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Regional Distribution of Individual Forms of Cytochrome P450 mRNA in Normal Adult Human Brain

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ABSTRACT. The cytochromes P450 are a large family of haemoproteins which have a major role in the oxidative metabolism of a wide range of xenobiotics and some endogenous compounds. In this study the presence of individual members of the CYP1, CYP2 and CYP3 P450 families has been investigated by reverse transcriptase polymerase chain reaction in different regions of normal human brain consisting of frontal and temporal cortices, mid brain, cerebellum, pons and medulla. All the P450s were identified in specific regions of brain with CYP1A1 and CYP2C being the most frequently expressed forms of P450. Sequencing identified the CYP2C PCR product as CYP2C8. This study indicates that individual P450 mRNAs are present in human brain and are found in specific brain regions. The distribution of individual P450s in different regions of human brain is likely to be highly important in determining the response of the brain to toxic foreign compounds. BIOCHEM PHARMACOL **55**;6:825–830, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. brain, cytochrome P450, polymerase chain reaction

The cytochromes P450 (P450)§ are a multi-gene family [1] of constitutive and inducible haem-containing enzymes with a critical role in the oxidative metabolism of a wide range of environmental chemicals (xenobiotics) including carcinogens, toxins and therapeutic drugs [2, 3, 4]. In addition, P450s may have a role in cell regulation, in view of their involvement in the metabolism of physiological chemicals active in inter- and intra-cellular signaling, including steroid hormones, eicosanoids and fatty acids [5, 6]. The P450s can be defined as falling within two broad classes; a large group of P450s that are involved primarily in the metabolism of xenobiotics and a second smaller group of P450s involved specifically in steroid hormone synthesis [1]. The major xenobiotic metabolizing families of P450 (CYP1, CYP2 and CYP3) have been well characterized in liver which is the primary organ involved in the metabolism of xenobiotics [7]. Individual forms of P450 have also been identified in a variety of extrahepatic tissues including kidney [8], lung [9] and small intestine [10, 11] and the presence of P450s in particular extrahepatic tissues is important in influencing the tissue specific toxicity of many xenobiotics [12].

A range of compounds which are metabolized by P450 are potentially neurotoxic [12]. Furthermore P450 metabolism of endogenous compounds including neurosteroids and eicosanoids within brain may contribute to endogenous brain function [13]. Several forms of P450 have already been identified in rodent brain and these studies have indicated that there is differential expression of individual P450s in particular regions of brain [14–19]. However, there has been very limited study of individual P450s in human brain. A characteristic carbon monoxide binding spectra of P450 has been obtained from both microsomes and mitochondria prepared from human cerebral cortex and quantitation of total P450 showed a higher level of P450 in the mitochondrial fraction compared with the microsomal fraction [20]. Several catalytically active but incompletely characterised P450s have been purified from a single whole human brain [21] while several P450 associated mono-oxygenase activities have been identified in subcellular fractions prepared from different regions of human brain [20, 22]. In this study we have undertaken a comprehensive investigation of the expression of individual P450 mRNAs in normal human brain by reverse transcriptase polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Tissue

Normal human brain was obtained, with permission, from autopsies carried out in the Department of Pathology, University of Aberdeen. The clinical details and causes of death are summarised in Table 1. None of the patients displayed any sign of neurological disease and they were non-smokers who had not been treated with any drugs known to induce P450 expression. All the brains appeared

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§ *Abbreviations:* dNTP, deoxynucleotide triphosphate; P450, cytochrome P450; RT-PCR, reverse transcriptase polymerase chain reaction.

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TABLE 1. Clinical data for human brain samples

Case no.	Age	Sex	Cause of death
1	72	F	Bronchopneumonia
2	80	F	Pulmonary thrombo-embolism
3	59	M	Hepatic cirrhosis
4	75	F	Pancreatic carcinoma
5	87	F	Ischaemic heart disease

normal at post-mortem examination and tissue samples were obtained within 24 hr of death from the following areas of each brain: medulla, pons, cerebellum, mid brain, basal ganglia, frontal cortex and temporal cortex. Normal human liver was obtained from transplant donors. All tissue samples were frozen in liquid nitrogen and stored at −70° prior to use.

Preparation of RNA

Total RNA was isolated from individual samples of tissue from each of the five brains. Sufficient RNA was isolated from each sample and it was unnecessary to pool any of the samples. Total RNA was prepared from 10–100 mg of every tissue sample according to the method of Chomczynski and Sacchi [23]. Briefly the tissue was rapidly homogenized in 12 vol of denaturing solution [4 M guanidium thiocyanate, 25 mM sodium citrate, 0.5% sodium lauroylsarcosine (Sigma), 100 mM 2-mercaptoethanol (Sigma)]. The following reagents were then added sequentially: 0.1 vol of 2 M sodium acetate (Sigma) pH 4, 1 vol water saturated phenol (Oncor Appligene) and 0.2 vol chloroform/isoamylalcohol (49:1). The samples were then vortexed, placed on ice for 20 min and centrifuged at 13,500 *g* in a microcentrifuge for 10 min. The aqueous phase containing the RNA was removed to a fresh microfuge tube and the extraction was repeated a further twice to obtain RNA of high purity free of contaminating genomic DNA. The RNA was precipitated by the addition of an equal volume of ice cold isopropanol to the final aqueous phase and incubated at −70° for 60 min. This was followed by centrifugation at 13,500 *g* for 10 min and the resulting pellet was washed twice with ice cold ethanol before air drying. Finally the RNA was resuspended in 10 μL of diethyl pyrocarbonate (DEPC, Sigma) treated water and the concentration of RNA determined spectrophotometrically at 260 nm.

cDNA Synthesis

cDNA was synthesized from the isolated RNA using a reverse transcriptase system (Promega) with the following reaction conditions as recommended by the supplier: 1 μg of RNA, 1 × reverse transcriptase buffer (10 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% Triton X-100), 5 mM $MgCl_2$ 1 mM of each dNTP, 0.5 unit RNasin, 15 units of avian myeloblastosis reverse transcriptase and 0.5 μg oligo dT_{15} primer in a final volume of 20 μL. Synthesis of cDNA was performed at 42° for 60 min and the reaction was stopped by heating to 99° for 5 min followed by chilling on ice. The cDNA was then stored at −70° until required.

Oligonucleotides

The primer sequences, nucleotide location, product size and GenBank accession numbers for each P450 are detailed in Table 2. All the P450 primers were obtained from Life Technologies while β-actin primers were purchased from Stratagene.

PCR

PCR was carried out in 50 μL reaction volumes with the following reaction conditions: 2 μL of cDNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.25 units of Amplitaq Gold (PE Applied Biosystems), 400 μM of each dNTP and 2.5 mM $MgCl_2$ (β-actin) or 3 mM $MgCl_2$ (CYP1A1 and CYP1B1), 3.5 mM $MgCl_2$ (CYP2C, CYP2D6, CYP2E1, CYP3A4, CYP3A5) or 4 mM $MgCl_2$ (CYP1A2). The amount of each primer in the PCR reaction was 80 pmol for β-actin and 85 pmol for each P450 primer. PCR with Amplitaq gold requires an initial precycling step at 95° for 15 min to activate the enzyme prior to commencing the thermocycling. The following thermocycling conditions were used for β-actin: 94° for 5 min, 60° for 5 min followed by forty cycles of amplification consisting of 72° for 1.5 min, 94° for 48 sec, 60° for 48 sec and a final 72° extension for 10 min. Thermocycling conditions for each P450 were as follows: forty cycles of amplification consisting of 94° for 30 sec, 60° for 30 sec and 72° for 1 min, followed by a final 2 min extension at 72°. Both negative and positive controls were included in the PCR reaction. The negative control was DEPC treated water in place of the template cDNA while the positive control for all the P450s except CYP1B1 was normal human liver. The positive control for CYP1B1 was a plasmid containing a full length CYP1B1 cDNA which was a gift from Dr. W. F. Greenlee, University of Massachusetts. The PCR products (10 μL) were separated by gel electrophoresis using a 1.5% agarose gel, stained with ethidium bromide (Sigma) and visualised by transillumination with ultra-violet light. The gels were photographed with Polaroid type 665 black and white film and the negatives are presented in the relevant figures. Fluorescence based DNA sequencing was performed to confirm the identity of the individual PCR products.

Sequencing of PCR Products

Amplification products from the PCR were sequenced directly using an Applied Biosystems model 373A automated DNA sequencer. PCR samples were purified for sequencing by using centricon C-100 columns (Amicon) and approximately 150 ng of template were sequenced using a Taq dye deoxy terminator sequencing kit using the

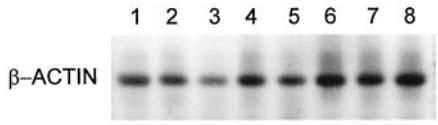

FIG. 1. The presence of β-actin mRNA in individual regions of human brain (lane 1, medulla; lane 2, pons; lane 3, cerebellum; lane 4, mid brain; lane 5, basal ganglia; lane 6, frontal cortex; lane 7, temporal cortex and lane 8, liver).

protocol recommended by the manufacturer (PE Applied Biosystems).

RESULTS

All the samples of brain showed the presence of β-actin mRNA (Fig. 1) and thus were subjected to PCR for individual P450s. The presence and distribution of individual P450s in the specific regions of the five different brains was similar. CYP1A1 mRNA was detected in cerebellum, mid brain, basal ganglia, frontal cortex and temporal cortex but was not detected in either the medulla or pons while CYP1A2 mRNA was detected only weakly in the basal ganglia and not in any of the other regions of brain (Table 2, Fig. 2). The presence of CYP1B1 mRNA was identified weakly in the medulla and was not identified in any of the other brain regions studied. CYP 2C mRNA was detected in cerebellum, midbrain, basal ganglia and frontal and temporal cortices while it was not identified in medulla, pons and cerebellum. Sequencing of the CYP2C PCR products each showed identity with CYP2C8. CYP2D6 was detected in only the mid brain while CYP2E1 mRNA was only identified in the medulla (Fig. 3). CYP3A4 mRNA was identified in both basal ganglia and frontal cortex while CYP3A5 mRNA was identified in mid brain basal ganglia and very weakly in frontal cortex (Fig. 4).

DISCUSSION

The P450s have a critical role in the activation and deactivation of many toxic foreign chemicals and the relative expression of specific forms of cytochrome P450 in a particular tissue is important in determining the toxicity of an individual chemical [12]. It is therefore important to determine the expression of different forms of P450 in specific areas of the brain to further understand the effect of toxic substances in brain. In rodent brain multiple forms of P450 have been shown to be constitutively expressed including members of the CYP1, CYP2 and CYP3A P450 families with specific expression of different forms of P450 in particular areas [14–19].

In this study the presence of individual forms of P450 mRNA in different regions of normal human brain have been investigated by RT-PCR. Normal human brain tissue can only be obtained at post-mortem and this has limited the direct study of P450 in human brain. All the cases included in this study were obtained from patients who had no evidence of neurological disease and whose brains appeared normal at post-mortem. Although all the brain tissue was obtained at post-mortem amplification for β-actin indicated that the RNA that had been isolated from all the brain samples had not undergone any significant degradation and was suitable for investigating the presence of individual forms of P450.

All the P450 forms in this study were identified in at least one area of brain and each P450 showed a distinct distribution in different regions of brain. The previous

TABLE 2. Primer sequences of individual P450s

P450	Primer sequence (5′ to 3′)	Nucleotide location	Product size	GenBank accession no.
CYP1A1	TGGATGAGAACGCCAATGTC TGGGTTGACCCATAGCTTCT	967–986 FOR 1358–1339REV	394 bp	K03191
CYP1A2	ACAGCACTTCCCGAGAGTA TCTGGATCTTCCTCTGTATC	626–645 FOR 1041–1022REV	416 bp	M55053 M38504
CYP1B1	CCACTATCACTGACATCTTC GCCTCTTGCTTCTTATTG	1311–1330FOR 1990–1973REV	680 bp	U03688
CYP2C	GCTAAAGTCCAGGAAGAGATTG GCTGAGAAAGGCATGAAGTA	1003–1024FOR 1331–1312REV	329 bp	M61853*
CYP2D6	TGATGAGAACCTGCGCATAG ACCGATGACAGGTTGGTGAT	873–892 FOR 1207–1186REV	333 bp	M20403
CYP2E1	AGCACAACTCTGAGATATGG ATAGTCACTGTACTTGAACT	925–944 FOR 1271–1290REV	365 bp	J02625
CYP3A4	CTGTGTGTTTCCAAGAGAAGTTTAC TGGTTGAAGAAGTCCTCCTAAG	782–805 FOR 1523–1502REV	742 bp	M14096
CYP3A5	GTCTCTCTGTTTCCAAAAGATACC TGAAGAAGTCCTTGCGTGTC	799–822 FOR 1535–1516REV	737 bp	J04813
β-Actin	TGACGGGGTCACCCACACTGTGCCCATCTA CTAGAAGCATTGCGGTGGACGATGGAGGG	1038–1067FOR 1876–1905REV	661 bp	X00351

* Primers were designed based on the region of identical sequence of all known CYP2C forms (CYP2C8, CYP2C9, CYP2C10, CYP2C17, CYP2C18 and CYP2C19) and did not differentiate between them. The location of the sequence and the size of the insert referred to is CYP2C18. Accession numbers in GenBank for other forms are: M17397 (CYP2C8), M15331 (CYP2C9), M61855 (CYP2C10), M61858 (CYP2C17) and M61854 (CYP2C19).

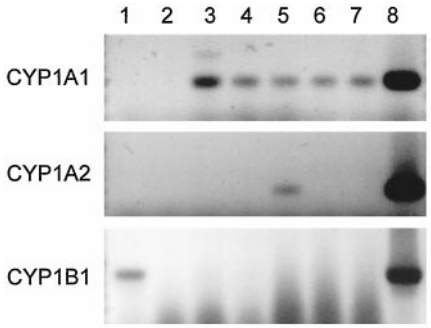

FIG. 2. The presence of CYP1A1, CYP1A2 and CYP1B1 mRNAs in different regions of normal human brain (lane 1, medulla; lane 2, pons; lane 3, cerebellum; lane 4, mid brain; lane 5, basal ganglia; lane 6, frontal cortex; lane 7, temporal cortex and lane 8, liver for CYP1A1 and CYP1A2 while the positive control for CYP1B1 is CYP1B1 containing plasmid).

studies of human brain P450, although very limited, have shown the presence of P450 associated mono-oxygenase activities [20, 22]. Moreover, several forms of P450 have been purified from a single brain and partially characterised [21]. Low levels of ethoxyresorufin deethylase, benzyloxy dealkylase and pentoxyresorufin dealkylase which are all P450 associated activities have also been identified in different regions of human brain [20]. Activities using all three alkoxyresorufin substrates were identified in both microsomal and mitochondrial fractions of brain subcellular homogenates. However, there was no consistent pattern of activity for the different substrates in different regions of brain. None of the alkoxyresorufin substrates are absolutely specific for individual P450s [24] and activity towards the different substrates would suggest the presence of different P450s including CYP1A, CYP2C and CYP3 P450 forms. In our study both CYP1A1 and CYP1A2 mRNA were identified with CYP1A1 mRNA being present in most regions of the brain examined. The presence of CYP1A2 mRNA was detected only in the basal ganglia and a P450 which reacts with an antibody to human CYP1A2 has been previously purified from microsomes prepared from the cerebral cortex of a single human brain [21].

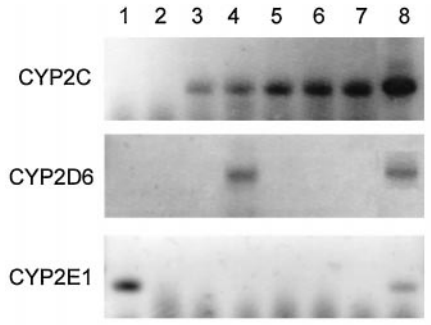

FIG. 3. The identification of CYP2C, CYP2D6 and CYP2E1 mRNAs in individual regions of brain (lane 1, medulla; lane 2, pons; lane 3, cerebellum; lane 4, mid brain; lane 5, basal ganglia; lane 6, frontal cortex; lane 7, temporal cortex and lane 8, liver).

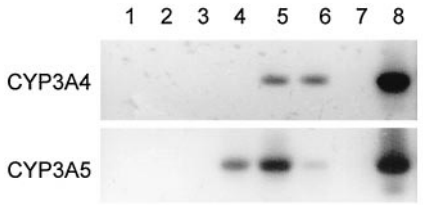

FIG. 4. The presence of CYP3A4 and CYP3A5 mRNAs in specific areas of normal human brain (lane 1, medulla; lane 2, pons; lane 3, cerebellum; lane 4, mid brain; lane 5, basal ganglia; lane 6, frontal cortex; lane 7, temporal cortex and lane 8, liver).

In this study there was a widespread distribution of CYP2C mRNA with all regions of the brain except medulla and pons containing CYP2C mRNA. The primers used to amplify CYP2C represented sequences common to all the known forms of the human CYP2C sub-family and did not differentiate between the different forms of this subfamily. Sequencing of the CYP2C PCR products was used to establish the identity of the CYP2C forms present in brain and this indicated the presence of CYP2C8. CYP2C8 has recently been shown to be an arachidonic acid epoxygenase [25] and this suggest that CYP2C8 may have an endogenous function in brain as arachidonic acid metabolites are involved in the regulation of the modulation of cerebral vascular tone [26].

The mid brain was the only region in which CYP2D6 mRNA was identified and this finding is consistent with a recent study of human brain which localized CYP2D6 mRNA by *in situ* hybridization to the mid brain in particular to neurons located within the substantia nigra [27]. The substantia nigra is the area of mid brain which is damaged in Parkinson's disease and this disease has been linked with polymorphisms of the CYP2D6 gene [28]. It has also been suggested that Parkinson's disease may be caused by compounds which are metabolized by CYP2D6 [27].

CYP2E1 mRNA was only identified in medulla and was not present in cerebral cortex or cerebellum. This finding corresponds well to the work of Bhamre *et al.* [21] in which purification of P450 from microsomes prepared from human cerebral cortex and cerebellum did not identify any P450 which showed immunoreactivity with an antibody to CYP2E1. However, medulla was not examined in that study [21].

CYP3A4 mRNA and CYP3A5 mRNA were both detected in the basal ganglia and frontal cortex while CYP3A5 mRNA was also detected in the mid brain. Although CYP3A5 has been identified in about 25% of human livers [29], it has been shown to be much more frequently expressed in extrahepatic tissues [30, 31]. A P450 protein which is reactive weakly with an antibody raised to human hepatic CYP3A4 has previously been purified from the cerebral cortex of a single human brain [21] although it is not clear which specific form was being detected in that study.

TABLE 3. Presence of individual P450 mRNAs in different regions of human brain

P450	Medulla	Pons	Cerebellum	Mid brain	Basal ganglia	Frontal cortex	Temporal cortex
CYP1A1	−	−	+	+	+	+	+
CYP1A2	−	−	−	−	+ (weak)	−	−
CYP1B1	+ (weak)	−	−	−	−	−	−
CYP2C	−	−	+	+	+	+	+
CYP2D6	−	−	−	+	−	−	−
CYP2E1	+	−	−	−	−	−	−
CYP3A4	−	−	−	−	+	+	−
CYP3A5	−	−	−	+	+	+ (weak)	−

A band of the correct size observed on ethidium bromide stained agarose gel is indicted with +. Weakly positive bands are indicated and a negative result is marked with −.

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