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CHARACTERIZATION OF CYTOCHROME P450 2E1 INDUCTION IN A RAT HEPATOMA FGC-4 CELL MODEL BY ETHANOL*

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Abstract—The hepatic microsomal ethanol-oxidizing system (MEOS) has been well characterized as an important pathway in ethanol metabolism. Cytochrome P450 2E1 (CYP 2E1), the principal component of MEOS, is ethanol inducible and has been implicated in hepatotoxicity associated with alcohol abuse and exposure to organic solvents. Results of chronic *in vivo* experiments have shown that ethanol induction of hepatic CYP 2E1 occurs by a two-step mechanism. The first step of induction is associated with low blood alcohol concentrations (BACs) and appears to be post-transcriptional, whereas high BACs observed in step-two induction are associated with increased CYP 2E1 gene transcription. The mechanisms underlying these induction steps are under intense investigation. Progress in this area has been limited due to lack of hepatic cell culture models that express CYP 2E1. We report here an *in vitro* tissue culture cell model, the FGC-4 hepatoma cell line, that exhibits basal levels of CYP 2E1 apoprotein that are inducible by ethanol treatment. Total cellular RNA and microsomal fractions were isolated from control or ethanol-treated confluent cells, and CYP 2E1 mRNA and apoprotein levels were characterized by northern blot or immunoblot analysis, respectively. Initial experiments on isolated microsomes revealed detectable levels of CYP 2E1 apoprotein in control cells that were induced 5-fold in cells treated with 100 mM ethanol for 24 hr. Concentration–response experiments demonstrated that the maximal 24-hr induction in CYP 2E1 apoprotein level was 5-fold and was attained at a concentration of 10 mM ethanol. Interestingly, while the steady-state mRNA levels encoding CYP 2E1 were detectable, they remained unchanged in identically treated cells. Furthermore, there was no observed increase in CYP 2E1 mRNA levels in an extended time course to 72 hr or at higher alcohol concentrations (up to 1500 mM), providing preliminary evidence that the induction is post-transcriptional. The time course of CYP 2E1 apoprotein induction by exposure to 100 mM ethanol demonstrated maximal induction at 8 hr. Measurement of CYP 2E1 apoprotein levels after removal of ethanol from pretreated cells demonstrated the half-life of the apoprotein to be 12.7 hr, in good agreement with previous reports using primary hepatocytes. The half-life of the induced protein after ethanol removal in the presence of cyclohexamide (10 μg/mL) was biphasic with a rapid 1.8 hr first phase followed by a slower 44.7 hr second phase. However, if the pre-induced cells were allowed to remain in the presence of ethanol and cyclohexamide, the half-life was monophasic, consisting of only the slow phase. These data provide preliminary evidence that the mechanism of stabilization of the ethanol-induced CYP 2E1 apoprotein is post-translational. Combined, these experiments demonstrate that the FGC-4 hepatoma cell line expresses CYP 2E1 and that the apoprotein is inducible by ethanol by post-transcriptional mechanisms.

Key words: ethanol; cytochrome P450; hepatoma cell line

Metabolism of ethanol *in vivo* is thought to be regulated predominantly by three enzymatic components: alcohol dehydrogenase, MEOS‖, and catalase [1]. While the relative contribution made by each of these components toward the complete metabolism of ethanol is still unknown, it is well accepted that the role of hepatic MEOS is an important one. CYP 2E1, the principal component of MEOS, has been demonstrated previously to be inducible by ethanol and many other solvents as well as a number of physiological conditions including fasting, obesity and diabetes [2, 3]. CYP 2E1 induction has been implicated in the mechanism of alcohol-induced cirrhosis [4], hepatotoxicity associated with acetaminophen, carbon tetrachloride

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‖ Abbreviations: MEOS, microsomal ethanol-oxidizing system; BACs, blood alcohol concentrations; and ADUs, arbitrary densitometric units.

and halothane [5–7], and the activation of nitrosamines and benzene to carcinogenic metabolites [8, 9]. Thus, the regulatory mechanism of this enzyme has been the subject of intense study. The CYP 2E1 apoprotein has been reported to be stabilized by chronic exposure to alcohol in rabbit and rat liver [10, 11] and in primary hepatocyte cultures [12, 13]. In addition to ethanol induction at the level of the apoprotein, the steady-state CYP 2E1 mRNA levels are also induced *in vivo* by chronic ethanol exposure in rats [3] and hamsters [14]. Furthermore, it has been demonstrated *in vivo* that ethanol is capable of increasing the transcriptional rate of the CYP 2E1 gene [15], and this transcriptional effect is associated with blood alcohol concentrations [3]. We have proposed that CYP 2E1 induction by chronic alcohol exposure occurs by a two-step mechanism: the first step of induction is associated with low BACs and is post-transcriptional, whereas higher BACs observed in step-two induction are associated with increased CYP 2E1 mRNA levels and CYP 2E1 gene transcription [3]. Currently, little is known about the mechanisms of either of these induction steps. Investigation of these mechanisms has proven difficult because *in vitro* hepatic cell culture models have limited induction of CYP 2E1.

In the current study, we have identified an *in vitro* cell culture model, the FGC-4 hepatoma cell line developed by Angrand *et al.* [16], that expresses both the CYP 2E1 mRNA and apoprotein. We also report that the CYP 2E1 apoprotein level in the FGC-4 cells was inducible upon treatment with ethanol and that this induction appeared to be mediated, in part, by post-translational mechanisms of the apoprotein.

MATERIALS AND METHODS

Cell culture and ethanol treatment. FGC-4 cells [16] were a gift provided by Mary C. Weiss at the Pasteur Institute (Paris, France). Cells were grown in Nutrient Mixture Ham's F12 Coon's Modification medium (Sigma, St. Louis, MO) and supplemented with 5% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin in a 7% CO_2 humidified atmosphere. For ethanol treatment, cells were grown to confluence in 100 mm tissue culture plates and refed with fresh medium containing the appropriate dose of 95% ethanol. To prevent evaporation of ethanol, plates were wrapped in Parafilm "M" (American National Can, Greenwich, CT). In our laboratory, these cells have been passaged 30 times without loss of ethanol-inducible CYP 2E1 immunoreactivity.

RNA isolation and northern blotting. Total cellular RNA was isolated from cells at each experimental point by: washing three times with ice-cold PBS (2.7 mM KCl, 137 mM NaCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4 [pH 7.4]); lysed by scraping in 9 mL of extraction buffer (4 M guanidinium isothiocyanate, 10 mM EDTA, 50 mM sodium acetate [pH 5.0], 1 M 2-mercaptoethanol); layered over a 5.7 M CsCl cushion containing 50 mM sodium acetate (pH 5.0) and centrifuged at 130,000 *g* in an SW-40 rotor (Beckman Instruments, Fullerton, CA). Pellets were resuspended in 0.3 M LiCl, 0.1% SDS; RNA was ethanol precipitated and resuspended at a concentration of ~5–6 μg/μL. Samples (30 μg) were subjected to electrophoresis on 1% agarose/6% formaldehyde gel and transferred to Magna NT nylon-supported membranes (Micron Separations, Westboro, MA) by capillary blotting using standard procedures. After transfer, the filters were prehybridized at 65° in 10 mL of 25 mM Na_2HPO_4 (pH 7.4), 0.1% SDS, 1 mM EDTA, 250 mM NaCl, 50 μg/mL wheat germ tRNA, 50 μg/mL polyadenylic acid, 500 μg/mL salmon sperm DNA, and 50% formamide. The prehybridization solution was replaced with an identical mixture containing 10^6 cpm/mL of ^{32}P-labeled probe and hybridized overnight at 48°. Filters were washed at 55° in 0.1× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]) and exposed to Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) overnight at −70°. The probe used was a complete cDNA clone encoding rat CYP 2E1 (the gift of Dr. Frank

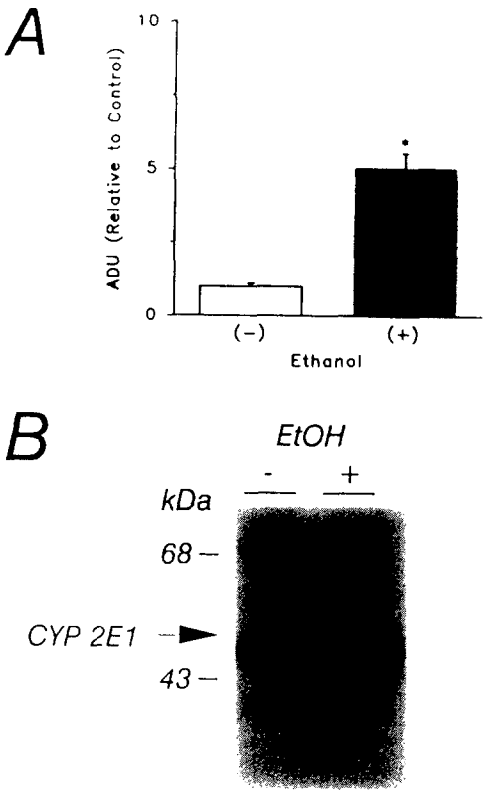

Fig. 1. Effects of ethanol on CYP 2E1 apoprotein levels. Microsomal protein (100 μg) prepared from FGC-4 cells treated with (+) or without (−) 100 mM ethanol for 24 hr were separated by size with 8% SDS–PAGE, transferred to nitrocellulose, and used for immunoblot analysis using a CYP 2E1 polyclonal antisera. (A) Following autoradiography, specific CYP 2E1 hybridization signals were quantitated by scanning densitometry. Control (−) cells were assigned an arbitrary densitometric unit (ADU) of 1.0, and the asterisk indicates a significant difference ($P < 0.002$) from control. Values are means ± SEM, $N = 9$. (B) Representative autoradiogram from one experiment with the migration of molecular weight standards indicated to the left in kilodaltons (kDa).

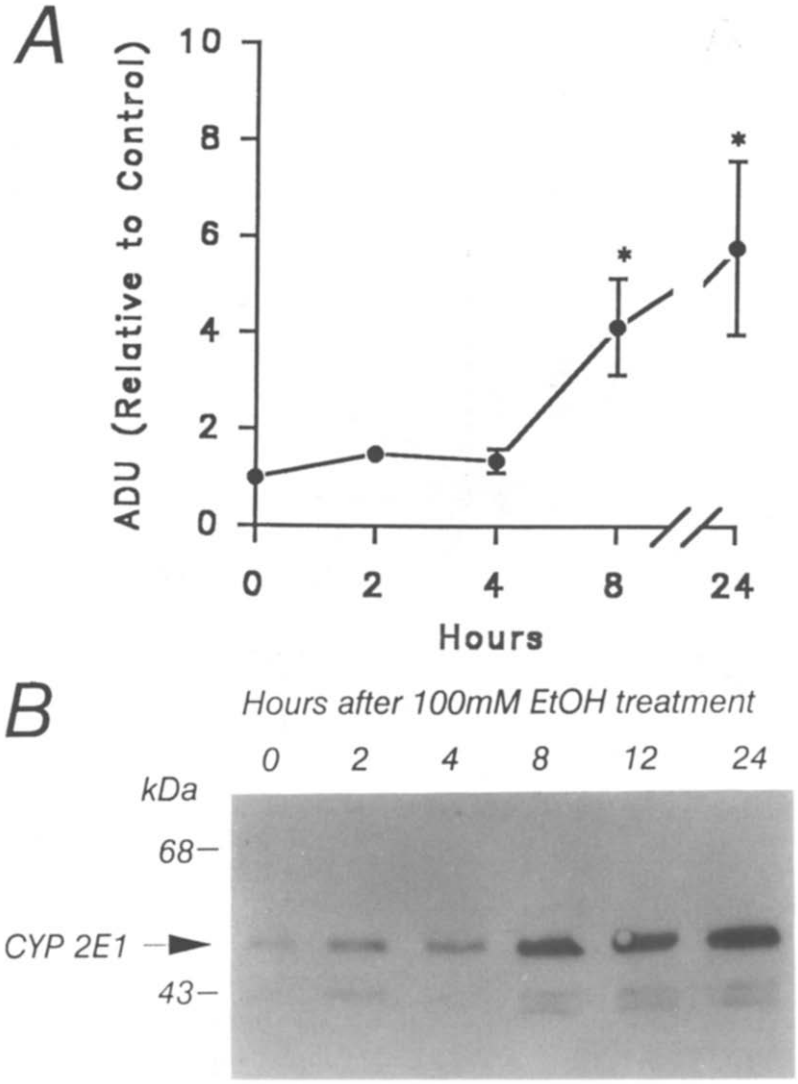

Fig. 2. Time course of CYP 2E1 apoprotein induction by ethanol. Microsomal protein (100 μg) was prepared from FGC-4 cells at the indicated times following treatment without (time 0) or with 100 mM ethanol and analyzed as described in the legend to Fig. 1. (A) Following autoradiography, specific CYP 2E1 hybridization signals were quantitated by scanning densitometry. Control (time 0) cells were assigned an arbitrary densitometric unit (ADU) of 1.0, and asterisks indicate significant differences ($P < 0.05$) from control. Values are means ± SEM, N = 3. (B) Representative autoradiogram from one experiment with the migration of molecular weight standards indicated to the left in kilodaltons (kDa).

Gonzalez, NIH, Bethesda, MD). Filters were subsequently stripped of CYP 2E1 probe and reprobed with a cDNA encoding mouse actin.

Isolation of microsomal fractions and immunoblot analysis. FGC-4 cell microsomal fractions were isolated using a procedure described by Eliasson *et al.* [13], with minor modifications. Briefly, plates were washed three times in ice-cold PBS, scraped in 1 mL of cold PBS, and gently pelleted in a microcentrifuge. Cells were resuspended in 2 mL of homogenization buffer (50 mM $NaPO_4$, 10 mM EDTA, pH 7.4), homogenized in a Kontes 2-mL miniature glass homogenizer (Kontes Glass, Vineland, NJ), and centrifuged at 10,000 *g* at 4° for 10 min. The supernatant was transferred to a new tube and centrifuged at 100,000 *g* at 4° for 60 min. Pellets were resuspended thoroughly in ~50 μL of resuspension buffer (50 mM $NaPO_4$ [pH 7.4], 0.1 mM EDTA, 10% glycerol) and quantitated with the Bio-Rad protein assay reagent (Bio-Rad, Richmond, CA). Microsomal protein (100 μg) isolated from each experimental point was separated by size by 8% SDS–PAGE and electroblotted onto pure nitrocellulose filters (Micron Separations) using standard procedures. CYP 2E1 was detected using a 1:10,000 dilution of a primary polyclonal antisera directed against CYP 2E1 [17]. The bound primary antibody was visualized by enhanced

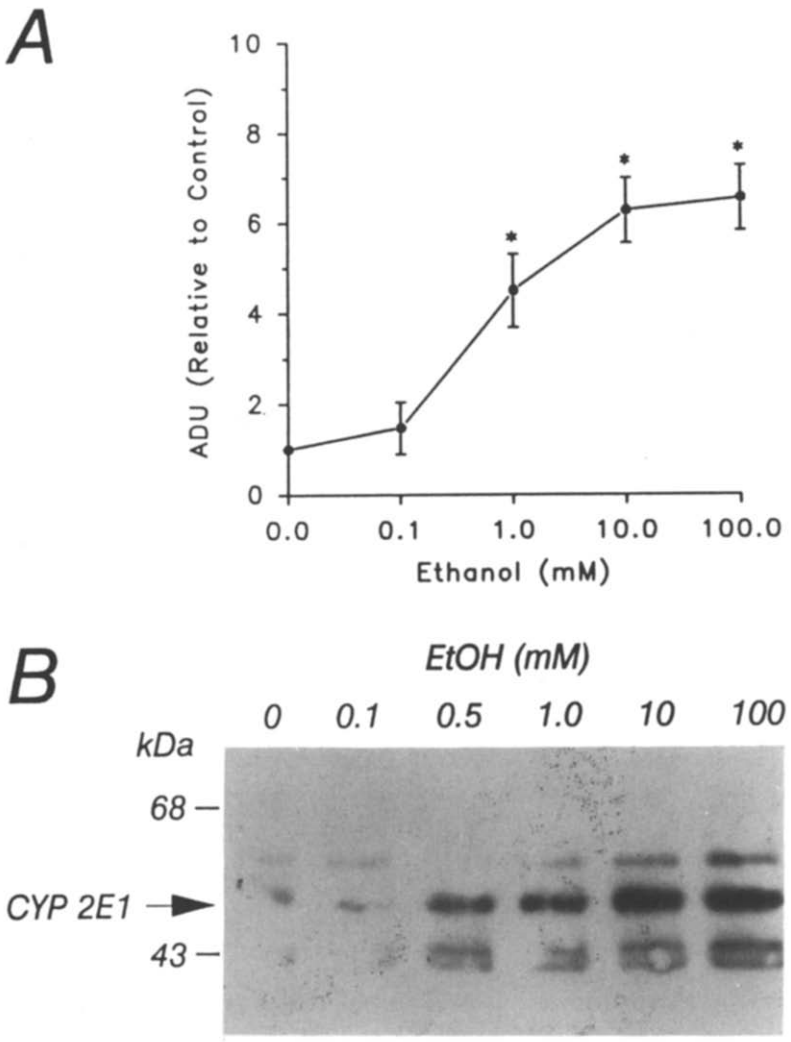

Fig. 3. Concentration–response of ethanol on the induction of CYP 2E1 apoprotein. Microsomal protein (100 μg) was prepared from FGC-4 cells treated for 24 hr with the indicated concentration of ethanol and analyzed as described in the legend to Fig. 1. (A) Following autoradiography, specific CYP 2E1 hybridization signals were quantitated by scanning densitometry. Control (0 concentration) cells were assigned an arbitrary densitometric unit (ADU) of 1.0, and asterisks indicate significant differences ($P < 0.05$) from control. Values are means ± SEM, N = 3. (B) Representative autoradiogram from one experiment with the migration of molecular weight standards indicated to the left in kilodaltons (kDa).

chemiluminescent detection (ECL kit; Amersham, Arlington Heights, IL) of secondarily bound horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) according to manufacturer's procedures.

Statistical analysis. Immunoblots were repeated three to nine times, exposed to Kodak X-OMAT AR film (Eastman Kodak), and scanned on a Molecular Dynamics IQ-3.0 laser densitometer (Molecular Dynamics, Sunnyvale, CA); the graphical data are expressed as means ± SEM. In the initial set of experiments to determine ethanol induction (Fig. 1), a Student's *t*-test was used to determine significant differences ($P < 0.002$) between control and ethanol-treated cells. In the studies on the effects of various times of treatment and concentrations of ethanol on CYP 2E1 apoprotein levels (Figs. 2–4), and the ethanol time course on CYP 2E1 mRNA (Fig. 7), a one-way ANOVA followed by Dunnett's post hoc analysis was used to determine significant differences ($P < 0.05$) between control and treated cells. Graphical data from the northern blots in Figs. 6 and 8 represent the averages of two experiments. Specific CYP 2E1 hybridization signals were normalized to actin to control for loading errors, and graphical analysis from all northern blots represents ADUs, obtained by scanning densitometry as described above.

RESULTS

Characterization of CYP 2E1 apoprotein in

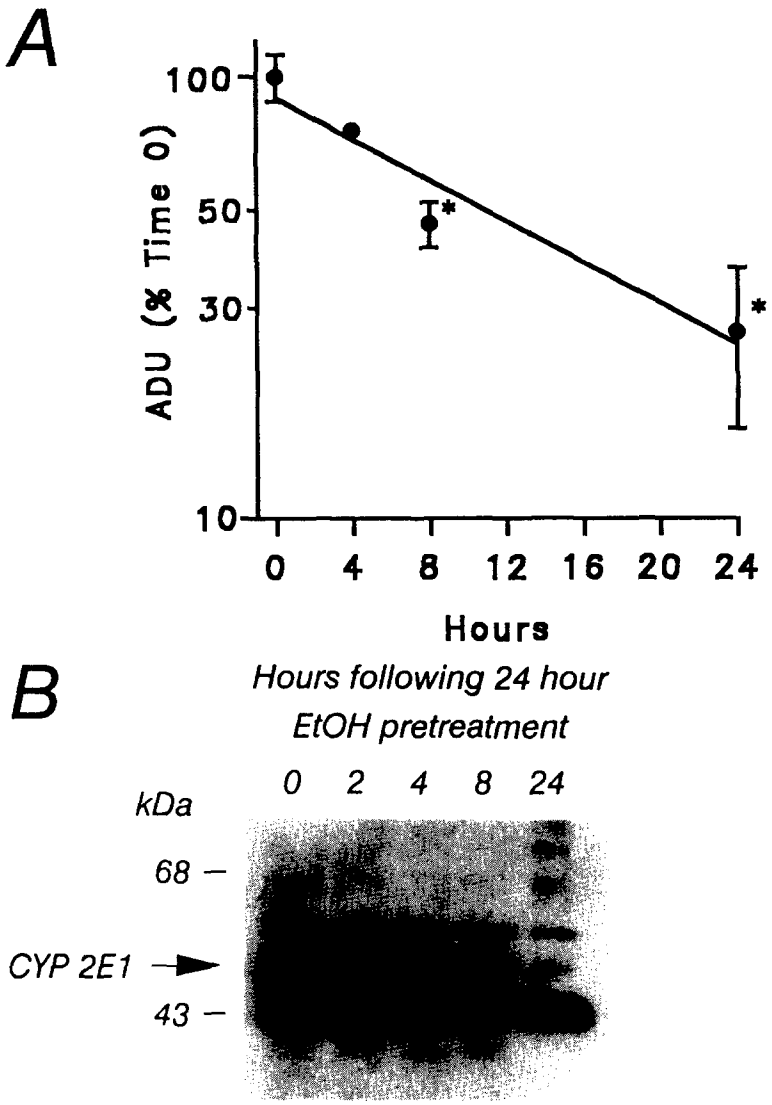

Fig. 4. Half-life determination of induced CYP 2E1 apoprotein. FGC-4 cells were pre-induced for 24 hr with 100 mM ethanol. Cells were subsequently washed and refed with medium containing no ethanol. The loss of CYP 2E1 immunoreactivity was followed by immunoblot analysis using 100 μg microsomal protein and analyzed as described in the legend to Fig. 1. (A) Data were plotted using first-order linear regression analysis (r value = 0.97). Control (time 0) cells were assigned a value of 100% and asterisks indicate significant differences ($P < 0.05$). Values are means ± SEM, N = 3. (B) Representative autoradiogram from one experiment with the migration of molecular weight standards indicated to the left in kilodaltons (kDa).

microsomal fractions of FGC-4 cells treated with ethanol. In preliminary experiments, FGC-4 cells were treated with 100 mM ethanol, a concentration equivalent to 450 mg/dL that we have demonstrated previously always results in a significant *in vivo* induction of the CYP 2E1 apoprotein [3]. Immunoblot analysis on microsomal fractions isolated from FGC-4 control untreated cells revealed basal levels of CYP 2E1 apoprotein expression, and treatment for 24 hr with 100 mM ethanol resulted in a 5-fold induction (Fig. 1). The level of expression of the induced CYP 2E1 apoprotein in microsomal fractions isolated from the FGC-4 cells was only about 1% (~0.5 pmol/mg microsomal protein) of that observed in microsomes from control rat liver, consistent detection of CYP 2E1 immunoreactivity required use of the highly sensitive chemiluminescence method of detection and 100 μg of microsomal protein was needed per lane. Enzyme activities were too low for detection using *p*-nitrophenol hydroxylase assays ([18], data not shown).

Subsequent experiments were performed to establish the time course and concentration–response of apoprotein induction observed in these cells. To establish the time course of induction of the CYP

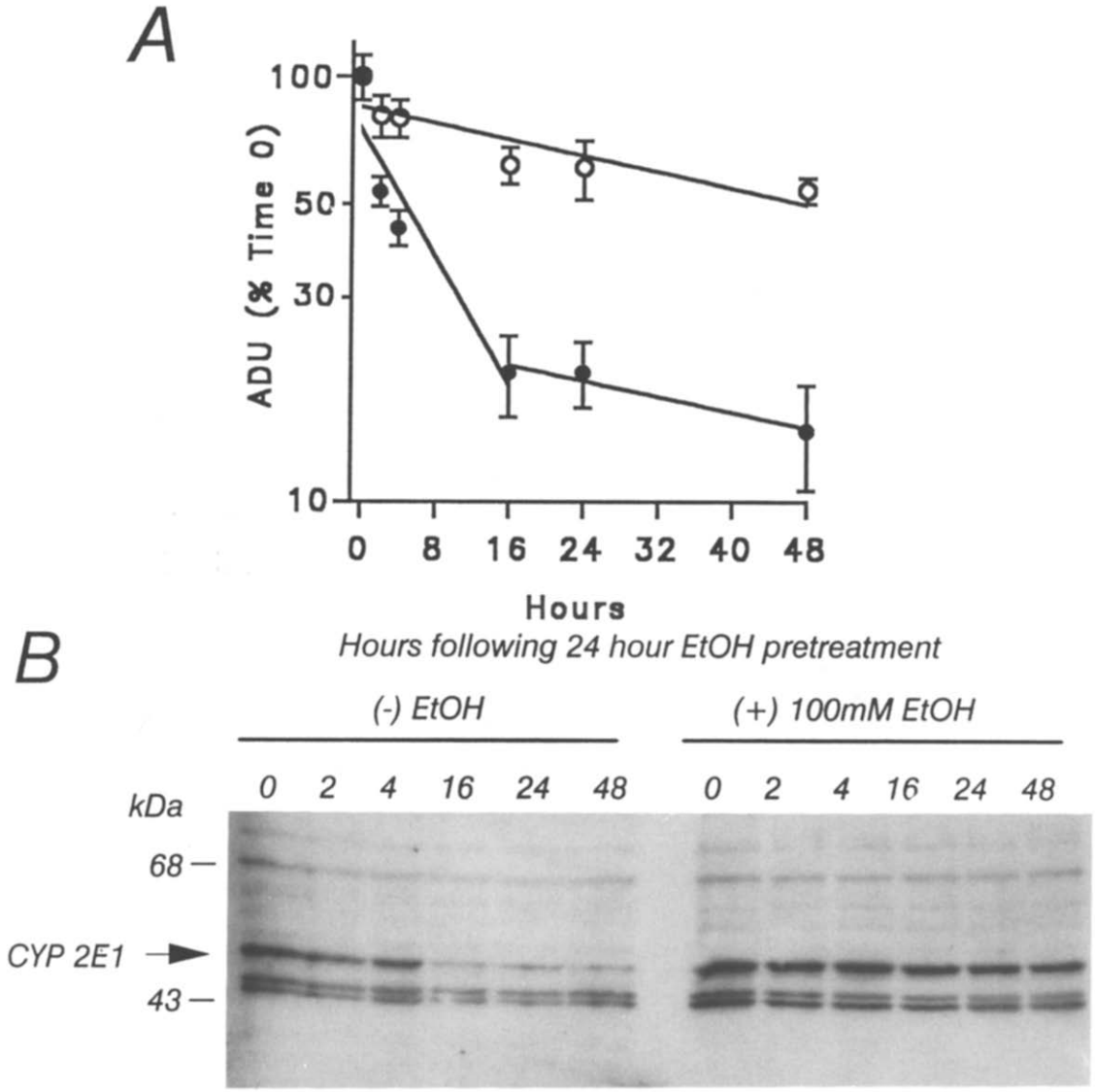

Fig. 5. Effect of ethanol on the half-life of induced CYP 2E1 apoprotein in the presence of cyclohexamide. FGC-4 cells were pre-induced for 24 hr with 100 mM ethanol. Cells were subsequently washed and treated with medium containing cyclohexamide (10 μg/mL) plus 100 mM ethanol (○) or cyclohexamide alone (●). The loss of CYP 2E1 immunoreactivity was followed by immunoblot analysis using 100 μg microsomal protein and analyzed as described in the legend to Fig. 1. (A) Data were plotted using first-order linear regression analysis (r value = 0.93) for plus ethanol values and second-order linear regression analysis (r value = 0.96) for minus ethanol values. Control (time 0) cells were assigned a value of 100%. Values are means ± SEM, N = 3. (B) Representative autoradiogram from one experiment with the migration of molecular weight standards indicated to the left in kilodaltons (kDa).

2E1 apoprotein, cells were treated with 100 mM ethanol and harvested at various times for isolation of microsomes. Immunoblot analysis from these experiments demonstrated a maximal induction of the CYP 2E1 apoprotein at 24 hr (Fig. 2). Treatment beyond 24 hr did not result in a further ethanol-induced increase in apoprotein expression (data not shown). Immunoblots on isolated microsomal protein from FGC-4 cells treated for 24 hr with increasing concentrations of ethanol revealed a concentration-dependent increase in CYP 2E1 apoprotein that was maximal at 10 mM (Fig. 3). This observation is in good agreement with our *in vivo* results in which BACs in rat of ~50–300 mg/dL (~10 mM) were high enough to consistently result in induction of CYP 2E1 apoprotein [3]. The faster and slower migrating immunoreactive signals in this figure (and subsequent figures) were observed consistently given sufficient exposure times. The identity of these signals is not absolutely known; however, the lower molecular weight signals may represent degradation products and the immediate upper band may represent a glycosylated variant of CYP 2E1 (Ingelman-Sundberg M, unpublished observation).

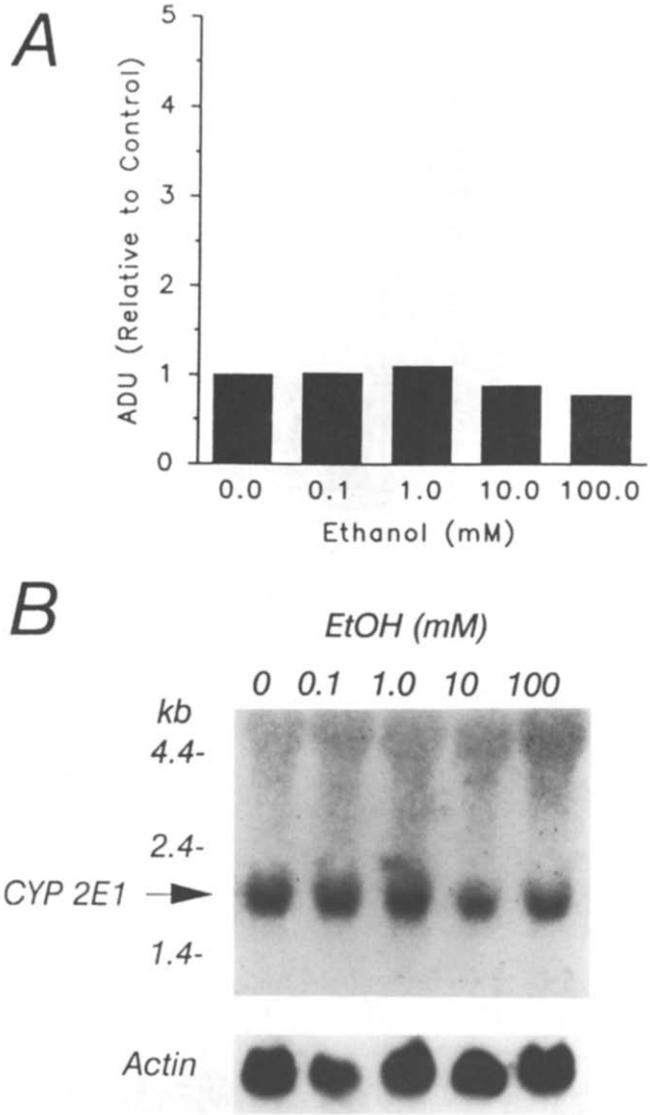

Fig. 6. Concentration–response of ethanol on CYP 2E1 mRNA levels. Total cellular RNA (40 μg) was isolated from FGC-4 cells treated for 24 hr with the indicated concentration of ethanol and subjected to northern blot analysis using a complete cDNA encoding CYP 2E1 as a probe. CYP 2E1 and actin hybridization signals were quantitated by densitometric scanning. (A) CYP 2E1 hybridization signals were normalized to values for actin and plotted. Values represent the averages of two separate experiments, and control (time 0) cells were assigned an arbitrary densitometric unit of 1.0. (B) Representative autoradiogram from one experiment with the migration of the RNA ladder indicated to the left in kilobases (kb).

After establishing the basal and ethanol-inducible expression of the CYP 2E1 apoprotein, experiments were performed to characterize the stability and half-life of the induced apoprotein. In these experiments, FGC-4 cells were pre-induced for 24 hr after which time the cells were re-exposed to either medium with 100 mM ethanol or medium alone, and the CYP 2E1 apoprotein was assessed by immunoblot analysis at various times after the 24-hr induction period. Removal of ethanol from pretreated cells demonstrated that degradation of the induced apoprotein exhibited first-order kinetics with a half-life of 12.7 hr (Fig. 4), in good agreement with previous reports using primary hepatocytes [13]. CYP 2E1 apoprotein levels remained at the elevated state in cells that were left in the presence of ethanol (data not shown). In similar experiments, ethanol was removed from pre-induced cells and treated with 10 μg/mL cyclohexamide to block protein translation. Data from these experiments revealed that in the absence of continued protein synthesis, the degradation of CYP 2E1 was biphasic and exhibited second-order kinetics with a rapid first phase half-life of 1.8 hr followed by a much slower second phase half-life of 44.7 hr (Fig. 5A, closed circles). However, if pre-induced cells translationally

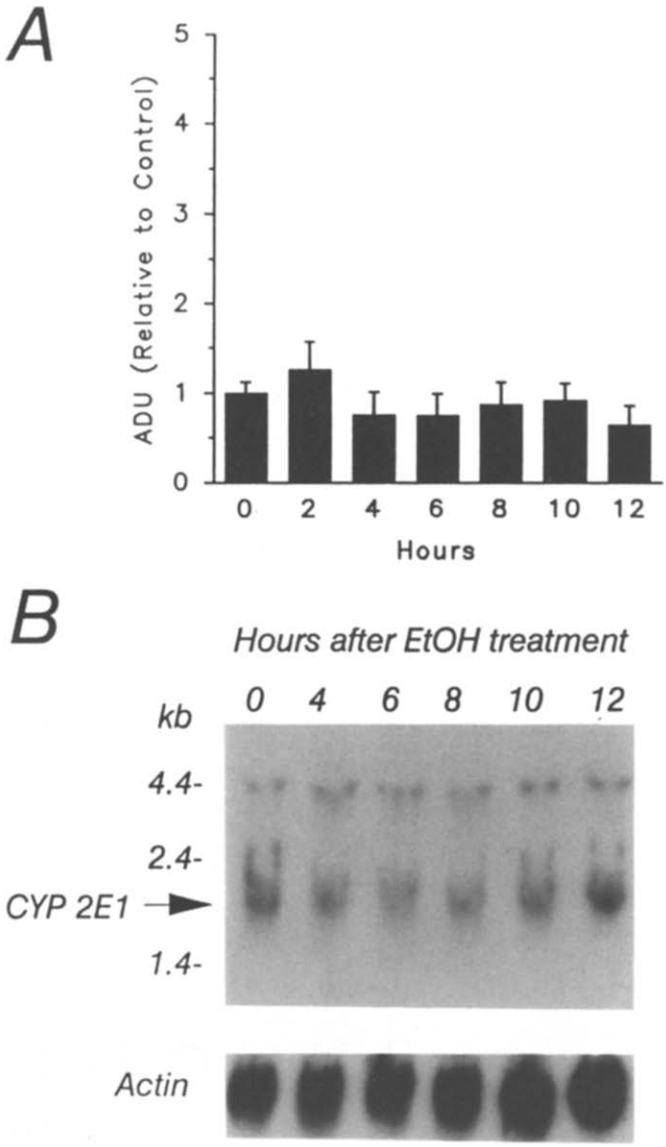

Fig. 7. Time course of ethanol treatment on CYP 2E1 mRNA levels. Total cellular RNA (40 μg) was isolated from FGC-4 cells at the indicated times following treatment without (time 0) or with 100 mM ethanol and analyzed as described in the legend to Fig. 6. (A) CYP 2E1 hybridization signals were normalized to values for actin and plotted. Values are means ± SEM, N = 3 (there were no significant differences). Control (time 0) cells were assigned an arbitrary densitometric unit of 1.0. (B) Representative autoradiogram from one experiment with the migration of the RNA ladder indicated to the left in kilobases (kb).

blocked with cyclohexamide were left in the presence of ethanol, the half-life of the CYP 2E1 apoprotein exhibited only a monophasic half-life of 45.5 hr (Fig. 5A, open circles) that closely paralleled the slow phase observed after removal of ethanol. This observation is in very close agreement with previously published observations in which acetone induced CYP 2E1 apoprotein post-translationally by ablation of the rapid turnover phase, leaving only the slow phase [19]. These combined results suggest that apoprotein induction occurs at least partially at the level of post-translational protein stabilization and establish that the FGC-4 cell line should serve as a good model to investigate the mechanisms associated with ethanol-induced CYP 2E1 apoprotein stabilization.

Characterization of steady-state mRNA levels encoding CYP 2E1 in ethanol-treated FGC-4 cells. Since ethanol was observed to induce CYP 2E1 apoprotein levels, it was of interest to determine if similar effects would be seen in steady-state mRNA levels. Northern blot analysis on total cellular RNA isolated from FGC-4 cells following a 24 hr ethanol concentration–response procedure revealed detectable levels of CYP 2E1 mRNA that remained essentially unchanged in response to ethanol (Fig.

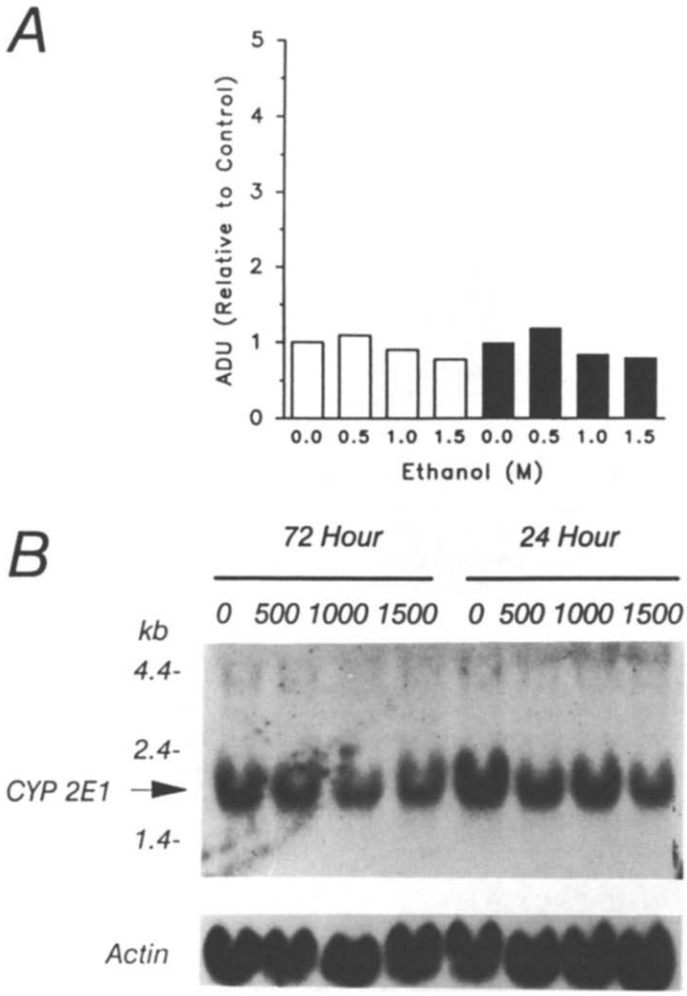

Fig. 8. Effects of high concentrations and increased time of ethanol exposure on CYP 2E1 mRNA levels. Total cellular RNA (40 μg) was isolated from FGC-4 cells treated at the indicated times and concentrations of ethanol and analyzed as described in the legend to Fig. 6. (A) CYP 2E1 hybridization signals were normalized to values for actin and plotted. Values represent the averages of two separate experiments (open bars: 72 hr and closed bars: 24 hr). Control cells were assigned an arbitrary densitometric unit of 1.0. (B) Representative autoradiogram from one experiment with the migration of the RNA ladder indicated to the left in kilobases (kb). Ethanol concentration (mM) is indicated above the blot.

6). The identity of the signal detected at 4.4 kb is unknown; however, it most likely represents precursor RNA or non-specific hybridization to 28S RNA. Furthermore, a 12-hr time course experiment using 100 mM ethanol to determine if there was a transient effect on CYP 2E1 mRNA also revealed no significant alterations in mRNA levels, as assessed by northern blot analysis (Fig. 7). Finally, there was no observed increase in CYP 2E1 steady-state mRNA levels when assessed by northern blot analysis on RNA isolated from FGC-4 cells treated with extremely high levels of ethanol (500, 1000, 1500 mM) for both 24 and 72 hr (Fig. 8).

DISCUSSION

CYP 2E1 is the principal component of the MEOS [1, 2]. The importance of the role played by CYP 2E1 in ethanol metabolism is underscored by its significant inducibility by alcohol. It has long been known *in vivo* that alcohol is capable of increasing CYP 2E1 apoprotein levels [17, 20, 21]. More recently, it has been demonstrated *in vivo* that high BACs resulting from chronic ethanol treatment are capable of increasing steady-state mRNA levels, at least in part, at the level of increased gene transcription [3, 15]. Combined, these observations led us to propose a model of CYP 2E1 induction comprised of two distinct steps. Step one of this model occurs *in vivo* at BACs below 300 mg/dL and is associated with an increase in CYP 2E1 apoprotein levels and enzyme activity in the absence of increased steady-state levels of CYP 2E1 mRNA. Step two occurs *in vivo* at BACs greater than 300 mg/dL and

is associated with an increase in CYP 2E1 gene transcription and mRNA levels and an even further elevation in CYP 2E1 apoprotein levels and enzyme activity. Unfortunately, little is known about the mechanisms underlying these events. Progress has been impeded because of the necessity to carry out these experiments *in vivo*. Primary hepatocyte cultures are difficult because of high inter-experimental variation and continuous loss of liver-specific function. Established and stable cell lines have proven invaluable in studying several molecular mechanisms within a given cell type. However, in the process of establishing stable cell lines, many of them "dedifferentiate" or lose some of the phenotype associated with fully differentiated cells. This observation has proven to be the case for CYP 2E1 (as well as other cytochromes P450) expression in hepatocyte or hepatoma cell lines. Our laboratory has screened numerous hepatocyte cell lines, and we have not been able to detect CYP 2E1 apoprotein (unpublished observations). To our knowledge there have been no literature reports of hepatic cell lines that express CYP 2E1, an observation that is consistent with the notion that CYP 2E1 expression serves as a suitable marker for well differentiated hepatocytes.

In this study, we demonstrated that CYP 2E1 is expressed in the rat hepatoma FGC-4 cell line. We originally focused our attention on these cells because Angrand *et al.* reported mRNA expression of cytochrome P450 2B1 as an assessment criteria for liver-specific function [16]. We report here that this cell line expressed CYP 2E1 and that ethanol was capable of inducing the apoprotein 5-fold in a concentration-dependent fashion. We also demonstrated that the induced CYP 2E1 apoprotein had a half-life of 12.7 hr upon removal of ethanol (in untreated FGC-4 cells the amount of immunodetectable CYP 2E1 is low, making it difficult to study degradation of the uninduced protein). This observation is in close agreement with Eliasson *et al.* [13], who demonstrated in microsomes isolated from untreated cultures of primary hepatocytes that the CYP 2E1 apoprotein half-life was ~9 hr. These investigators further report that in the presence of inducer (1-butanol), the half-life is extended to greater than 24 hr. In FGC-4 cells left in the presence of ethanol, CYP 2E1 levels remained elevated up to 48 hr (data not shown).

To better address the kinetics of degradation, we performed half-life experiments in the presence of cyclohexamide to block continued protein synthesis. These experiments revealed that upon removal of ethanol, the induced CYP 2E1 apoprotein exhibited a biphasic degradative pathway consisting of a rapid first phase with a half-life of 1.8 hr, followed by a much slower second phase with a half-life of 44.7 hr. However, in the presence of both cyclohexamide and ethanol, degradation of the apoprotein exhibited a monophasic decay in which the rapid first phase was ablated leaving only a slower phase with a half-life of 45.5 hr. This finding is in excellent agreement with the results of Song *et al.* [19], who demonstrated *in vivo* that in control rats, hepatic microsomal CYP 2E1 apoprotein turnover is biphasic with a rapid ~7-hr phase coupled to a slower ~37-hr phase and, furthermore, that treatment of rats with acetone completely ablated the rapid phase, leaving only the slower phase.

The observed increase in ethanol-inducible CYP 2E1 apoprotein in FGC-4 cells was not associated with an increase in CYP 2E1 mRNA levels. This finding is consistent with results using *in vivo* or primary hepatocyte experiments from several laboratories that have observed induction of CYP 2E1 apoprotein without increased mRNA levels [10–13, 20], as well as our laboratory in rats with BACs lower than 300 mg/dL [3]. It should be pointed out, however, that this observation is in contrast to other groups that have demonstrated *in vivo* ethanol-induced CYP 2E1 apoprotein and mRNA using rats pair-fed on the Lieber–DeCarli diet [17, 22, 23] and by our group using the total enteral nutrition model for rats with BACs over 300 mg/dL (the second step of the aforementioned two-step induction model). Thus, while FGC-4 cells appear to be an excellent model of step-one induction of CYP 2E1, these cells do not behave similarly to *in vivo* models with respect to step-two induction.

In summary, the effects of ethanol on CYP 2E1 in the FGC-4 cell line described in this study agree well with many of the observations made by others using primary hepatocyte cultures and some *in vivo* models. Based on these findings, the FGC-4 cells may become a valuable model in which to investigate mechanisms of induction of CYP 2E1 apoprotein in the absence of increases in steady-state mRNA levels, as well as basal transcriptional regulation of the CYP 2E1 gene.

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