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Expression analysis of the mixed function oxidase system in rat brain by the polymerase chain reaction

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Abstract

Metabolism of therapeutic drugs in the body by the mixed function oxidase system is an important consideration in the analysis of a drug's effectiveness. P450-dependent metabolism within the brain of a neuro-specific drug may affect the drug's course of action. To determine whether cytochrome P450 was expressed in brain, RNA was isolated from the whole brains of rats treated with a variety of known hepatic P450 inducers, including amitriptyline, imipramine, isosafrole, phenobarbital, and β-naphthoflavone. The RNA was analyzed for the presence of P450 isozymes by the PCR technique. Differential expression of P450IA1, P450IIB1, P450IIB2, P450IID, and P450IIE1 was detected in the brain samples, depending on the treatment. Cytochrome P450 reductase expression was also detected in the brain samples, giving strong evidence that the brain contains a competent mixed function oxidase system under all conditions studied. (Mol Cell Biochem **120:** 171–179, 1993)

Key words: cytochrome P450, P450 reductase, brain

Introduction

Cytochrome P450 (P450) is the designation for a family of mixed function oxidases which are involved in a variety of reactions in mammalian systems, including metabolism of a diverse group of exogenous and endogenous compounds, carcinogen deactivation and pre-carcinogen activation. The general mechanism for this metabolism catalyzed by cytochrome P450 is:

$$RH + NADPH + H^+ + O_2 \rightarrow ROH + NADP^+ + H_2O$$

where RH represent a variety of chemical compounds including alkanes, environmental pollutants, fatty acids, polycyclic aryl hydrocarbons, steroids and *N*- and *O*-alkyl drugs. These activities also require other com-

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ponents of the mixed function oxidase system. NADPH, phosphatidylcholine (1) and NADPH cytochrome P450 reductase (reductase). There are many isoforms of P450, each with its own unique substrate profile. Adding to the complexity, many substrates are metabolized by multiple isoforms, including many therapeutically significant drugs [2, 3, 4]. Activation of some of these drugs requires the MFO system; efficacy of others is dependent on the time course of inactivation by P450.

The mixed function oxidase (MFO) system has been extensively studied utilizing hepatic microsomes where large concentrations of P450 and reductase exist. More recently cytochrome P450-mediated metabolism has been analyzed in a variety of other organs including colon [5], lung [6], and brain [7, 8]. The presence of the MFO system in a given tissue, therefore, is prima facie evidence that some or all of the activities described above occur at that site. Xenobiotics, which because of either the method of administration or tissue tropism are found in a given site, may have dramatic effects on that tissue prior to hepatic exposure. Hence, to determine the full effects a particular drug may have on a given tissue, it is necessary to detect the presence of the MFO in that tissue and biochemical action of the MFO on that drug. The specific biochemical action depends upon which P450 isozyme(s) are expressed in the given tissue.

Although the P450 activity in the brain is 1–10% of that found in the liver, the MFO system in brain is intriguing because of the dramatic cognitive and behavioral effects its activity may have on brain function. The importance of identifying specific isoforms of P450 in brain is underscored by studies linking certain diseases to differences in levels of specific P450 isoforms. For example, at least one study [9] has demonstrated a correlation between differential P450 isoform expression and the presence of brain tumors in mice. Several studies have suggested a link between a deficiency of P450 IID1 (P450 db1) and idiopathic Parkinson's disease [10, 11, 12].

Isoforms which have been shown previously to demonstrate activity in brain tissue preparations include IA1 [13], IIB1 [13], and IID1 [14, 15]. Immunohistochemical analysis has demonstrated the presence of isoforms IA1 [16, 17, 18], IA2 [17], IIB [18], and IIE1 [19]. The other major enzymatic component of the MFO system, P450 reductase, has also been found to be active in the brain. The presence of reductase in brain has been demonstrated using immunohistochemical analysis [20] and activity studies [21]. Bergh and Strobel purified rat brain reductase and demonstrated activity with artificial electron acceptors as well as in a MFO reconstituted system [22]. They found similar MFO turnover numbers in brain and liver.

The above studies indicate the presence and activity of multiple P450 isozymes and reductase in brain, suggesting the activity of a brain MFO system. These studies are limited, however, in their sensitivity and specificity. As previously stated, each isoform of P450 metabolizes a broad spectrum of substrates, which are not necessarily unique to that isoform. Therefore, activity studies, while indicating expression of a certain isoform, are not specific because other isoforms may contribute to the total observed activity. Additionally, there is evidence that not all forms of P450 expressed in the brain have been identified. For example spectral levels demonstrate higher levels of P450 than can be accounted for by those isozymes shown to be present by activity studies [13].

In order to improve sensitivity, specificity and to identify isoforms not yet demonstrated we have screened brain tissue with the most sensitive analytical expression technique: the polymerase chain reaction (PCR). Using PCR, DNA can be amplified up to a billionfold from very small amounts of DNA. PCR has been used successfully to amplify the DNA from a single cell [23]. PCR has been used to identify two isoforms of P450 in the brain: XIA1 and XXVIA1 [24]. We have designed oligonucleotide PCR primers specific for a number of other P450 isoforms and reductase. Each set of primers was used to amplify the specific isoforms from brain tissue of induced or untreated rats.

Materials and methods

Tissue collection

Four week old Sprague-Dawley rats (75–100 g) were obtained from Harlan Labs (Houston, TX). Male rats were used in all experiments except for the studies involving amitriptyline which were all female. The rats were treated in lots of 50 with inducers specific for P450 isozymes in the liver, as follows: 75 mg/kg body weight sodium phenobarbital with 50 mg/kg body weight hydrocortisone. Rats were injected twice daily for three days prior to sacrifice. Other induction protocols were: 10 mg/kg body weight amitriptyline or imipramine, injected i.p. daily for 10 days; 80 mg/kg body weight β-

napthoflavone injected i.p. once daily for 3 days; 150 mg/kg body weight isosafrole injected i.p. once daily for 3 days; 100 mg/kg body weight pyrazole injected i.p. once daily for 2 days. On the day following the end of the injection protocol, rats were killed by decapitation. Livers and complete brains were placed separately in containers of liquid N_2. Cells were lysed by high speed homogenization in 4 M guanidinium isothiocyanate with 0.1 M Tris-HCl (pH 7.6) and 1% 2-mercaptoethanol. The lysate was placed on 5.7 M CsCl containing 4 mM EDTA and centrifuged at 100,000 × g for 18 hr at 12° C. The RNA pellets were resuspended in 10 mM Tris-HCl (pH 7.6), 1 mM EDTA and stored at − 80° C until use.

PCR analysis

Total RNA (25 μg) from brain or liver was used in the reverse transcription reaction. mRNA was reverse transcribed using oligo (dT) as the primer by the method of Krug and Berger [25]. The resulting RNA : cDNA hybrid was treated with 0.3 N NaOH, 30 mM EDTA and boiled for 5 min, then neutralized with HCl before use in PCR. A small fraction (less then 4%) was subjected to PCR. Primers were designed using the following criteria: there must be little or no complementarity with self or the other primer used for amplification; the two primers must amplify a region of message which originally contained an intron, where determinable; and the primers must hybridize to only a very limited number of isozyme messages in order to be able to distinguish the isozyme(s) being detected. Oligonucleotide primers were synthesized by Genosys (The Woodlands, TX). A 40 μl PCR was carried out using 0.5 μM each primer, 20 μM cDNA, 0.2 mM of each the four deoxynucleotides (dATP, dCTP, dGTP, dTTP) (Boehringer Mannheim), 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.5 units of Taq DNA Polymerase (Perkin-Elmer Cetus) and an optimal $MgCl_2$ concentration individually determined for each set of primers using cDNA made from liver. Amplification of P450 isoform IIE1 (j) included the addition of 1 μl of Perfect Match Polymerase Enhancer (Stratagene) into the PCR solution. Thirty cycles of the following PCR reaction were carried out in an Eppendorf Thermocycler: 94° C for 1 min for denaturation, 72° C for 2 min for annealing and extension. PCR products were analyzed by electrophoresis on 1% agarose gels stained with ethidium bromide. Band sizes of P450 isoforms b&e were determined from M_R vs. distance traveled plots.

Materials

Unless otherwise stated all reagents were obtained from Sigma Chemical Co.

Results

In order to amplify P450 mRNA in the brain, we initially established functional PCR primer sequences (Table 1) and hybridization conditions for RNA derived from liver as described in Materials and Methods. The length of the primers varied from 19–38 nucleotides. All of the primers are 100% homologous to their respective isoforms except for db1 which contains one redundancy resulting in a calculated percent homology of 96%. This means that db1 is actually a mixture of two sequences, one containing a C and one containing a T at position 15. Therefore, the actual homology to IID is 100% for half of the primer molecules in the db mixture (Table II).

Figure 1 demonstrates the ability of each primer set (IA1, IA2, IIA1, IIB, IIC7, IIC11, IID, IIE1, and IIA1) to amplify male rat liver mRNA under the given conditions. The band sizes do not deviate from the predictions presented in Table 1. The isoforms IIB1 and IIB2 are very homologous, differing mainly in an additional twenty-four base pairs in IIB2. These isoforms are therefore amplified with the same primers and differentiated by size on agarose gels (Figs 1 & 3).

Results of PCR amplification of brain tissue RNA that produced bands visible by agarose gel analysis are shown in Figs 2–6. Primers specific for isoforms IA1, IIB1, IIB2, IID, IIE1 and reductase all produced bands of the correct size in at least some of the differently treated brain tissue. Primers specific for isoforms IA2, IIA1, IIC7, IIC11, and IIIA1 did not produce the expected size band in any of the types of treated brain tissues in these studies (results not shown). In order to improve the PCR specificity, these isoforms were also subjected to PCR using the Perfect Match Polymerase Enhancer (as stated in Materials and Methods). Under these enhanced conditions, no additional bands were amplified.

Among the isoforms expressed in the brain, isoform

IA1 produces the greatest variety in its induction pattern (Fig. 2). All of the brain samples, treated and untreated produced the appropriate band size except for the imipramine treated rats (lane IMI). The fact that imipramine does not produce any band may be a result of deregulation of the IA1 gene in the brain. Since all of the rats were male except for the amitriptyline treated rats (lane AMI), we can conclude that both sexes maintain the ability to express IA1 under at least some given conditions.

The expression of P450 isoforms IIB1 and IIB2 is demonstrated in Fig. 3. Primers specific for isoform IIB (b1 & b2) in liver (lane LV) generate the predicted 380 and 404 bp PCR product as reported in Tables 1 and 2. The 380 bp band is visible in all of the brain samples, including the untreated brain tissue (lane UN). The same primers (b1 & b2) generate the predicted band size of 404 bp in the liver control (lane LV) as predicted in Table 1 and 2. All of the induced brain tissues produce the 404 bp product (lanes PB, AMI, ISO, IMI, BNF). The uninduced brain (lane UN) does not appear to produce the 404 bp band. We conclude that isoform IIB2 is not present or is present at extremely low levels in untreated rat brain.

Figure 4 shows the results of the PCR with primers db1 and db2, specific for isoform IID. The liver control (lane LV) produced the expected size band of 512 bp, as did all of the brain samples (lanes UN, PB, AMI, ISO, IMI, BNF). Because of the positive results for the uninduced brain (lane UN), we must conclude that at least one form of IID listed in Table 2 (IID 1, 2, 4 & 5) is expressed constitutively in rat brain. All rats were male except for the amitriptyline treated rats, therefore IID is expressed in both male and female rats.

The expression of P450 isoform IIE1 is shown in Fig. 5. PCR of this isoform was performed uniquely as stated in Methods and materials. Lane M contains 123 bp marker (Bethesda Research Labs). The liver control (lane LV) produced the expected band size of 847 bps. All of the other brain samples also produced the appropriate size band, untreated (lane UN) and treated (lanes PB, AMI, ISO, IMI, BNF), male (lanes UN, PB, ISO, IMI, BNF) and female (lane AMI).

Brain P450 reductase, in earlier work in this laboratory [22], has been purified to homogeneity. We have shown the brain reductase to function with the same catalytic ability as liver P450 reductase. Figure 6 shows, as expected, the amplification of rat brain P450 reductase mRNA in male and female rat brains. The liver control shows the expected PCR product size of 597 bp (lane LV), as do the brain samples (lanes UN, PB, AMI, ISO, IMI, BNF). In lanes (UN, PB, ISO,

Table 1. PCR primer sequences. The name of each PCR primer is recorded to the left of the DNA sequence. The name of the specific isoform that each set of primers will amplify is recorded between the names of each set of primers. Oligonucleotide sequences are listed in the 5′ to 3′ direction. The expected size in base pairs of the amplified PCR product is listed under EXPECTED BAND SIZE

P450	Primer name	Sequence	Expected band size
IIA1	a1	GAGGACACGGAGTTCCTGTCACTGC	679 bp
	a2	CCCTTGTCATCCAGGAAGTTCTGGG	
IIB1 &	b1	CCAAGCGCTCCACGAGACTT	380 bp-b
IIB2	b2	TTGGGAAGCAGGTACCCTC	404 bp-e
IA1	c1	GATGCTGAGGACCAGAAGACCGC	679 bp
	c2	CAGGAGGCTGGACGAGAATGC	
IA2	d1	CTTGGAGAAGCGTGGCCAGG	664 bp
	d2	CTACAAAGACAACGGTGGTCT	
IIC7	f1	CCCCATGGATGCAGGTCTGCAATAG	886 bp
	f2	ATTGCAGAAGGCAACACAGTAGAAGCC	
IIC11	h1	AAACTTCAGGCTTTTCAGCTCCCCATGGC	827 bp
	h2	AATGGCCAAATCCACTGATAGCTGGTG	
IID	db-1	CCTGAGAGCAGCTTYAATGATG	512 bp
	db-2	GAAGAGGAAGAGCTCCATGCGG	
IIE1	j1	CTGCCCCCAGGACCTTTTCCC	847 bp
	j2	ATCAGGAGCCCATATCTCAGAGTTGTGCTGGTGGTCTC	
IIIA1	PCN 1	CAGCTCTCACACTGGAAACCTGGG	689 bp
	PCN 2	CTCATATATTGGGGTGAGGAATGG	
reductase	OR1	GAGCACTTCAATGCCATGGGCAAG	597 bp
	OR2	GATGTCCAGGTAGTAGGTGAGGGCC	

IMI, BNF) we can observe reductase expression in differently treated male brains: untreated (lane UN), phenobarbital (lane PB), imipramine (lane IMI), isosafrole (lane ISO), β-naphthoflavone (lane BNF). Amitriptyline treated female brain (lane AMI) also shows reductase expression.

Table 2. Primer homology search. The University of Wisconsin GCG program was used to search all of the P450 sequences registered in GenBank for homology to the primers [26]. A match is noted when a set of primers will anneal to the appropriate strands of mRNA (*i.e.* the first primer of a set anneals to the coding strand of mRNA and the second primer of the set anneals to the reverse compliment (RC) strand). The size of the expected PCR product is listed after the Match number. The first number in parentheses represents the overall percent homology between the primer and the P450 sequence named; the second number represents the percent homology between the 3′ 14 bases of the primer and the P450 sequence named. Each match represent a different site in a given P450 sequence of a different sequence. All matches above 90% are listed. OR = cytochrome P450 reductase

	Match 1: 679 bp	
Primer a1	IIA1 (100;100)	
Primer a2	IIA1RC (100;100)	
	Match 1: 380 bp	Match 2: 404 bp
Primer b1	IIB1 (100;100)	IIB2 (100;100)
Primer b2	IIB1RC (95;93)	IIB2RC (95;93)
	Match 1: 679 bp	
Primer c1	IA1 (100;100)	
Primer c2	IA1RC (100;100)	
	Match 1: 664 bp	
Primer d1	IA2 (100;100)	
Primer d2	IA2RC (100;100)	
	Match 1: 886 bp	
Primer f1	IIC7 (100;100)	
Primer f2	IIC7RC (100;100)	
	Match 1: 827 bp	
Primer h1	IIC11 (100;100)	
Primer h2	IIC11RC (100;100)	
	Match 1: 847 bp	
Primer j1	IIE1 (100;100)	
Primer j2	IIE1RC (100;100)	
	Match 1: 689 bp	
Primer pcn1	IIIA1 (100;100)	
Primer pcn2	IIIA1RC (100;100)	
	Match 1: 597	
Primer OR1	OR (100,100)	
Primer OR2	ORRC (100,100)	
	Match 1: 512 bp	Match 2: 512 bp
Primer db1	IID1 (96;93)	IID2 (96;93)
Primer db2	IID1RC (100;100)	IID2RC (100;100)
	Match 3: 512	Match 4: 512 bp
Primer db1	IID4 (96;93)	IID5 (96;93)
Primer db2	IID4RC (100;100)	IID5RC (100;100)

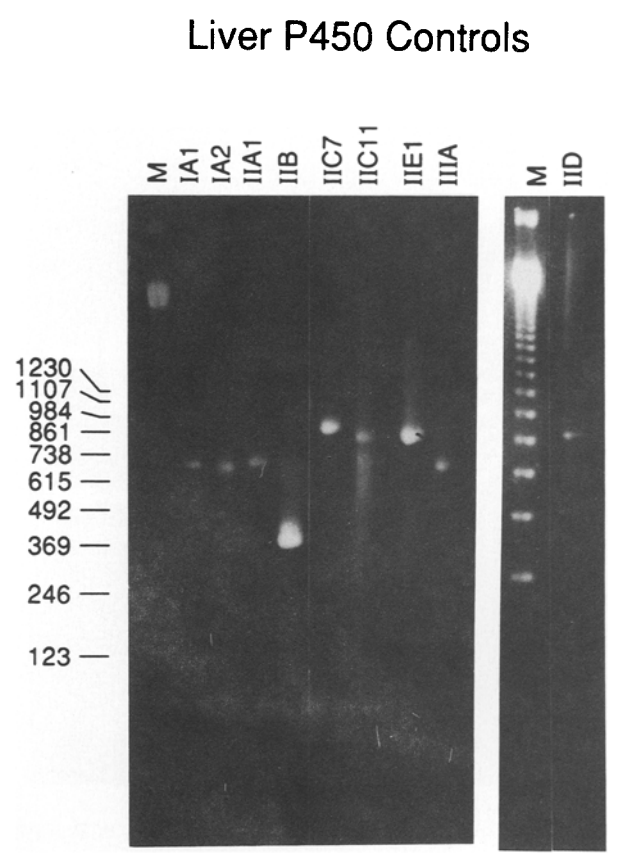

Fig. 1. mRNA expression analysis by PCR of liver tissue. mRNA was extracted from rat livers and amplified by PCR as described in Materials and methods. Primer sequences used to amplify each P450 isoform are listed in Table 1. Expected band sizes are listed in Table 2. 4 μl of each PCR were loaded in each experimental lane. From left to right lanes were loaded as follows: 1 μg of 123 bp molecular weight marker (M), P450 IA1 amplified with primers c1/c2 (IA1), P450 IA2 amplified with primers d1/d2 (IA2), P450 IIA1 amplified with primers a1/a2 (IIA1), P450 IIB amplified with primers b1/b2 (IIB), P450 IIC7 amplified with primers f1/f2 (IIC7), P450 IIC11 amplified with primers h1/h2 (IIC11), P450 IIE1 amplified with primers j1/j2 (IIE1), P450 IIIA1 amplified with primers pcn1/pcn2 (IIIA1), 1 μg of 123 bp molecular weight marker (M), and P450 IID amplified with primers db-1/db-2 (IID).

Discussion

Ten sets of PCR primers were designed for analysis of RNA isolated from rat brain to identify various P450 isozymes and reductase which may be present in that tissue. Using the GCG program [26], each primer was examined for homology to all P450 sequences registered in GenBank to determine the specificity of each set of primers. One of the primers in a single set must be able to hybridize to the complementary strand of DNA in order to produce a complete PCR product. In our

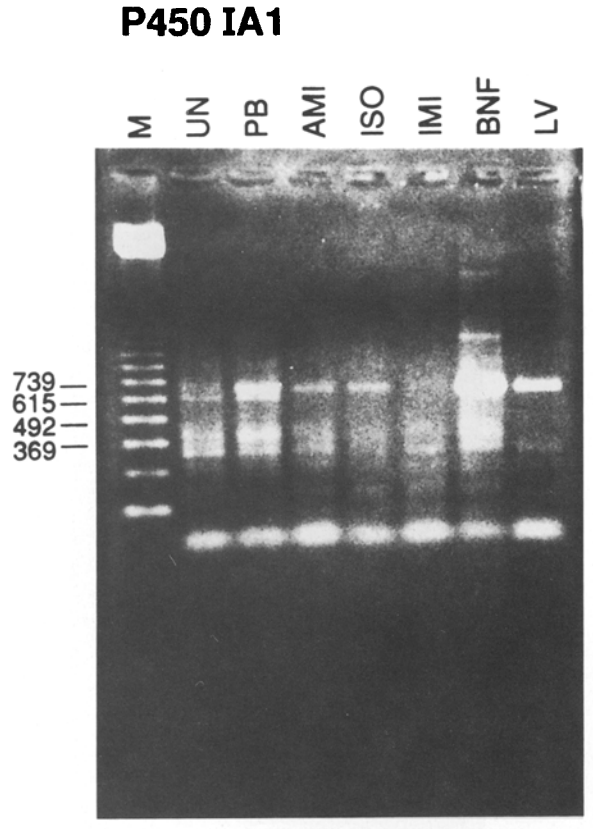

Fig. 2. mRNA expression analysis of rat brain tissue by PCR of P450 isoform IA1 (c). mRNA was extracted from rat brains treated as designated by lane markers below and amplified by PCR as described in Materials and Methods. Primers c1 and c2 (Table 1) were used to amplify P450 isoform IA1 (c). 1 μg of 123 bp molecular weight marker was loaded in lane marked (M). 4 μl of each PCR were loaded in each experimental lane as follows: UN = untreated, PB = phenobarbital, AMI = amitriptyline, ISO = isosafrole, IMI = imipramine, β-NF = β-naphaflavone. A rat liver control was amplified using the same primers and conditions (lane LV).

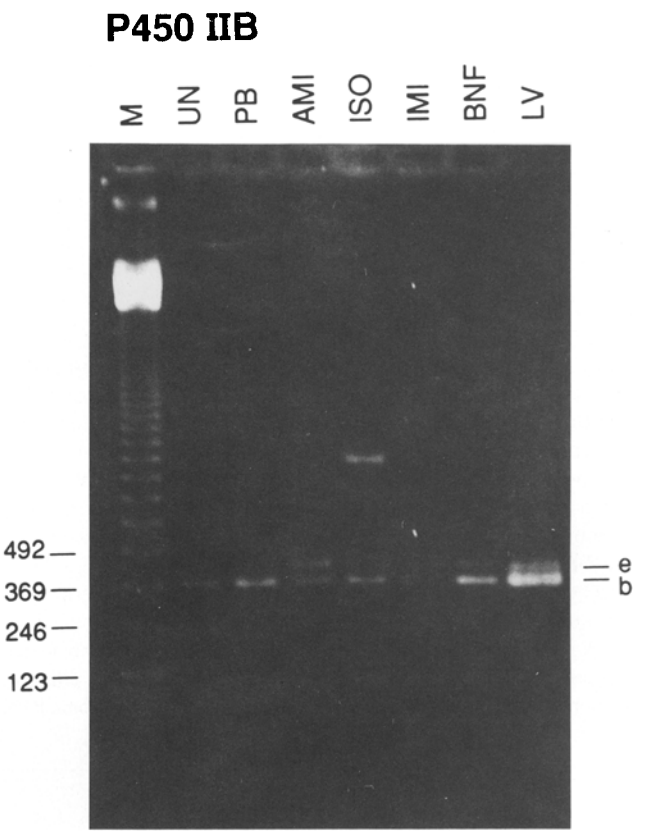

Fig. 3. mRNA expression analysis of rat brain tissue by PCR of P450 isoforms IIB (b & e). mRNA was extracted from rat brains and amplified by PCR as described in Materials and Methods. Primers b1 and b2 (Table 1) were used to amplify P450 isoform IIB1 & 2 (b & e). 1 μg of 123 bp molecular weight marker was loaded in lane marked (M). 4 μl of each PCR were loaded in each experimental lane as follows: UN = untreated, PB = phenobarbital, AMI = amitriptyline, ISO = isosafrole, IMI = imipramine, β-NF = β-naphaflavone. A rat liver control was amplified using the same primers and conditions (lane LV).

search, each primer in a set was compared to both strands for homology. Both the entire primer sequence and the 14 bases closest to the 3′ end were examined for homology to the P450 sequence. As a result of the high temperature (72° C) used in the annealing step of the amplification, only homology equal to or greater than 90% was considered to be significant. The validity of the hypothesis was borne out by the fact that predictions of PCR products using lower homology limits were not supported by the results in liver (Fig. 1). Even with the very stringent annealing PCR conditions that we have optimized for liver mRNA, occasionally a primer may anneal to a specific site in the brain cDNA with 6–8 bp homology at the 3′ end. For example, in the ISO lane of Fig. 3 there is a 791 bp band that has been amplified in addition to the 380 and 404 bp products. It is very possible that primer b1 has annealed to a site 386 bp upstream (at base 370) of the intended site of 756 in P450 IIB2. The alternate annealling site (base 370) contains seven bp of perfect homology at the 3′ end of the primer. The occasional presence of additional bands not withstanding the appearance of a good band of the predicted size gives strong evidence that the particular isoform is represented in the messenger RNA of the brain. Although it might be possible to select a unique set of primers by lowering the stringency conditions for the GCG searches, the degree of homology among the isoforms makes overlaps and therefore cross-hybridization among isoforms more likely (i.e. primers for IIE1 might recognize IIB1).

The results of the GCG searches (Table 2) showed the variety of PCR products possible from a given set of primers. For example, these searches implied that the

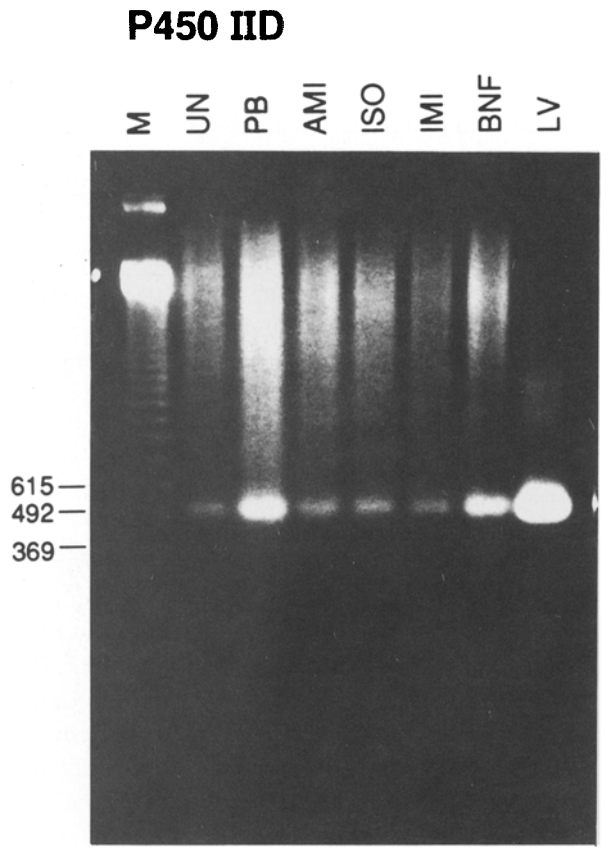

Fig. 4. mRNA expression analysis of brain tissue by PCR of P450 isoforms IID (db). mRNA was extracted from rat brains and amplified by PCR as described in Materials and Methods. Primers db1 and db2 (Table 1) were used to amplify P450 isoform IID (db). 1 μg of 123 bp molecular weight marker was loaded in lane (M). 4 μl of each PCR were loaded in each experimental lane as follows: UN = untreated, PB = phenobarbital, AMI = amitriptyline, ISO = isosafrole, IMI = imipramine, β-NF = β-naphaflavone. A rat liver control was amplified using the same primers and conditions (lane LV).

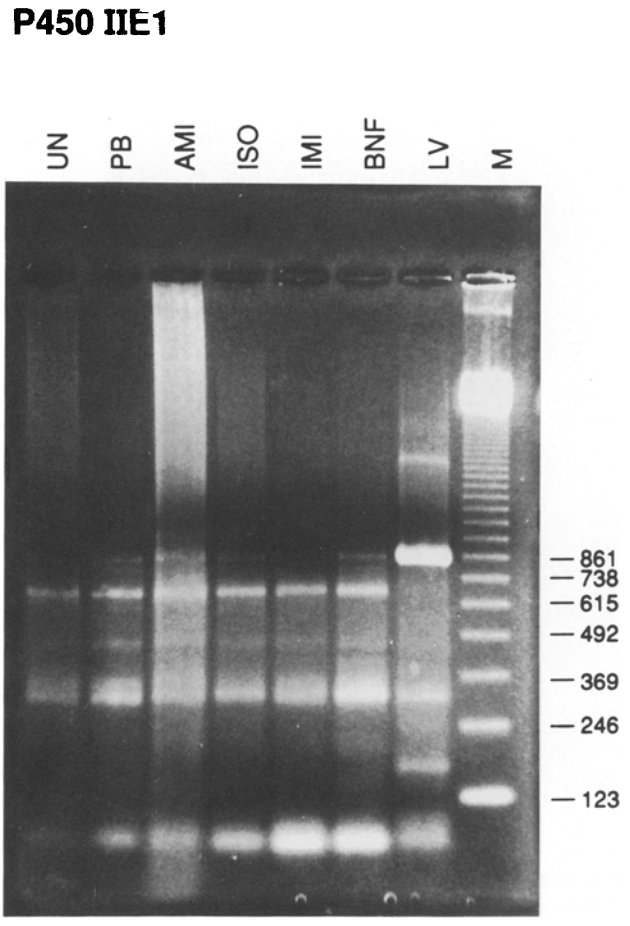

Fig. 5. mRNA expression analysis of brain tissue by PCR of P450 isoforms IIE1 (j). mRNA was extracted from rat brains and amplified by PCR as described in Materials and Methods. Primers j1 and j2 (Table 1) were used to amplify P450 isoform IIE1 (j). 1 μg of 123 bp molecular weight marker was loaded in lane (M). 4 μl of each PCR were loaded in each experimental lane as follows: UN = untreated, PB = phenobarbital, AMI = amitriptyline, ISO = isosafrole, IMI = imipramine, β-NF = β-naphaflavone. A rat liver control was amplified using the same primers and conditions (lane LV).

primer set c1/c2 should detect only IA1 messages and the primer set d1/d2 should detect only IA2 messages, although these two isozymes are very homologous in DNA sequence. The success of this approach is suggested by the fact that d1/d2 is unable to detect message in brain, although c1/c2 was successful in amplification of a message in brain. The primer set d1/d2 was, however, able to detect IA2 message in liver. The homology search also showed that the primer set db1/db2 should detect a number of IID isozymes (IID1, 2, 4 and 5) which must then be differentiated on the basis of restriction mapping or sequencing of the PCR product.

Using the predictions from Table 2, and the PCR results shown above, we conclude that the brain is capable of producing several isozymes of P450, including IA1, IIB1, IIB2, IID and IIE1 (b, e, c, db, and j) and reductase as summarized in Table 3. The detection of IIB1 [13, 18], IA1 [13, 16, 17, 18], IID [14, 15], IIE1 [19] and reductase [22] agrees with previous findings using immunohistochemistry and/or activity (as specified in the introduction) showing that these enzymes are present in rat brain. The detection of IIB2 specifically has not been reported previously, although IIB1 and IIB2 are so closely related that immunological or Northern techniques used to demonstrate the presence of IIB1 are not able to discriminate between these two forms. Kohler *et al.* [17] and Warner *et al.* [18] have reported the presence of P450IA2 (d) in brain tissue using an immunological probe which detects both P450 isoforms IA1 and IA2. Therefore, with their positive results,

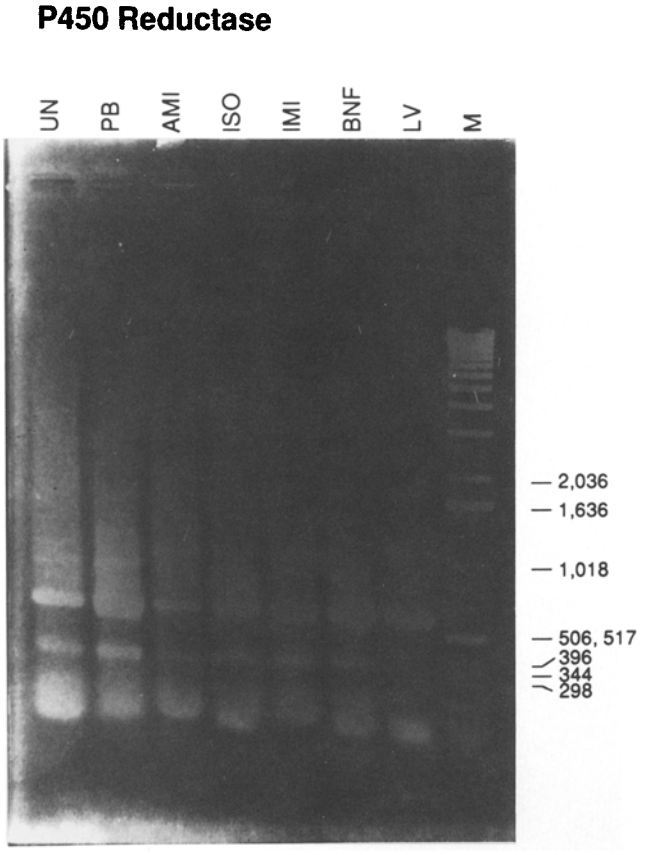

Fig. 6. mRNA expression analysis of brain tissue by PCR of P450 reductase. mRNA was extracted from rat brains and amplified by PCR as described in Materials and Methods. Primers OR1 and OR (Table 1) were used to amplify P450 reductase. 4 μl of each PCR were loaded in each experimental lane as follows: UN = untreated, PB = phenobarbital, AMI = amitriptyline, ISO = isosafrole, IMI = imipramine, β-NF = β-naphaflavone. A rat liver control was amplified using the same primers and conditions (lane LV). 1 μg of 1 kd molecular weight marker was loaded in lane (M).

they concluded that both isoforms were present. We were able to differentiate between the isoforms in liver by the amplification of different size PCR products using primers specific for each isoform. In brain we observed the expression of only one form, P450IA1. The inability to detect other isozymes using the PCR primers can be attributed to either a total absence or extremely minute levels of those members of the P450 family in our system, since each set of primers used produced the predicted results from the liver cDNA. We infer that those messages which were demonstrated to be present are significant, since P450 reductase message has also been found to be present in rat brain by PCR (Fig. 6) and since we were able to isolate and purify reductase which is fully active in a reconstituted MFO system [22].

Table 3. Summary of mRNA expression analysis. Results for each P450 isozyme and reductase that produced positive expression results by PCR analysis (Figs 2–6). Treatment of rats are listed under inducers. Each P450 isoform and reductase are listed on the top of the grid. A (+) indicates a PCR product of the appropiate size generated by primers specific for the indicated isoform. A (−) indicates lack of the appropiate PCR product of that isoform for the indicated primers.

	P450 isozyme or reductase					
Inducers	IA1 (c)	IIB1 (b)	IIB2 (e)	IID (db)	IIE1 (j)	reductase
Untreated	+	+	−	+	+	+
Phenobarbital	+	+	+	+	+	+
Amitriptyline	+	+	+	+	+	+
Isosafrole	+	+	+	+	+	+
Imipramine	−	+	+	+	+	+
β-naphthoflavone	+	+	+	+	+	+

From the above results we may conclude that reductase as well as many forms of P450 including IA1, IIB1, IIB2, IID and IIE1 are expressed in rat brain. Several of these isoforms (IA1, IIB1, IID and IIE1) and reductase are expressed even in the brains of untreated animals. It appears that at least two forms of cytochrome P450 (IA1 & IIB2) in the brain are under various levels of control by different P450 inducers. Thus, the brain contains a mixed function oxidase system capable of metabolizing an extensive array of xenobiotics which are inducible by some treatments.

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