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Expression of Cytochrome P4502E1 in Rat Fetal Hepatocyte Culture

DEFENG WU and ARTHUR I. CEDERBAUM

Department of Biochemistry, Mount Sinai School of Medicine, City University of New York, New York, New York 10029

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SUMMARY

Cytochrome P450 (CYP) 2E1 is present at very low levels or cannot be detected in rat fetal liver. Experiments were carried out to develop an *ex vivo* model of CYP2E1 expression in fetal liver. Fetal hepatocytes were prepared from pregnant rats on gestation days ranging from 12 to 19 and placed into culture for 2 days. Expression of CYP2E1 was observed at all gestational periods as evident from immunoblots and oxidation of *para*-nitrophenol and *N*,*N*-dimethylnitrosamine by fetal liver microsomes. Northern blot analysis indicated production of CYP2E1 mRNA by the fetal hepatocytes cultured for 2 days but not by freshly isolated fetal rat hepatocytes. The addition of ethanol to the hepatocyte cultures did not have a significant effect on CYP2E1 catalytic oxidation of substrates or CYP2E1 mRNA levels. The content of CYP2E1, CYP2E1 mRNA levels, and CYP2E1 catalytic activity was greater in the fetal cultures grown in the presence of 2.5% fetal calf serum than in that grown with 15% fetal calf serum, suggesting that factors present in the serum limit expression or stability of CYP2E1. CYP2E1 was not detectable in two human fetal livers; however, expression did occur when human fetal hepatocytes were placed into culture for 4 days. These results suggest that cultures of rat and human fetal hepatocytes may be a valuable model with which to study factors that regulate expression of CYP2E1 and the influence of ethanol and other inducers on expression and stabilization of CYP2E1.

CYP2E1 activates a variety of compounds that are of toxicological significance (1–3). Induction of the enzyme can occur through several different mechanisms (2, 4). CYP2E1 mRNA or protein was not detectable in rat fetal liver (5–10). Activation of the CYP2E1 gene in the liver occurs within hr after birth and seems to be accompanied by demethylation of cytosine residues located at the 5′ end of the gene (7). Once activated, CYP2E1 mRNA levels remain relatively constant or slowly increase. Increases in CYP2E1 mRNA levels are associated with elevations in the content of CYP2E1 protein and catalytic activity with effective 2E1 substrates such as aniline, DMN, and PNP (5, 7, 11–13).

Hong *et al.* (8) reported that transplacental induction of CYP2E1 by acetone occurred when the acetone was administered on the 19th and 20th days of gestation. However, Casazza *et al.* (14) found that treating pregnant rats with drinking water containing 1% acetone did not result in a premature induction of CYP2E1 activity in fetuses killed on the 20th day of gestation. Feeding pregnant rats a liquid diet containing ethanol did not result in production of CYP2E1 mRNA, protein, or catalytic activity in fetal liver, suggesting that transplacental induction of CYP2E1 by ethanol did not occur (15). An important finding by Casazza *et al.* (14) was that CYP2E1 mRNA, protein, and activity were lower in livers of the pregnant rats than in livers of control female rats, indicating pretranslational suppression of the CYP2E1 gene during pregnancy by some unknown factor or factors. It is possible that this pretranslational suppression of the CYP2E1 gene during pregnancy and the inability of fetal liver to express detectable amounts of CYP2E1 are due to similar mechanisms or factors. In view of these findings, and the rapid rise in CYP2E1 gene transcription shortly after birth, the current study was carried out to evaluate whether fetal rat hepatocytes would express CYP2E1 when placed into culture and thus no longer be under the influence of the mechanisms or factors responsible for suppression of the CYP2E1 gene in the pregnant mother or the fetal liver. Indeed, fetal rat hepatocytes isolated even relatively early during the gestational period that did not express CYP2E1 when initially isolated were found to be capable of expressing CYP2E1 when placed into culture for 2 days.

Materials and Methods

Sprague-Dawley rats at 12–19 days' gestation (Taconic Farm, German Town, NY) or human fetal liver at 18–19 weeks' gestation (Liver Transplant Division, Mount Sinai School of Medicine, New York, NY) were used to isolate fetal hepatocytes through a collagenase (Worthington, NJ) and trypsin digestion method. Most experi-

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ABBREVIATIONS: PNP, *para*-nitrophenol; DMN, *N*,*N*-dimethylnitrosamine; PBS, phosphate-buffered saline; CYP, cytochrome P450.

ments were carried out with 18-day gestation rats. Briefly, the fetal liver was isolated and cleaned from other tissues. Livers were cut to pieces of ~1 mm^3 and digested in a solution consisting of 3 mg/ml collagenase, 0.075% trypsin, and 1 mM $CaCl_2$ at 37° for 15 min with stirring. The digested solution was passed through four layers of gauze to filter out undigested tissue. Hepatocytes were collected from the filtered solution through centrifugation at 500 × *g* for 5 min. Hepatocytes (6×10^6 cells/100-mm disk) were seeded onto matrigel-coated disks (Collaborative Research, Waltham, MA) (16, 17). Rat fetal hepatocytes were cultured in Williams' E medium supplemented with an antibiotic mixture of 1% penicillin/streptomycin/fungizone (v/v/v); a trace element mixture containing 10^{-8} M $CuSO_4$, 10^{-6} M $ZnSO_4$, and 10^{-8} M H_2SeO_3; 2 mM glutamine; 5 μg/ml insulin; 50 ng/ml epidermal growth factor; 0.4 μg/ml dexamethasone; 1 μg/ml glucagon; and 10 μg/ml linoleic acid/albumin complex. The medium contained 2.5% fetal calf serum for most experiments; some comparisons were made with 15% fetal calf serum. The cells were cultured for 48 hr with or without various concentrations of ethanol in the medium. The medium was changed every 24 hr. A typical monolayer of rat fetal hepatocytes, prepared after the 15th day of gestation and cultured for 2 days, is shown in Fig. 1A.

Human fetal hepatocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml glucagon, 50 ng/ml epidermal growth factor, 3.5×10^{-6} M hydrocortisone, 6.5 ng/ml somatotropin, 5 ng/ml transferrin, 0.5 μg/ml linoleic acid complexed to 1 mg/ml bovine serum albumin, 1×10^{-7} M $CuSO_4 \cdot 5H_2O$, 5×10^{-10} M $ZnSO_4 \cdot 7H_2O$, and 3×10^{-8} M SeO_2 (18–20). The cells were usually cultured for 4 days; a typical monolayer of human fetal hepatocytes, prepared after the 18th week of gestation, is shown in Fig. 1B.

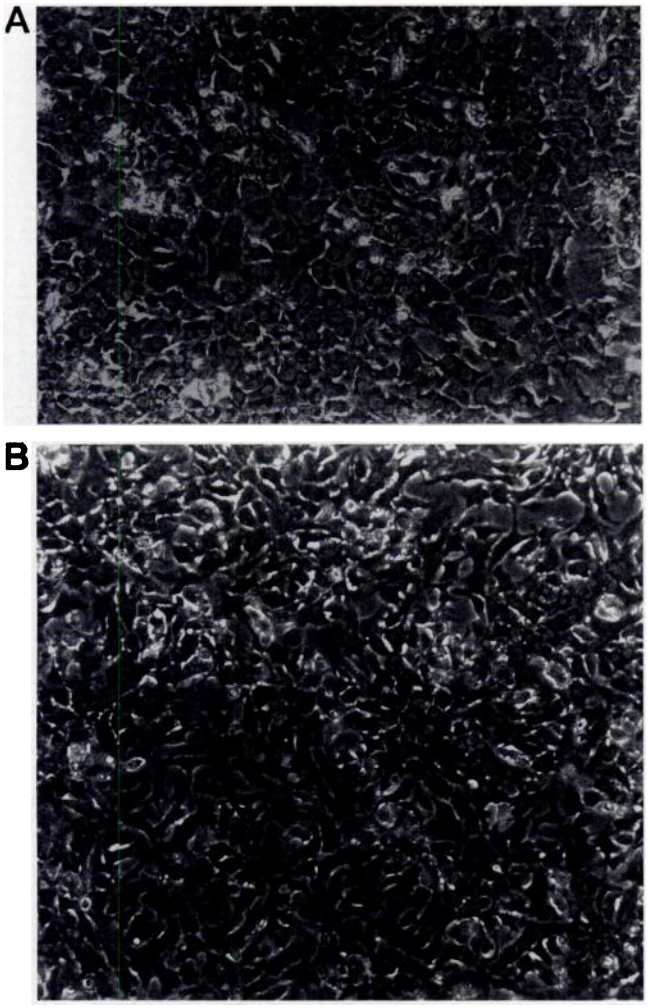

Fig. 1. A, Monolayer of rat fetal hepatocytes prepared on the 15th day of gestation and cultured for 2 days as described in Materials and Methods (10 × 10). B, Monolayer of human fetal hepatocytes (18th week of gestation) cultured for 4 days in RPMI 1640 medium as described in Materials and Methods (10 × 10).

Microsomes were isolated through differential centrifugation after the rat or human fetal cells were first disrupted with sonication. Microsomal PNP hydroxylase activity was determined in a 100-μl reaction system containing 0.1 M PBS buffer, pH 7.2, 200 μg of microsomal protein, 0.2 mM PNP, and 1 mM NADPH. Reactions were initiated with NADPH and were carried out for 1 hr at 37° in a shaking water bath. The reaction was terminated by the addition of 30 μl of 30% (w/v) trichloroacetic acid. After the precipitated protein was spun down, the supernatant was collected and incubated with 10 μl of 10 N NaOH. PNP hydroxylase activity was determined by measuring the absorbance at 546 nm (21). DMN demethylase activity was determined in a 130-μl reaction system containing 0.1 M PBS buffer, pH 7.2, 4 mM DMN, 200 μg of microsomal protein, and 1 mM NADPH. The system was allowed to react for 1 hr at 37° in a shaking water bath and was stopped by the addition of 30 μl of 30% trichloroacetic acid. After removal of precipitated protein by centrifugation, the supernatant was mixed with 150 μl Nash reagent and incubated at 60° for 15 min. The amount of formaldehyde produced was determined from the absorbance at 412 nm (22). Immunoblots were carried out as described previously (11, 15) with 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and loading 20 μg/lane fetal microsomal protein or 10 μg/lane adult microsomal protein. The CYP2E1 antibody was raised in rabbits with CYP2E1 purified from hamster liver (kindly provided by Dr. J. Lasker, Department of Biochemistry, Mount Sinai School of Medicine, New York, NY) and used at a concentration of 2.5 μg of IgG protein/ml. Goat anti-rabbit IgG conjugated with horseradish peroxidase (1:3000 dilution) was used as the second antibody. After incubation for 1 hr and several washes with PBS containing 0.5% Tween 20, the membranes were incubated with chemiluminescence reagent (Amersham, Life Sciences, Buckinghamshire, UK) for 1 min and exposed to Kodak X-OMAT X-ray film (Eastman Kodak, Rochester, NY) for 10–20 sec. The CYP2E1 content was determined with Photoshop and ImageQuant computer software. Northern blotting was carried out as described previously (11, 15). Total fetal hepatocyte RNA was isolated according to a guanidinium thiocyanate/phenol/chloroform extraction procedure (23), and 15 μg of total RNA was electrophoresed in a 1.2% agarose/formaldehyde gel. The RNA was immobilized onto nitrocellulose membranes by being baked at 80° in a vacuum oven for 2 hr, and the membranes were incubated with 6× standard saline citrate (1× = 150 mM sodium chloride and 17 mM sodium citrate) prehybridization buffer containing 50 μg/ml salmon sperm DNA at 68° with a CYP2E1 oligonucleotide probe that was labeled at the 5′ end with [γ-^{32}P]ATP and T4 polynucleotide kinase (24). The oligonucleotide probe corresponded to the DNA coding for the first 19 amino acids of CYP2E1 (25), as determined from the sequence (nucleotides 16–72) reported by Song *et al.* (5). The sequence of the probe was 5′-TATGACGAGGAGGGTGGCCACCCACACCAGCAAGGCAATGGTGATGCCAAGAACCGC, 57 mer. After several washings, the membranes were exposed to X-OMAT AR Kodak diagnostic X-ray film at −70° for 5 days and analyzed with the Photoshop and ImageQuant computer software. After analysis, the membranes were placed in a boiling water bath for 10 min, cooled, and rinsed to strip off the CYP2E1 oligonucleotide probe. The membranes were then hybridized with the use of a 40-mer β-actin oligonucleotide probe (NEN Research Products, Boston, MA), and Northern blot analysis was carried out essentially as described above.

It was necessary to pool several fetal livers to have sufficient material to assay for CYP2E1 activity, content, and mRNA levels under various conditions. Typically, fetal livers from two mothers were combined for the isolation of sufficient hepatocytes for a complete experiment. Data are given as mean ± standard deviation and

are from three separate experiments (except for data in Table 1). Statistical analysis were carried out with Student's *t* test for unpaired data.

Results

Microsomes prepared from rat fetal liver isolated on the 19th day of gestation displayed little or no CYP2E1 catalytic activity when assayed with either PNP or DMN as substrates (Table 1), confirming the absence of CYP2E1 in fetal rat liver (5–11). Cultures were prepared from fetuses isolated on days 12, 13, 15, 17, or 19 of gestation; 6×10^6 fetal hepatocytes were seeded onto matrigel-coated dishes and incubated in hormone-supplemented medium for 2 days (Fig. 1A). At this time, microsomes or RNA was isolated, and assays were carried out to evaluate the content and activity of CYP2E1 protein or mRNA. Regardless of the day of gestation, incubation of fetal hepatocytes in culture for 2 days resulted in expression of PNP hydroxylase and DMN demethylase activities (Table 1). The gestation day did not seem to have a major effect on expression of PNP hydroxylase activity as similar ranges of activities were found for midterm gestation (12 and 13 days of gestation) and late-term gestation (17 and 19 days of gestation). The oxidation of these two substrates by liver microsomes isolated from the pregnant mother (19th day of gestation) was considerably lower than their oxidation by nonpregnant rats (Table 1), confirming results of Casazza *et al.* (14) and our previous results (15).

Immunoblots to assay for the content of CYP2E1 were in agreement with the catalytic activity assays. Freshly prepared fetal hepatocytes isolated on the 17th day of culture had a very low, barely detectable amount of CYP2E1 (Fig. 2, *lanes 5 and 6* and Table 1). Once placed into culture for 2 days, clear expression of CYP2E1 was observed (Fig. 2, *lanes 7–10*, and Table 1). Regardless of the day of gestation, fetal hepatocytes expressed CYP2E1 when cultured for 2 days.

The effect of ethanol on the expression of CYP2E1 by the fetal hepatocyte cultures was determined by incubating the cultures with 25–200 mM ethanol over the 2-day experimental period. Ethanol treatment did not result in an increase in the oxidation of PNP or DMN by microsomes isolated from the fetal hepatocyte cultures (Table 2).

TABLE 1

Activity and content of CYP2E1 in fetal liver microsomes

Oxidation of PNP or DMN was determined as described in Materials and Methods using microsomes isolated from livers of pregnant or nonpregnant rats, from fetal hepatocytes (19th day of gestation), or from fetal hepatocytes isolated on the indicated day of gestation and cultured for 2 days. Immunoblots to determine the content of CYP2E1 were carried out as described in Materials and Methods using microsomes from the above preparations except that the fetal hepatocytes that were not cultured were from day 17 of gestation. Data are calculated from the immunoblot shown in Fig. 2. Results are expressed as arbitrary units. Fetal calf serum was used at a concentration of 2.5%.

Preparation	Day of gestation	PNP oxidation	DMN oxidation	CYP2E1 content
		pmol/min/mg of microsomal protein		
Adult liver		1500 ± 160	1770 ± 370	
Pregnant liver		501 ± 80	432 ± 297	6221
Fetal liver	19	11 ± 2	0	9
Fetal culture	12	127		
	13	105		100
	15	94		147
	17	112	25	230
	19	74	34	183

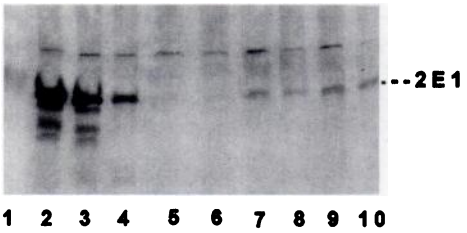

Fig. 2. Content of CYP2E1 in microsomes isolated from fetal hepatocytes. Immunoblots were carried out as described in Materials and Methods with 10 μg (*lanes 2–4*) or 20 μg (*lanes 5–10*) of microsomal protein. *Lane 1*, molecular weight standards. *Lanes 2–4*, liver microsomes from pregnant rats. *Lanes 5 and 6*, microsomes from fetal liver isolated on the 17th day of gestation. *Lanes 7–10*, microsomes from fetal liver isolated on 13th, 15th, 17th, and 19th day of gestation, respectively, and placed into culture for 2 days. The CYP2E1 band corresponded to molecular mass of 51,000–52,000 kDa.

TABLE 2

Effect of ethanol on the activity of CYP2E1 in fetal liver microsomes

Fetal hepatocytes were prepared from rats on the 18th day of gestation and either used immediately to prepare microsomes or placed into culture for 2 days in the presence or absence of the indicated concentrations of ethanol. Fetal calf serum was used at a concentration of 2.5%. Oxidation of PNP or DMN was determined as described in Materials and Methods. Results are from three experiments.

Reaction condition	PNP oxidation	DMN oxidation
	pmol/min/mg microsomal protein	
Fetal hepatocytes	3.3 ± 4.7	6.7 ± 6.2
2-day culture	70.7 ± 16.2[a]	43.3 ± 11.8[a]
+ 25 mM ethanol	59.7 ± 16.7	35.7 ± 6.2
+ 50 mM ethanol	57.7 ± 25.6	35.7 ± 6.2
+ 100 mM ethanol	56.5 ± 27.6	32.3 ± 4.7
+ 200 mM ethanol	60.0 ± 9.0	58.6 ± 17.5

[a] $p < 0.001$ compared with fetal hepatocytes.

The expression of CYP2E1 protein and catalytic activity in cultured fetal hepatocytes most likely was a result of transcription of the *CYP2E1* gene. Northern blotting to determine CYP2E1 mRNA levels was carried out with an oligonucleotide probe specific for CYP2E1 mRNA (5, 25). In freshly prepared fetal hepatocytes isolated on the 18th day of gestation, CYP2E1 mRNA was not detectable (Fig. 3, *lanes 1 and 2*, and Table 3). However, when the fetal hepatocytes were placed into culture for 2 days, CYP2E1 mRNA was expressed (Fig. 3, *lane 3*, and Table 3). The addition of 25–200 mM ethanol to the fetal hepatocyte cultures did not produce any significant change in CYP2E1 mRNA levels or the ratio of CYP2E1 to actin mRNA (Fig. 3, *lanes 5–8*, and Table 3), when three different fetal preparations were evaluated.

The concentration of fetal calf serum in the tissue culture medium was found to strongly influence the content of CYP2E1. Fetal hepatocytes from day 18 of gestation were incubated for 2 days with either 2.5% or 15% fetal calf serum. The oxidation of PNP or DMN by microsomes isolated from the fetal hepatocytes incubated with 2.5% fetal calf serum was 2–3-fold higher than activity by microsomes prepared from fetal hepatocytes incubated with 15% fetal calf serum (Table 4). Similarly, the content of CYP2E1 was ~4-fold higher after incubation with 2.5% fetal calf serum compared with the use of 15% fetal calf serum (Table 4 and Fig. 4). CYP2E1 mRNA levels and the CYP2E1/actin mRNA ratio was ~2.5–3-fold higher after incubation with 2.5% fetal calf serum compared with the use of 15% fetal calf serum (Fig. 3, *lanes 3 and 4*, and Table 4).

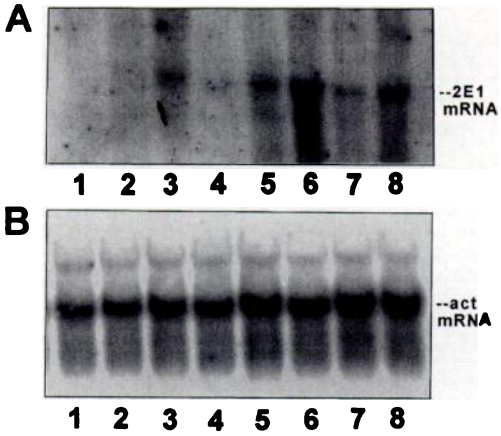

Fig. 3. A, Northern blot analysis to determine the content of CYP2E1 mRNA levels in fetal hepatocytes. Total RNA (15 μg) was electrophoresed as described in Materials and Methods. Fetal hepatocytes were prepared on the 18th day of gestation. *Lanes 1 and 2*, RNA isolated from fetal hepatocytes. *Lane 3*, fetal hepatocytes cultured for 2 days with 2.5% serum. *Lane 4*, fetal hepatocytes cultured for 2 days with 15% serum. *Lanes 5–8*, fetal hepatocytes cultured for 2 days with 2.5% serum and 25, 50, 100, or 200 mM ethanol, respectively. The size of the CYP2E1 mRNA band corresponded to 1.8 kb. In this preparation, some increase in CYP2E1 mRNA was observed at the ethanol concentration of 50 mM;, however, such increases were not found in two other experiments (see Table 3). B, Northern blot analysis to determine the content of actin mRNA levels in fetal hepatocytes. Conditions and lanes are identical to those in A, as the same membrane used to blot for CYP2E1 mRNA was used to blot for actin mRNA.

TABLE 3

CYP2E1 mRNA levels in fetal liver

Fetal hepatocytes were prepared from rats on the 18th day of gestation and assayed directly or placed into culture for 2 days in the presence of 2.5% serum. Cultures were carried out in the absence or presence of the indicated concentrations of ethanol. Northern blot analysis was carried out using 15 μg of RNA. Results are expressed either as arbitrary units or as the ratio of CYP2E1/actin mRNA. Results are calculated from the blot shown in Fig. 3 plus two other blots and are from three separate experiments.

Reaction condition	CYP2E1 mRNA	Actin mRNA	CYP2E1/Actin
Fetal hepatocytes	10 ± 4	94 ± 5	0.10 ± 0.02
2-day culture	100[a]	100	1.0[a]
+ 25 mM ethanol	101 ± 9	127 ± 7	0.80 ± 0.30
+ 50 mM ethanol	145 ± 19	123 ± 9	1.18 ± 0.40
+ 100 mM ethanol	80 ± 18	120 ± 12	0.67 ± 0.20
+ 200 mM ethanol	147 ± 19	118 ± 13	1.25 ± 0.30

[a] $p < 0.01$ compared with fetal hepatocytes.

Preliminary experiments were carried out with human fetal hepatocytes. Fetal hepatocytes were prepared (18th or 19th week of gestation) and cultured as described in Materials and Methods; a monolayer culture was formed after 2–4 days (Fig. 1B). There was no detectable oxidation of PNP or DMN by microsomes isolated from these two human fetal livers. However, after the fetal hepatocytes were in culture for 4 days, microsomes isolated from these cells oxidized PNP and DMN at rates of 61 ± 27 and 53 ± 22 pmol/min/mg protein, respectively.

Discussion

CYP2E1 is either absent in rat fetal liver (5–8, 11, 15) or expressed at very low levels (26, 27). Fetal hepatocytes isolated from rats on gestational days ranging from 13 to 19 did not display detectable or significant levels of CYP2E1 mRNA, protein, or catalytic activity. However, when these hepatocytes were placed into culture for 2 days, expression of CYP2E1 was initiated, as demonstrated by the formation of CYP2E1 mRNA and protein and oxidation of substrates such as PNP and DMN. Essentially similar levels of expression were found regardless of the gestational time in which the fetal hepatocytes were isolated and subsequently cultured, suggesting that the *CYP2E1* gene can become activated almost immediately on release of the fetal liver from regulation by factors present in the maternal environment. Experiments are planned to evaluate whether activation of the *CYP2E1* gene in this culture model reflects demethylation of specific cytosine residues at the 5′ end, analogous to what occurs soon after birth (7).

The ability of ethanol to induce CYP2E1 has been of long-standing interest because CYP2E1 may play a role in the mechanisms by which ethanol is hepatotoxic (28–30). Earlier studies suggested that the mechanism by which ethanol induced CYP2E1 was at the level of protein stabilization because ethanol did not increase CYP2E1 mRNA levels under conditions in which CYP2E1 protein or activity was elevated (5, 9, 31). More recent reports indicated increases in CYP2E1 mRNA levels after chronic ethanol consumption by rats (32–34). In the fetal hepatocyte cultures, 25–200 mM ethanol had no significant effect on the CYP2E1 catalytic activity or CYP2E1 mRNA levels. This suggests regulation of CYP2E1

TABLE 4

Comparison of the effect of 2.5 and 15% fetal calf serum on induction of fetal CYP2E1

Rat fetal hepatocytes were prepared on the 18th day of gestation and cultured for 2 days in medium containing either 2.5 or 15% fetal calf serum. Activity refers to pmol per min per mg microsomal protein for PNP and DMN oxidation and to arbitrary units for CYP2E1 content and mRNA levels. Results are from three separate experiments.

Reaction	Activity	
	2.5% serum	15% serum
PNP oxidation	70.7 ± 16.2	23.0 ± 16.1[a]
DMN oxidation	43.3 ± 11.8	21.0 ± 11.3[b]
CYP2E1 content	24 ± 6	6 ± 2[c]
CYP2E1 mRNA	100	32 ± 14[b]
CYP2E1/actin	1.0	0.36 ± 0.3[b]

[a] $p < 0.02$.
[b] $p < 0.05$.
[c] $p < 0.01$.

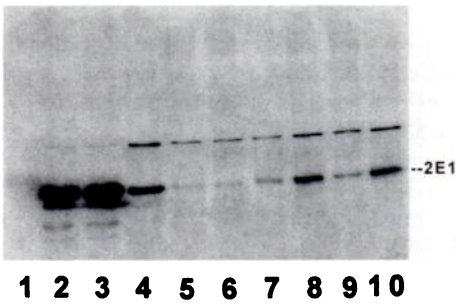

Fig. 4. Effect of serum on the expression of CYP2E1 in fetal liver cultures. Immunoblots were carried out as described in Materials and Methods with 10 μg (*lanes 2 and 3*) or 20 μg (*lanes 4–10*) of microsomal protein. *Lane 1*, molecular mass standards. *Lanes 2 and 3*, liver microsomes from pregnant rats. *Lanes 4, 8, and 10*, microsomes from three separate fetal livers cultured with 2.5% serum for 2 days. *Lanes 5, 7, and 9*, microsomes from three separate fetal livers cultured with 15% serum for 2 days. *Lane 6*, microsomes from a fetal liver isolated on the 18th day of gestation.

largely or solely by transcriptional rather than post-transcriptional events in this model.

In view of the apparent derepression of the *CYP2E1* gene when fetal hepatocytes were placed into culture, the question was raised whether factors present in serum limited expression of the *CYP2E1* gene. We therefore compared the effectiveness of low (2.5%) versus high (15%) levels of fetal calf serum in the culture medium in supporting expression of CYP2E1 in fetal hepatocytes isolated on the 18th day of gestation. Oxidation of PNP and DMN, content of CYP2E1, and CYP2E1 mRNA levels were higher in preparations isolated from fetal hepatocytes cultured in the low serum medium. The nature of the factors present in the fetal calf serum that seem to repress CYP2E1 expression and whether similar types of factors may be present in serum from pregnant rats remain to be determined.

As summarized in a recent review (26), human fetal tissues can metabolize xenobiotics as well as endogenous substrates (35–39). Some substrates metabolized by human fetal preparations are effective substrates for CYP2E1, e.g., acetaminophen bioactivation, aniline hydroxylation, and DMN demethylation (35, 36). The absence of detectable CYP2E1 in human fetal liver has been reported (39–41). Although CYP2E1 mRNA transcripts of 3.8, 1.9, and 1.7 Kb were detected in adult human livers after hybridization with a full-length CYP2E1 cDNA, no transcripts were detected in human fetal livers (40). Substantial methylation at the 3′ region of the *CYP2E1* gene was found in both adult and fetal liver samples; two small DNA fragments at the 5′ end of the *CYP2E1* gene were found after cleavage with the restriction enzyme *HPa*II in adult liver but not fetal liver, leading to the suggestion that methylation of specific 5′ residues in the *CYP2E1* gene seemed to be responsible for the lack of transcription of the *CYP2E1* gene in human fetal liver (40). We could not detect CYP2E1 in two human fetal preparations (18th and 19th weeks of gestation) as assessed with assays of catalytic activity with PNP or DMN. However, analogous to results with rat fetal hepatocytes, CYP2E1 was expressed when the human fetal hepatocytes were placed into culture. Therefore, human fetal hepatocyte cultures may represent a valuable model for studies evaluating the regulation of expression and stabilization of human CYP2E1 and the influence of ethanol on these reactions.

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Send reprint requests to: Dr. Arthur I. Cederbaum, Department of Biochemistry, Box 1020, Mount Sinai School of Medicine, New York, NY 10029. E-mail: acederb@smtplink.mssm.edu