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Expression and Distribution of Cytochrome P450 Enzymes in Male Rat Kidney: Effects of Ethanol, Acetone and Dietary Conditions

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ABSTRACT. Ethanol, acetone, diet and starvation, known modulators of the hepatic cytochrome P450 (CYP)-dependent microsomal monooxygenase system, were assessed for their effects on cytochrome P450 isozyme content and monooxygenase activities in the male rat kidney. In acute experiments, rats were either treated with acetone, fasted or given a combination of the two treatments. Acetone treatment alone induced CYP2E1-dependent *p*-nitrophenol hydroxylase activity in kidney microsomes by 8-fold. This was accompanied by a 6-fold increase in CYP2E1 apoprotein as determined by Western blot analysis. There was, however, no significant increase in steady-state levels of CYP2E1 mRNA as measured by Northern blot analysis. Starvation also induced CYP2E1 apoprotein in the kidney and, as has been reported previously in the liver, had a synergistic inductive effect with acetone. CYP2B and CYP3A apoproteins were also induced by acetone, starvation and starvation/acetone combinations in the kidney. Immunohistochemical analysis revealed localization of CYP2E1 and CYP2B principally in the cortex associated with tubular cells. This distribution was maintained upon starvation/acetone treatment. Two induction experiments were performed in which the ethanol was administered as part of a system of total enteral nutrition (TEN). A short-term study was conducted in which ethanol was administered for 8 days in two liquid diets of different composition, and a chronic experiment was performed in which ethanol was administered for 35 days. A diet-independent 6-fold increase in CYP2E1 apoprotein was observed in the short-term experiment. Expression of CYP3A and CYP2A cross-reactive apoproteins in kidney microsomes appeared to be affected by alterations in diet but, were unaffected by ethanol treatment. In the chronic 35-day ethanol exposure experiment, CYP2E1 apoprotein was also elevated 6-fold and this was found to be accompanied by a significant 3-fold increase in CYP2E1 mRNA. In the same study, no ethanol effects were apparent on expression of CYP2B and CYP3A apoproteins. Thus, acetone induced a variety of renal cytochrome P450 forms in addition to CYP2E1, while ethanol appeared to be a much more specific renal CYP2E1 inducer. Furthermore, as reported in the liver, acetone and ethanol appeared to induce CYP2E1 in the kidney by different mechanisms. BIOCHEM PHARMACOL **55**;2:123–129, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. kidney; cytochrome P450; ethanol; diet; acetone; starvation

The inductive effects of ethanol on oxidative drug metabolism have been intensively studied over the past 20 years. The major enzymes involved in these reactions are the cytochrome P450[‖] (CYP)-dependent microsomal monooxygenases which are located primarily in the liver. The principal ethanol-inducible enzyme of rat, hepatic cytochrome P450 CYP2E1, has been purified in a number of laboratories [1–4] and its regulation described in detail [5–8]. CYP2E1 is the major component of the microsomal ethanol-oxidizing system (MEOS) and is capable of oxidizing a large variety of small organic molecules including acetone, carbon tetrachloride, aniline and acetaminophen [1, 2, 5, 9, 10]. MEOS is also the major enzyme system involved in the activation of nitrosamines such as *N*-nitrosodimethylamine (NDMA) to carcinogenic metabolites [11–13].

A number of compounds other than alcohol induce CYP2E1 in the liver, and one of the most studied is acetone [1, 6]. Acetone is also an excellent inducer of another hepatic P450 isozyme, CYP2B1 [1, 6]. By contrast, ethanol is reported to be either a noninducer [14, 15] or only a weak inducer of CYP2B1 [1].

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‖ *Abbreviations:* CYP, cytochrome P450; MEOS, microsomal ethanol-oxidizing system, NMDA, *N*-nitrosodimethylamine; TEN, total enteral nutrition.

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CYP2E1 has been suggested to have a physiological role in gluconeogenesis through the metabolism of ketone bodies [5, 16]. Hepatic CYP2E1 and CYP2B1 have been demonstrated to be regulated by diet [14, 17–19] and to be induced by conditions such as starvation and diabetes that are accompanied by ketosis [1, 5, 6]. The mechanisms whereby starvation and acetone regulate hepatic CYP2E1 and CYP2B1 appear to be different, since a combination of the two treatments results in synergistic induction of the two P450 isozymes [1].

Much less is known about the effects of ethanol, acetone, diet and starvation on extrahepatic expression of CYP2E1 or other P450-dependent monooxygenases. These extrahepatic systems may not play a major role in drug clearance; however, they may be of importance in the local activation of carcinogens and in organ-specific toxicity. For example, CYP2E1 in the kidney have been implicated in the nephrotoxicity of acetaminophen [20], and alterations in other renal P450 enzymes have been associated with cyclosporin nephrotoxicity [21]. CYP2E1 is present in kidney microsomes at approximately 5% of the concentration in rat liver microsomes and has been reported to be induced by isoniazid [12], acetone [13], diethyl ether [22], pyridine [23], ethanol [24] and starvation [13, 22]. However, it is not known if the mechanism of induction of renal cytochrome P450 enzymes by ethanol and acetone is the same as that of the hepatic enzymes, or if diet and physiological regulation of extrahepatic P450s is identical to that in the liver. In addition, cellular distribution of P450 enzymes is heterologous in different tissues. In the liver, CYP2E1 and many other P450s are only expressed in perivenous hepatocytes [25]. In the colon, CYP2E1 expression is restricted to crypt cells [26]. It is not known if renal P450 enzymes are also expressed in a cell-specific fashion or if this cellular distribution is altered during induction.

In order to address these questions, we developed a rat model to study the interactive effects of ethanol and diet on the tissue-specific regulation of cytochrome P450 enzymes. Ethanol was administered as part of a chemically defined liquid diet utilizing a total enteral nutrition (TEN) delivery system [14, 15]. In the current study, we examined the effects of starvation, acetone, two liquid diets, short-term and chronic ethanol treatment (8 days and 35 days at 35% of calories) in the TEN model on a number of microsomal monooxygenase activities and cytochrome P450 apoproteins in male rat kidney, including: CYP2E1, CYP2B, CYP3A, and CYP2A. In addition, we examined the cellular distribution of CYP2E1 and CYP2B apoprotein expression in the kidney using immunohistochemical analysis.

MATERIALS AND METHODS

Experiment 1

Male Sprague–Dawley rats (180 g, $n = 4$/group) were induced by acetone treatment, starvation or a combination of the two as described by Johansson et al. [1]. Acetone (5 mL/kg/day) was administered by gavage as a 30% solution in saline for two days. Starvation consisted of the removal of food for 72 hr with ad lib access to water.

Experiment 2

Male Sprague-Dawley rats (350 g, $n = 6$/group) were implanted with chronic indwelling gastric cannulae which were attached to a spring and swivel allowing the rats free movement as described previously [14]. After 7 days to recover from surgery, one group of animals was infused intragastrically with saline and allowed ad lib access to standard rat chow and water (operated controls). The remaining groups were infused with chemically defined liquid diets (TEN) with or without ethanol substitution for carbohydrate to a level of 35% of calories as described previously [14]. Two groups were infused with Diet A (a low-fat diet in which free amino acids form the principal source of nitrogen) [14], and two groups were infused with Diet B (a high-fat diet using peptides as a nitrogen source) [14]. Both diets were infused at a rate of 3 mL/hr, allowing administration of a mean of 167 kcal/kg$^{0.75}$/day. In the ethanol-treated groups (Diet A + ethanol and Diet B + ethanol), carbohydrate calories were replaced by ethanol starting at 10% of calories and increased 5% each day to a final level of 35% calories. The animals were killed after 8 days.

Experiment 3

Groups of 6–8 Sprague–Dawley rats (300 g) were infused a previously described TEN diet with or without ethanol for 35 days [15]. Blood alcohol concentrations were measured using an assay kit from Sigma and microsomes were prepared from kidneys by differential ultracentrifugation [27]. Kidney microsomes were assayed for monooxygenase activities using the following P450 selective substrates: *p*-nitrophenol (CYP2E1) [28], lauric acid (CYP4A) [29] and androstenedione (CYP 2C11, CYP 2A1 and CYP 3A) [14, 15] and were probed in Western blot analysis with rabbit polyclonal antibodies directed against rat liver cytochrome P450 enzymes CYP2E1 [1], CYP2B1, CYP3A2 and CYP2A (gifts from Dr. Anders Åström, Huddinge, Sweden) [30], using a ^{125}I-labelled goat anti-rabbit secondary antibody [14]. Immunoquantitation of the Western blots was accomplished by densitometric scanning of the resulting autoradiographs. Immunohistochemistry of kidney sections was conducted using anti-CYP2E1 and -CYP2B1 antibodies by the method of Hsu *et al.* [31]. Northern blot analysis of kidney CYP2E1 mRNA was conducted as described previously [15] using a full-length cDNA probe directed against rat CYP2E1 (gift from Dr. Frank Gonzalez, Bethesda, MD).

All of these assays were extremely reproducible with CV $< 10\%$ for replicates. All the data were analyzed by one-way ANOVA and compared with control groups using Dunnett's post hoc analysis with $P < 0.05$ considered significant.

TABLE 1. Effect of acetone and starvation on expression of renal CYP2E1, CYP2B and CYP3A[d]

	CYP2E1		CYP2B		CYP3A
Treatment	pNP[e]	Western	Northern	Western	Western
Control	104 ± 16	100 ± 60[d]	100 ± 35[d]	100 ± 10[d]	100 ± 5[d]
Acetone	820 ± 93[a]	630 ± 110[a]	134 ± 20	335 ± 90[a]	270 ± 50[a]
Starvation	1010 ± 186[a]	900 ± 360[a]	543 ± 80[a]	254 ± 44[a]	350 ± 100[a]
S/Ac	1400 ± 269[a,b,c]	25,500 ± 200[a,b,c]	245 ± 30[a]	595 ± 200[a]	575 ± 50[a,b]

[a] Significant at $P < 0.05$ vs. operated control.
[b] Significant at $P < 0.05$ vs. acetone-treated group.
[c] Significant at $P < 0.05$ vs. starvation group.
[d] Values are means ± SEM of arbitrary densitometric units expressed as percent of controls for $n = 4$ animals/group. S/Ac, starvation plus acetone.
[e] pmol/mg microsomal protein/min, mean ± SEM.

RESULTS

Effects of Acetone, Starvation and Acetone Plus Starvation

Cytochrome P450 CYP2E1 activity was found in kidney microsomes of untreated male rats at a level of 10–20% that found in liver microsomes. Hydroxylation of the CYP2E1-dependent substrate *p*-nitrophenol occurred at a mean value of 0.10 nmol/mg microsomal protein/min in kidney microsomes and 0.6 nmol/mg/min in liver microsomes. A cross-reactive protein band of identical molecular weight to hepatic CYP2E1 was observed in Western blot analysis of kidney microsomes with an antibody monospecific for rat liver CYP2E1. The effects of acetone, starvation and starvation plus acetone on kidney microsomal *p*-nitrophenol hydroxylation, CYP2E1 apoprotein and CYP2E1 steady-state mRNA levels are summarized in Table 1. Acetone induced an 8-fold increase in CYP2E1-dependent *p*-nitrophenol hydroxylase activity and a 6-fold increase in renal CYP2E1 apoprotein levels. However, no significant effects were observed on expression of renal CYP2E1 mRNA. Renal expression of CYP2E1 in starved rats was approximately equal to that of acetone-treated animals. Starvation plus acetone treatment synergistically induced renal CYP2E1 apoprotein expression by over 20-fold. Western blot analysis with antibodies directed against two other P450 enzymes, rat hepatic CYP2B1 and CYP3A2, revealed cross-reactive apoproteins in renal microsomes from untreated animals at the same molecular weight as seen in the liver. Signals with both antibodies were significantly increased following starvation and acetone treatment. Starvation and acetone treatments combined appeared to be additive on expression of both these P450 enzymes (Table 1).

Immunohistochemical Localization of CYP2E1 and CYP2B in the Kidney

Immunohistochemical analysis of fixed kidney slices with CYP2E1 and CYP2B1 antisera revealed localization of cross-reactivity to both P450 enzymes predominantly in the kidney cortex (Fig. 1). Preimmune sera showed no signal (data not shown). Staining was observed in cortical tubules, but not in the medulla. Differential staining with the two antisera was observed only in the glomeruli. Following starvation plus acetone treatment, localization of CYP2E1 and CYP2B in the kidney remained essentially unchanged.

Effect of Diet and Short-Term Ethanol Treatment

Eight days of ethanol treatment at 35% calories in the TEN model [14] resulted in a diet-independent, 6-fold induction of CYP2E1 in kidney microsomes (Table 2, Fig. 2). Examination of cross-reactive bands with antibodies to rat hepatic CYP3A2 and CYP2A in Western blot analysis revealed a significant suppression of expression of these two apoproteins with Diet B compared to operated controls. However, ethanol treatment had no effect on expression of either P450 enzyme. Metabolism of androstenedione and lauric acid by kidney microsomes from these animals is presented in Table 3. Significant hydroxylation of androstenedione occurred at positions 16α-, 6β- and 7α-; however, no significant effects of diet or ethanol were observed on any of these activities. Lauric acid was also hydroxylated by renal microsomes at positions 11 (ω-1) and 12 (ω) in a ratio of 1:2. Again, diet and ethanol-treatment had no effect on these parameters.

Effect of Chronic Ethanol Treatment

At the beginning of the experiment, the mean body weight of the TEN group was 291 ± 3 g and that of the ethanol group was 289 ± 4 g. At sacrifice the body weights were 342 ± 4 and 324 ± 6 g, respectively. Urine alcohol levels were monitored throughout the experiment and as we have previously reported [32] were found to cycle between 0 and greater than 500 mg/dL (data not shown). At the time of sacrifice, blood alcohol concentrations in the ethanol-treated group were all greater than 300 mg/dL. Results of Western immunoblot and Northern blot analysis are shown in Table 4. CYP2E1 apoprotein was induced 6-fold ($P < 0.005$) by chronic ethanol treatment, and this was accompanied by a significant 3-fold increase ($P < 0.005$) in CYP2E1 mRNA. In contrast, no effects of chronic ethanol treatment were observed on expression of CYP2B or CYP3A apoproteins in kidney microsomes.

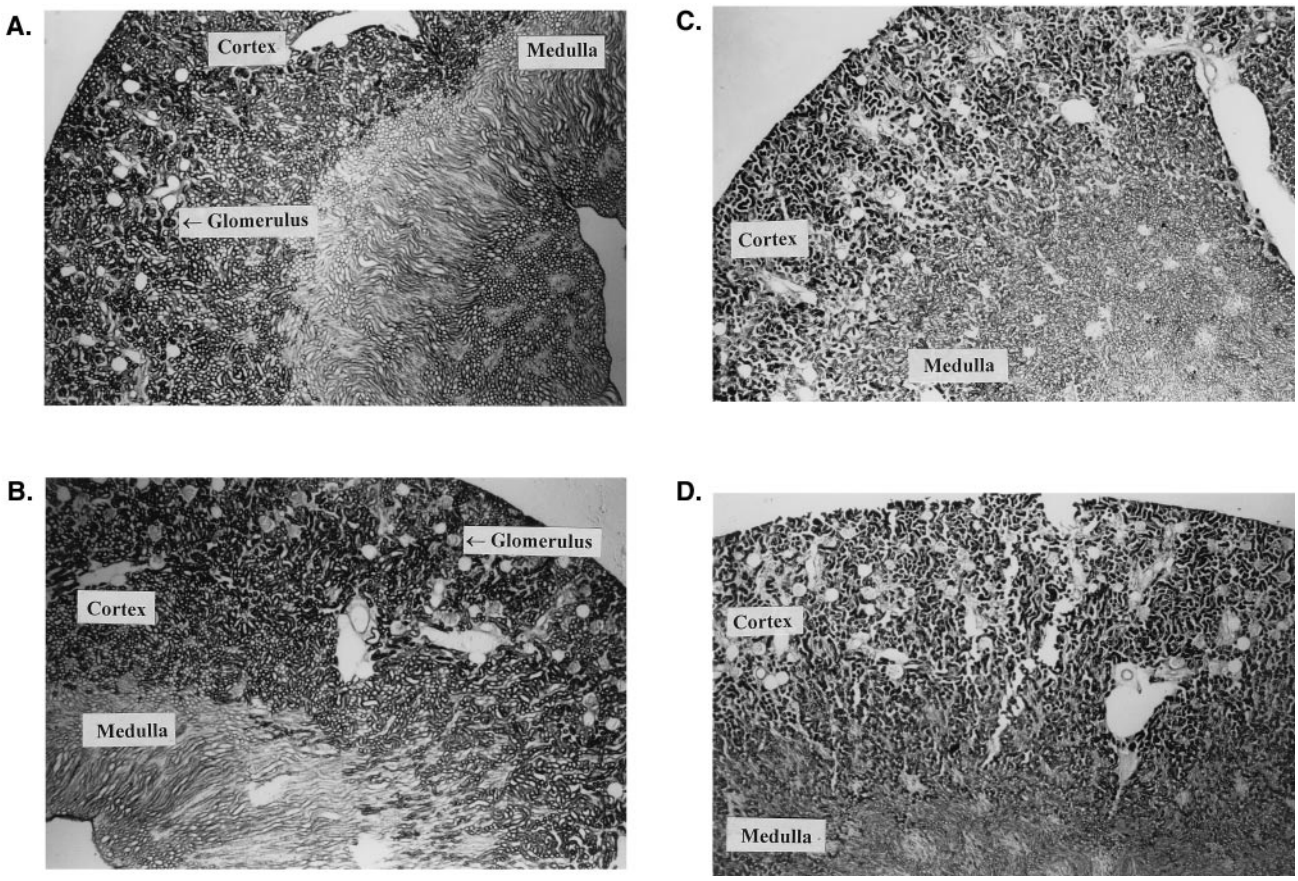

FIG. 1. Immunohistochemical localization of CYP2E1 and CYP2B in rat kidney. CYP2B1 (A) and CYP2E1 (B) in untreated rat kidney; CYP2B1 (C) and CYP2E1 (D) in rat kidney following treatment with a combination of starvation and acetone.

DISCUSSION

In this study we have examined the cytochrome P450-dependent microsomal monooxygenase system of the male rat kidney and the response of this system to conditions known to modulate hepatic drug metabolism, namely diet and starvation, acetone and ethanol treatment.

TABLE 2. Effect of diet and short-term ethanol treatment on expression of renal CYP2E1, CYP3A and CYP2A apoproteins[d]

Treatment	CYP2E1	CYP3A	CYP2A
Operated Control	100 ± 21	100 ± 28	100 ± 6
Diet A	143 ± 14	93 ± 27	106 ± 23
Diet A + EtOH	681 ± 80[a]	110 ± 14	189 ± 24
Diet B	124 ± 17	35 ± 6[b]	63 ± 4[b]
Diet B + EtOH	642 ± 72[c]	46 ± 3	63 ± 6

Diet A: low-fat, free amino acid nitrogen source, Diet B: high-fat, short chain peptide nitrogen source [14].

[a] Significant at $P < 0.005$ Diet A + EtOH vs. Diet A.

[b] Significant at $P < 0.05$ Diet B vs. operated control.

[c] Significant at $P < 0.005$ Diet B + EtOH vs. Diet B.

[d] Values are means ± SEM arbitrary densitometric units expressed as percent of controls for $n = 6$ animals/group.

Cellular Localization and Regulation of Renal CYP2E1

Immunohistochemical analysis with antibodies to CYP2E1 revealed a heterologous distribution of this enzyme within the kidney with expression principally limited to the kidney cortex. CYP2E1 was present in proximal and distal tubular cells. This cell-specific distribution was maintained follow-

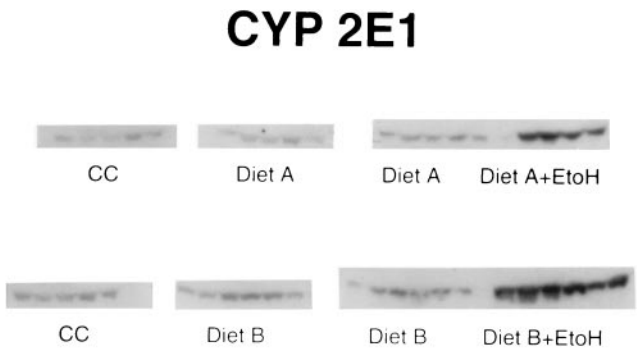

FIG. 2. Western blot analysis of CYP2E1 apoprotein expression in rat kidney. Data presented as blots of kidney microsomes from individual animals loaded at 25 μg protein/well. CC = operated chow control, Diet A, Diet B = liquid diets given as total enteral nutrition for 8 days [14], Diet A + EtOH, Diet B + EtOH = different enteral diets with ethanol substitution for 35% of calories for 8 days as described previously [14].

TABLE 3. Effect of diet and short-term ethanol on renal metabolism of androstenedione and lauric acid[a]

	Androstenedione hydroxylation[b]			Lauric acid hydroxylation[b]	
Treatment	16α	6β	7α	(ω-1)-OH	ω-OH
Operated control	115 ± 49	190 ± 47	178 ± 49	301 ± 19	603 ± 36
Diet A	177 ± 62	162 ± 35	135 ± 28	236 ± 18	464 ± 52
Diet A + EtOH	104 ± 45	168 ± 36	159 ± 42	308 ± 10	625 ± 23
Diet B	165 ± 52	171 ± 41	149 ± 29	312 ± 27	617 ± 40
Diet B + EtOH	138 ± 39	163 ± 40	141 ± 48	339 ± 26	590 ± 59

[a] Data presented as mean ± SD of assays using renal microsomes from $n = 5$ animals/treatment group. 16α-OH (CYP2C11), 6β-OH (CYP3A), 7α-OH (CYP2A1), ω-OH(CYP 4A), (ω-1)-OH (CYP2E1 in human liver).

[b] pmol product formed/mg/min.

ing induction. With the exception of diet and diet/ethanol interactions, regulation of CYP2E1 expression in the kidney resembled that observed in liver. Both ethanol- and acetone-induced renal CYP2E1 and a combination of starvation and acetone treatment resulted in synergistic induction to levels over 20-fold those seen in control renal microsomes. This is identical to the response previously reported for hepatic CYP2E1 [1]. As described previously in liver renal induction of CYP2E1 by acetone appeared to be via post-transcriptional mechanisms, since no concomitant effects on CYP2E1 steady-state mRNA expression were observed [1, 6, 16]. Our data are in contrast to a recent report in which another CYP2E1 inducer, pyridine, was examined in rat liver and kidney [23]. Whereas pyridine induction of hepatic CYP2E1 was reported to be entirely post-transcriptional, induction of CYP2E1 in renal microsomes was reported to be accompanied by significant increases in CYP2E1 mRNA levels. Although we previously reported a suppression of hepatic CYP2E1 expression in rats fed TEN diet B and a differential induction response to short-term ethanol treatment in animals receiving different TEN diets [14], expression of renal CYP2E1 was not significantly affected by diet and was induced to the same degree by 8-day ethanol treatment. Chronic ethanol treatment for 35 days using the TEN model resulted in a similar level of induction of renal CYP2E1 as 8-day treatment and identically to our previous observations in liver, was accompanied by a significant elevation in CYP2E1 mRNA levels [15, 33, 34].

Other Renal Cytochrome P450 Enzymes

Cross-reactivity in kidney microsomes was observed with antibodies directed against hepatic CYP2B1, CYP3A2 and CYP2A. In addition, significant activities were observed with a steroid substrate, androstenedione, and with lauric acid, a substrate for the P450 enzyme CYP4A1. The cellular distribution of renal CYP2B apoprotein was similar to that of CYP2E1, being localized primarily to the proximal and distal tubular cells of the kidney cortex. CYP2B, however, was also present in the glomeruli. CYP2B and CYP3A enzymes were found to be inducible by acetone, starvation and combinations of the two. This was previously described for CYP2B1 in the liver [1]. In agreement with our observations in hepatic microsomes, chronic ethanol treatment had no effect on expression of renal CYP2B apoprotein. Expression of renal CYP2A and CYP3A apoproteins was observed to be significantly affected by manipulations of the TEN diets. However, no significant dietary effects were observed on the rates of hydroxylation of androstenedione at positions 6β- or 7α-, activities reported to be specific for hepatic CYP3A and CYP2A [35]. It is possible that other renal cytochrome P450 enzymes contribute significantly to these steroid hydroxylations or that the substrate specificity of renal CYP3A and CYP2A enzymes is different from that of hepatic forms. Also in contrast to the liver [14, 15], no significant effects of ethanol treatment were observed on expression of renal CYP3A apoproteins. In addition, no significant effects of diet or ethanol were observed on lauric acid hydroxylation by kidney microsomes at either the ω or ω-1 position. This is interesting in light of recent identification of lauric acid ω-1 hydroxylation as an activity catalyzed by human CYP2E1 [36] and induction of CYP 4A (lauric acid ω-hydroxylase) by ethanol in liver and colon microsomes from ethanol-treated rats [26, 37].

In conclusion, the above data suggest that diet, dietary restriction such as starvation, and ketone bodies such as

TABLE 4. Effect of chronic ethanol treatment on renal CYP2E1, CYP2B and CYP3A[b]

Treatment	pNP[c]	CYP2E1 apoprotein	CYP2E1 mRNA	CYP2B1 apoprotein	CYP3A apoprotein
TEN	378 ± 197	100 ± 16	100 ± 9	100 ± 11	100 ± 7
TEN + EtOH	4816 ± 262[a]	557 ± 44[a]	275 ± 40[a]	77 ± 13	111 ± 8

[a] Significant at $p < 0.005$ vs. TEN group.

[b] Data are arbitrary denistometric units expressed as % TEN control, mean ± SEM for $n = 10$ animals/group.

[c] pmol/mg/min, mean ± SEM.

Ethanol treatment in the TEN model at 35% calories for 35 days.

acetone can affect the expression of a number of P450 enzymes in the kidney. This in turn may affect local activation/detoxification patterns of nephrotoxins or carcinogens. By contrast, ethanol consumption selectively induces renal CYP2E1. This P450 enzyme may be the most important factor underlying ethanol-enhanced nephrotoxicity of compounds such as acetaminophen [20].

The mechanism of CYP2E1 induction appears to be inducer-dependent. In both liver and kidney, acetone induces via a purely posttranscriptional mechanism probably involving posttranslational stabilization of the CYP2E1 apoprotein and alterations in translational efficiency [1, 6–9, 16, 31], whereas ethanol induction of both renal and hepatic CYP2E1 involves an additional transcriptional component when blood ethanol concentrations exceed a threshold value of 200–300 mg/dL [31, 32].

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