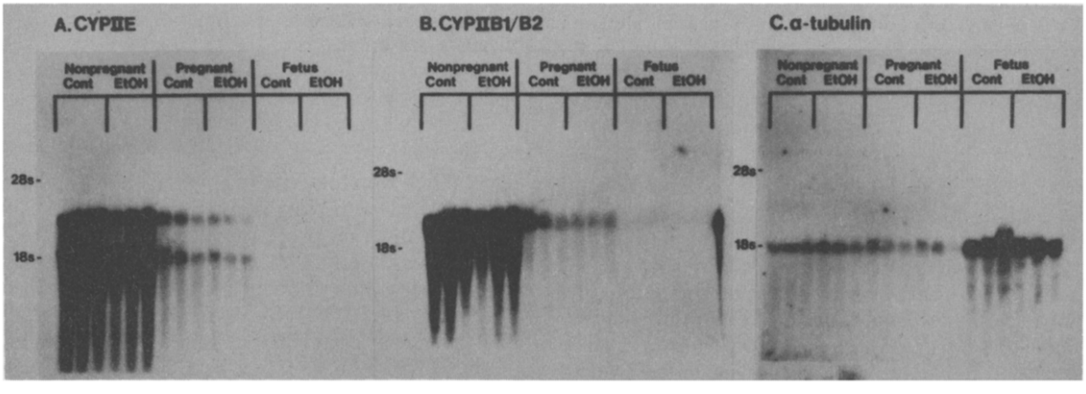

Fig. 1. Expression of hepatic CYP2E (CYPIIE) and 2B (CYPIIB) RNAs in nonpregnant, pregnant, and fetal hamsters. Glyoxal-denatured RNA (20 μg) was fractionated on a 1% agarose gel and blotted onto a nylon membrane. The membranes were probed with labeled plasmids to: (A) pP450j, (B) pSP450 oligo, and (C) pαT14. The autoradiographs shown have been overexposed in order to visualize the fainter RNA bands.

Table 2. Quantitation of CYP2E and 2B RNA expression by densitometric scanning

	Control	% Nonpregnant	Ethanol	% Control
Liver, 2E				
Nonpregnant	8.27 ± 1.56	100	6.28 ± 3.23	76
Pregnant	2.22 ± 0.35	27	1.77 ± 0.36	80
Fetal	0.44 ± 0.25	5	0.43 ± 0.14	96
Liver, 2B				
Nonpregnant	4.06 ± 1.49	100	3.50 ± 1.40	86
Pregnant	0.59 ± 0.10	15	0.46 ± 0.11	78
Fetal	Values too low to be accurately quantitated.			
Lung, 2E				
Nonpregnant	9.15 ± 1.64	100	7.00 ± 2.68	77
Pregnant	8.84 ± 3.08	97	9.37 ± 2.25	106
Fetal	0.10 ± 0.03	1	0.17 ± 0.08	164
Lung, 2B				
Nonpregnant	1.26 ± 0.55	100	1.12 ± 0.40	89
Pregnant	2.55 ± 0.88	202	3.69 ± 0.87	144
Fetal	0.36 ± 0.10	28	0.44 ± 0.12	122

Eighteen, six, and three micrograms of total cellular RNA were blotted directly onto nylon membranes and probed with the pP450j, pSP450 oligo, and pαT14 plasmids. RNA levels were quantitated by densitometric scanning of the autoradiographs using an LKB laser densitometer. Multiple exposures of each blot were scanned to ensure quantitation over the linear range of the film and are reported as relative absorbance ± the standard deviation. Values obtained for CYP2E and 2B were corrected for the amount of α-tubulin signal obtained. Values represent the mean ± SD from three individual adult hamsters or three litters.

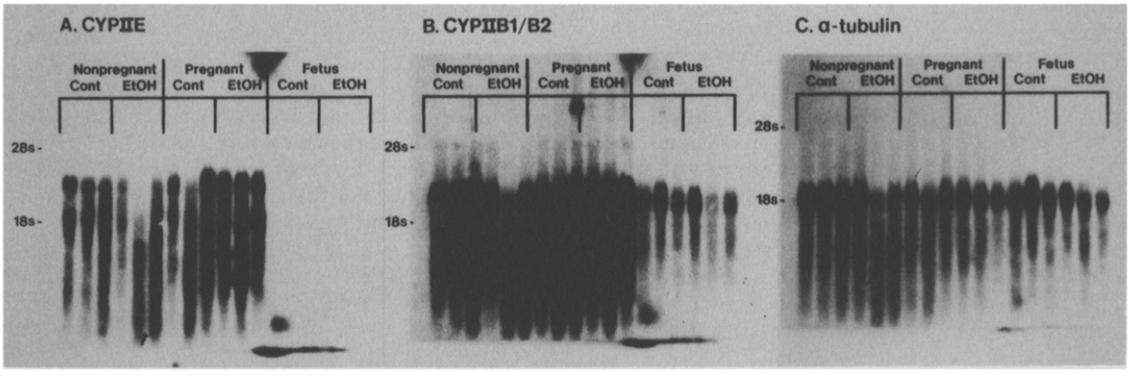

Fig. 2. Expression of pulmonary CYP2E (CYPIIE) and 2B (CYPIIB) RNAs in nonpregnant, pregnant, and fetal hamsters. Glyoxal-denatured RNA (20 μg) was fractionated on a 1% agarose gel and blotted onto a nylon membrane. The membranes were probed with labeled plasmids to: (A) pP450j, (B) pSP450 oligo, and (C) pαT14. The autoradiographs shown have been overexposed in order to visualize the fainter RNA bands.

levels of CYP2E RNA in any of the ethanol-treated lung and liver groups (Table 2). Because of the difficulty in obtaining good quantitative data for the very faint fetal CYP2E RNAs, it is hard to tell if the slight 1.6-fold increase seen in the fetal lung following ethanol treatment is of any importance.

Hamsters on day 15 of gestation showed a large decrease in the level of CYP2B RNA in the liver to 15% of nonpregnant values (Fig. 1B, Table 2), in keeping with the marked decrease in CYP2B1 protein (Table 1). However, the levels of CYP2B2 protein appeared to be similar in pregnant and nonpregnant hamsters. The levels of the CYP2B isozymes were extremely low in fetal liver samples, and were barely detectable by both Northern and immunoblot analysis. Whereas the pregnant liver demonstrated a marked decrease in CYP2B1 protein and 2B RNA, the pregnant lung showed a 2-fold increase in the amount of CYP2B RNA over nonpregnant values (Fig. 2B, Table 2) and little change at the protein level (Table 1). At the RNA level, fetal lung demonstrated 28% of the CYP2B levels found in nonpregnant adults (Table 2) but had extremely low amounts of immunodetectable CYP2B1 protein (Table 1). Ethanol treatment had little or no effect on the levels of CYP2B protein or RNA in the lungs or livers of pregnant and

fetal hamsters, and caused a nearly 2-fold increase of CYP2B2 protein in nonpregnant liver samples.

The results reported in Table 2 have been corrected for expression of the constitutively expressed α-tubulin gene. As can be seen in the liver and lung blots in Figs. 1C and 2C, respectively, the results obtained cannot be attributed to differences in the amount of RNA loading across the different samples.

Studies in rats have identified a single CYP2E1 RNA species of 1.7 kb detectable by Northern analysis [22]. The rabbit, however, contains two members of the CYP2E family, designated E1 (2.2 kb) and E2 (1.9 kb), which appear to be the result of a gene duplication that occurred after the evolutionary speciation of rabbits and rats [26]. As can be seen from the results obtained in Figs. 1A and 2A, both liver and lung RNAs contain two bands of RNA species, as detected by Northern analysis with the rat CYP2E1 probe, of 1.8 and 2.8 kb. Further studies will be needed to determine the relationship of these two transcripts to each other and to rat and rabbit members of the CYP2E subfamily, and to determine whether the hamster CYP2E genes are the hamster equivalent of the already characterized CYP2E1 and 2E2 genes or should be classified as distinct genes in the 2E subfamily. The results reported in Table 2 are from densitometric scans of slot blots and thus represent the combined signal from both RNA species that hybridize to the rat CYP2E1 probe, as the two transcripts appear to be coordinately regulated in hamster tissues. The rat CYP2B1 probe gave a single 2.3 to 2.4 kb band with hamster RNAs. As discussed above for the hamster CYP2E genes, the relationship of members of the hamster CYP2B subfamily to the rat CYP2B1 and 2B2 genes has not been established.

DISCUSSION

Several studies have demonstrated the importance of fetal and maternal metabolism in modulating the susceptibility of fetuses to the potential carcinogenic effects of various compounds [27, 28]. One of the best studied animal model systems for transplacental carcinogenesis is the genetic C57BL/6 and DBA/2 mouse models which differ in their inducibility for the CYP1A1 isozyme in response to polycyclic hydrocarbons. Recent studies using biochemical, antibody inhibition, Western blot, and Northern blot analyses have shown the importance of the CYP1A1 isozyme in modulating tumor susceptibility in different fetal tissues from fetuses of differing genetic backgrounds residing in different maternal environments [19, 29, 30]. Similar approaches have been used in this study to determine the molecular mechanisms regulating the previously observed alterations in NNK metabolism and immunodetectable P450 isozyme content in pregnant and fetal hamsters [15].

The results presented in this report demonstrate the age-, pregnancy-, and tissue-specific regulation of the levels of CYP2E and 2B RNA in Syrian Golden hamsters. In comparison to nonpregnant control hamsters, pregnant hamsters showed markedly decreased hepatic levels of both CYP2E and CYP2B RNA, which correlated with the results seen in immunoblot studies of protein levels for these isozymes ([15] and Table 1), although the protein levels for hepatic CYP2E were decreased very little compared to the large decrease seen at the RNA level. Some of this discrepancy may be due to the post-transcriptional regulation of CYP2E gene expression normally observed in other rodent species [22, 31]. In addition, it is unclear whether pregnancy differentially affects the regulation of this isozyme at the protein and RNA levels. In the lung, however, the levels of these two P450 RNAs remained either unchanged (CYP2E) or increased (CYP2B) in pregnant hamsters compared to nonpregnant controls, and apparently were not greatly affected by pregnancy at the protein level. Thus, the levels of these two enzymes in pregnant hamsters appears to be regulated by both transcriptional and post-transcriptional processes. Whether the CYP2B and 2E genes are differentially regulated in the lungs and liver or represent different members of the same P450 subfamilies in different tissues remains to be determined. The fact that the rat monoclonal antibody failed to detect any CYP2E-related protein in the lung, despite the relatively high RNA transcript levels, favors the interpretation that the liver and lung isozymes are different members of the same gene subfamily.

The levels of both the CYP2E and CYP2B proteins and RNAs were much lower in late gestational fetal tissues than in nonpregnant adult tissues. CYP2E RNA levels were 1 and 5% of nonpregnant values in lung and liver tissues, respectively. RNAs for members of the CYP2B family were barely detectable in the fetal hamster liver but were 28% of nonpregnant adult values in the lung. These observations are similar to results obtained in other rodent species showing either low expression or the absence of detectable RNA transcripts during the fetal period. CYP2E1 RNA and protein were not detected in the livers of fetal rats [22]. Subsequent studies have shown that expression of this isozyme in newborn rat livers correlates with enhanced transcription of the gene as a result of demethylation of cytosine residues in 5′ upstream regulatory regions of the DNA [32] and enhanced protein binding to this upstream region [33].

Similarly in the rabbit, neither CYP2E1 nor 2E2 RNAs were detectable in fetal livers or lungs [34]. However, these investigators used small oligonucleotide probes to screen for CYP2E expression that can distinguish between the different isozymic forms of rabbit CYP2E but may not be sensitive enough to detect low levels of fetal RNA expression. A recent study examining the expression of CYP1A1 in fetal rats has demonstrated that larger, full-length probes may be needed to detect P450 RNAs during gestational development [35]. Interestingly, the two members of the CYP2E gene family appeared to be differentially regulated in rabbit tissues. Such does not appear to be the case for the two members of the hamster CYP2E gene family (Figs. 1A and 2A).

In the hamster, as in the rat, hybridization with a rat CYP2B1 probe yields a single RNA species. The 2.3 to 2.4 kb band detected in fetal hamster tissues in this study agrees well with the 1.8 kb band

previously reported in fetal rat livers [36]. As was the case with the CYP2E genes, studies in other rodent species have reported either very low or no detectable expression of CYP2B RNAs during gestation [22, 36]. However, recent studies employing the sensitive polymerase chain reaction have demonstrated the expression of constitutive and phenobarbital-inducible CYP2B1 and B2 RNAs as early as day 15 of gestation in rats [37], thus demonstrating that, in fetal tissues, regulatory gene mechanisms are already in place that allow induction of fetal CYP2B isozymes in response to xenobiotics.

In summary, these studies clearly show the age-, pregnancy-, and tissue-specific regulation of RNAs from the CYP2E and CYP2B subfamilies in adult and fetal hamsters. The hamsters, like other rodent species, appear to regulate expression of ethanol-inducible P450 genes through a combination of transcriptional and post-transcriptional processes. The lack of induction of either RNA species following ethanol treatment clearly implicates post-transcriptional regulatory pathways in the modulation of P450 protein levels in response to inducing agents. These results contradict studies in the hamster suggesting that ethanol pretreatment results in an increase in translatable mRNAs [38]. Using the same hamster strain and treatment protocol as this study, the authors showed that treatment with 10% ethanol in the drinking water for 10 days results in a 2.6-fold increase in translatable RNAs as measured by an *in vitro* translation assay. However, since this group did not measure RNA levels directly by Northern or slot blot analysis, it is possible that the amount of total RNA remained relatively constant but the percentage of translatable mRNA species increased upon ethanol treatment. Further studies comparing the levels of poly $(A)^+$ and poly $(A)^-$ CYP2E RNAs in the hamsters will be needed to resolve this issue.

Unlike the rat, but similar to the rabbit, there are two CYP2E RNA species that hybridize to the rat CYP2E1 probe. Whereas these are differentially regulated in the rabbit, they appear to be coordinately regulated in the hamster. Whether these two transcripts are differentially spliced transcripts from the same gene or represent separate gene transcripts remains to be determined. The hamster CYP2E gene thus exhibits many similarities and some interesting differences with the CYP2E genes of other rodent species.

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