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cDNA sequence, deduced amino acid sequence, predicted gene structure and chemical regulation of mouse Cyp2e1

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The cDNA encoding the mouse Cyp2e1 protein has been isolated and sequenced, and shown to share 92%, 79%, 80% and 79% sequence similarity over the coding region with rat, human, rabbit 1 and rabbit 2 *CYP2E1* cDNA sequences respectively. The predicted Cyp2e1 protein contains 493 amino acids, with a molecular mass of 56781 Da. The protein contains many features common to other cytochrome *P*450s, including a potentially phosphorylatable serine residue at position 129 within a canonical cyclic AMP-dependent protein kinase site. Southern blot analysis of genomic DNA prepared from C57BL/6 and DBA/2N mice suggests the presence of only a single *Cyp2e1* gene. The *Cyp2e1* gene was isolated and its organization was established by PCR using oligonucleotides to its predicted intron/exon boundaries. These results showed that the mouse *Cyp2e1* gene is approx. 11000 bp in length and has a similar structure to the human and rat *CYP2E1* genes. Cyp2e1 protein expression was studied in a variety of tissues and a sexual dimorphism in its levels in some tissues was noted. Acetone treatment induced the Cyp2e1 protein in all of the tissues studied in both sexes, but this Cyp2e1 protein induction was not accompanied by an increase in Cyp2e1 mRNA levels. Indeed, mRNA levels were seen to be decreased on treatment, suggesting that acetone administration affects either mRNA translation efficiency or protein stability. Of a wide range of drugs known to modify other cytochrome *P*450 levels only diethylnitrosamine had a significant effect on Cyp2e1, causing a decrease in protein levels.

INTRODUCTION

The cytochrome *P*450 mono-oxygenases (*P*450s) represent a large group of related enzymes encoded by a multigene family distributed widely in many species. The genes can be grouped according to sequence relatedness into several families (Nebert *et al.*, 1991). In vertebrates, the members of some families (e.g. 4, 11 and 21) encode proteins involved in the catalysis of very specific reactions, e.g. in steroid hormone biosynthesis and metabolism. Other families (1, 2 and 3) encode proteins displaying a much broader range of substrate specificity and appear to have evolved in order to metabolize environmental lipophilic xenobiotics to facilitate their removal from the cell. The capacity of these groups of *P*450s to activate a broad range of foreign compounds has been intimately linked with the process of chemical carcinogenesis. Cytochrome *P*450 nomenclature (Nebert *et al.*, 1991), molecular biology (Gonzalez, 1990), evolution (Nelson & Strobel, 1987; Gonzalez & Nebert, 1990), purification (Astrom & DePierre, 1986; Ryan & Levin, 1990), metabolism (Juchau, 1990) and role in carcinogenesis (Wolf, 1986; Guengerich, 1988) have been extensively reviewed.

Certain *P*450s appear to be part of an adaptive response system to chemical challenge, and exposure to a foreign compound may thus induce a *P*450 active in its metabolism (reviewed in Okey, 1990). In this regard, members of the CYP2E1 subfamily were shown to be active in the metabolism of, and induced by, a variety of organic compounds, e.g. acetone, ethanol and chloroform (reviewed in Yang *et al.*, 1990). In addition, they are also seen to be the major enzymes responsible for the activation of several carcinogenic nitrosamine compounds, e.g. *N*-nitrosodimethylamine (NDMA). Nitrosamines represent a cogent carcinogenic risk: they are potent carcinogens, with broad environmental occurrence and are easily formed *in vivo* from nitrosatable amino compounds (Bartsch & Montesano, 1984). CYP2E1 proteins have also been shown to be responsible for the metabolism of several hepatotoxins, such as acetominophen, carbon tetrachloride and benzene (Koop & Coon, 1986; Lindros *et al.*, 1990). Although previously identified in the liver, CYP2E1 has also been seen to be present in the bone marrow in rabbits (Schnier *et al.*, 1989) and the brain in rats (Hansson *et al.*, 1990). In the latter case, the location of CYP2E1 in the basal ganglia, frontal cortex and hippocampus is intriguing, as these regions display biochemical and morphological changes following ethanol consumption in experimental animals.

The pathophysiological states of diabetes (both spontaneous and chemically induced) and starvation have been shown to induce CYP2E1 in rats (Past & Cook, 1982; Johansson *et al.*, 1988). Roles for circulating acetone, which is elevated in these conditions, together with hormonal influences, have been suggested in generating the induction (Miller & Yang, 1984).

It has been proposed that the reason for CYP2E1 induction by acetone and other solvents and in diabetes/starvation relates to a constitutive role for this enzyme in gluconeogenesis. CYP2E1 is suggested to play a central role in the conversion of acetone, produced by decarboxylation of acetoacetate, to acetol and methylglyoxal, which is then metabolized by glyoxylases I and II to produce D-lactate and finally pyruvate. CYP2E1 is thought to represent the acetone and acetal mono-oxygenase component of this pathway (Casazza *et al.*, 1984; Koop & Casazza, 1985; Argeles, 1986). In studies in fasting humans, figures suggested that 11% of the gluconeogenic demand could be satisfied by acetone-derived regeneration pathways, assuming that all acetone produced could be converted into glucose (Reichard *et al.*, 1979).

CYP2E1 has been purified from rat (Ryan *et al.*, 1985), rabbit (Koop *et al.*, 1982) and human (Wrighton *et al.*, 1987) sources.

Abbreviations used: NDMA, nitrosodimethylamine; i.p., introperitoneal, TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)benzene]; UTR, untranslated region.
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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ databases under the accession number X62595.

Nucleotide sequences at both the cDNA and the genomic levels have been obtained for rat and human *CYP2E1* genes (Song *et al.*, 1986; Umeno *et al.*, 1988*a,b*). In these species there is only one *CYP2E1* gene present. In rabbit, however, two genes are present (Khani *et al.*, 1987, 1988*a,b*).

Studies on the regulation and function of *P*450 in the mouse are of particular importance, as this species is genetically well characterized and advances in transgenic and gene targeting technology allow the potential generation of virtually any desired genotype. In order to investigate both the possible endogenous metabolic function and the role of CYP2E1/Cyp2e1 in carcinogenesis, the mouse *Cyp2e1* cDNA and gene were isolated. Studies on Cyp2e1 protein regulation in this species are reported.

MATERIALS AND METHODS

Nomenclature

The nomenclature used throughout is that recommended for *P*450 by Nebert *et al.* (1991).

Chemicals and reagents

All chemicals were of an analytical grade or better and were obtained from either Sigma (Poole, Dorset, U.K.) or BDH Ltd. (Glasgow, U.K.). Restriction endonucleases, T_4 DNA ligase and DNA polymerase I (Klenow fragment) were from Boehringer. *Taq* DNA polymerase was from Promega. [α-^{35}S]thio-dATP, [α-^{32}P]dCTP, ^{125}I-conjugated Protein A and nylon membranes used for DNA and RNA transfer in all techniques were from Amersham International. Oligonucleotides were made on an Applied Biosystems 380A DNA synthesizer. Autoradiography employed Kodak X-OMAT AR5 X-Ray film. RNA and DNA markers were purchased from Bethesda Research Laboratories Life Technologies.

Animals

Adult male BALB/c mice used in the cDNA library preparation were starved for 24 h. DBA/2N male and female mice used in induction studies were fed 1 % (v/v) acetone in drinking water for 14 days. C57BL/6 and DBA/2N mice used in regulation studies were treated daily for 3 days with dexamethasone [100 mg/kg, intraperitoneal (i.p.)], pyrazole (200 mg/kg, i.p.), diethylnitrosamine (200 mg/kg, i.p.), β-naphthoflavone (80 mg/kg, i.p.), 3-methylcholanthrene (200 mg/kg, i.p.), 1,4-bis[2-(3,5-dichloropyridyloxy)benzene] (TCPOBOP) (2 mg/kg, i.p.) or phenobarbital (80 mg/kg, intragastric). Other animals were also treated with aflatoxin B1 (one dose of 3 mg/kg, i.p.) and 2-acetylaminofluorene (four doses of 50 mg/kg, i.p.). After the last day of treatment, animals were killed by cervical dislocation. The organs were removed, rinsed in phosphate-buffered saline (Na_2HPO_4, 1.5 g/l; NaCl, 8 g/l; $MgCl_2$,$6H_2O$, 0.1 g/l; KH_2PO_4, 0.2 g/l; KCl, 0.2 g/l; $CaCl_2$,$2H_2O$, 0.132 g/l) and frozen in liquid nitrogen. These tissues were maintained at −70 °C.

Isolation and nucleotide sequencing of clones

A full-length human *CYP2E1* cDNA clone (Song *et al.*, 1986) was used to screen an adult male C57BL/6 liver cDNA library in λgt11 (Clontech, Palo Alto, CA, U.S.A.) using standard protocols (Benton & Davis, 1977). Restriction fragments for use as probes were radioactively labelled with [α-^{32}P]dCTP (3000 Ci/mmol) by nick-translation, by the method of Rigby *et al.* (1977). Positive clones were plaque-purified, DNA was prepared by the method of Grossberger (1987), and *Eco*RI inserts were cloned into pUC18 and M13 mp18/19 and sequenced. In addition, a cDNA library was constructed in λZAPII (Stratagene) using mRNA from livers of 24 h-starved adult male BALB/c mice. RNA was isolated by the guanidine thiocyanate method (Chirgwin *et al.*, 1979) and library construction was according to the manufacturer's (Stratagene) protocols using *Eco*RI/*Not*I adaptors. Clones were identified using the mouse partial *Cyp2e1* clones isolated from the commercially produced library. Single-stranded DNA for sequencing was prepared from these clones according to the manufacturer's protocols (Stratagene). The cDNA insert was cut out as an *Xba*I/*Hind*III fragment and subcloned into pTZ18R/19R multifunctional phagemids (Pharmacia LKB Biotechnology), and single-stranded DNA for sequencing was prepared according to the manufacturer's protocol. Using the mouse *Cyp2e1* cDNA, several clones were isolated from an adult male BALB/c mouse genomic library in λEMBL 3 (Clontech) and DNA was prepared. *Sal*I fragments derived from these clones were subcloned into pTZ18R/19R.

DNA sequence analysis

The Sanger dideoxy termination method was employed, using [α-^{35}S]thio-dATP (600 Ci/mmol) to sequence cloned DNA in the various vectors (Sanger *et al.*, 1977). Overlapping sequences were determined in both directions using a series of synthetic oligonucleotides. Sequences were compiled and analysed using the GCG (Devereux *et al.*, 1984) and Genejockey (Biosoft) software packages.

Microsome preparation and immunoblotting

Microsomes were prepared by differential centrifugation as described by Adams *et al.* (1985). Protein concentration was determined according to the method of Lowry *et al.* (1951). The microsomal proteins were separated by SDS/PAGE according to the method of Laemmli (1970), using 9 % separating gels. After separation the proteins were electrophoretically transferred to nitrocellulose membranes and probed with polyclonal antisera to rat *P*450s. Immunoblotting was performed as described in Towbin *et al.* (1979). These antibodies have been used previously in immunoblotting studies with mice microsomal material, and their specificity has been demonstrated (Hong *et al.*, 1989; Henderson *et al.*, 1990; Hu *et al.*, 1990). A horseradish peroxidase-labelled second antibody was used to reveal immunoreactive polypeptides using 4-chloronaphthol as the substrate, and the signal was enhanced with ^{125}I-conjugated Protein A (40 mCi/mg) with subsequent autoradiography.

Northern blotting

RNA was isolated using guanidine hydrochloride by the method of Cox (1968). The concentration and purity of the samples were measured spectrophotometrically. After electrophoresis on denaturing formaldehyde gels, RNA integrity and loading equivalence were assessed by ethidium bromide staining. Blotting and hybridization conditions were as described by Meehan *et al.* (1984). Densitometric scanning of autoradiographs was performed using a Joyce–Loebl Chromoscan 3 densitometer.

Other techniques

Genomic DNA preparation, restriction endonuclease digestion and Southern blotting were carried out according to standard protocols (Sambrook *et al.*, 1989). Membranes were hybridized at 65 °C. Washing was carried out to a final salt concentration of 0.2 × SSC/0.1 % SDS (1 × SSC = 0.15 M-NaCl/0.015 M-sodium citrate) at 65 °C. The PCR protocol employed on isolated phage DNA was as described by Innis & Gelfand (1990) using the oligonucleotide primers specified in Table 1.

Table 1. PCR oligonucleotide primers used in gene structure characterization

Region	Exon	Sequence
3′	1	CAGCTGGATTTGAAGGATAT
5′	2	TGGCCCGAAGCGCTTTGCCA
3′	2	TCCTGTGTTCCAGGAGTACA
5′	3	TTCCATGTGGGTCCATTATT
3′	3	CCTGGTGGAGGAGCTCAAAA
5′	4	CCAATCAGAAAGGTAGGGTC
3′	4	TTCTACCTGCTGAGTACTCC
5′	5	TTGTAGATAATCCGAAAAGT
3′	5	GACTGTCTCCTCATAGAGAT
5′	6	CATTGTGTACATGGGTTCTT
3′	6	GATTCTCATGAAATACCCAG
5′	7	ATAACCCTGTCAATTTCTTC
3′	7	GTGTTCCGAGGATATGTCAT
5′	8	AAGGGAGTCCAGAGTTGGAA
3′	8	CAGTGACTATTTCAAGGCGT
5′	9	GCCAGGCCTTCTCCAACACA

RESULTS AND DISCUSSION

Using the full-length human *CYP2E1* cDNA as a probe (Song *et al.*, 1986), two inserts of approx. 800 bp and 600 bp of the mouse *Cyp2e1* cDNA were cloned from a cDNA library in λgt11 constructed from an adult male C57BL/6 mouse. The 800 bp fragment (coding for exon 4 to exon 9, by comparison with published sequences from another species), was used as a probe to screen a liver library prepared in λZAPII from starved adult male BALB/c mouse liver. Three positive clones were isolated, all containing an electrophoretically identical insert of approx. 1700 bp. DNA was prepared from two of the clones and sequenced; fragments were also subcloned into pTZ18R and pTZ19R and sequenced (Fig. 1). The enzyme sequence coded for 493 amino acids, with a predicted molecular mass of 56781 Da. The mouse sequence displayed 92 %, 79 %, 80 % and 79 % identity over its nucleotide coding region, 88 %, 75 %, 75 % and 74 % identity over its total length, and 92 %, 77 % 79 % and 79 % identity at the amino acid level to the *CYP2E1* rat, human and rabbit 1 and 2 gene cDNA sequences respectively (Song *et al.*, 1986; Khani *et al.*, 1988*b*). The level of sequence conservation, particularly compared with the rat, is very interesting and suggests that the protein may play a very conserved role in the various species.

Analysis of the derived amino acid sequence in conjunction with findings gained from the crystallized bacterial CYP101 ($P450_{cam}$) and other physical techniques, such as quantitative antibody binding studies and e.p.r. spectroscopy, allow the putative assignment of structural and functional regions within the protein. The *N*-terminus of the protein is hydrophobic, and it is this portion that has been shown, in studies of other *P*450s, to span the membrane of the endoplasmic reticulum, thus holding the main bulk of the protein on the cytoplasmic side (Kemper & Szcesna-Skopura, 1989). Regions suggested to interact with the haem prosthetic group, sandwiched between the L and I α-helices in the CYP101 crystal, are indicated in Fig. 1 (∧∧∧). Two conserved clusters of charged amino acids suggested to interact with the *P*450 reductase are also indicated (∼ ∼ ∼), although it is still not clear if a defined docking region exists or a more random interaction occurs between the two proteins. The mouse sequence retains a tryptophan residue at position 122 (indicated as ··· in Fig. 1), which is conserved in all vertebrate *P*450s with its related positively charged group four residues away (arginine in the mouse Cyp2e1). In CYP101 this arginine interacts with a

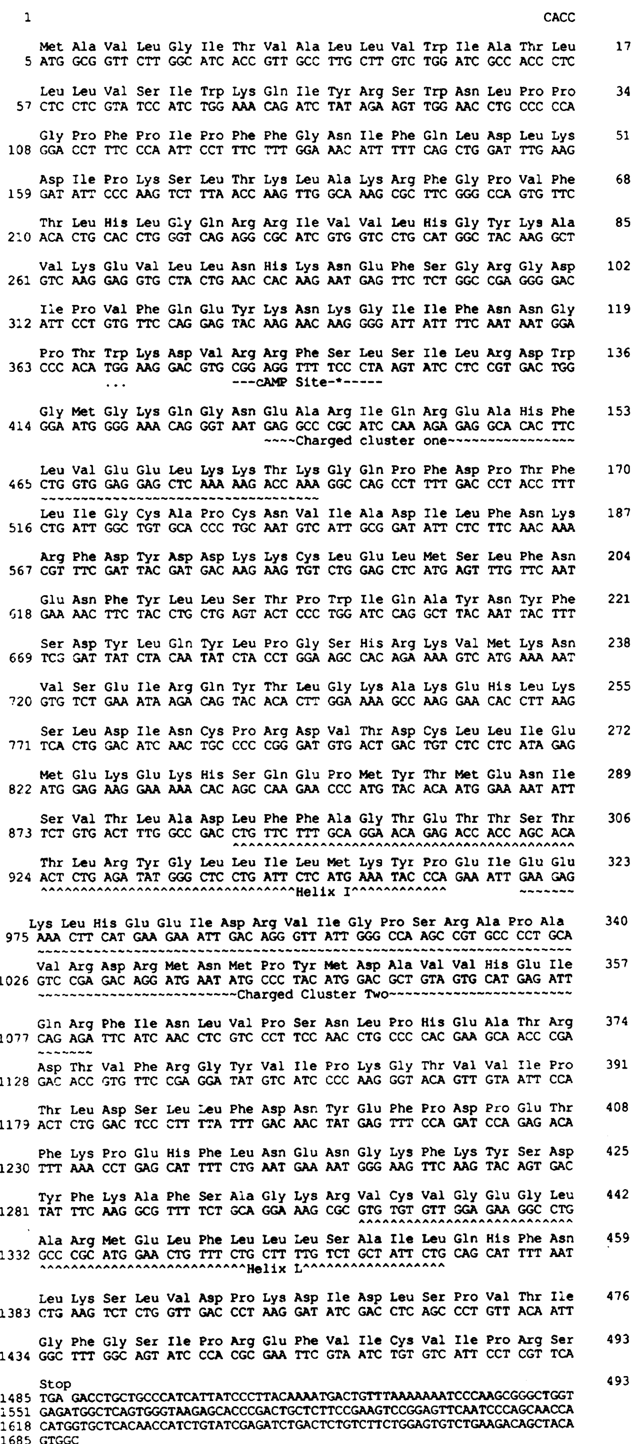

Fig. 1. Nucleotide and deduced amino acid sequence of Cyp2e1 protein

Nucleotides are numbered to the left and amino acids to the right of the diagram. Underlined and annotated portions of the sequence are referred to in the Results and discussion section. Abbreviation: cAMP, cyclic AMP.

haem propionate group, suggesting a role for the conserved tryptophan in electron transfer between the *P*450 reductase and haem (Poulos *et al.*, 1985, 1987; Nelson & Strobel, 1988). The predicted mouse Cyp2e1 protein sequence also retains a potentially phosphorylatable serine residue at position 129 (indicated by * in Fig. 1), within a canonical cyclic AMP-dependent protein kinase recognition site Arg-Arg-Xaa-Ser-Xaa,

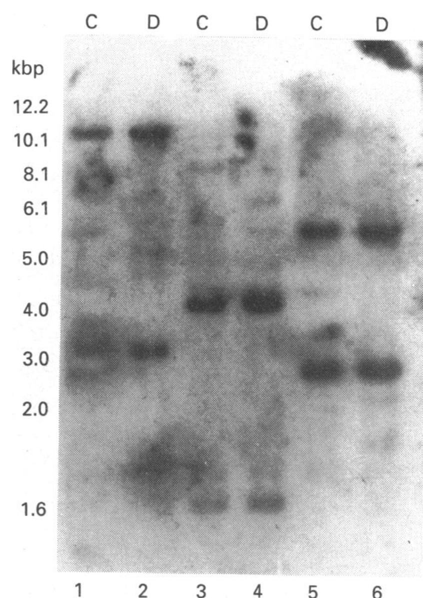

Fig. 2. Southern blot analysis of the *Cyp2e1* gene

Genomic DNA prepared from C57BL/6 (C) and DBA/2N (D) mice was digested with *Eco*RI (lanes 1 and 2). *Bam*HI (lanes 3 and 4) and *Bgl*II (lanes 5 and 6), subjected to agarose gel electrophoresis and transferred to a nylon filter. The filter was hybridized with ^{32}P-labelled *Cyp2e1* probe. DNA size markers are indicated.

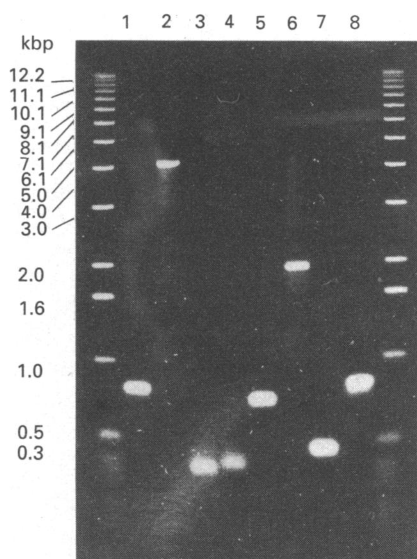

Fig. 3. Characterization of the *Cyp2e1* gene structure

Oligonucleotides to the predicted intron/exon junctions of the *Cyp2e1* gene were used in a PCR mixture to prime DNA prepared from genomic *Cyp2e1* clones in λEMBL3. The products of these reactions were subjected to agarose gel electrophoresis in conjunction with DNA markers to allow the estimation of intron size. DNA markers are indicated. Numbers above lanes indicate introns (e.g. 1 represents the intron between exon 1 and exon 2).

where Xaa is hydrophobic (indicated as -----). This is seen in all other family 2 *P*450s but is lost due to an 8-amino-acid insertion in family 1 (Muller *et al.*, 1985; Nelson & Strobel, 1988). The proximity of this potential phosphorylation site and the conserved tryptophan 122 is intriguing, and the functional implications remain to be elucidated.

Table 2. Comparison of the predicted *Cyp2e1* gene organization with known *CYP2E1* gene structures

Comparison of the PCR-evaluated *Cyp2e1* gene intron sizes and the known intron sizes of human and rat *CYP2E1*. Intron 1, for example, represents the intron between exon 1 and exon 2. Exon sizes of *Cyp2e1* were predicted on the basis of the known human and rat *CYP2E1* gene exon sizes. Sizes are in base pairs and all mouse intron sizes are approximate.

	Size (bp)		
	Human	Rat	Mouse
Exon 1	176	176	176
Intron 1	904	761	800
Exon 2	159	159	159
Intron 2	2938	3404	4100
Exon 3	149	149	149
Intron 3	388	304	250
Exon 4	160	160	160
Intron 4	406	325	300
Exon 5	176	176	176
Intron 5	881	788	750
Exon 6	141	141	141
Intron 6	2836	1873	1900
Exon 7	187	187	187
Intron 7	498	496	450
Exon 8	141	141	141
Intron 8	883	766	850
Exon 9	184	184	184

Mouse genomic DNA prepared from DBA/2N and C57BL/6 mouse livers was digested with a series of restriction endonucleases, subjected to agarose gel electrophoresis, transferred by Southern blotting to a nitrocellulose filter and probed using the full-length radioactively labelled mouse *Cyp2e1* clone. A simple banding pattern was obtained, suggesting the presence of only one *Cyp2e1* gene (Fig. 2). No differences in banding patterns were seen between the two mouse strains, indicating that the gene is not polymorphic within the sites of those restriction endonucleases studied.

Using the *Cyp2e1* cDNA as a probe, several positive clones were isolated from a BALB/c male mouse liver library in λEMBL3. DNA was prepared from three of these clones. A 5000 bp *Sal*I fragment was subcloned into pTZ18R/19R and portions were sequenced. This analysis confirmed the clone as genomic mouse *Cyp2e1* (results not shown). Alignment of the mouse *Cyp2e1* cDNA sequence against that of the published *CYP2E1* gene of rat and human (Umeno *et al.*, 1988*a*,*b*) allowed the prediction of intron/exon boundaries. Based on these data, oligonucleotide primers were synthesized to the most 5′ and 3′ regions of the nine exons. These primers, in conjunction with the purified phage DNA containing the *Cyp2e1* gene, were used to determine the organization of the *Cyp2e1* gene by use of PCR. The sizes of the products of the PCR reactions, after agarose gel electrophoresis, gave the intron sizes. Smaller fragment sizes were more accurately assessed on higher-percentage agarose gels (results not shown). The results from this analysis demonstrated that the mouse gene has the same basic structure as that seen in the human and rat genes, and that the mouse *Cyp2e1* gene was approx. 11000 bp in length (Fig. 3 and Table 2).

The effects on Cyp2e1 expression of a series of compounds known to modulate hepatic *P*450 levels were investigated in C57BL/6 and DBA/2N male mice (Fig. 4*a*). Cyp2e1 protein levels were not changed by inducers of CYP1A (3-methylcholanthrene and β-naphthoflavone), CYP2B/Cyp2b (phenobarbital and dexamethasone) or CYP3A

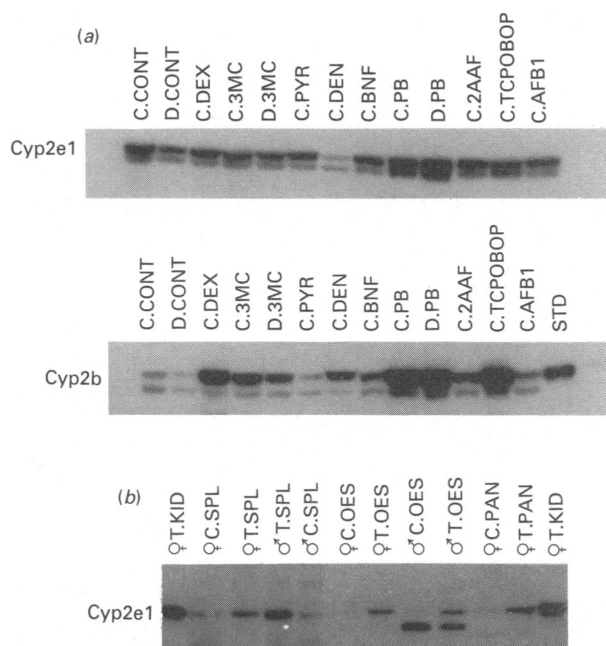

Fig. 4. (a) Effect of foreign compound administration on hepatic Cyp2e1 expression, and (b) effect of acetone administration on Cyp2e1 expression in a variety of tissues

(a) Hepatic microsomal protein was prepared from C57BL/6 (C) and DBA/2N (D) male mice treated with the following compounds: DEX (dexamethasone), 3MC (3-methylcholanthrene), PYR (pyrazole), DEN (diethylnitrosamine), BNF (β-naphthoflavone), PB (phenobarbital), 2AAF (2-acetylaminofluorene), TCPOBOP, AFB1 (aflatoxin B1). CONT., control animals. Microsomal protein (15 μg) was separated by SDS/PAGE, transferred to nitrocellulose and probed with polyclonal rat antiserum to rat CYP2E1 and CYP2B1. The CYP2B1 standard (Std) was isolated from rat liver. (b) Microsomal protein was prepared from control (C) and acetone-treated (T) male (♂) and female (♀) DBA/2N mouse kidney (KID), spleen (SPL), oesophagus (OES), and pancreas (PAN). Microsomal protein from spleen, oesophagus and pancreas (100 μg) and from kidney (15 μg) was separated by SDS/PAGE, transferred to nitrocellulose and probed with rat polyclonal antisera to CYP2E1. In both cases Cyp2e1 is the upper band, the nature of the lower band is discussed in the text.

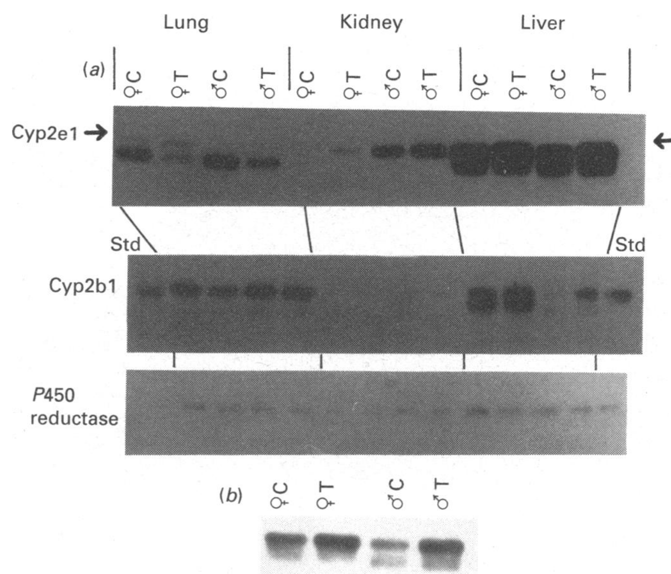

Fig. 5. Effect of acetone treatment on Cyp2e1 protein

Microsomal proteins (15 μg) from control (C) and acetone-treated (T) male and female DBA/2N mice from the tissues indicated were separated by SDS/PAGE, immunoblotted and probed with polyclonal antisera raised to rat P450 enzymes. In (a) the upper arrowed band represents Cyp2e1; the lower band is discussed in the text. Shorter exposure of the liver samples from the same immunoblot, allowing better assessment of induction level. No bands were detected on Western blots other than those shown. P450 reductase and CYP2B1 standards (Std) were isolated from rat liver. No CYP2E1 standard was available.

(dexamethasone), or by general Cyp2/3 inducers (TCPOBOP and 2-acetylaminofluorene). In contrast, levels of Cyp2b are induced by dexamethasone, 3-methylcholanthrene, β-naphthoflavone, diethylnitrosamine, phenobarbital, 2-acetylaminofluorene and TCPOBOP. A dramatic decrease in Cyp2e1 is seen on treatment with diethylnitrosamine. This last observation has been shown to be a common feature of all Cyp2 members, with the exception of Cyp2b, and occurs at the level of both protein and mRNA (A. L. Russell, unpublished work). Pyrazole, an inducer and substrate of CYP2E1 in rats and rabbits, has no effect on Cyp2e1 levels in the mouse. Pyrazole is thought to induce member(s) of the Cyp2a family (Honkakoshi *et al.*, 1988) in the mouse.

Microsomal fractions and total RNA were prepared from various tissues from male and female control mice and mice given 1% acetone in their drinking water for 14 days. Cyp2e1 protein, of approximate molecular mass of 52000 Da, was found to be constitutively expressed in many tissues in the mouse, including lung, liver, kidney, spleen, pancreas and oesophagus. The presence of Cyp2e1 in the oesophagus has been confirmed by immunohistochemical studies (results not shown) and is particularly interesting in view of the susceptibility of this tissue to cancers related to ethanol consumption (Tuyns *et al.*, 1979; Garro & Lieber, 1990). Cyp2e1 was also shown to be present, and inducible by acetone, in testes (results not shown). Treatment with acetone generates an increase in Cyp2e1 protein levels in both sexes (Figs. 4*b* and 5). The lower band seen on the Cyp2e1 immunoblots in the liver, male oesophagus and lung may represent the effect of a common epitope(s) between Cyp2e1 and another P450, or the result of Cyp2e1 degradation products, and has been noted in previous studies employing this antibody (Hong *et al.*, 1989). The upper band representing Cyp2e1 is indicated by arrows in Fig. 5(*a*).

A sexual dimorphism in Cyp2e1 protein expression occurs in the mouse kidney with higher levels in males than in females. This is not seen in CYP2E1 expression in other species and is controlled at a transcriptional level by testosterone (Hong *et al.*, 1989; Henderson *et al.*, 1990; Hu *et al.*, 1990). Interestingly, in this context, it has been noted that nitrosamines induce renal neoplasms predominantly in male mice (Noronha, 1977). In the lung, in contrast to the kidney, a reversal of the sexual dimorphism is seen, with higher levels in the female (Fig. 5).

Acetone treatment generates no perturbation in the levels of cytochrome P450 reductase. Cyp2b levels are increased in the liver of both sexes, but decreased in the lung following acetone treatment. Similar studies in rats revealed that such treatment caused a marked increase in the levels of hepatic CYP2B1 and 2 at both mRNA and protein levels (Song *et al.*, 1989). Analysis of *Cyp2e1* mRNA prepared from acetone-treated animals showed that *Cyp2e1* generated a single mRNA of approx. 1900 bp. The induction of the protein seen is not a result of increased transcription, as the levels of Cyp2e1 mRNA are not elevated in the treated tissues, but are decreased by 75% in female kidney, 66% in female liver and 25% in male kidney (Fig. 6). This treatment had no effect on the level of actin mRNA in the tissues

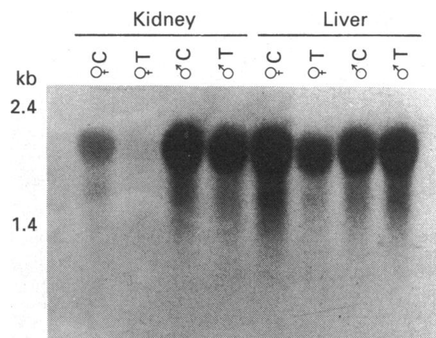

Fig. 6. Effect of acetone treatment on Cyp2e1 mRNA

RNA was isolated from control (C) and acetone-treated (T) DBA/2N mouse tissues as indicated, and 15 μg of each sample was loaded per track. Northern blotting was performed and the filters were probed with a ^{32}P-labelled *Cyp2e1* probe. Marker sizes are indicated.

studied (results not shown). Decreases in CYP2E1 mRNA have been observed previously in pyridine-treated rats (Kim & Novak, 1990) and ethanol-treated rabbits (Porter *et al.*, 1989). Induction of mouse Cyp2e1 protein appears to be the result of an increased translation of the mRNA, stabilization of the protein, or both. In the rat the induction by acetone is a result of protein stabilization (Song *et al.*, 1989). Proteins which have been shown to be regulated by modifications in the mRNA translation rate have in general a 5′ untranslated region (UTR) differentially bound by regulatory elements controlling translational levels (e.g. Thireos *et al.*, 1984; McGarry & Lindquist, 1985). In all CYP2E1 mRNAs studied to date a very short leader sequence is present, suggesting that an increase in protein levels by previously recognized mechanisms of increased protein synthesis is unlikely. However, a number of studies suggest a role for an increase in CYP2E1 mRNA translation following chemical induction (Kubota *et al.*, 1988; Porter *et al.*, 1989; Kim & Novak, 1990; Kim *et al.*, 1990).

In starvation and diabetes, induction of CYP2E1 occurs via mRNA stabilization in rats (Song *et al.*, 1987). Acetone had no effect on mRNA levels in the rat, suggesting that other factors play a role in induction of CYP2E1 in these conditions. A potential involvement for the CYP2E1 3′ UTR interacting with stabilizing proteins present in these states is possible, as seen in the case of other mRNAs where stabilization has been observed (e.g. Casey *et al.*, 1989). Sequence comparisons of the *Cyp2e1* cDNA 3′ UTR and other *CYP2E1* cDNA 3′ UTR sequences known to date did not reveal any conserved motifs which may potentially be bound by stabilizing proteins, although clearly a global three-dimensional conservation of structure rather than a specific primary nucleotide order may be important. Roles for hormonal regulation of CYP2E1, mediated via phosphorylation cascade pathways, have been proposed, and hormonal changes may generate the intracellular changes which led to the increased mRNA stability seen in these states (Johansson *et al.*, 1989).

In view of the proposed gluconeogenic role of CYP2E1/Cyp2e1 it is feasible that, in an energetically compromised state, a cell could increase the flux through a salvage gluconeogenic system involving CYP2E1/Cyp2e1 by blocking CYP2E1/Cyp2e1 protein degradation. It could be envisaged that such an induction system would be employed, as this type of mechanism requires no further significant energy input and thus would not further compromise the cell.

Interesting species differences in the induction of CYP2E1/Cyp2e1 between the rat and the mouse have been noted. Studies from this laboratory and others suggest that although rat CYP2E1 expression is suppressed by growth hormone, no such effect is observed in the mouse (Henderson *et al.*, 1990; Hong *et al.*, 1990). Clearly, further studies at the molecular level in order to better characterize the systems regulating Cyp2e1, and how regulation has evolved to generate the differences seen between the mammalian species, is needed. These studies will allow a better understanding of the endogenous role of CYP2E1/Cyp2e1 and the induction systems impinging upon it, and so give insight into the corresponding modifications of xenobiotic metabolism.

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REFERENCES

Adams, D. J., Seilman, S., Ameliazad, Z., Oesch, F. & Wolf, C. R. (1985) Biochem. J. **232**, 869–876

Argeles, J. M. (1986) Trends Biochem. Sci. **11**, 61–63

Astrom, A. & DePierre, J. W. (1986) Biochim. Biophys. Acta **853**, 1–27

Bartsch, H. & Montesano, R. (1984) Carcinogenesis **5**, 1381–1393

Benton, W. D. & Davis, R. W. (1977) Science **196**, 180–182

Casazza, J. P., Felver, M. E. & Veech, R. L. (1984) J. Biol. Chem. **259**, 231–236

Casey, J. L., Koeller, D. M., Ramin, V. C., Klausner, R. D. & Harford, B. H. (1989) EMBO J. **8**, 3693–3699

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) Biochemistry **18**, 5294–5299

Cox, R. A. (1968) Methods Enzymol. **12**, 120–129

Devereux, J., Haeberli, P. & Smithies, O. (1984) Nucleic Acids Res. **12**, 387–395

Garro, A. J. & Lieber, C. S. (1990) Annu. Rev. Pharmacol. Toxicol. **30**, 219–249

Gonzalez, F. J. (1990) Pharmacol. Ther. **45**, 1–38

Gonzalez, F. J. & Nebert, D. W. (1990) Trends Genet. **6**, 182–186

Grossberger, D. (1987) Nucleic Acids Res. **15**, 6737

Guengerich, F. P. (1988) Can. Res. **48**, 2946–2954

Hansson, T., Tindberg, N., Ingelman-Sunberg, M. & Kohler, C. (1990) Neuroscience **34**, 451–463

Henderson, C. J., Scott, A. R., Yang, C. S. & Wolf, C. R. (1990) Biochem. J. **266**, 675–681

Hong, J. Y., Pan, J., Ning, S. M. & Yang, C. S. (1989) Can. Res. **49**, 2973–2979

Hong, J. Y., Pan, J., Ning, S. M., Ma, B.-L., Lee, M.-J., Pan, J. & Yang, C. S. (1990) Arch. Biochem. Biophys. **281**, 132–138

Honkakoshi, P., Autio, S., Juvonen, R., Ruanio, H., Gelboin, H. V., Park, S. S., Pelkonen, O. & Lang, M. A. (1988) Arch. Biochem. Biophys. **267**, 589–598

Hu, J. J., Rhoten, W. B. & Yang, C. S. (1990) Biochem. Pharmacol. **40**, 2597–2602

Innis, M. A. & Gelfand, D. H. (1990) in PCR Protocols (Innis, M. A., Gelfand, D. H., Sminsky, J. J. & White, T. J., eds.), pp. 3–13, Academic Press, San Diego

Johansson, I., Eskman, G., Scholte, B., Puzychi, D., Jornvall, H. & Ingelman-Sundberg, M. (1988) Biochemistry **27**, 1925–1934

Johansson, I., Eliasson, E., Johansson, A., Hagbjork, A.-L., Lindros, K. & Ingelman-Sunberg, M. (1989) in Cytochrome *P*450 Biochemistry and Biophysics (Schuster, I., ed.), pp. 592–595, Taylor and Francis, London

Juchau, M. R. (1990) Life Sci. **47**, 2385–2394

Kemper, B. & Szcesna-Skopura, E. (1989) Drug. Metab. Rev. **20**, 811–820

Khani, S. C., Zaphiropoulos, P. G., Fujita, V. S., Porter, T. D., Koop, D. R. & Coon, M. J. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 638–642

Khani, S. C., Porter, T. D. & Coon, M. J. (1988*a*) Biochem. Biophys. Res. Commun. **150**, 10–17

Khani, S. C., Porter, T. D., Fujita, V. S. & Coon, M. J. (1988*b*) J. Biol. Chem. **263**, 7170–7175

Kim, S. G. & Novak, R. F. (1990) Biochem. Biophys. Res. Commun. **166**, 1072–1079

Kim, S. G., Shenkin, S. E., States, J. C. & Novak, R. F. (1990) Biochem. Biophys. Res. Commun. **172**, 767–774

Koop, D. R. & Casazza, J. P. (1985) J. Biol. Chem. **260**, 13607–13612
Koop, D. R. & Coon, M. J. (1986) Alcohol Clin. Exp. Res. (1986) **10** (suppl.), 44S–51S
Koop, D. R., Morgan, E. T., Tarr, G. C. & Coon, M. J. (1982) J. Biol. Chem. **257**, 8472–8480
Kubota, S., Lasker, J. M. & Lieber, C. S. (1988) Biochem. Biophys. Res. Commun. **150**, 304–310
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lindros, K. O., Cai, Y. & Pentilla, K. E. (1990) Hepatology **12**, 1092–1097
Lowry, O. H., Rosebrough, N. J., Far, A. L. & Randall, R. L. (1951) J. Biol. Chem. **193**, 265–275
McGarry, T. J. & Lindquist, S. (1985) Cell **42**, 903–911
Meehan, R. R., Barlow, D. P., Hill, R. E., Hogan, B. L. M. & Hastie, N. D. (1984) EMBO J. **3**, 1881–1885
Miller, K. W. & Yang, C. S. (1984) Arch. Biochem. Biophys. **229**, 483–491
Muller, R., Schmidt, W. E. & Stier, A. (1985) FEBS Lett. **187**, 21–24
Nebert, D. W., Nelson, D. R., Coon, M. J., Estabrook, R. W., Feyereisen, R., Fujii-Kuriyama, Y., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R. & Waxman, D. J. (1991) DNA Cell Biol. **10**, 1–14
Nelson, D. R. & Strobel, H. W. (1987) Mol. Biol. Evol. **4**, 572–593
Nelson, D. R. & Strobel, H. W. (1988) J. Biol. Chem. **263**, 6038–6050
Noronha, R. F. X. (1977) J. Surg. Oncol. **9**, 463–468
Okey, A. B. (1990) Pharmacol. Ther. **45**, 241–298
Past, M. R. & Cook, D. E. (1982) Biochem. Pharmacol. **31**, 3329–3334
Porter, T. D., Khani, S. C. & Coon, M. J. (1989) Mol. Pharmacol. **36**, 61–65
Poulos, T. L., Finzel, B. C., Gunsalus, I. C., Wagner, G. C. & Kraut, J. (1985) J. Biol. Chem. **260**, 16122–16130
Poulos, T. L., Finzel, B. C. & Howard, A. J. (1987) J. Mol. Biol. **195**, 687–700
Reichard, G. A., Jr., Hoff, A. C., Skutches, C. L., Paul, P., Holroyde, C. P. & Owen, O. E. (1979) J. Clin. Invest. **63**, 619–626
Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) J. Mol. Biol. **113**, 237–251
Ryan, E. R. & Levin, W. (1990) Pharmacol. Ther. **45**, 153–239
Ryan, D. E., Ramanathan, L., Lida, S., Thomas, P. E., Haniu, M., Shively, J. E., Lieber, C. S. & Levin, W. (1985) J. Biol. Chem. **260**, 6385–6393
Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual (Ford N., Nolan, C. & Ferguson, M. eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor
Sanger, F., Nicklen, S. & Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 5463–5467
Schnier, G. G., Laethem, C. L. & Koop, D. R. (1989) J. Pharmacol. Exp. Ther. **251**, 790–796
Song, B. J., Gelboin, H. V., Park, S. S., Yang, C. S. & Gonzalez, F. J. (1986) J. Biol. Chem. **261**, 16689–16697
Song, B. J., Matsunga, T., Hardwick, J. P., Park, S. S., Veech, R. L., Yang, C. S., Gelboin, H. V. & Gonzalez, F. J. (1987) Mol. Endocrinol. **1**, 542–547
Song, B. J., Veech, R. L., Park, S. S., Gelboin, H. V. & Gonzalez, F. J. (1989) J. Biol. Chem. **264**, 3568–3572
Thireos, G., Driscoll, M. D. & Greer, H. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 5096–5100
Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
Tuyns, A. J., Pequignot, G. & Abbatucci, J. S. (1979) Int. J. Cancer **23**, 443–447
Umeno, M., McBride, O. W., Yang, C. S., Gelboin, H. V. & Gonzalez, F. J. (1988*a*) Biochemistry **27**, 9006–9013
Umeno, M., Song, B. J., Kozak, C., Gelboin, H. V. & Gonzalez, F. J. (1988*b*) J. Biol. Chem. **263**, 4956–4962
Wolf, C. R. (1986) Trends Genet. **2**, 209–214
Wrighton, S. A., Thomas, P. E., Ryan, D. E. & Levin, W. (1987) Arch. Biochem. Biophys. **258**, 292–297
Yang, C. S., Yoo, J. S. H., Ishizaki, H. & Hong, J. (1990) Drug Metab. Rev. **22**, 147–159

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