J. Exp. Biomed. Sci. 11 (2005) 109-113

Complete Nucleotide Sequence of Cytochrome P450 2E1 Expressed in the Rat Brain

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From the RT-PCR amplifications using mRNA templates isolated from Sprague-Dawley rat brains, we isolated a cDNA fragment of 1,524 bp which covered the full coding information of the rat brain CYP2E1. Its nucleotide sequence was identical to the previously reported rat liver CYP2E1 mRNA except for the difference of one base (A to C at the nucleotide position 73). This difference also altered the amino acid Lys to Gln. However, no insertion or deletion of nucleotide(s) which could alter the reading frame was found within the structure of this rat brain CYP2E1. This study should provide the molecular basis regarding the pathophysiological function of CYP2E1 in the brain.

Key Words: CYP2E1, Rat brain, Alcohol

INTRODUCTION

Cytochrome P450 2E1 (CYP2E1) has been the focus of intense studies in the liver because it is involved in the bioactivation of many metabolites and carcinogens (Tu et al., 1983; Yang et al., 1990). It is known to be induced by ethanol and chronic ingestion of alcohol has been linked to several cases of liver diseases and cancers (Tsutsumi et al., 1983; Koop et al., 1989). A recent report by Cederbaum *et al.* also indicates that CYP2E1 mediated metabolism of its substrates is involved in the process of apoptosis when it is overexpressed in human hepatocarcinoma cells (Chen et al., 1997). Previous characterization of cDNA clones for CYP2E1 suggested that it was well conserved in various animal species (Song et al., 1986; Khani et al., 1988; Davis et al., 1993). Interestingly, CYP2E2 which differs in only 16 amino acids from CYP2E1 has also been reported in the rabbit (Khani etal, 1988).

Previous reports have also indicated the presence of CYP2E1 in human and rat brains (Adams et al., 1992; Geng et al., 1993; Yoo et al., 1997). Juchau *et al.* also demonstrated the expression of CYP2E1 during embryogenesis and fetogenesis in human cephalic tissues (Boutelet-Bochan et al., 1997). Therefore, detailed characterization of CYP2E1 expressed in the brain at nucleotide level would be very important for the better understanding of brain toxicology possibly mediated by various chemicals including alcohol.

Although the presence of CYP2E1 in the rat brain has been well documented by PCR, RT-PCR and western blot analysis over 10 years (Hansson et al., 1990; Geng et al., 1993; Yoo et al., 1997), little information is available regarding its nucleotide sequence. We now report the complete nucleotide sequence of CYP2E1 expressed in the rat brain.

*Received: February 22, 2005
Accepted after revision: April 9, 2005
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MATERIALS AND METHODS

1. Materials

Adult Sprague-Dawley rats were from Dongsan Medical

Center (Taegu, Korea). Eight oligonucleotides (P1-P8) were designed and synthesized (Bioneer, Korea) as described in Table 1 based on the sequence of the rat liver CYP2E1 mRNA (Song et al., 1986). A *BglII-PstI* fragment of the rat liver CYP2E1 cDNA that corresponds to the sequence coding for amino acids 26~432 was used as a hybridization probe for Southern blot analysis. DIG DNA labeling and detection kit was purchased from Boehringer Mannheim.

2. RT-PCR

Rats were anesthetized by the injection of sodium pentobarbital (25 mg/kg, i.p.). After perfusion with phosphate-buffered saline (pH 7.4) containing 2 units/ml of heparin, brain was removed and frozen in isopentane (-70℃) followed by homogenization in a solution containing RNAzol B (BioTex Laboratories, Inc.). After extraction with chloroform once, the supernatant was collected and total RNA was precipitated by adding an equal volume of isopropanol RNA pellet was dissolved in DEPC-treated ddH_2O. First cDNA strand was generated by incubating 1 Fg of total RNA with the reverse transcription mixture containing 2 mM dNTPs, 100 pmoles of oligo$(dT)_{16}$, 20 units of RNasin 200 units of M-MuLV reverse tranascriptase. PCR program (40 cycles) was as follows: 1 min at 94℃, 1 min at 68℃, 1 min at 72℃.

Table 1. Primers used for amplifications

Primer	Sequence and localization (number*)
P1	ATGGCGGTTCTTGGCATCAC (sense, 1~20)
P2	GGGGATATCCTTCAAATCCAGCTG (antisense, 139~162)
P3	GTGAACACTGGCCCGAAGCGC (antisense, 186~206)
P4	TATGACGTTGCAGGGCGCGCAGCC (antisense, 517~540)
P5	TCCGCAAAGTTATTGTAAAGCTGGA (antisense, 644~668)
P6	CCATATCTCAGAGTTGTGCTGGTGGT (antisense, 907~932)
P7	CAATTCCATGCGGGCCAGGCCTTCTCC (antisense, 1315~1341)
P8	TAAGGGATAACAATGGAAGGGATATC (antisense, 1499~1524)

*Numbers represent the relative positions of primers in Fig. 2.

3. Southern blot analysis

DNA membrane was prehybridized at 60℃ for 2 h, then hybridized with DIG labeled rat liver CYP2E1 cDNA probe at 65℃ for at least 8 h. Color detection of hybridization signals was carried out by the manufacturer's instructions.

4. DNA sequencing

PCR products were subcloned into pT7Blue T-vector (Novagen) and sequenced by a method of primer extension.

RESULTS AND DISCUSSION

1. Isolation of CYP2E1 cNDA in rat brain

To isolate cDNA which contains the complete coding sequence for the rat brain CYP2E1 we performed RT-PCR

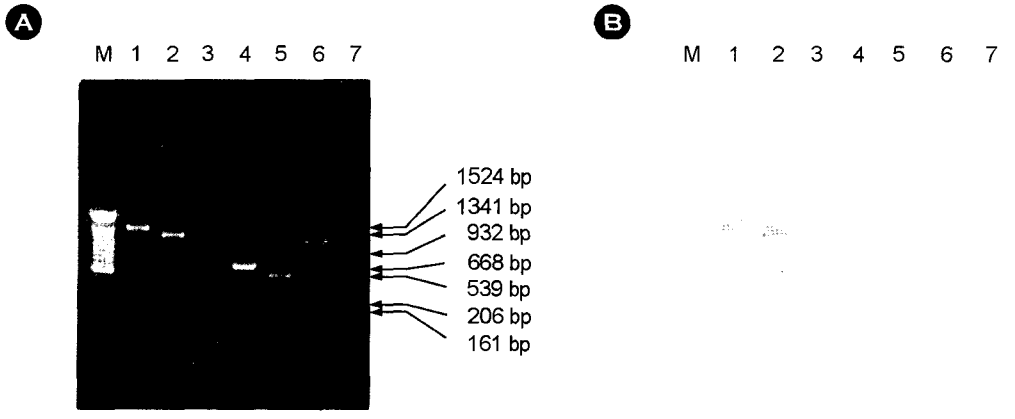

Fig. 1. Results of RT-PCR followed by agarose gel electrophoresis (**A**) and Southern blot analysis (**B**). Total RNA isolated from the brains of adult Sprague-Dawley rats were used as templates for RT-PCR. Primer pairs used for lanes 1~7 were P1/P8, P1/ P2, P1/P3, P1/P4, P1/P5/, P1/P6, P1/P7, respectively. The 100 bp ladder was used as a size marker (M) and was also used as a negative control for Southern blot analysis.

amplifications with specific primers designed, based on the rat liver CYP2E1 mRNA sequence. Initial amplification with primers P1 and P8 resulted in about 1,500 bp DNA band (Fig. 1A, lane 1). The size of this DNA band corresponded to the expected size, compared to the sequence of the rat liver CYP2E1 mRNA. Nested PCR amplifications were carried out to further examine the internal structure of this PCR product, with different pairs of primers (P1 and P2, P1 and P3, P1 and P4, P1 and P5, P1 and P6, P1 and P7). Again, DNA bands of about 1340, 930, 670, 540, 210, and 160 bp, respectively, were amplified as expected from the sequence of the rat liver CYP2E1 mRNA (Fig. 1A, lanes 2~7). From this initial analysis it was concluded that the brain form of CYP2E1 should be identical or very similar to the liver counterpart. Southern blot analysis using rat liver CYP2E1 cDNA as a hybridization probe also showed strong signals for these DNA bands (Fig. 1B), suggesting again that CYP2E1 expressed in the brain was identical or very similar to the liver CYP2E1 at nucleotide level.

2. Analysis of CYP2E1 cDNA sequence

For further analysis, the nucleotide sequence of the lar-

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ATGGCGGTTCTTGGCATCACCATTGCCTTGCTGGTGTGGGTGGCCACCCTCCTCGTCATATCCATCTGGAAGCAGATCTATAACAGTTGG   90
 M  A  V  L  G  I  T  I  A  L  L  V  W  V  A  T  L  L  V  I  S  I  W  K  Q  I  Y  N  S  W
AACCTGCCCCCAGGACCTTTCCCTCTTCCCATCCTTGGGAACATTTTTCAGCTGGATTTGAAGGATATCCCCAAGTCTTTCACCAAGTTG  180
 N  L  P  P  G  P  F  P  L  P  I  L  G  N  I  F  Q  L  D  L  K  D  I  P  K  S  F  T  K  L
GCAAAGCGCTTCGGGCCAGTGTTCACACTGCACCTTGGCTCAAGGCGCATCGTGGTCCTGCATGGCTACAAGGCTGTCAAGGAGGTGCTA  270
 A  K  R  F  G  P  V  F  T  L  H  L  G  S  R  R  I  V  V  L  H  G  Y  K  A  V  K  E  V  L
CTGAACCACAAGAATGAGTTTTCTGGACGGGGGGACATTCCTGTGTTCCAGGAGTACAAGAACAAGGGGATTATTTTCAATAATGGGCCC  360
 L  N  H  K  N  E  F  S  G  R  G  D  I  P  V  F  Q  E  Y  K  N  K  G  I  I  F  N  N  G  P
ACATGGAAGGATGTGCGGAGGTTTTCCCTAAGCATTCTCCGAGACTGGGGAATGGGGAAACAGGGTAATGAGGCCCGCATCCAAAGGGAG  450
 T  W  K  D  V  R  R  F  S  L  S  I  L  R  D  W  G  M  G  K  Q  G  N  E  A  R  I  Q  R  E
GCGCAATTCCTGGTGGAGGAGCTCAAAAAGACCAAAGGCCAGCCTTTTGACCCCACATTTCTGATTGGCTGCGCACCCTGCAATGTCATT  540
 A  Q  F  L  V  E  E  L  K  K  T  K  G  Q  P  F  D  P  T  F  L  I  G  C  A  P  C  N  V  I
GCGGATATCCTCTTCAACAAACGTTTCGACTACAATGACAAGAAGTGTCTGAGGCTCATGAGTTTGTTCAATGAAAACTTCTACCTGCTG  630
 A  D  I  L  F  N  K  R  F  D  Y  N  D  K  K  C  L  R  L  M  S  L  F  N  E  N  F  Y  L  L
AGCACCCCCTGGATCCAGCTTTACAATAACTTTGCGGATTATCTACGATACCTACCTGGAAGCCATAGAAAAATCATGAAAAATGTGTCT  720
 S  T  P  W  I  Q  L  Y  N  N  F  A  D  Y  L  R  Y  L  P  G  S  H  R  K  I  M  K  N  V  S
GAAATAAAACAGTACACACTTGAAAAAGCCAAGGAACACCTTCAGTCACTGGACATCAACTGCGCCCGGGATGTGACTGACTGTCTCCTC  810
 E  I  K  Q  Y  T  L  E  K  A  K  E  H  L  Q  S  L  D  I  N  C  A  R  D  V  T  D  C  L  L
ATAGAGATGGAGAAGGAAAAACACAGCCAAGAACCCATGTACACAATGGAAAATGTTTCTGTGACTTTGGCCGACCTGTTCTTTGCAGGA  900
 I  E  M  E  K  E  K  H  S  Q  E  P  M  Y  T  M  E  N  V  S  V  T  L  A  D  L  F  F  A  G
ACTGAGACCACCAGCACAACTCTGAGATATGGGCTCCTGATCCTCATGAAATACCCAGAAATTGAAGAGAAACTTCATGAAGAAATTGAC  990
 T  E  T  T  S  T  T  L  R  Y  G  L  L  I  L  M  K  Y  P  E  I  E  E  K  L  H  E  E  I  D
AGGGTTATTGGGCCAAGCCGCGTCCCTGCTGTCAGAGACAGACTGGATATGCCCTACATGGATGCTGTGGTGCATGAGATCCAGAGATTC 1080
 R  V  I  G  P  S  R  V  P  A  V  R  D  R  L  D  M  P  Y  M  D  A  V  V  H  E  I  Q  R  F
ATCAATCTTGTCCCTTCCAACCTACCCCATGAAGCAACCAGAGATACTGTGTTCCAAGGATATGTCATCCCCAAGGGTACAGTTGTGATT 1170
 I  N  L  V  P  S  N  L  P  H  E  A  T  R  D  T  V  F  Q  G  Y  V  I  P  K  G  T  V  V  I
CCAACTCTGGACTCCCTCTTATATGACAGCCATGAGTTTCCAGATCCAGAGAAGTTTAAACCTGAGCATTTCCTGAATGAAAATGGGAAG 1260
 P  T  L  D  S  L  L  Y  D  S  H  E  F  P  D  P  E  K  F  K  P  E  H  F  L  N  E  N  G  K
TTCAAGTACAGTGACTATTTCAAGGCATTTTCTGCAGGAAAGCGTGTGTGTGTTGGAGAAGGCCTGGCCCGCATGGAATTGTTTCTGCTC 1350
 F  K  Y  S  D  Y  F  K  A  F  S  A  G  K  R  V  C  V  G  E  G  L  A  R  M  E  L  F  L  L
CTGTCTGCTATTCTGCAGCATTTTAACCTGAAGTCTCTGGTTGACCCTAAGGATATCGACCTCAGTCCTGTCACAGTTGGCTTTGGCAGT 1440
 L  S  A  I  L  Q  H  F  N  L  K  S  L  V  D  P  K  D  I  D  L  S  P  V  T  V  G  F  G  S
ATCCCACCCCAATTTAAACTCTGTGTCATTCCCCGTTCATGAGACCTGAAAACTTCCTGATATCCCTTCCATTGTTATCCCTTA 1524
 I  P  P  Q  F  K  L  C  V  I  P  R  S  *
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Fig. 2. Nucleotide and deduced amino acid sequences of the rat brain CYP2E1. Boxed area represents the difference when compared to the rat liver CYP2E1 mRNA.

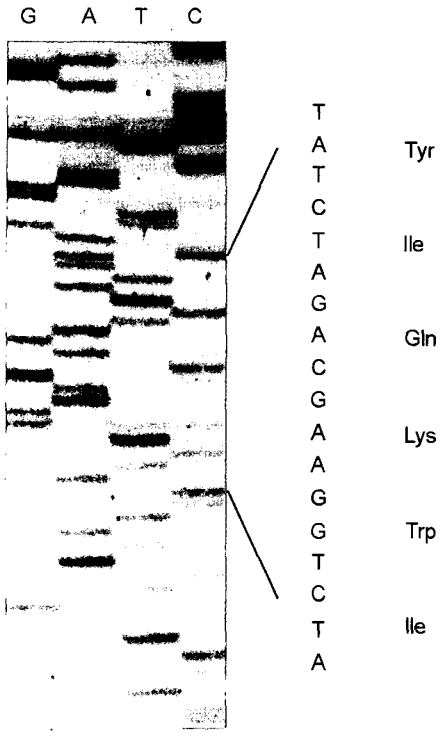

Fig. 3. DNA sequencing of the area that shows the difference.

gest PCR product (1,500 bp) was determined (Fig. 2). Its length was actually 1,524 bp and the sequence was identical to the previously reported rat liver CYP2E1 mRNA (Song et al., 1986) except for one base difference (A to C at the nucleotide position 73). This nucleotide mismatch altered the amino acid Lys to Gln, too. However, no insertion or deletion of nucleotide(s) which could alter the reading frame was found within the structure of this rat brain CYP2E1. We assume that this difference might represent a polymorphism between individual rats because the homology between this product and the liver CYP2E1 is almost 100% at both nucleotide and deduced amino acid levels. This difference was confirmed by DNA sequencing as shown in Fig. 3. The significance of this amino acid change (Lys to Gln) is not known at the present time.

Our results confirm the presence of CYP2E1 in the rat brain and verify its actual sequence. Results also confirm that there is only one gene in rat genome as indicated by Song *et al.* and the brain CYP2E1 is basically same as the liver one (Song et al., 1986). In the brain, the mechanism to regulate the level of CYP2E1 mRNA and/or protein, which can be also induced by ethanol, seems to be very important because the expression of CYP2E1 is very limited. This study should provide the molecular basis regarding the pathophysiological function of CYP2E1 in the brain.

Acknowledgments

This work was supported by Ministry of Science & Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University.

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