ABSTRACT:

Cytochrome P450 2E1 (CYP2E1) and 2B (CYP2B) mRNA and protein expression was examined in primary cultured rat hepatocytes under basal cell culture conditions and in response to three prototypic CYP2E1 inducers, i.e. ethanol, acetone, and pyrazine. Xenobiotic treatment for 24 hr, initiated after hepatocytes had been maintained in culture for 72 hr, resulted in 2–6-fold increases in CYP2E1 protein levels, relative to untreated cells. A ≥2-fold increase in CYP2E1 protein levels was detected at the lowest concentration (1 mM) of each of the xenobiotics examined. The increase in CYP2E1 protein expression was not accompanied by any significant increase in 2E1 mRNA expression. In contrast, CYP2B protein and mRNA levels were increased by acetone or pyrazine at concentrations greater than 1 mM. Ethanol (up to 100 mM) failed to significantly increase CYP2B protein or mRNA levels. The maximal increases measured for CYP2B protein and mRNA (∼25-fold and −90-fold, respectively) after treatment of hepatocytes with acetone were comparable to those measured in our laboratory, and reported by others, for phenobarbital treatment of primary cultured rat hepatocytes. The results of this study show that the pattern of expression of CYP2E1 and 2B in this primary cultured rat hepatocyte system and the magnitude of induction parallel those reported for rat liver in vivo in response to these xenobiotics. This primary hepatocyte culture system provides opportunities for studies of the role of CYP2E1 in the metabolism and bioactivation of drugs, chemicals, and putative carcinogens, as well as mechanistic studies on xenobiotropic-mediated regulation of CYP2E1 expression.

CYP2E1 is a major catalyst in the bioactivation of a number of procarcinogens, including N-nitrosodimethylamine (Levin et al., 1986; Yamazaki et al., 1992), N-nitrosodiethylamine (Yamazaki et al., 1992), benzene (Johansson and Ingelman-Sundberg, 1988; Guengerich et al., 1991), vinyl chloride, trichloroethylene, and acrylonitrile (Guengerich et al., 1991). CYP2E1 has also been shown to be involved in the metabolism of a number of low-molecular weight solvents and drugs, including pyridine (Kim et al., 1988), EtOH (Ekstrom et al., 1989), carbon tetrachloride (Ekstrom et al., 1989; Johansson and Ingelman-Sundberg, 1985), and acetaminophen (Rauch et al., 1989; Snawder et al., 1994; Lee et al., 1996). A recent report (Chen et al., 1997) has suggested a role for CYP2E1 in apoptosis and cell toxicity via lipid peroxidation resulting from CYP2E1-mediated arachidonic acid metabolism. CYP2E1 levels are elevated in response to xenobiotics, including EtOH, pyridine, and ACE, or in response to altered physiological states, such as fasting and diabetes, which results in increased toxicity after exposure to compounds bioactivated by this P450 (Johansson and Ingelman-Sundberg, 1985; Evarts et al., 1983; Watkins et al., 1988).

Treatment of animals with a variety of xenobiotics, including pyridine, EtOH, ACE, and PYZ, results in increases in hepatic CYP2E1 protein levels of 2–8-fold, in the absence of a concomitant increase in CYP2E1 mRNA levels, suggesting that posttranscriptional mechanisms are involved in the regulation of this P450 (Kim et al., 1988, 1993; Kim and Novak, 1993; Johansson et al., 1988). Mechanistic studies conducted in vivo have provided evidence for increased de novo synthesis of CYP2E1 protein, implicating increased translational efficiency (Kim and Novak, 1990; Kim et al., 1990; Tsutsumi et al., 1993), whereas other reports have suggested that stabilization of CYP2E1 protein is responsible for the xenobiotropic-mediated increase in CYP2E1 protein (Song et al., 1989; Roberts et al., 1995). Thus, the results from in vivo mechanistic studies suggest that multiple pathways may be active in the regulation of CYP2E1 protein expression in response to xenobiotics.

Several of these CYP2E1-inducing agents (EtOH, ACE, and pyridine) have also been shown to enhance CYP2B expression at both the mRNA and protein levels in vivo (Kim et al., 1993; Johansson et al., 1988; Louis et al., 1994). In contrast, PYZ treatment of rats, at doses that enhanced CYP2E1 protein levels, failed to increase CYP2B protein levels (Japenga et al., 1993).

The limited ability to maintain or enhance CYP2E1 expression in primary cultured hepatocytes has restricted the scope of mechanistic studies examining the regulation of hepatic 2E1 expression. Previous reports demonstrated that xenobiotropic treatment of primary cultured rat hepatocytes could result in increased levels of CYP2E1 protein, relative to untreated cells, but those studies used hepatocytes prepared from animals treated with CYP2E1 inducers in vivo before culture (Hunt et al., 1991; Eliasson et al., 1988) or involved xenobiotropic
treatment of hepatocytes from the initiation of culture (Hunt et al., 1991; Eliasson et al., 1988; Perrot et al., 1991), eliminating the minimal 48-hr equilibration period. CYP2E1 protein expression has been reported to be increased in primary cultured rat hepatocytes by 48-hr treatment with 200 mM EtOH (Sinclair et al., 1991), a concentration that is substantially greater than that which occurs in vivo.

Kocarek et al. (1993) reported that ciprofibrate treatment of primary rat hepatocytes cultured on Matrigel enhanced the expression of CYP2E1 mRNA, and Waxman et al. (1990) demonstrated that primary hepatocytes cultured on Vitrogen in serum-free, modified Chee’s medium displayed substantial levels of induction (>50-fold) of CYP2B1/2 after PB treatment. Our laboratory subsequently examined the effect of ciprofibrate and pyridine on the expression of CYP2E1 mRNA and protein in primary cultured rat hepatocytes by using modified Chee’s medium and Vitrogen substratum (Zangar et al., 1995). That study demonstrated that ciprofibrate treatment elevated CYP2E1 mRNA and protein levels, whereas pyridine increased 2E1 protein, but not mRNA, expression relative to untreated cells.

Questions remain, however, regarding the response of this system to other CYP2E1 inducers, as well as the effects on CYP2B. Thus, the objective of the present study was to examine the enhancement of CYP2E1 and CYP2B expression in this primary rat hepatocyte culture system by agents known to increase 2E1 and 2B expression in vivo, to determine whether these agents produce changes that correspond to those reported in vivo. In this report, we demonstrate that treatment of primary cultured rat hepatocytes with known xenobiotic inducers of CYP2E1 elevated CYP2E1 protein expression by 2–8-fold, with no corresponding increase in 2E1 mRNA levels. This magnitude of induction of CYP2E1 protein by these xenobiotics is comparable to that previously reported in vivo. CYP2B expression was enhanced at both the mRNA and protein levels by ACE and PYZ, but not by EtOH. Moreover, CYP2E1 protein levels were increased at lower xenobiotic concentrations than those required to increase CYP2B protein levels. The changes in CYP2E1 mRNA and protein in response to the xenobiotics in this study are mechanistically comparable to those previously reported in hepatic tissue in vivo after treatment with these chemicals.

Materials and Methods

Materials. Modified Chee’s medium, gentamicin, L-glutamine, and tricine were obtained from GIBCO/BRL (Gaithersburg, MD). Insulin (NovolinR) was purchased from Novo-Nordisk (Princeton, NJ). Collagenase (type I) was purchased from Worthington Biochemicals (Freehold, NJ). Vitrogen (95–98% type I collagen/2–5% type III collagen) was obtained from Collagen Corp. (Palo Alto, CA). Tissue culture flasks (Corning) were purchased from Baxter (McGaw Park, IL). Anti-rat CYP2B1 was purchased from Human Biologics (Phoenix, AZ), horse radish peroxidase-conjugated goat anti-rabbit antibody was obtained from Bio-Rad (Melville, NY), and horseradish peroxidase-conjugated rabbit anti-goat antibody was from Jackson Laboratories (West Grove, PA). Enhanced chemiluminescence reagents were purchased from Amersham (Arlington Heights, IL). PB and ACE (HPLC grade) were obtained from Mallinckrodt (Chesterfield, MO), and PYZ was purchased from Aldrich (Milwaukee, WI). EtOH (HPLC grade) and all other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Primary Rat Hepatocyte Cultures. Hepatocytes were isolated from the livers of male Sprague-Dawley rats (200–300 g) by using collagenase perfusion, as described previously (Zangar et al., 1995; Seglen, 1982). Hepatocytes were plated onto flasks that had been covalently coated with Vitrogen as described previously (Waxman et al., 1990; Zangar et al., 1995). Cells were plated at densities of 12 × 10^6 cells/75-cm^2 flask or 4 × 10^6 cells/25-cm^2 flask. Modified Chee’s medium was fortified as described (Waxman et al., 1990), except that it was supplemented with 0.7 μM insulin and 0.1 μM dexamethasone. Medium was changed 4 hr after plating and every 24 hr thereafter.

Xenobiotic treatment was initiated after the cells had been maintained in culture for 72 hr, and treatment duration was 24 hr. Treatment duration was selected based on preliminary studies, which resulted in optimal xenobioto-mediated increases in CYP2E1 and 2B protein expression after 24-hr treatment.

PYZ was prepared as a 6 M stock solution in sterile water. EtOH and ACE were added directly to the medium. Because these treatments involved volatile chemicals, the tissue culture flasks were sealed to prevent loss of the chemicals. Thus, after medium change, loosely capped flasks were returned to the incubator for 1–2 hr, to allow equilibration of medium with the incubator chamber atmosphere. Chemicals were then added, and flasks were sealed. In several experiments, some untreated flasks were left open during the treatment period, and these hepatocytes expressed CYP2E1 and 2B mRNA and protein at the same levels as did the untreated flasks with closed lids (data not shown), thus indicating that closing the flask lids did not affect CYP2E1 or 2B expression. Lactate dehydrogenase levels in the medium were determined as described by Wroblewski and LaDue (1955).

Immunoblot Analysis. Hepatocyte microsomes were prepared essentially as described previously (Zangar et al., 1995). Cells were washed twice with 5 ml of 4°C phosphate-buffered saline (pH 7.4), suspended in 6 ml of phosphate-buffered saline/flask (using three flasks for each treatment), and pelleted by centrifugation at 200g for 3 min. To minimize the possibility of proteolytic degradation of CYP2E1 protein during isolation, cells were suspended in 1 ml of buffer containing protease inhibitors [20 mM Tris-HCl, pH 7.4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 5 μg/ml pepstatin A, 50 mM spermine, 50 mM spermidine, 250 mM sucrose, 10 mM β-mercaptoethanol, 2 mM EDTA, and 2 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid] and were repelleted. Cells were then suspended in 250 μl of this buffer, homogenized for 30 sec using an Omni 1000 homogenizer (Waterbury, CT) set on high, and centrifuged for 10 min at 10,000g at 4°C. The resulting supernatant was centrifuged for 90 min at 105,000g at 4°C. The pellet from this step was washed with 1 ml of microsome storage buffer (50 mM Tris-acetate, 20% glycerol, 1 mM EDTA, pH 7.4) and then suspended in 100 μl of this buffer very gently sonicated with a probe sonicator. Microsomal proteins (20 μg/ lane) were separated on 10% polyacrylamide gels and transferred as described previously (Laemmli, 1970).

CYP2E1 antibody was prepared as described previously (Kim et al., 1991). Detection of the bound primary antibodies was performed using horseradish peroxidase-conjugated secondary antibody, followed by chemiluminescence detection. Densitometry was performed using a Molecular Dynamics (Sunnyvale, CA) laser densitometer and the ImageQuant analysis program.

Northern Blot Analysis. Total hepatocyte RNA was prepared, separated (10 μg/lane) on formaldehyde-agarose gels, and capillary blotted as described (Xie and Rothblum, 1991; Sambrook et al., 1989). A CYP2E1 cDNA probe was prepared as previously described (Zangar et al., 1995). A full-length rat CYP2B1 cDNA (Doehner et al., 1988) was generously provided by Dr. Milton Adensnick (New York University Medical Center, New York, NY), and the cytoplasmatic 7S cDNA (Balmain et al., 1982) was generously provided by Dr. Allan Balmain (Beatson Institute for Cancer Research, Glasgow, UK). The cDNAs were labeled using a random primer kit (GIBCO/BRL), according to manufacturer’s instructions.

Membranes were prehybridized, incubated with labeled probe (final activity, 10^6 cpm/ml), and washed as previously described (Zangar et al., 1995). The blots were then exposed to X-ray film (Kodak XAR) for 2 hr to 4 days, and band volume was determined using a Molecular Dynamics laser densitometer and the ImageQuant analysis program.

Statistical Analysis. Significant differences between groups were determined using the Tukey-Kramer test, with p < 0.05 indicating significance.

Results

A prerequisite before initiation of experiments on xenobioto-mediated expression of CYP2E1 and 2B in primary cultured hepatocytes was determination of CYP2E1 and 2B protein and mRNA levels with time in culture after plating. CYP2E1 protein levels in primary cultured rat hepatocytes declined to approximately 89, 54, and 2% of the level detected in freshly isolated cells after 24, 48, and 72 hr in culture, respectively (fig. 1A). These changes are comparable to those
reported by others for primary cultured hepatocytes obtained from female rats (Hunt et al., 1991). In contrast, CYP2B protein levels declined to approximately 46, 12, and 1% of the levels in freshly isolated cells at the same time points (fig. 1A). Thus, CYP2B protein levels declined at a more rapid rate than did CYP2E1 protein levels, with half-lives of ~24 hr for CYP2B and ~50 hr for CYP2E1. Both CYP2E1 and 2B protein levels exhibited patterns of continuous decline until 72 hr in culture, by which time they both reached a steady-state level of ~1–2% of that in freshly isolated cells.

The expression of CYP2E1 and 2B mRNA over time in culture was also determined. CYP2E1 mRNA levels declined to ~33% of the level in freshly isolated hepatocytes after 24 hr in culture and reached a stable level of expression, at ~3–4% of that in freshly isolated cells, by 72 hr in culture (fig. 1B). This parallels the findings reported by other investigators, using various substrata and media, for the decline in CYP2E1 mRNA levels in hepatocytes over time in culture (Hunt et al., 1991; Eliasson et al., 1988; Kocarek et al., 1993). In contrast, CYP2B mRNA levels declined to ~2% of the level in freshly isolated cells after 24 hr in culture, followed by a subsequent increase to a plateau level, which at 72 hr was approximately 25% of that in freshly isolated hepatocytes (fig. 1B). This pattern of CYP2B expression is also comparable to that reported by other investigators (Kocarek et al., 1993). Thus, a critical difference between CYP2E1 and CYP2B expression in primary cultured rat hepatocytes is the rebound in CYP2B mRNA that occurs after 24 hr in culture. In contrast, CYP2E1 mRNA levels continue to decline over the initial ~48 hr and reach a steady-state level at 48–72 hr after plating (fig. 1A).

Thus, clearly different expression patterns emerge for CYP2E1 and 2B mRNA and protein levels after the plating of hepatocytes. CYP2E1 mRNA levels declined rapidly over the first 48 hr in culture, whereas CYP2E1 protein levels declined at a slower rate over the initial 72 hr, with both mRNA and protein levels reaching a steady state, at a level of ~1–2% of that in freshly isolated cells, after 72 hr in culture. In contrast, whereas CYP2B mRNA levels declined precipitously over the first 24 hr, this was followed by a rebound over the next 48 hr, such that by 72 hr in culture CYP2B mRNA levels were ~25% of the level in freshly isolated hepatocytes. CYP2B protein levels, however, declined continuously over 72 hr to a steady level of ~1–2% of that in freshly isolated cells. Based on these data, we initiated all subsequent experiments at 72 hr after plating, because this represented the time point for steady-state CYP2E1 and CYP2B protein and mRNA expression.

To determine the optimal conditions for maximal enhancement of CYP2E1 and CYP2B protein expression, the concentration dependence of xenobiotic-mediated CYP2E1 and 2B expression was determined. The concentrations used (1–100 mM) were chosen based on preliminary experiments in our own laboratory (Zangar et al., 1995), as well as previous literature reports examining the maintenance of CYP2E1 protein expression in primary cultured rat hepatocytes (Hunt et al., 1991; Eliasson et al., 1988; Perrot et al., 1991).

Based on preliminary experiments examining CYP2E1 protein expression in response to these xenobiotics in the 1–100 mM concentration range, for which ~2–8-fold increases in CYP2E1 protein levels were measured, concentrations of 1 and 50 mM EtOH, ACE, and PYZ were used to examine CYP2E1 protein levels for statistically significant increases. EtOH treatment produced a concentration-dependent increase in CYP2E1 protein expression, from ~2-fold with 1 mM to ~8-fold with 50 mM (fig. 2). The enhancement of CYP2E1 protein expression in response to ACE treatment, however, was not concentration dependent, with ~4-fold increases in CYP2E1 protein levels being measured at 1 and 50 mM ACE (fig. 2). PYZ treatment resulted in an ~2-fold increase in CYP2E1 protein levels at 1 mM and an ~5-fold increase at 50 mM (fig. 2).

These results indicate that expression of CYP2E1 protein is readily enhanced in primary cultured rat hepatocytes, even at relatively low xenobiotic concentrations (i.e. 1 mM). Furthermore, the 2–8-fold increases in CYP2E1 protein levels, relative to untreated cells, measured in this study are similar to those reported for rat liver in vivo in response to these xenobiotics. Because treatment with these xenobiotics in vivo results in an increase in CYP2E1 protein expression, with no corresponding increase in CYP2E1 mRNA levels, CYP2E1 mRNA expression was measured in primary cultured rat hepatocytes in response to these agents. A continuous concentration-dependent decrease in CYP2E1 mRNA

FIG. 1. Time-dependent alterations in basal expression of CYP2E1 and CYP2B protein and mRNA in primary cultured rat hepatocytes.

CYP2E1 and CYP2B protein (A) and mRNA (B) levels are presented as a percentage of the protein or mRNA levels in freshly isolated hepatocytes (100%). mRNA and protein data represent the means of Northern blot analysis of two separate preparations of total RNA or data from Western blot analysis of a single preparation of microsomes for each time point, respectively, from a single hepatocyte culture. mRNA values were normalized for loading using 7S RNA.
mRNA levels was observed with EtOH treatment (fig. 3). In contrast, no change in CYP2E1 mRNA expression was observed with ACE (fig. 3), compared with untreated cells. Interestingly, PYZ treatment (5 and 50 mM) appeared to cause a slight (<2-fold), but not statistically significant, increase in 2E1 mRNA expression (fig. 3). These results illustrate that, with the possible exception of 50 mM PYZ, xenobiotic treatment of primary cultured rat hepatocytes does not result in an increase in CYP2E1 mRNA expression, consistent with published observations in vivo.

The effects of EtOH, ACE, or PYZ treatment on CYP2B protein and mRNA expression in primary cultured hepatocytes were also examined, because some of these agents (EtOH and ACE) have been shown to enhance CYP2B subfamily expression in vivo. EtOH treatment failed to significantly alter CYP2B protein levels at either 1 or 50 mM (fig. 4). Lower concentrations of ACE failed to enhance CYP2B protein levels, but 50 mM ACE increased CYP2B expression by ~25-fold, relative to untreated cells (fig. 4). Similarly, PYZ treatment did not increase CYP2B protein expression at 1 mM but produced an ~15-fold enhancement of CYP2B expression at 50 mM (fig. 4).

CYP2B protein data are presented as combined 2B1/2B2 values, because the two proteins were not consistently detectable as separate bands. When they were both detectable, however, the changes in 2B2 levels paralleled the changes in 2B1 levels; therefore, the two forms of CYP2B appeared to be coordinately expressed in primary cultured rat hepatocytes in this system in response to these xenobiotics.

CYP2B mRNA expression was enhanced by these xenobiotics in a manner parallel to that seen for CYP2B protein expression. EtOH treatment, at up to 100 mM, failed to increase CYP2B mRNA levels, relative to untreated cells (fig. 5). PYZ, at concentrations greater than 5 mM, resulted in increased CYP2B mRNA levels up to 10-fold greater than those of untreated cells (fig. 5). ACE, at either 50 or 100 mM, produced the greatest increases in CYP2B mRNA levels (~70–90-fold) (fig. 5) of the agents examined. The lower concentrations of ACE increased CYP2B mRNA expression to a much lesser degree (~2- and 8-fold at 1 and 10 mM, respectively) (fig. 5). These results demonstrate that CYP2B expression is altered in response to xenobiotics in this system of primary cultured rat hepatocytes in a manner parallel to that reported for other xenobiotics in vivo, with increases in both CYP2B mRNA and protein and coordinate elevation of CYP2B1/2B2 expression.

Discussion

Previous research in our laboratory (Zangar et al., 1995) demonstrated that primary rat hepatocytes cultured in Chee’s medium on Vitrogen substratum expressed detectable basal levels of CYP2E1 mRNA and protein and that pyridine treatment of hepatocytes enhanced CYP2E1 protein levels, with no concomitant increase in 2E1 mRNA levels. In the present study, we have examined the effects of prototypic agents that have been reported to enhance the expression of CYP2E1 and CYP2B in vivo, to determine whether corresponding fold increases in CYP2E1 and 2B expression are produced in this system of primary cultured rat hepatocytes, compared with results observed in vivo.

CYP2E1 and CYP2B mRNA and protein levels in primary cultured rat hepatocytes reached steady-state levels of expression at 72 hr after plating (fig. 1). CYP2E1 mRNA and protein reached levels of expression of ~2–4% of those in freshly isolated hepatocytes, as did CYP2B protein. CYP2B mRNA levels initially declined, followed by a re-
bound to a level of ~25% of that in freshly isolated hepatocytes. These results support those previously reported by other investigators for expression of CYP2E1 or CYP2B mRNA or protein in primary hepatocytes over the first few days in culture, when various substrata and media were used. Hunt et al. (1991), using modified Waymouth MB-752 medium and Matrigel substratum, reported that CYP2E1 mRNA and protein levels declined to 4 and 3% of levels in freshly isolated cells by 48 hr and 96 hr, respectively. Eliasson et al. (1988), using Waymouth medium and rat tail collagen as substratum, reported declines in CYP2E1 mRNA and protein levels to <0.5% and 2%, respectively, of the levels in freshly isolated cells after 72 hr in culture. Kocarek et al. (1993), using a modification of Waymouth MB-752 medium and Matrigel substratum, reported decreases of CYP2E1 mRNA levels to ~4% and ~2% of the levels in freshly isolated hepatocytes after 48 and 96 hr in culture, respectively. Those authors also reported a decline in CYP2B mRNA levels to ~1% of the levels in freshly isolated hepatocytes after 48 hr in culture, followed by an increase to ~22% after 96 hr in culture. These findings indicate that decreases in CYP2E1 and 2B expression in primary rat hepatocytes with time in culture are consistent among different substrata and media, although the absolute levels of these P450s appear to vary with different media.

CYP2E1 protein expression was enhanced 2–8-fold by EtOH, ACE, or PYZ treatment of primary cultured rat hepatocytes (fig. 2). These fold increases in CYP2E1 protein levels are comparable to those reported by numerous investigators for the same chemicals in rat hepatic tissue in vivo (Kim and Novak, 1993; Kim et al., 1990; Johansson et al., 1988; Tsutsumi et al., 1993; Louis et al., 1994). Increased CYP2E1 protein expression (~2-fold) was measured at the lowest xenobiotic concentration examined (1 mM), whereas maximal expression occurred at concentrations of 10 mM (data not shown) to 50 mM for all chemicals examined in this study. Similar concentrations (1–100 mM) of CYP2E1 inducers (e.g. EtOH, isopropanol, pyrazole, dimethylsulfoxide, and isoniazid) have been effectively used by numerous other investigators examining the maintenance of CYP2E1 expression in primary cultured rat hepatocytes (Hunt et al., 1991; Eliasson et al., 1988), and concentrations of 100–200 mM EtOH were previously used to enhance CYP2E1 protein levels in primary cultured rat hepatocytes (Sinclair et al., 1991; Louis et al., 1993).

The increased CYP2E1 protein expression observed in these hepatocytes occurred in the absence of any significant increase in CYP2E1 mRNA levels (fig. 3). Thus, the expression of CYP2E1 in primary cultured rat hepatocytes, maintained with Chee’s medium on Vitrogen substratum, in response to the xenobiocles examined in this study is qualitatively and quantitatively comparable to that reported for the same agents in vivo (Kim and Novak, 1993; Johansson et al., 1988; Song et al., 1989; Louis et al., 1994), with comparably enhanced CYP2E1 protein levels (~2–8-fold) occurring in the absence of corresponding increases in CYP2E1 mRNA levels.

CYP2B expression was also enhanced in primary cultured rat hepatocytes by ACE and PYZ treatment (figs. 4 and 5). In contrast to CYP2E1 expression, the increases in CYP2B protein levels were accompanied by increases in CYP2B mRNA levels. ACE treatment of primary hepatocytes increased CYP2B mRNA and protein levels up to 70-fold (fig. 5) and 25-fold (fig. 4), respectively, whereas treatment of rats with ACE in vivo has been reported to increase CYP2B mRNA and protein levels 10–40-fold (Johansson et al., 1988). Interestingly, PYZ treatment of cultured hepatocytes enhanced CYP2B mRNA and protein expression 10–15-fold (figs. 4 and 5), whereas previous in...
vivo studies found that treatment of rats with PYZ did not increase CYP2B protein levels (Japenga et al., 1993). In those in vivo studies, however, a single dose of PYZ, lower than that required for maximal enhancement of CYP2E1 protein levels in vivo, was examined for effects on CYP2B; as demonstrated in figs. 2 and 4, a higher concentration of PYZ was required for enhanced CYP2B protein expression, compared with CYP2E1 protein. Thus, the difference between our findings in cultured hepatocytes and previous in vivo findings may be related to xenobiotic concentrations. Alternatively, there may be hormonal/metabolic influences in vivo that prevent the induction of CYP2B by PYZ that are not present in cultured hepatocytes.

The primary hepatocyte culture system used in this study can be of value for distinguishing between primary effects of xenobiotics on hepatocytes and secondary effects associated with hormonal imbalances or pathophysiological conditions that may occur as a result of in vivo xenobiotic treatment. This is exemplified in the present study by the experiments on EtOH treatment of primary cultured hepatocytes, in which no increases in CYP2B protein or mRNA levels were observed (figs. 4 and 5). It was reported that administration to rats of EtOH in the Lieber-DeCarli diet for 20 days produced ~10-fold and ~3-fold increases in CYP2B protein and mRNA levels, respectively (Johansson et al., 1988). Long-term dietary administration of EtOH, however, results in increased levels of fatty acids and ketone bodies, and our laboratory has demonstrated that treatment of primary cultured rat hepatocytes with fatty acids and ketone bodies results in enhanced expression of CYP2B protein and mRNA (Zangar and Novak, 1997). Thus, increased CYP2B expression after dietary EtOH administration may be the result of secondary effects of long-term EtOH exposure, rather than a direct effect of EtOH on hepatocytes. On the other hand, 48-hr treatment of primary cultured rat hepatocytes with high concentrations of EtOH (>100 mM) has been reported to increase CYP2B protein levels to a small extent (Sinclair et al., 1991; Louis et al., 1993), and the combination treated of hepatocytes with EtOH and isopentanol has been reported to result in increases in CYP2B protein levels approaching those resulting from PB treatment of the same cells (Louis et al., 1993). Thus, the lack of induction of CYP2B by EtOH in the present study may reflect the lower EtOH concentrations and shorter duration of treatment.

The maximal increases in CYP2B mRNA and protein levels obtained in primary cultured rat hepatocytes in this study in response to inducers (25–90-fold with 50 or 100 mM ACE) are comparable to those obtained in previous studies in our laboratory with PB treatment (data not shown). Comparable levels of induction (>50-fold) of CYP2B after PB treatment of cultured hepatocytes, using the same medium and substratum as in the present study, have been reported by Waxman et al. (1990). Thus, CYP2B mRNA and protein expression is highly responsive to xenobiotic-mediated increases in hepatocytes cultured on Vitrogen substratum using Chec’s medium.

The results of this work demonstrate that xenobiotic-enhanced CYP2E1 and CYP2B expression occurs in our primary cultured rat hepatocyte system in a manner mechanistically comparable to that reported in vivo (i.e., elevation of CYP2E1 protein levels in the absence of a concomitant increase in CYP2E1 mRNA levels, and elevation of CYP2B mRNA and protein levels). In addition, the magnitude of induction of CYP2E1 protein levels in response to the chemicals used in this study is comparable to that reported in vivo and is sufficient to allow further detailed mechanistic studies of xenobiotic-mediated regulation of CYP2E1 expression. This primary cultured rat hepatocyte model provides an excellent system with which to study mechanisms regulating CYP2E1 expression, as well as the role of CYP2E1 in the metabolism and bioactivation of drugs, chemicals, and putative carcinogens.

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