Insulin Differentially Affects Xenobiotic-Enhanced, Cytochrome P-450 (CYP)2E1, CYP2B, CYP3A, and CYP4A Expression in Primary Cultured Rat Hepatocytes

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ABSTRACT
Uncontrolled diabetes results in enhanced expression of cytochrome P-450 (CYP)2E1, CYP2B, CYP3A, and CYP4A. Because of the simultaneous and confounding metabolic and hormonal changes that occur in vivo as a consequence of diabetes, primary cultured rat hepatocytes provide an excellent model system for examination of the effects of insulin on P-450 expression and on xenobiotic-mediated P-450 expression. In the present study, we examined the effects of insulin on pyridine-, phenobarbital-, and ciprofibrate-mediated expression of CYP2E1, CYP2B, CYP3A, and CYP4A in primary cultured rat hepatocytes. Pyridine addition to primary rat hepatocytes cultured in the presence of 1 nM insulin or in the absence of insulin resulted in a 3.5-fold and 3-fold enhancement in CYP2E1 protein expression, respectively, in the absence of any pyridine-mediated increase in mRNA expression. In contrast, hepatocytes cultured in the standard concentration of 1 μM insulin resulted in only a 2-fold increase in protein expression. Thus, the fold-induction of CYP2E1 protein in response to pyridine was 1.5- to 1.8-fold greater in either the absence of insulin or in the presence of 1 nM insulin, respectively, than that monitored in the presence of 1 μM insulin. To examine whether insulin effects on xenobiotic-mediated CYP2E1 expression were selective, insulin effects on xenobiotic-mediated expression of transcriptionally regulated CYP2B, CYP3A, and CYP4A were examined. Pyridine- or phenobarbital-mediated induction of CYP2B mRNA and protein expression in hepatocytes was suppressed by as much as 80% at lower insulin levels (0 and 1 nM), relative to the level monitored in the presence of 1 μM insulin. Omitting insulin from the medium resulted in a 50% decrease in CYP3A mRNA levels in response to phenobarbital treatment and a 30% decrease in CYP4A mRNA levels in response to ciprofibrate treatment, relative to the level obtained in response to these treatments in the presence of 1 μM insulin. The results of this study demonstrate that decreasing the insulin level in the primary hepatocyte culture medium enhanced xenobiotic-mediated CYP2E1 expression, whereas lower insulin levels suppressed xenobiotic-mediated CYP2B, CYP3A, and CYP4A expression in this cell culture system.

Pathophysiologic alterations such as diabetes, fasting, and high-fat diet increase cytochrome P-450 (CYP)2E1 expression by ~3- to 8-fold at both the mRNA and protein levels (Hong et al., 1987; Song et al., 1987; Bellward et al., 1988; Dong et al., 1988; Favreau and Schenkman, 1988; Johansson et al., 1988; Shimojo et al., 1993). CYP2E1 mRNA and protein are elevated in both chemically induced (Dong et al., 1988; Favreau and Schenkman, 1988; Thummel and Schenkman, 1990; Shimojo et al., 1993) and spontaneous (Bellward et al., 1988; Dong et al., 1988) diabetic rats. Elevation of CYP2E1 mRNA levels in the diabetic state in vivo has been attributed to mRNA stabilization (Song et al., 1987). CYP2B1 (Yamazoe et al., 1989b; Barnett et al., 1990a; Donahue and Morgan, 1990), CYP3A (Barnett et al., 1990b; Shimojo et al., 1993), and CYP4A (Barnett et al., 1990b; Shimojo et al., 1993) protein and activity levels have also been reported to be increased ~2- to 5-fold in rats made diabetic by alloxan or streptozotocin treatment. The elevated expression of these P-450s has been largely attributed to diabetes-induced alterations in metabolism (elevated ketone body levels; Bellward et al., 1988; Dong et al., 1988; Barnett et al., 1990a; Barnett et al., 1990b) or hormone secretion (decreased growth hormone and testosterone levels; Yamazoe et al., 1989a,b; Thummel and Schenkman, 1990; Richardson et al., 1992). Although insulin administration to diabetic rats has been shown to lower CYP2E1, CYP2B, CYP3A, and CYP4A to control levels (Dong et al., 1988; Favreau and Schenkman, 1988; Yamazoe et al., 1989b; Barnett et al., 1990b; Donahue and Morgan, 1990; Shimojo et al., 1993), these effects have been attributed to the normalization of ketone body and/or growth hormone levels. We have demonstrated, using primary cultured rat hepatocytes, that CYP2E1 mRNA and

ABBREVIATIONS: CYP, cytochrome P-450; PYR, pyridine; PB, sodium phenobarbital; CIPRO, ciprofibrate.
protein expression are primarily regulated by insulin, with lower insulin concentrations (which reflect the diabetic state) elevating CYP2E1 mRNA and protein levels ~4- to 11-fold (Woodcroft and Novak, 1997). In contrast, lower insulin levels were found to have minimal effect on basal CYP2B, CYP3A, or CYP4A expression in primary cultured rat hepatocytes (Woodcroft and Novak, 1997).

Treatment of rats with xenobiotics such as pyridine, acetone, or alcohols (ethanol and isopropanol) results in increased hepatic CYP2E1 protein levels in the absence of a concomitant increase in CYP2E1 mRNA levels, indicating that posttranscriptional mechanisms are involved in the regulation of this P-450 (Johansson et al., 1988; Kim et al., 1988). Mechanistic studies in vivo have implicated both translational efficiency (Kim and Novak, 1990; Kim et al., 1990) and protein stabilization (Song et al., 1989; Roberts et al., 1995) in xenobiotic-mediated elevation of CYP2E1 protein levels.

It has been reported that administration of the CYP2E1 inducer 4-methylpyrazole to rats made diabetic by streptozotocin treatment results in an additive increase in hepatic CYP2E1 protein levels relative to the increases obtained by either 4-methylpyrazole or streptozotocin treatment alone, indicating that xenobiotic-mediated CYP2E1 protein expression is enhanced during diabetes (Wu and Cederbaum, 1993). Thus, the mechanisms governing regulation of CYP2E1 expression are complex and involve transcriptional, post-transcriptional, translational, and post-translational events. Moreover, the superimposition of xenobiotic effects upon metabolic and/or hormonal changes further complicates delineation of the factors and/or mechanisms regulating CYP2E1 expression.

In the present study, the effect of insulin on xenobiotic-mediated CYP2E1 mRNA and protein expression in primary cultured rat hepatocytes was examined for the purpose of further delineating the contribution of insulin to CYP2E1 expression. Moreover, the effects of insulin (or diabetes) on xenobiotic-mediated expression of CYP2B, CYP3A, or CYP4A have not been examined either in vivo or in cultured hepatocytes, despite reports that these P-450s are elevated during diabetes (Yamazoe et al., 1989b; Barnett et al., 1990a, 1990b; Donahue and Morgan, 1990; Shimojo et al., 1993). Thus, xenobiotics [e.g., pyridine (PYR), phenobarbital (PB), and ciprofibrate (CIPRO)] known to enhance the expression of CYP2E1, CYP2B, CYP3A, and CYP4A were used to assess the effect of insulin on xenobiotic-enhanced expression of these P-450s in primary cultured rat hepatocytes. Treatment of primary rat hepatocytes with a CYP2E1-inducing agent, in combination with lower insulin concentrations in the medium (to mimic the diabetic state), results in greater CYP2E1 protein levels than occurs with either of these treatments alone. CYP2E1 protein levels are elevated in response to the inducing agent by 1.5- to 1.8-fold in the presence of lower insulin concentrations over the levels monitored in the presence of the standard concentration of 1 μM insulin. In contrast, xenobiotic-mediated CYP2B, CYP3A, and CYP4A induction, which occurs primarily through transcriptional activation, was suppressed by 30 to 80% by lowering the insulin concentration. Thus, insulin differentially regulates xenobiotic-mediated expression of CYP2E1 relative to CYP2B, CYP3A, and CYP4A.

Materials and Methods

Chemicals. Modified Chee's medium, l-glutamine, tricine, and gentamicin were obtained from Life Technologies, Inc. (Gaithersburg, MD). Insulin (NovolinR) was purchased from Novo-Nordisk (Princeton, NJ). Collagenase (type I) was purchased from Worthington Biochemicals (Freehold, NJ). Culture dishes (Falcon) or flasks (Corning) were obtained from Baxter (McGaw Park, IL). Vitrogen (95–98% type I collagen, 2–5% type III collagen) was obtained from Celsitrix Pharmaceuticals (Santa Clara, CA). Anti-rat CYP2B1 was purchased from Xenotech (Kansas City, KS). Horseradish peroxidase-conjugated goat anti-rabbit antibody was obtained from BioRad (Melville, NY). Enhanced chemiluminescence reagents were purchased from Amersham (Arlington Heights, IL). PB was obtained from Mallinkrodt (Chesterfield, MO). PYR (technical grade) was obtained from Fisher (Pittsburgh, PA). CIPRO was a gift from Sterling-Winthrop Pharmaceuticals (Rensselaer, NY). All other reagents 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) using collagenase perfusion as described previously (Seglen, 1982; Zangar et al., 1995). Hepatocytes were plated onto dishes or flasks coated with Vitrogen as described previously (Waxman et al., 1990; Zangar et al., 1995). Cells were plated at densities of 12 × 10⁶ cells/75 cm² flask or 4 × 10⁶ cells/25 cm² flask. Modified Chee's medium was fortified as described (Waxman et al., 1990), except that it was supplemented with 0.1 μM dexamethasone and 1 μM insulin, the standard concentration of insulin used in primary hepatocyte culture. Four hours after plating, medium was replaced with medium containing various concentrations of insulin (no insulin, 1 nM, and 1 μM). The medium was changed every 24 h thereafter.

Treatment of hepatocytes with PYR (7.5 mM), PB (0.1 mM), or CIPRO (30 μM) was initiated 72 h after cell plating and lasted 24 h. These optimal concentrations and times were chosen based on previous studies performed in our laboratory using this primary hepatocyte culture system (Zangar et al., 1995; Zangar and Novak, 1997; Woodcroft and Novak, 1998). All cells were harvested 96 h after initiation of culture. When treatment involved the volatile chemical PYR, the tissue culture flasks were sealed to prevent loss of the chemical. Thus, after media change, loosely capped flasks were returned to the incubator for 1 to 2 h to allow equilibration of media with the incubator chamber atmosphere. Chemicals were then added, and flasks were sealed. We have reported previously that sealing of the flasks for the duration of the xenobiotic treatment period did not affect CYP2E1 or 2B expression (Woodcroft and Novak, 1998). PYR was prepared as a 5 M stock solution in sterile water. PB was prepared as a 100 mM stock solution in sterile water. CIPRO was prepared as a 30 mM stock solution in 100 mM HEPEES (pH 7.2). Data for statistical analysis were obtained from three separate microsome or total RNA preparations from a single hepatocyte preparation, and reproducibility of results were confirmed in at least two hepatocyte preparations (data not shown).

The xenobiotic and insulin concentrations used in this study have previously been demonstrated by our laboratory to be nontoxic to primary cultured rat hepatocytes, as measured by lactate dehydrogenase release (Zangar et al., 1995; Woodcroft and Novak, 1997).

Immunoblot Analyses. Hepatocyte microsomes were prepared essentially as described previously (Zangar et al., 1995; Woodcroft and Novak, 1998). Cells were washed twice with 5 ml of 4°C PBS (pH 7.4), suspended in 6 ml of PBS/flask using three flasks/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-Cl, 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 EGTA) and repelleted. Cells were then suspended in 250 μl of this buffer, homogenized for 30 s using an Omni 1000 homogenizer.
(Waterbury, CT) set on high, and centrifuged for 10 min at 10,000g, 4°C. The resulting supernatant was centrifuged for 1.5 h at 105,000g, 4°C. The pelleted microsomes were 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 by very gentle sonication with a probe sonicator (setting 2 on a Tekmar Sonic Disruptor for 20 to 30 s). Microsomal proteins (20 μg/lane) were separated on 10% polyacrylamide gels and transferred to nitrocellulose for immunoblot analysis 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 detection by chemiluminescence. Densitometry was performed using a Molecular Dynamics (Sunnyvale, CA) laser densitometer and the ImageQuant analysis program.

Northern Blot Analysis. Total RNA from hepatocytes was isolated and separated (10 μg/lane) on formaldehyde-agarose gels and capillary blotted as described previously (Sambrook et al., 1989; Xie and Rothblum, 1991). A CYP2E1 cDNA probe was prepared as described previously (Zangar et al., 1995). cDNAs for CYP2B1 (Doehmer et al., 1988), CYP3A1 (pDex12) (Wrighton et al., 1985), and CYP4A1 (Hardwick et al., 1987) were generously provided by Drs. Milton Adesnik (New York University Medical Center, NY), Philip Guzelian (University of Colorado, Denver, CO), and Frank Gonzalez (National Cancer Institute, Bethesda, MD), respectively, and the cytoplasmic 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 (Life Technologies, Inc.) according to the manufacturer’s instructions.

Membranes were prehybridized, incubated with labeled probe (106 CPM/ml final activity), and washed as described previously (Zangar et al., 1995). The blots were then exposed to X-ray film (Kodak XAR) for 2 h to 4 days, and bands were quantified using a Molecular Dynamics (Sunnyvale, CA) laser densitometer and the ImageQuant analysis program.

Statistics. Significant differences between treatment groups were determined by ANOVA, followed by Tukey-Kramer, p < .05.

Results

Insulin Effects on CYP2E1 mRNA and Protein Expression. Basal expression of CYP2E1 mRNA was monitored in primary hepatocytes cultured for 96 h in the presence of the standard concentration of 1 μM insulin or 1 nM insulin or in the absence of insulin. Relative to the level of CYP2E1 mRNA in hepatocytes cultured in the presence of 1 μM insulin, hepatocytes maintained in culture in the presence of 1 nM insulin exhibited an ~4-fold increase in the basal expression of CYP2E1 mRNA, and those cultured in the absence of insulin exhibited an ~12-fold increase in CYP2E1 mRNA expression (Fig. 1).

Treatment of hepatocytes with PYR failed to alter CYP2E1 mRNA expression relative to the corresponding untreated cells, regardless of the insulin concentration used in the culture medium (Fig. 1).

Basal CYP2E1 protein levels were also elevated in response to lower insulin concentrations in the hepatocyte culture medium. Relative to cells cultured in the presence of 1 μM insulin, CYP2E1 protein levels were elevated 1.4-fold in hepatocytes cultured in the presence of 1 nM insulin, and ~6-fold in hepatocytes cultured in the absence of insulin (Fig. 2).

PYR-induced CYP2E1 protein levels increased ~2.5- to 9-fold with decreasing insulin concentration (Fig. 2). Treatment of hepatocytes with PYR in the presence of 1 μM insulin, 1 nM insulin, and in the absence of insulin increased CYP2E1 protein expression ~2-, 3.5-, and 3-fold, respectively, relative to the corresponding untreated cells at each...
Insulin concentration (Fig. 2). Thus, the magnitude of induction of CYP2E1 protein after PYR treatment was 1.5- to 1.8-fold greater in the absence of insulin or in the presence of 1 nM insulin, respectively, as compared with that monitored in the presence of 1 μM insulin. Therefore, lowering the concentration of insulin in the medium, or removing it completely, enhanced the induction of CYP2E1 protein in response to PYR.

Insulin Effects on CYP2B mRNA and Protein Expression. Basal CYP2B mRNA expression was marginally increased (maximally 3.5-fold) by maintaining hepatocytes in the presence of 1 nM insulin or in the absence of insulin, relative to levels monitored in hepatocytes cultured in the presence of 1 μM insulin (Fig. 3).

PYR-induced CYP2B mRNA levels declined by ~15 to 25% with decreasing insulin concentration (Fig. 3). PYR treatment of hepatocytes resulted in a 27-fold increase in CYP2B mRNA in the presence of 1 μM insulin, a 6.5-fold increase in the presence of 1 nM insulin, and a 5-fold increase in the absence of insulin. Thus, the PYR-induced CYP2B mRNA expression decreased by 76% in the presence of 1 nM insulin and by 81% in the absence of insulin, relative to that monitored in the presence of 1 μM insulin.

Basal CYP2B protein levels were elevated slightly at lower insulin concentrations, relative to cells maintained in culture in the presence of 1 μM insulin, with ~2.5- and 4-fold increases in CYP2B protein monitored in cells cultured in the presence of 1 nM insulin and in the absence of insulin, respectively (Fig. 4).

CYP2B protein expression in response to PYR treatment, however, declined with decreasing insulin concentrations (Fig. 4), in parallel with the results obtained for CYP2B mRNA. PYR treatment produced an 18-fold elevation of CYP2B protein levels in the presence of 1 μM insulin, a 7-fold elevation of protein in the presence of 1 nM insulin, and a 3-fold elevation of protein in the absence of insulin (Fig. 4), which represents a 60 to 85% decline in PYR-mediated induction of CYP2B protein.

Because PB is the prototype inducer of CYP2B expression, the effects of insulin on PB-enhanced CYP2B mRNA expression were also examined in hepatocytes cultured in the presence or absence of 1 μM insulin (Fig. 5). PB treatment enhanced CYP2B mRNA expression ~38-fold in the presence of 1 μM insulin; in contrast, expression was enhanced only ~27-fold in the absence of insulin. Therefore, lower insulin concentrations suppressed PB-mediated induction of CYP2B mRNA by ~30%.

Insulin Effects on CYP3A and CYP4A mRNA Expression. Because the expression and activities of CYP3A and CYP4A have also been reported to be elevated in diabetic rats, and these P-450s are regulated primarily at the level of transcription, we examined the effect of insulin on xenobiotic-mediated CYP3A and CYP4A mRNA expression.

CYP3A mRNA levels were decreased slightly (~35%) in primary hepatocytes cultured in the absence of insulin compared with hepatocytes cultured in the presence of 1 μM insulin (Fig. 6). PB-induced CYP3A mRNA levels were significantly (~50%) lower in hepatocytes maintained in the absence of insulin relative to those maintained in the presence of 1 μM insulin (Fig. 6). The induction of CYP3A mRNA by PB decreased from ~6-fold in the presence of 1 μM insulin to ~4.5-fold in the absence of insulin. Thus, the absence of insulin significantly decreased PB-mediated expression of CYP3A mRNA.
Hepatocytes maintained in culture in the absence of insulin expressed 50% lower levels of CYP4A mRNA relative to those maintained in the presence of 1 mM insulin (Fig. 7).

CIPRO-induced CYP4A mRNA levels were significantly (~30%) lower in hepatocytes maintained in the absence of insulin relative to those maintained in the presence of 1 μM insulin (Fig. 7). Thus, the absence of insulin significantly decreased CIPRO-mediated expression of CYP4A mRNA.

Discussion

Primary rat hepatocyte culture offers an excellent system with which to examine the effects of insulin on xenobiotic-mediated expression of these P-450s, because it permits control of the insulin levels in the absence of simultaneous changes in metabolic or hormonal factors (e.g., ketone bodies, growth hormone, and testosterone), which occur during diabetes in vivo. We have shown that a primary rat hepatocyte culture system that provides detectable basal expression of CYP2E1, CYP2B, CYP3A, and CYP4A mRNA and protein is also responsive to xenobiotic-mediated increases in these P-450s in a manner that parallels that monitored in vivo (Zangar et al., 1995; Zangar and Novak, 1997; Zangar and Novak, 1998; Woodcroft and Novak, 1998). We have also used this primary rat hepatocyte culture system to examine insulin effects on basal expression of CYP2E1, CYP2B, CYP3A, and CYP4A (Woodcroft and Novak, 1997).

The basal expression of CYP2E1, and to a lesser extent CYP2B, mRNA and protein was enhanced in primary cultured rat hepatocytes by either lowering the insulin concentration or completely excluding insulin from the medium (Figs. 1–4; Woodcroft and Novak, 1997). In contrast, exclusion of insulin from the culture medium resulted in a slight
decrease in the basal levels of CYP3A and CYP4A mRNA (Figs. 6 and 7; Woodcroft and Novak, 1997).

The substantial increase in CYP2E1 mRNA and protein levels in hepatocytes cultured in lower concentrations of insulin observed in the present study and in previous studies by our laboratory (Woodcroft and Novak, 1997) demonstrate that insulin is primarily responsible for the increase in CYP2E1 expression. In more detailed concentration-response experiments performed in primary cultured rat hepatocytes in our laboratory, the level of insulin monitored in the diabetic Sprague-Dawley rat (<0.02 nM; Donahue and Morgan, 1990) corresponds to the insulin concentration at which we begin to observe a positive effect on CYP2E1 expression (0.01 nM; Woodcroft and Novak, unpublished observations). That insulin appears to be the primary regulator of the increase in CYP2E1 expression is supported further by additional studies from our laboratory, which demonstrated that ketone bodies (3-hydroxybutyrate and acetoacetate) did not increase the expression of CYP2E1 mRNA or protein in primary cultured rat hepatocytes (Zangar and Novak, 1997).

Whereas insulin plays a primary role in regulating CYP2E1 expression in primary cultured rat hepatocytes, it does not appear to be a primary mediator of CYP2B expression, because minimal increases in basal expression were observed in this study in the presence of lowered insulin levels (Figs. 3–5). Concomitant research, however, has demonstrated that 3-hydroxybutyrate and acetoacetate do cause substantial increases in CYP2B expression (Zangar and Novak, 1997). Our studies also indicate that insulin is not a major factor in regulating CYP3A or CYP4A expression, because maintaining hepatocytes in the absence of insulin resulted in minimal decreases in the basal levels of these two P-450s (Figs. 6 and 7).

The effects of insulin on xenobiotic-enhanced CYP2E1 expression observed in this study suggest that insulin can also affect protein expression independent of effects on mRNA expression. Maintaining hepatocytes in the presence of 1 nM insulin or in the absence of insulin, compared with the presence of the standard concentration of 1 µM insulin, resulted in an increase in basal expression of CYP2E1 mRNA (Fig. 1) and CYP2E1 protein (Fig. 2). However, lowering the insulin levels resulted in a greater increase in the expression of CYP2E1 protein after PYR treatment than that monitored in the presence of 1 µM insulin (Fig. 2), with no concomitant xenobiotic-mediated increase in CYP2E1 mRNA (Fig. 1) (i.e., lowering the insulin concentration enhanced the degree of CYP2E1 protein induction mediated by PYR). These findings support similar observations in rats by Wu and Cederbaum (1993), wherein hepatic CYP2E1 protein levels were reported to be increased to a greater degree by 4-methylpyrazole treatment of streptozotocin-induced diabetic rats than by either 4-methylpyrazole or streptozotocin treatment alone without additional effect on CYP2E1 mRNA levels other than that produced by streptozotocin alone.

In contrast to the elevation of xenobiotic-mediated CYP2E1 protein levels produced by lower insulin concentrations, CYP2B mRNA and protein expression in response to PYR or PB treatment were suppressed by lower insulin levels (Figs. 3–5). Interestingly, lower insulin concentrations exerted opposing effects on basal and xenobiotic-mediated CYP2B expression, enhancing basal levels while suppressing xenobiotic-mediated CYP2B expression. The insulin effect on CYP2B is similar to that reported with dexamethasone, which at low concentrations suppresses basal expression of CYP2B mRNA in primary cultured rat hepatocytes while enhancing PB-mediated induction (Kocarek et al., 1994). This may be a result of xenobiotic inducers of CYP2B acting on intermediary components of the insulin receptor-mediated signal transduction pathway, leading to an enhancement of the insulin effect on CYP2B gene expression.

The absence of insulin decreased expression of CYP3A and CYP4A mRNA in both untreated and xenobiotic-treated hepatocytes, a result that differs from that of CYP2E1 or CYP2B, illustrating that insulin can have multiple and varied effects on gene expression in primary cultured hepatocytes. This is consistent with the effects of insulin on other genes. For example, insulin has been reported to inhibit the expression of cholesterol 7a-hydroxylase, sterol 27-hydroxylase, aspartate aminotransferase, phosphoenolpyruvate carboxykinase, growth hormone, and glucagon while stimulating the expression of glyceraldehyde-3-phosphate dehydrogenase, c-Fos, α-amylase, and glucokinase (O’Brien and Granner, 1991; Twisk et al., 1995; and references therein).

Mechanistic studies on the regulation of expression of CYP2E1 have been limited by the lack of a cell culture system that expresses detectable basal CYP2E1 mRNA and protein and that mimics the in vivo response of CYP2E1 to xenobiotics. Our laboratory has used primary rat hepatocytes cultured in modified Chee’s medium on Vitrogen substratum to achieve both measurable basal expression of CYP2E1 and a significant induction response to xenobiotics known to enhance the hepatic expression of CYP2E1 in vivo (Zangar et al., 1995; Woodcroft and Novak, 1998). The results of the present study using this cell culture model indicate that insulin differentially regulates the xenobiotic-enhanced expression of CYP2E1, CYP2B, CYP3A, and CYP4A, enhancing that of CYP2E1 while suppressing the others. We have also demonstrated that insulin can affect the level of xenobiotic-mediated expression of CYP2E1 protein exclusive of any effect of insulin on CYP2E1 mRNA. Thus, this cell culture system provides an excellent model for additional mechanistic investigations into the insulin-mediated regulation of P-450 expression.

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