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Expression of Multiple Forms of Cytochrome P450 and Associated Mono-oxygenase Activities in Rat Brain Regions

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ABSTRACT. Cytochrome P450 (P450) content and P450-mediated mono-oxygenase activities were measured in microsomes prepared from various regions of rat brain. The regional P450 content in brain varied between 0.1 and 0.15 nmol/mg of protein, with the brainstem and cerebellum showing the highest levels. NADPH cytochrome *c* reductase activity was highest in the cortex followed by cerebellum and brainstem as compared with the whole brain. Mono-oxygenase activities also varied among the various brain regions. Southern blot analysis of the cDNA synthesized from the poly(A)RNA isolated from rat brain regions and hybridized with cDNA to rat liver P4502B or P4502E1 revealed the presence of a transcript in untreated rat brain that had a molecular mass similar to that of the corresponding transcript from rat liver. Immunoblot analyses using antisera to purified rat liver P4502E1, P450(2B1/2B2), and a phenobarbital-inducible form of rat brain P450 revealed the presence of corresponding immunoreactive protein bands in all the brain regions examined. The present study demonstrated the diversity in the distribution of P450 and associated mono-oxygenase activities in brain and thus may reflect the differential capability of various regions of the brain to detoxify or bioactivate diverse xenobiotics. BIOCHEM PHARMACOL **56**;3:371–375, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. cytochrome P450; mono-oxygenase; brain; drug metabolism; cytochrome P4502B1; cytochrome P4502E1

P450§ (EC 1.14.14.1) and associated mono-oxygenases are involved in the oxidation of a wide variety of xenobiotics as well as endogenous compounds [1]. The broad substrate specificity of P450 and the variety of chemical reactions mediated by P450 are attributed to the presence of multiple isoforms within this hemeprotein family [2, 3].

P450 and associated mono-oxygenase activities are found predominantly in liver, although their presence in a variety of extrahepatic tissues [4–7] including the brain has been well documented [8–10]. In addition to the biotransformation of xenobiotics, cerebral P450-mediated metabolism of endogenous compounds such as steroids and prostaglandins has also been detected [11]. Multiple forms of P450 in brain are selectively induced by a variety of chemicals [10, 12]. A phenobarbital-inducible form of P450 has been purified from rat brain, and the activity has been reconstituted *in vitro* [13]. Collectively, such studies have firmly established the presence of an integral P450 system in the brain.

The specific content of P450 in brain is about one-tenth of that of the liver [10]. Considering the extraordinary regional and cellular heterogeneity within the brain, variations in the distribution of P450 within certain cells in specific regions conceivably could confer differential vulnerability to damage through bioactivation mediated by this enzyme. Such observations have been made in a variety of other extrahepatic tissues, for example the lung, where the pulmonary P450 levels are only one-tenth of corresponding hepatic levels, and the enzyme is present mostly in Clara and Type II cells [14]. Consequently, there is selective vulnerability and, hence, damage of these cells by environmental toxins [15].

The present study was carried out, therefore, to examine the constitutive expression and differential distribution of multiple forms of P450 and associated mono-oxygenase activities in various regions of the rat brain.

MATERIALS AND METHODS

Male Wistar rats (3–4 months old) were obtained from the NIMHANS Animal Research Facility. Animals had access to pelleted diet (Lipton India Ltd.) and water *ad lib.*

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§ *Abbreviations:* APD, aminopyrine *N*-demethylase; ECD, 7-ethoxycoumarin *O*-deethylase; MND, morphine *N*-demethylase; NDMAD, *N*-nitrosodimethylamine *N*-demethylase; P450, cytochrome P450; and RT-PCR, reverse transcriptase/polymerase chain reaction.

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TABLE 1. Cytochrome P450 and associated mono-oxygenase activities in various rat brain regions

Region	P450 (nmol/mg protein)	Reductase (nmol cytochrome *c* reduced/min/mg protein)	APD	MND	ECD	NDMAD
			(nmol product formed/min/mg protein)			
Cortex	0.10 ± 0.002	27.0 ± 0.8*	169.0 ± 6.3	119.0 ± 8.5	2.2 ± 0.3*	0.80 ± 0.08
Cerebellum	0.14 ± 0.006*	23.0 ± 0.4*	159.0 ± 8.5	109.3 ± 6.0	1.5 ± 0.2	0.70 ± 0.01*
Brainstem	0.15 ± 0.003*	23.0 ± 0.8*	151.4 ± 5.0	119.0 ± 6.8	2.6 ± 0.6*	0.60 ± 0.05*
Striatum	0.10 ± 0.002	20.5 ± 0.8	133.8 ± 6.0	105.0 ± 9.5	1.0 ± 0.01	0.60 ± 0.03*
Hippocampus	0.10 ± 0.002	19.5 ± 0.6	147.0 ± 10.0	127.3 ± 8.5	0.9 ± 0.1	0.70 ± 0.03*
Thalamus	0.11 ± 0.002	20.5 ± 0.8	201.5 ± 13.0*	125.3 ± 13.0	2.4 ± 0.4*	0.62 ± 0.06*
Whole brain	0.10 ± 0.004	21.0 ± 0.1	178.0 ± 5.0	128.4 ± 2.1	1.5 ± 0.2	0.80 ± 0.03

P450 content is expressed as mean ± SEM, while the other activities are expressed as mean ± SD (N = 4–6 experiments carried out with different batches of microsomes prepared by pooling brain regions from 30 rats).

*Significantly different from whole brain ($P < 0.05$), as determined by ANOVA followed by Duncan's test.

Animals were anesthetized with ether and perfused transcardially with 30 mL of 0.1 M Tris–HCl (pH 7.4) prior to decapitation. The brain was removed immediately and six regions, namely the cortex, cerebellum, brainstem, striatum, hippocampus, and thalamus, were dissected as described [16].

Microsomes were prepared from whole brain and various regions of the brain by the calcium precipitation method [17]. Brain regions from 30 animals were pooled for each experiment. Microsomes were stored at −70° until used. Protein content was determined by the dye-binding method [18]. All experiments were carried out with at least three different batches of microsomes, each prepared from brain regions pooled from 30 rats.

P450 levels [19], NADPH cytochrome *c* reductase activity [20], and levels of APD and MND [21], ECD [22], and NDMAD [23] were measured according to the referenced procedures. All enzyme assays were performed in duplicate, using each batch of microsomes. Statistical analyses were done using ANOVA followed by Duncan's multiple range test.

Microsomal proteins from various brain regions were subjected to SDS–PAGE [24]. The proteins were transferred from the gel to nitrocellulose paper as described [25]. The transferred proteins were immunostained using antisera to purified rat liver (1) P450(2B1/2B2), (2) P4502E1, and (3) a purified phenobarbital-inducible form of rat brain P450 [13].

Rats were killed by cervical dislocation, and the total RNA was prepared immediately from brain regions and liver using acid guanidinium thiocyanate-phenol-chloroform extraction [26]. The poly(A^+)RNA was prepared from the total RNA by purification, using oligo(dT)cellulose. The cDNA was synthesized from the mRNA prepared from rat brain regions and liver using random hexamer primers [27] and processed for Southern blots [28] following electrophoresis on 1.2% agarose gels. The DNA was transferred from agarose gels to nylon membranes, UV cross-linked, and hybridized with digoxigenin-labeled cDNA to rat liver P4502B1 or P4502E1 (obtained as gifts from Dr. Frank Gonzalez and B. J. Song, respectively). The hybridized cDNA was visualized using alkaline phosphatase-labeled antibody to digoxigenin and appropriate chromophores.

RESULTS

P450 content and various P450-associated mono-oxygenase activities in rat brain regions are given in Table 1. P450 levels were significantly higher in brainstem and cerebellum as compared with the whole brain. NADPH cytochrome *c* reductase activity in the brain was approximately 30% of the hepatic level (data not shown), and its activity was significantly higher in the cerebral cortex, cerebellum, and brainstem than in the whole brain.

P450-mediated mono-oxygenase activities also showed regional variations within the brain. APD activity was significantly higher in the thalamus as compared with the whole brain. ECD activity was significantly higher in cortex, brainstem, and thalamus than in the whole brain. NDMAD activity in the cortex was comparable to that of the whole brain, whereas all the other brain regions showed significantly lower NDMAD activity (Table 1). There also appeared to be some regional differences in MND activity; however, these differences did not reach statistical significance in the present investigation.

SDS–PAGE of microsomal proteins from various regions of the rat brain followed by immunoblotting with antiserum to rat liver P450(2B1/2B2) revealed the presence of a single protein band in all the regions examined (Fig. 1A). Similar SDS–PAGE followed by immunostaining with antisera to a phenobarbital-inducible form of rat brain P450 revealed the presence of a single immunoreactive band in all the regions examined (Fig. 1B). Immunoblot analyses using antisera to rat brain P4502E1 also revealed the presence of a single immunoreactive band in all the regions examined (Fig. 1C).

Rat brain mRNA hybridizable with the cDNA corresponding to rat liver P4502B (Fig. 2, left panel) and P4502E (Fig. 2, right panel) was visualized in Southern blots performed using the cDNAs to rat brain regions and liver poly(A)RNA. The molecular masses of the transcripts coding for rat brain P4502B and P4502E were similar to those seen in rat liver (Fig. 2). Constitutive expression of

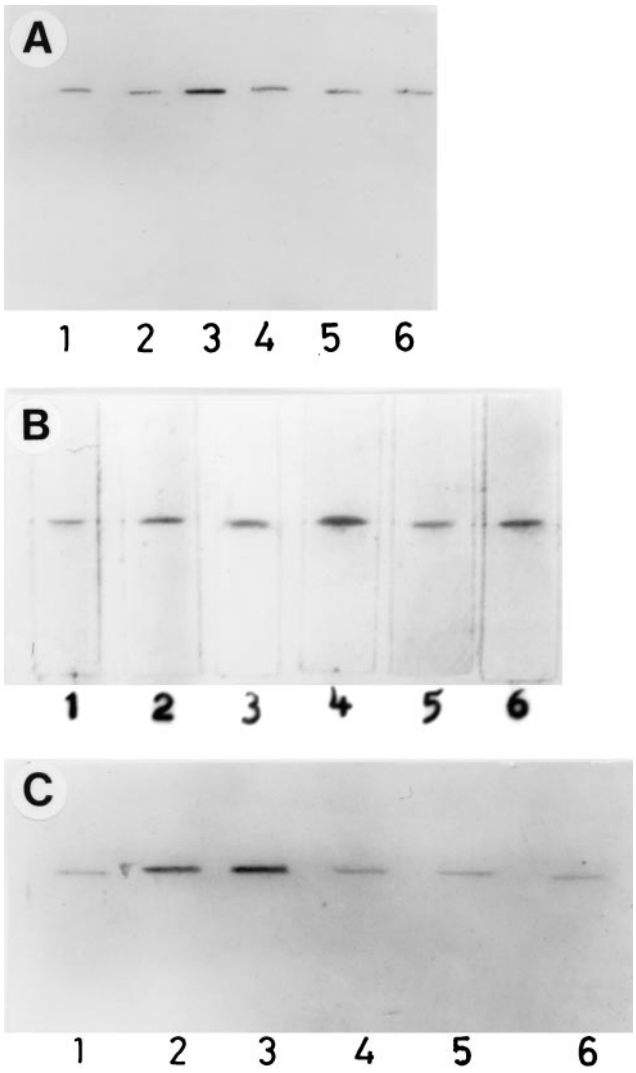

FIG. 1. Immunoblots of microsomes from rat brain regions and liver following staining with antisera to: (A) rat liver P450(2B1/2B2). Each lane was loaded with 40 μg of brain microsomal protein; (B) a purified phenobarbital-inducible form of cytochrome P450 from rat brain. Each lane was loaded with 40 μg of brain microsomal protein; and (C) rat liver P4502E1. Each lane was loaded with 180 μg of brain microsomal protein. The microsomes from different brain regions were loaded onto the gel in the following lanes: (1) cortex, (2) cerebellum, (3) brainstem, (4) striatum, (5) hippocampus, and (6) thalamus.

P4502B and P4502E was detected in all brain regions examined. In the blots hybridized with cDNA to P4502B (Fig. 2, left panel), another band of a higher molecular mass (~3.0 kb) was detected in brain regions as well as in the liver.

DISCUSSION

The present study revealed some distinct regionality in the distribution of P450 and associated mono-oxygenase activities in rat brain. Although the P450 levels were significantly higher in cerebellum and brainstem, it is interesting that the brainstem region contains relatively low levels of glutathione compared with other brain regions [29, 30]. A relatively high P450 content combined with low glutathione levels might render this region particularly vulnerable to damage through P450-mediated bioactivation of xenobiotics to reactive electrophilic metabolites.

The comparably high specific content of P450 in the cerebellum and brainstem was paralleled by relatively high levels of reductase activity in these regions; however, the highest reductase activity was observed in the cortex. The distribution of mono-oxygenase activities also showed regional differences, but these differences did not consistently parallel the P450 differences. For example, in the thalamus, APD and ECD activities were higher and NDMAD activity was lower than in the whole brain, yet the P450 content of this region was not significantly different from the whole brain. Similarly, in the cortex, the ECD activity was higher than in the whole brain, yet the P450 content of this region was not significantly different from the whole brain. Such variances may reflect regional differences in the presence of P450 forms other than those examined in the present study. Likewise, although P450 levels in cerebellum were significantly higher than in all the other regions of the brain with the exception of brainstem, this was not reflected in any of the mono-oxygenase activities tested.

The present study also indicates a considerable regional variation in the distribution of various isoforms of P450 in brain. Gross P450 content alone does not necessarily reflect the xenobiotic-metabolizing capability of a particular region, as illustrated by the substantial region-to-region variation in the capacity to metabolize a variety of substrates (Table 1). Determination of a variety of mono-oxygenase activities in this study provided a more adequate assessment of the xenobiotic-metabolizing capability of distinctive regions of the brain.

Immunoblots from brain regions showed immunological cross-reactivity between certain forms of hepatic P450 and cerebral P450, further suggesting that multiple forms of P450 are constitutively but differentially distributed in rat brain regions. An interesting observation in the present study was that the intensity of the immunoreactive bands from brain regions varied among the blots immunostained with antiserum to rat liver P4502B (the phenobarbital-inducible form of rat liver P450) and the antiserum to the phenobarbital-inducible form of rat brain P450. Upon visual inspection, immunostaining with the antiserum to rat liver P4502B showed maximal intensity in brainstem and cerebellum, whereas the antiserum to rat brain P450 showed increased staining in the striatum and thalamus, although quantitative analysis of data are not available. In our earlier studies using microsomes prepared from whole brain of rats, we demonstrated that brain P450s co-migrate with the corresponding bands in liver microsomes [10, 12, 13].

Earlier studies on the constitutive expression of multiple forms of P450 in rat brain regions have typically used slot-blot analysis or RT-PCR. The data presented here provide the first direct evidence for the expression of

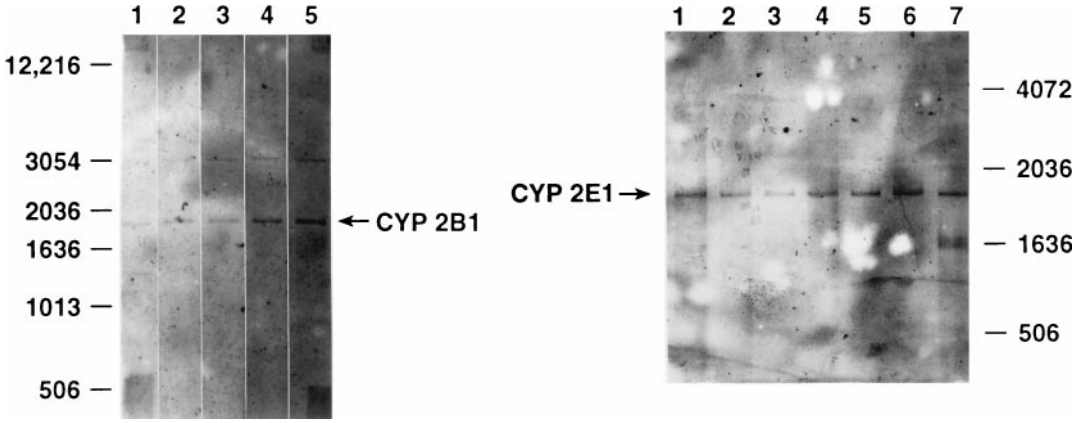

FIG. 2. Southern blot of cDNA synthesized from poly(A)RNA of rat brain regions and liver and hybridized with cDNA to rat liver P4502B (left panel) and P4502E (right panel). Poly(A)RNA (2 μg from brain regions) was used for the synthesis of cDNA using random hexamer primers, and all of the cDNA synthesized was used for Southern blot. Poly(A)RNA from rat liver (1 μg) was used for cDNA synthesis, and one-half of the cDNA synthesized was loaded onto the gel. The cDNA was subjected to electrophoresis on 1.2% agarose gel and transferred to nylon membrane for hybridization with cDNA to rat liver P4502B or P4502E. Lanes represent the following: (Left panel): (1) cortex, (2) cerebellum, (3) thalamus, (4) brainstem, and (5) liver. (Right panel): (1) cortex, (2) cerebellum, (3) thalamus, (4) hippocampus, (5) striatum, (6) brainstem, and (7) liver. The apparent molecular mass of the standard DNA ladder is also depicted.

full-length cDNA of P4502B and P4502E in different regions of rat brain and demonstrate that the molecular masses of the transcripts are similar to those from the liver. Because the blots were performed using cDNA synthesized from mRNA, the relative quantitation of the intensity of bands could not be carried out because this would require the assumption that the cDNA synthesis proceeded to the same extent in all the samples. It was not possible to quantitate the cDNA prior to loading onto the gels.

Earlier slot-blot analyses of untreated rat brain poly(A^+)RNA using the cDNA for rat liver P4502B1 (P450b) as a probe revealed the presence of detectable amounts of hybridizable RNA [31, 32]. The PCR of reverse transcriptase-treated mRNA from whole brain using specific oligonucleotide primers for CYP2B1 and CYP2E1 has revealed the constitutive presence of P4502B1 and P4502E1 in rat brain [33, 34]. The RT-PCR analysis of rat whole brain has also revealed the constitutive presence of P4501A1.

A certain amount of inconsistency is apparent upon further review of the available information on the presence of various forms of P450 in brain (as detected immunologically or by mRNA analyses). Immunological studies have been carried out by various laboratories with polyclonal or monoclonal antibodies, which may have contributed to variability in the observations reported. Similarly, the RT-PCR studies were performed using a variety of oligonucleotide primers, probably lending certain variations to the resulting observations. For example, Hodgson and coworkers [33] were unable to detect P4501A2 by RT-PCR analysis of brain mRNA, but other workers using different oligonucleotide primers subsequently detected it by RT-PCR [35]. Limitations in homologies between brain and liver P450 might additionally account for divergence of experimental results. A more detailed characterization of the individual forms of brain P450 therefore assumes greater importance for future investigations.

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