0026-895X/93/050990-07$03.00/0

MOLECULAR PHARMACOLOGY, 44:990-996

Regional Distribution and Expression Modulation of Cytochrome P-450 and Epoxide Hydrolase mRNAs in the Rat Brain

BENOIT SCHILTER and CURTIS J. OMIECINSKI

Department of Environmental Health, School of Public Health and Community Medicine, SC-34, University of Washington, Seattle, Washington 98195

Received April 14 , 1993; Accepted August 10, 1993

SUMMARY

In the present study, we developed a very sensitive, semiquantitative assay based on the reverse transcriptase-coupled polymerase chain reaction to measure, in a region-selective manner, mRNA expression patterns within the brain for microsomal epoxide hydrolase and several cytochrome P-450s (P-450s) known to be induced by prototypic agents in other tissues. The P-450s assessed included the polyaromatic hydrocarbon-inducible CYP1A1 and CYP1A2 systems, together with the phenobarbital-inducible P-450s, CYP2B1, CYP2B2, CYP3A1, which were examined 18 hr after a single intraperitoneal dose of the respective inducing agents. Highly region-specific patterns of expression were evident for P-450 mRNAs within the rat brain. In the control, uninduced brain, CYP1A1 mRNAs were readily detected in the striatum and in the hypothalamus, and to a lesser extent in the other regions examined. The regional pattern of expression was similar for CYP1A2; however, a major difference was noted in the olfactory bulbs, characterized by a relatively high level of CYP1A2 mRNA but correspondingly low levels of CYP1A1. Within the brain regions examined, the highest content of CYP2B1 and CYP2B2 mRNAs were present in the striatum and in the cerebellum, whereas CYP3A1 levels varied only slightly across the respective regions. In contrast to the P-450s, microsomal epoxide hydrolase mRNAs were expressed at relative homogeneous amounts throughout the brain. β-Naphthoflavone markedly increased the CYP1A1 and CYP1A2 mRNA contents of each brain region investigated, although this agent did not affect levels of epoxide hydrolase. At 18 hr post-treatment with phenobarbital, an optimal time period for hepatic induction, brain expression was characterized by a complex pattern of effects, with increased levels noted for CYP2B1 mRNA content in the medulla oblongata, midbrain, and cortex, but decreased contents measured in the cerebellum, the hypothalamus, and the striatum. In each of these respective regions, CYP2B2 content was profoundly decreased whereas epoxide hydrolase expression was slightly increased by the same treatment. These results establish that the central nervous system actively expresses a number of different biotransformation gene products in a regional specific and inducer-dependent manner, and suggest that for tissues exhibiting low regenerative capacity, like the brain, such reactions are likely to be of critical toxicological significance.

The P-450s are a superfamily of genes encoding an array of enzymes involved in the oxidation of endogenous compounds such as steroids, fatty acids, and prostaglandins, as well as of exogenous chemicals including drugs and environmental pollutants (1, 2). The P-450-dependent biotransformation of xenobiotics may result in the production of reactive metabolites that are often detoxified by conjugation with molecules such as glucuronic acid, sulfate, or glutathione (1). However, the reactive intermediates produced by P-450 enzymes frequently bind to proteins, lipids, or nucleic acids, leading to cytotoxicity, mutations, and/or cancer (1, 3). Many P-450 genes are under complex and distinct control by both exogenous and endogenous compounds (1, 2). For example, in the rat liver, the aromatic hydrocarbons induce genes in the CYP1A subfamily, whereas phenobarbital increases the transcriptional rates of particular genes in the CYP2B, CYP2C, and CYP3A subfamilies (4). The highest P-450 content is found in the liver; however, substantial expression occurs in many extrahepatic tissues, especially in tissues with high environmental exposure like intestine and lung (3).

Several P-450-catalyzed oxidations of endogenous (5, 6) as well as exogenous substrates have been reported to occur in the mammalian brain. With respect to xenobiotic biotransformation, low but significant activities of P-450-catalyzed reactions have been measured in the brain of man (7), mouse (8), and rat (9). These include the formation of carcinogens and mutagens, the activation of neurotoxic insecticides and the biotransformation of diverse drugs such as ethylmorphine, morphine, codeine, bufurarol, benzamphetamine, and aminopyrine (10).

This study was supported by National Institutes of Health Grants GM32281 and ES04978. B.S. is a recipient of a Swiss National Research Foundation fellowship award.

ABBREVIATIONS: P-450, cytochrome P-450; CNS, central nervous system; PB, phenobarbital; βNF, β-naphthoflavone; PAH, polyaromatic hydrocarbon; EH, microsomal epoxide hydrolase; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-coupled polymerase chain reaction; bp, base pair(s).

Immunohistochemical studies using antibodies raised against hepatic P-450s revealed the presence of CYP1A-, CYP2B-, and CYP2E-like immunoreactivity in the rat brain (11–14). However, the cerebral localization of these proteins is still uncertain because conflicting results were obtained, apparently due to discrepancies in the antisera used which may recognize different P-450 epitopes and/or cross-react with other uncharacterized brain P-450s (14).

Nevertheless, previous reports demonstrate the capability of the CNS to metabolize foreign chemicals, and this feature may have important consequences regarding the pharmacokinetics of neuroactive drugs. Also, the P-450-dependent transformation of substrates to reactive intermediates may be of high neurotoxicological significance in the brain, because this organ has very limited regenerative ability. For example, mouse cerebral P-450s have been implicated in the neurotoxicity of phenytoin, an anticonvulsant drug in wide therapeutic use (8). Furthermore, several lines of evidence suggest a possible role of brain P-450s in the development of neurodegenerative processes, such as Parkinson's disease (15, 16).

Despite the potentially important implications of P-450 expression in the CNS, little is known about the particular forms of P-450s expressed in the brain, their location, or their molecular regulation. The main goal of the present study was to examine specific brain expression profiles for several of the major rat P-450s involved in the xenobiotic metabolism; the PAH-inducible P-450s, CYP1A1 and CYP1A2, as well as the PB-inducible P-450s, CYP2B1, CYP2B2, and CYP3A1. In parallel we also measured the expression of EH transcripts, as a marker of xenobiotic metabolism distinct from the P-450s. Because of the low level of P-450 and EH expression in the CNS, we developed a semiquantitative RT-PCR strategy. This extremely sensitive methodology was shown to be useful for studying P-450 mRNAs in fetal tissues in which mRNAs were not readily detectable with other techniques (17, 18). Moreover, the use of specific primers during the amplification process permitted the discrimination of very closely related genes products, such as CYP2B1 and CYP2B2, which share 97% sequence identity (17). This strategy was used to compare the contents of different P-450s and EH mRNAs in distinct brain regions and to determine the cerebral effects of chemicals known to induce P-450s in the liver.

Materials and Methods

Animals. Adult male Sprague-Dawley rats (280–320 g) were obtained from Simonsen (Gilroy, CA). The animals were housed in plastic cages and maintained on a 12-hr light-dark cycle. Food and water were freely available. To assess the effects of PB or βNF, rats were treated with a single intraperitoneal injection of either 80 mg/kg PB in saline solution, or 80 mg/kg βNF dissolved in corn oil. Control rats received equivalent volumes of the respective vehicle alone. The rats were sacrificed 18 hr after the individual treatments.

Tissue preparation and RNA isolation. The animals were killed by decapitation under light ether anesthesia. The brains were rapidly removed and washed with ice-cold saline solution. Livers also were excised to independently assess the effects of inducer treatment. For regional distribution studies, brains were dissected into seven regions: cerebellum, medulla oblongata, olfactory bulbs, hypothalamus, striatum, midbrain, and cortex, according to Glovinski and Iversen (19). Some of these regions include more than one distinct anatomical structure: the "medulla oblongata" corresponds to the medulla oblongata and pons; the "striatum" contains the putamen nucleus, the caudate nucleus, and the globus pallidus nucleus. The "midbrain" corresponds to the midbrain, the thalamus, the subthalamus, and the hippocampus. Tissue samples were immediately frozen in liquid nitrogen and stored at −80° until RNA extraction.

The RNAs were purified according to the method of Chomczynski and Sacchi (20). The RNA concentrations were quantified by UV absorbance at 260 nm using a Beckman DU-50 spectrophotometer.

cDNA synthesis. Before the reverse transcriptase step, any contaminating genomic DNA was eliminated from the RNA samples by treatment with DNase I. Five micrograms of total RNAs, dissolved in 11 μl of diethylpyrocarbonate-treated water, were incubated 30 min at 37° with 6 μl of a mix containing 4 μl of 5× reverse transcriptase buffer (250 mM Tris-HCl, pH = 8.3, 375 mM KCl, 15 mM $MgCl_2$, 0.1 M dithiothreitol, 20 units of RNasin (Promega), and 1 unit of RNase-free DNase I (USB). After the addition of 160 ng of oligo(dT) ($pd(T)_{12-18}$; Pharmacia), the samples were heated at 75° for 2 min and then placed on ice. The reverse transcriptase reaction was performed subsequently by adding 1.5 μl of a solution containing 100 units of Superscript™ Moloney virus RNaseH-reverse transcriptase (Gibco/BRL), and 0.25 mM of each deoxyribonucleotide triphosphate (Pharmacia). The reverse transcriptase incubation was conducted for 1 hr at 45°. The resulting cDNAs were stored at −20° until use.

PCR reaction. PCR reactions were performed in a 50-μl final volume consisting of 1× Taq polymerase buffer (50 mM KCl; 10 mM Tris-HCl, pH = 9.0; 0.1% Triton X-100; Promega), 0.2 mM of each deoxyribonucleotide triphosphate, 10 pmol of each forward and reverse primer, 1 unit of Taq polymerase (Promega), and 2 μl of appropriate cDNA dilutions. Following the addition of 3 drops of sterilized mineral oil (Sigma), the samples were heated to 94° for 4 min and immediately cycled using a programmable heating block (Coy Laboratories); 30 cycles for CYP1A1, CYP1A2, CYP3A1, and EH, and 33 cycles for CYP2B1 and CYP2B2. The sequence of temperatures consisted of 40 sec denaturation at 93°, 40 sec annealing at 54°, and 40 sec extension at 72°. To assess potential DNA contamination, within each PCR reaction set, a blank was cycled with the samples, containing all of the PCR reagents but devoid of cDNA. As a positive control for each PCR reaction set, a liver cDNA sample of known quality was assessed concurrently.

The characterization of the primers used in this study specific for CYP1A1, CYP2B1, CYP2B2, and CYP3A1 has been described previously (17, 18). For CYP1A2, the forward and reverse primers, 5′-TGCAGAAAACAGTCCAGGA-3′ and 5′-GGAAAAGGAACAAGGGTGGC-3′, respectively, were predicted to produce a 793-bp product, and directed against nucleotide positions 805–1598 of the respective CYP1A2 cDNA. The EH forward, 5′-CACTATGGCTTCAACTCC-3′, and reverse, 5′-CAGGCCTCCATCCTCCAG-3′, primer pair predicted a PCR product of 791 bp, and were targeted toward nucleotide positions 392–1183 of the corresponding rat cDNA. The oligomers were synthesized using an Milligen Cyclone Plus™ DNA synthesizer.

PCR product analysis. PCR products (12.5 μl) were directly separated through a 1.5% Nusieve/1.5% Seakem LE Agarose mixture (FMC) and visualized by ethidium bromide staining. Sizes of the PCR products were estimated from the concurrent migration of DNA size markers (Drigest III, Pharmacia) and also compared with PCR products obtained from the amplification of a positive control liver cDNA sample. To verify the identity of the PCR products and for quantification, the products were Southern-blotted and probed with independent ^{32}P-labeled hybridization oligomers targeted to expected internal sequences within the PCR products. The hybridization oligomers utilized to probe CYP1A1, CYP2B1, CYP2B2, and CYP3A1 products have been described previously (17, 18). For CYP1A2 and EH, the hybridization probes used were 5′-GTTGACCTGCCACTGCTTTA-3′ and 5′-TGCACCATCAGCAAGGGC-3′, respectively.

The reproducibility of the results was assessed by: a) repeating the PCR reactions and Southern hybridizations at least twice from each pool of cDNA synthesized, b) generating at least two separate cDNA reaction sets per tissue region from each animal and repeating the PCR

amplification on each of the resulting cDNA samples, and c) reproducing the RT-PCR experiments using two to four separate animals.

Results

Use of RT-PCR for semiquantitative analysis of the brain expression of P-450 RNAs. To overcome the detection problem due to low expression levels of cerebral P-450 mRNAs, we developed a sensitive, semiquantitative assay, based on a RT-PCR procedure. A critical point in quantitative PCR is defining conditions in which the cDNA is the only limiting factor of the reaction, while the other components are in saturating concentrations. In such a case, the yield of PCR product is directly proportional to the quantity of the input cDNA. Using the PCR conditions described in the previous section, for each of the genes and treatments investigated, we determined the concentration range of cDNAs ensuring a linear reaction. Fig. 1 illustrates this strategy using CYP1A1 as an example. In this experiment, total RNAs were extracted from control or βNF-treated rat brains. After a DNase I treatment to eliminate any genomic DNA contamination, which might influence the amplification efficiency of RNA-specific sequences, the RNAs were subjected to reverse transcription, and the cDNAs obtained were PCR-amplified for 30 cycles. The PCR products were analyzed by Southern hybridization and quantified by densitometry. The linearity of the assay was verified by using 3-fold serial dilutions of the starting cDNA. The results from an ethidium bromide-stained gel are presented in the Fig. 1A and show clearly the presence of a 341-bp band corresponding to the expected size of the CYP1A1 product. The specific hybridization of this product with a radiolabeled oligonucleotide probe targeted to the internal CYP1A1 sequence further established the authenticity of the product (Fig. 2B). Fig. 2C shows a good correlation between the highest cDNA dilutions and the PCR product signals quantified by densitometry. The least diluted βNF-treated sample yielded an underestimated signal indicating that this cDNA concentration was partially saturating. These results demonstrate the linearity and the accuracy of the assay in the range of cDNA concentrations used, and indicate the suitability of RT-PCR to measure CYP1A1 RNA expression in the rat brain. From these data, we conclude that CYP1A1 is expressed in the rat brain, and highly induced by βNF. For each of the genes studied; the PAH-inducible P-450s, CYP1A1 and CYP1A2, the PB-inducible P-450s, CYP2B1, CYP2B2, and CYP3A1, as well as EH, we applied this approach to determine the linear range of cDNA concentrations (not shown). From the resulting data appropriate cDNA dilutions were chosen for further studies to examine the region-specific brain expression of these genes.

Regional brain expression of polycyclic hydrocarbon-inducible genes. In Fig. 2, we present data obtained from RT-PCR analysis of CYP1A1 and CYP1A2 expression in different regions of control or βNF-treated rat brains. In control animals,

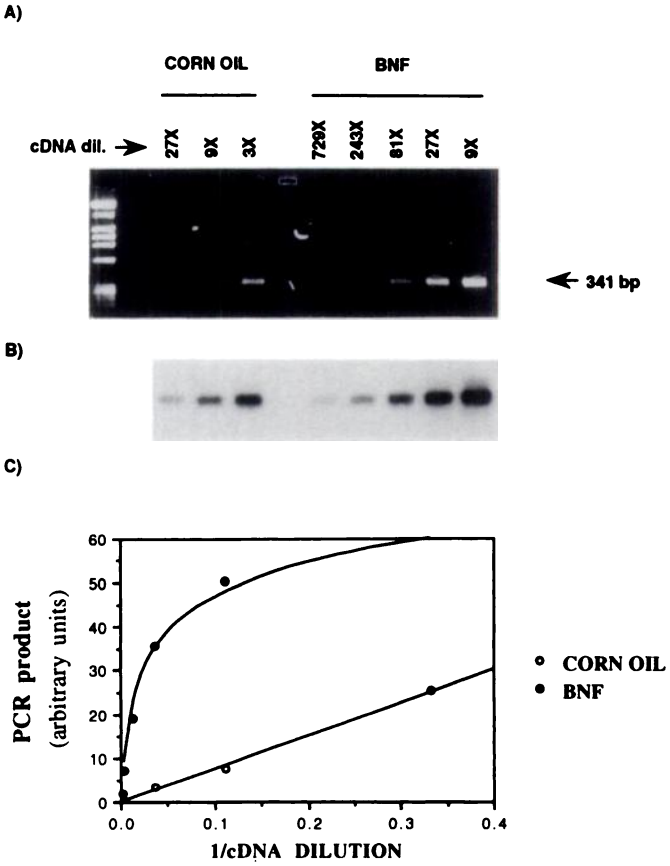

Fig. 1. Semiquantitative RT-PCR amplification of CYP1A1 mRNAs. Total RNAs were isolated from whole brain of control or BNF (βNF)-treated rats and subjected to reverse transcription reactions. The resulting cDNAs were diluted serially and subjected to 30 cycles of amplification using a primer set specific for CYP1A1 sequence. A, Ethidium bromide-stained gel; B, autoradiographic results of Southern-blotted membrane hybridized with an independent radiolabeled oligomer probe targeted to the expected internal sequence of the product; C, densitometric integration analysis demonstrating that RT-PCR amplification produced a linear autoradiogram signal (see text for details).

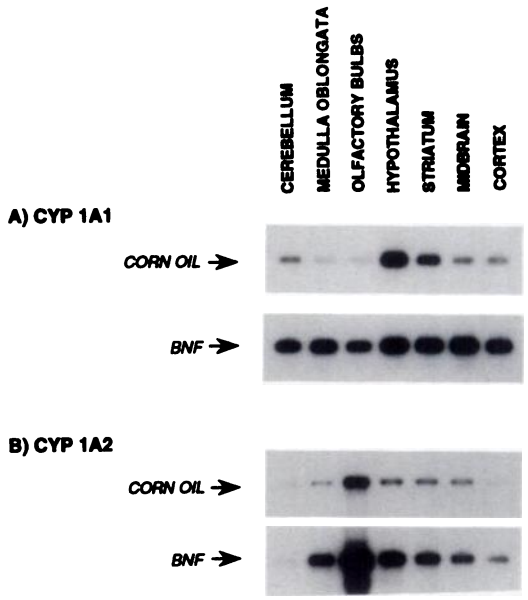

Fig. 2. Brain regional distribution of CYP1A1 (A) and CYP1A2 (B) mRNAs determined by RT-PCR amplification. RNAs were isolated from various regions of control (corn oil) or BNF (βNF)-treated brains. For the CYP1A1 assay, the dilutions of the input cDNAs were 20- and 80-fold, respectively, for the control (corn oil) and BNF samples. For CYP1A2 the control cDNAs were undiluted, whereas the βNF cDNA samples were diluted 4-fold. The cDNA dilutions were subjected to 30 amplification cycles. For comparative estimates, autoradiographic results of Southern-blotted membranes hybridized with specific independent internal probes are presented in this and subsequent figures using the same individual pools of cDNA synthesized from each respective brain region and treatment, but subjected to individual PCR amplification reactions and gel analysis.

specific bands corresponding to CYP1A1 and CYP1A2 mRNAs were present in each brain region examined. The level of CYP1A1 expression clearly varied in a region-selective manner. Comparatively higher levels of CYP1A1 mRNA were present in the hypothalamus and in the striatum, whereas the lowest levels of this transcript were measured in the olfactory bulbs and in the medulla oblongata (Fig. 2A). A similar pattern of regional distribution was obtained for CYP1A2 mRNAs which were detected in large quantities in the hypothalamus and in the striatum, and at relatively lower levels in the medulla oblongata, cerebellum, and the cortex (Fig. 2B). It is of interest to note that the highest CYP1A2 RT-PCR product level observed was in the olfactory bulbs, which were characterized by the lowest levels of CYP1A1 expression. Treating rats with βNF resulted in a large increase in CYP1A1 and CYP1A2 mRNA contents in each brain region. With this treatment, the highly heterogeneous regional pattern of CYP1A1 expression was altered into a more homogeneous distribution. However, the CYP1A2-specific regional profile observed was largely unaltered between the control and βNF-induced brain regions.

Fig. 3 presents data showing that EH was relatively evenly expressed throughout the brain of both control and βNF-treated animals. βNF did not appear to modulate EH mRNA levels in the brain regions examined.

Regional brain expression of PB-inducible genes. The regional distribution data for CYP2B1 and CYP2B2 mRNA expression in control and PB-treated rat brains are presented in Fig. 4. A single band corresponding to the CYP2B1 mRNAs was clearly apparent in each region of the control brains, but exhibited a large region-specific variation in intensity (Fig. 4A). The highest levels of CYP2B1 mRNA expression were detected in the striatum and in the hypothalamus. The cerebellum and the olfactory bulbs also were relatively rich in this transcript, whereas the lowest content was measured in the medulla oblongata. In the control brains, as for CYP2B1, high levels of CYP2B2-specific mRNAs were detected in the striatum and in the cerebellum, with lower levels in the cortex, midbrain, and medulla oblongata. A major difference in the expression pattern of these two homologous genes was observed in the hypothalamus which was rich in CYP2B1 mRNAs but lacking in CYP2B2 transcripts.

Treatment of animals with PB resulted in a complex pattern

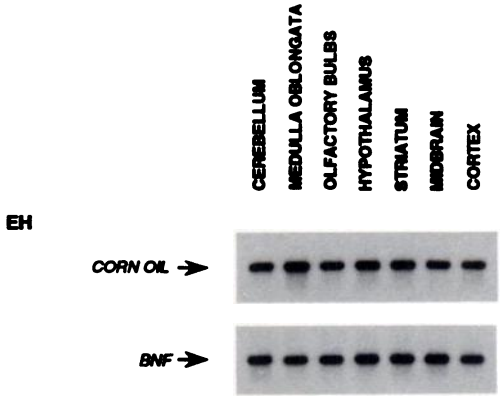

Fig. 3. Regional distribution of microsomal EH mRNA expression in the brain determined by RT-PCR amplification. RNAs were isolated from various regions of control (corn oil) or BNF (βNF)-treated brains. For this assay, 40-fold diluted cDNAs were subjected to 30 amplification cycles. Autoradiographic results of Southern-blotted membranes hybridized with a specific independent internal probe are presented.

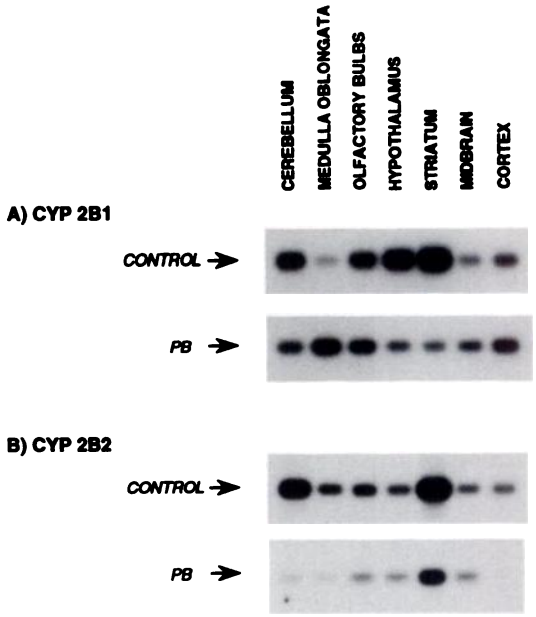

Fig. 4. Brain regional distribution of CYP2B1 (A) and CYP2B2 (B) mRNAs expression determined by PCR amplification. RNAs were isolated from various regions of control (saline) or PB-treated brains. For these assays, undiluted cDNAs were subjected to 33 amplification cycles. Autoradiographic results of Southern-blotted membranes hybridized with specific independent internal probes are presented.

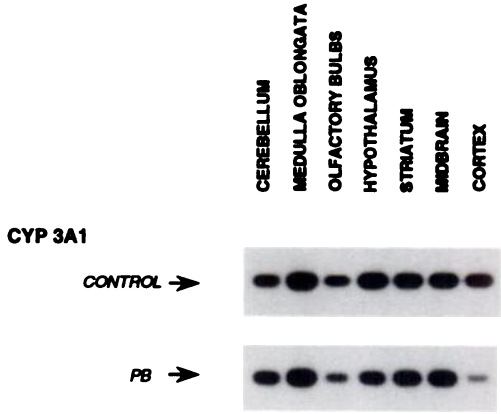

Fig. 5. Brain regional distribution of CYP3A1 mRNAs expression determined by PCR amplification. RNAs were isolated from various regions of control (saline) or PB-treated brains. For this assay, undiluted cDNAs were subjected to 30 amplification cycles. Autoradiographic results of Southern-blotted membranes hybridized with specific independent internal probes are presented.

of effects on the CYP2B1 expression. Compared to the respective brain regions of untreated animals, in PB-treated rat brain, slight increases in CYP2B1 mRNA content were measured consistently in the medulla oblongata, in the cortex, and less reproducibly in the midbrain. In contrast, levels of CYP2B1 transcripts were unaffected in the olfactory bulbs by 18 hr PB treatment but clearly reduced in the striatum, hypothalamus, and cerebellum in PB-exposed rats. The profile of PB effects on CYP2B2 was distinct; CYP2B2 mRNA content in each region of the PB-treated rat brains was sharply reduced in comparison with the corresponding regions of the control brains.

The data presented in Fig. 5 illustrate the expression of CYP3A1 mRNAs in the rat brain. Compared to the other P-

450s investigated, a less pronounced inter-regional variation was detected in the control untreated brains. However, relative to the other regions, higher amounts of these transcript were generally present in the medulla oblongata, hypothalamus, striatum, and the midbrain. Treatment with PB 18 hr before sacrifice either did not affect or appeared to slightly decrease the CYP3A1 mRNA contents of the different regions.

Results from RT-PCR analysis of EH mRNAs in brain regions of control or PB-treated rats are presented in Fig. 6. Very little inter-regional variation of this transcript level was observed. Consistently, PB treatment resulted in a slight increase of the EH RNA content in each region investigated. Due to the highly consistent inter-regional levels of EH mRNA within the brain, EH served as a valuable internal standard in these assays to which other mRNA levels could be referenced.

Expression in brain versus untreated liver. We used densitometry scanning to assess levels of the Southern-hybridized RT-PCR products to approximate comparative levels of P-450 and EH mRNAs in the control and βNF-treated rat brain samples with that of untreated control liver. The data obtained are presented in Table 1A. The results are indicated as percentage of control liver (control liver = 100%), and account for the cDNA dilutions used to maintain each PCR reaction in the respective linear ranges. According to these estimates, the content of CYP1A1 mRNAs in the control brain was approximately 50% that found in the untreated liver, whereas the level of CYP1A2 mRNA was less than 0.01% of the hepatic value. As demonstrated in the different brain regions, CYP1A1 as well as CYP1A2 mRNA contents of the whole brains were clearly increased on βNF treatment, reaching 1000% and 0.4%, respectively, of the control liver levels. However, to place these results in perspective, it should be noted that the untreated liver exhibits only extremely low levels of CYP1A1 mRNA, which are often undetectable with specific probes and using standard Northern or slot-blotting procedures (18).

In Table 1B data are presented for the PB-inducible genes examined in this study. In the control brain, levels of transcripts specific for CYP2B1, CYP2B2, and CYP3A1 were very low, corresponding to 1%, 0.01%, and 0.02%, of their respective amounts in control liver. As suggested by the regional study, 18 hr treatment of animals with PB markedly decreased the CYP2B2 mRNA content of the brain, but affected only slightly the contents of CYP2B1 and CYP3A1 mRNAs.

The level of EH-specific mRNA detected in the control brain with the RT-PCR procedure was approximately 20% that of the liver content. βNF treatment did not appear to affect EH mRNA expression; however, PB treatment resulted in slight increases (approximately 75% above control levels).

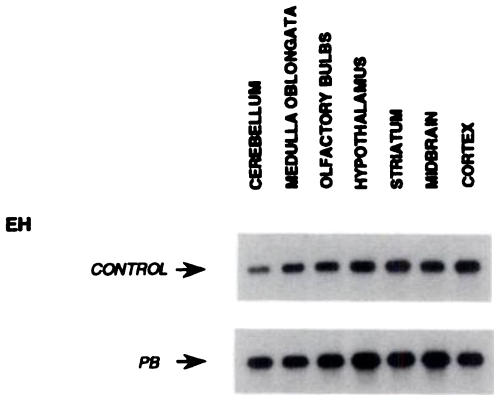

Fig. 6. Brain regional distribution of microsomal EH mRNA expression determined by PCR amplification. RNAs were isolated from various regions of control (saline) or PB-treated brains. For this assay, 40-fold diluted cDNAs were subjected to 30 amplification cycles. Autoradiographic results of Southern-blotted membranes hybridized with a specific independent internal probe are presented.

TABLE 1

Quantitative approximations of brain P-450 and EH-specific mRNA contents versus control liver contents using RT-PCR.

	A. βNF-inducible genes[a, b]		
	CYP1A1[c]	CYP1A2	EH
Liver control	100	100.00	100
Brain control	53	0.08	23
Brain βNF	1040	0.40	26

	B. PB-inducible genes[b, d]			
	CYP2B1	CYP2B2	CYP3A1	EH
Liver control	100.0	100.000	100.000	100
Brain control	1.0	0.010	0.020	16
Brain PB	0.7	0.003	0.017	28

[a] RNAs were isolated from whole brains of control (corn oil) or βNF-treated rats and from control rat liver.

[b] The dilutions of the input cDNAs for the control liver samples were 1000-fold (CYP1A2 and CYP2B2), 100-fold (CYP2B1), 40-fold (EH), and 20-fold (CYP1A1), respectively. For the control brains, the dilutions were 40-fold (EH) and 20-fold (CYP1A1), respectively; for the remaining genes (CYP1A2, CYP2B1, CYP2B2, CYP3A1), the cDNAs were used undiluted. For the βNF-treated brain samples, the dilutions used were 80-fold (CYP1A1), 40-fold (EH), and 3-fold (CYP1A2), respectively. For the PB-treated brain samples, the cDNA dilutions were 40-fold (EH), and for the other genes (CYP2B1, CYP2B2, CYP3A1) the cDNAs were used undiluted. The cDNA dilutions were subjected to 33 amplification cycles for the CYP2B1 and CYP2B2 assays, and to 30 cycles for the other assays. The results presented were derived from individual animals using densitometric integration of autoradiographs of Southern-blotted membranes hybridized with specific internal radiolabeled probes. Results are expressed as percentage of the untreated liver values (control liver = 100%). Data points presented were ascertained within treatment groups using the same pools of cDNA for each amplification reaction. The results presented were typical of repeated assays using several separate RT-PCR reactions from at least two animals per treatment group.

[c] Levels of CYP1A1 mRNA in untreated control rat liver are extremely low and difficult to detect using standard hybridization assays such as Northern blotting procedures (18, 24).

[d] RNAs were isolated from whole brains of control (saline) or PB-treated rats and from control liver.

Discussion

Previous studies performed in our laboratory have demonstrated that RT-PCR reaction strategies enabled assessment of P-450 mRNAs in tissues in which mRNAs were not readily detectable with other techniques (17, 18). Moreover, it was shown that the utilization of specific pairs of primers, in association with high stringency annealing conditions during the amplification process, permitted the discrimination of very closely related gene products, such as CYP2B1 and CYP2B2 mRNAs which share 97% sequence identity (17). Thus, the RT-PCR strategy was chosen for investigating expression of P-450s in the CNS, a tissue known to express low levels of various biotransformation isoforms. It is appreciated that RT-PCR is very sensitive, but that precise quantification is often difficult. However, by using conditions where the cDNA concentration is the only limiting factor in the reaction, several reports demonstrated the feasibility of using PCR for quantitative estimations of mRNA content (17, 21). In the liver, it was shown that PCR data of P-450 mRNA expression correlated well with mRNA levels ascertained with other methods such as Northern blotting (17, 18) and accurately reflected the

predicted changes in mRNA levels produced by treatment with known inducing agents (17, 18). In the case of the brain, as illustrated in the Fig. 1, for each gene studied we empirically determined a range of cDNA concentrations that produced a linear correlation between the cDNA input level and the resulting PCR amplification signal. These preliminary experiments resulted in the definition of appropriate dilutions of cDNA enabling the use of RT-PCR for comparing the relative levels of individual P-450 gene mRNAs in distinct regions of the rat brain.

In the uninduced brain, expression corresponding to each P-450 studied was detected in all the brain regions examined. The striatum was a region of particularly high P-450 mRNA content. Indeed, compared to the other regions, this structure was characterized by a very high expression of all the P-450s assayed. Hypothalamus also was rich in P-450 content, whereas the medulla oblongata and the cortex were characterized by the lowest levels of CYP1A and CYP2B gene transcripts. The other brain regions presented more complex P-450 composition profiles. The cerebellum was defined by relatively high expression of P-450s from the 2B subfamily, whereas the transcripts of the 1A subfamily were found only in low levels. It is important to note that in contrast to the high inter-regional variability in mRNA expression observed for the P-450s, levels of EH-specific transcripts were relatively homogeneous across the various portions of the brain. The EH data therefore served as an internal standard for comparison of P-450 levels which frequently varied in level among the various brain regions examined. These results, in accordance with data obtained in the human brain (22), indicate that remarkably distinct P-450 gene expression patterns exist inter-regionally in the CNS.

The P-450 expression patterns reported in this study are at some variance from those reported by others addressing the distribution of brain P-450s (14, 23). However, comparison of results obtained from methods possessing high discriminative power, like RT-PCR, with data stemming from less specific methods, are difficult. Immunological (14) as well as enzymological (23) evidence strongly suggests that most of the P-450s in the brain remain uncharacterized. According to estimates by Warner *et al.* (14), the contribution of CYP1A1/1A2 and CYP2B1/2B2 combined accounts for <1% of the total brain P-450s. Thus it should not be surprising that the distribution of specific P-450 mRNA representing potentially only a small fraction of the overall presence of P-450s in the brain may differ from P-450 distributions assessed by more general methods such as spectral quantification (14), or enzymatic assays employing substrates biotransformed by a spectrum of known and probably unknown P-450s (23). Similarly, results from immunocytochemical studies need to be interpreted cautiously, as it is conceivable that antibodies may cross-react with uncharacterized P-450 isoforms (14). Furthermore, it is important to keep in mind that our study focused on mRNA expression profiles, whereas most previous reports in this area have presented data corresponding to protein or enzymatic determinations. However, in a separate study by Farin and Omiecinski (22), a reasonably good correspondence was observed for CYP1A1 and EH expression profiles determined in the human brain assessed with both RT-PCR and immunohistochemical methods.

In untreated animals, tissue-specific expression of CYP1A as well as CYP2B subfamily genes is apparent. In contrast to CYP1A1, which is expressed in most induced cells and tissues, CYP1A2 is expressed constitutively in the liver but not in most extrahepatic tissues from either untreated or induced animals (24). Analogously, CYP2B1 mRNAs are detected in lung and testes but not in the untreated liver from Sprague-Dawley rats, and CYP2B2 is constitutively expressed in the liver but not in extrahepatic tissues (25, 26). With the exception of intestinal enterocytes (27), neither are CYP2B1 or CYP2B2 PB-inducible in most extrahepatic tissues (25, 26). Thus the results from the present study are striking in a number of respects.

For example, our data reveal the presence, within the CNS, of CYP1A2 and CYP2B2, two genes generally regarded as specific hepatic markers. Also, induction of both CYP1A1 and CYP1A2 genes was apparent in the brain upon βNF treatment. However, the highly heterogeneous inter-regional profile of CYP1A1 mRNA expression observed in control was altered after βNF treatment into a much more homogeneous pattern, whereas a similar alteration was not observed for CYP1A2. This marked and distinctive effect of βNF on the regional pattern of these two genes might be attributed to different mechanisms of induction; CYP1A1 is activated transcriptionally whereas CYP1A2 appears to be largely up-regulated through post-transcriptional processes (24).

With respect to activation by PB, we demonstrated an induction of mEH RNA in each of the examined brain regions of PB-treated rats. Furthermore, CYP2B1 mRNA expression was increased in several of the regions investigated. Taken together, these results suggest that the brain is a PB-responsive organ. However in contrast to CYP2B1, CYP2B2 expression was clearly reduced in each region upon PB challenge and CYP3A1 levels, inducible in the liver by PB (28), were not inducible in the brain structures examined. CYP2B1 levels were themselves reduced in some structures such as the hypothalamus and striatum upon PB exposures. Although the results are intriguing, the reason for these differential effects are not understood at this time. Several previous reports suggested an up-regulation of 2B genes by PB in the CNS of the rat (13, 29, 30). However, in the present study, the rats received an acute PB treatment of 18 hr, whereas in most of the other reports the rats were exposed chronically to PB through the food and/or by multiple intraperitoneal injections (13, 29, 30). Preliminary experiments conducted in our laboratory suggest that animals treated 4 days with PB expressed higher CYP2B1-, CYP2B2-, and CYP3A1-specific mRNA levels than the control (unpublished data).

It is recognized that compared with the liver, brain P-450 levels are extremely low. In attempting to estimate the levels of the different P-450 mRNAs assessed from whole brain samples as percentages of the respective values measured in control liver, we discovered marked variations in the mRNA levels among the different P-450s examined. CYP1A1 appeared as the most abundant form in the CNS of those measured. For example, a 27-fold cDNA dilution amplified 30 cycles yielded a visible band on ethidium bromide-stained gels that was specific for CYP1A1 products, whereas undiluted cDNA preparations, similarly amplified, were needed to yield visible bands specific for CYP1A2 or CYP3A1. CYP2B1 and CYP2B2 were expressed at very low levels; a 33-cycle amplification was necessary with undiluted cDNA to detect faint bands specific for these gene products. Using these comparisons, it appeared that CYP1A1 was expressed in the brain at a level approximately 20-fold

higher than CYP1A2 or CYP3A1, and apparently 100 to 200 times higher than CYP2B1 or CYP2B2. Upon βNF treatment the brain CYP1A1 mRNA content increased to a level approximately 10-fold higher than that of the untreated rat liver.

Thus, it appears that expression of CYP1A1 in the rat brain is relatively appreciable and potentially may be of neurotoxicological relevance. Such a role for brain P-450s was suggested previously in experiments with brain slices (15). *In vivo*, treatment with the CYP1A inducers βNF or cigarette smoke was shown to protect mice against the toxicity of *N*-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, a neurotoxin known to cause a syndrome of striato-nigral degeneration similar to Parkinson's disease (31). Furthermore, epidemiological studies have shown repeatedly that Parkinson's disease occurs less frequently in smokers than in nonsmokers (32). The results of the present study strongly support the hypothesis that the *in situ* expression of CYP1A1, and possibly other biotransformation systems, might play a critical role in toxication/detoxication processes for putative environmental or endogenous toxins involved in neurodegenerative disease etiology.

Acknowledgments

The authors wish to thank Dr. J. Sidhu, Dr. F. Farin, and L. Aicher for fruitful discussions and skillful technical assistance.

References

1. Okey, A. B., E. A. Roberts, P. A. Harper, and M. S. Denison. Induction of drug-metabolizing enzymes: mechanisms and consequences. *Clin. Biochem.* **19**:132–141 (1986).
2. Nebert, D. W. Proposed role of drug-metabolizing enzymes: regulation of steady-state levels of the ligands that affect growth, homeostasis, differentiation and neuroendocrine functions. *Mol. Endocrinol.* **5**:1203–1314 (1991).
3. Gram, T. E., L. K. Okine, and R. A. Gram. The metabolism of xenobiotics by certain extrahepatic organs and its relation to toxicity. *Annu. Rev. Pharmacol. Toxicol.* **26**:259–291 (1986).
4. Gonzalez, F. J. Molecular genetics of the P-450 superfamily. *Pharmacol. Ther.* **45**:1–38 (1990).
5. Warner, M., M. Stromstedt, L. Moller, and J.-A. Gustafsson. Distribution and regulation of 5a-androstane-2b,17b-diol hydroxylase in the rat central nervous system. *Endocrinology* **125**:2699–2706 (1989).
6. Miyairi, S., O. Sugita, S. Sassa, and J. Fishman. Aromatization and 19-hydroxylation of androgens by rat brain cytochrome P-450. *Biochem. Biophys. Res. Commun.* **150**:311–315 (1988).
7. Ghersi-Egea, J.-F., R. Perrin, B. Leininger-Muller, M.-C. Grassiot, C. Jeandel, J. Floquet, G. Cuny, G. Siest, and A. Minn. Subcellular localization of cytochrome P450, and activities of several enzymes responsible for drug metabolism in the human brain. *Biochem. Pharmacol.* **45**:647–658 (1993).
8. Volk, B., Z. Amelizad, J. Anagnostopoulos, R. Knoth, and F. Oesch. First evidence of cytochrome P-450 induction in the mouse brain by phenytoin. *Neurosci. Lett.* **84**:219–224 (1988).
9. Perrin, R., A. Minn, J.-F. Ghersi-Egea, M.-C. Grassiot, and G. Siest. Distribution of cytochrome P450 activities towards alkoxyresorufin derivatives in rat brain regions, subcellular fractions and isolated cerebral microvessels. *Biochem. Pharmacol.* **40**:2145–2151 (1990).
10. Minn, A., J.-F. Ghersi-Egea, R. Perrin, B. Leininger, and G. Siest. Drug metabolizing enzymes in the brain and cerebral microvessels. *Brain Res. Rev.* **16**:65–82 (1991).
11. Köhler, C., L. G. Eriksson, T. Hansson, M. Warner, and J.-Å. Gustafsson. Immunohistochemical localization of cytochrome P-450 in the rat brain. *Neurosci. Lett.* **84**:109–114 (1988).
12. Kapitulnik, J., H. V. Gelboin, F. P. Guengerich, and D. M. Jacobowitz. Immunochemical localization of cytochrome P-450 in rat brain. *Neuroscience* **20**:829–833 (1987).
13. Anandatheerthavarada, H. K., S. K. Shankar, and V. Ravindranath. Rat brain cytochromes P450: catalytic, immunochemical properties and inducibility of multiple forms. *Brain Res.* **536**:339–343 (1990).
14. Warner, M., C. Köhler, T. Hansson, and J.-Å. Gustafsson. Regional distribution of cytochrome P-450 in the rat brain: spectral quantitation and contribution of P-450b, e and P-450c, d. *J. Neurochem.* **50**:1057–1063 (1988).
15. Pai, K. S., and V. Ravindranath. Protection and potentiation of MPTP-induced toxicity by cytochrome P-450 inhibitors and inducers: *in vitro* studies with brain slices. *Brain Res.* **555**:239–244 (1991).
16. Shahi, G. S., N. P. Das, and S. M. Moochhala. Parkinson's disease and cytochrome P450: a possible link? *Med. Hypotheses* **32**:277–282 (1992).
17. Omiecinski, C. J., C. Hassett, and P. Costa. Developmental expression and *in situ* localization of the phenobarbital-inducible rat hepatic mRNAs for cytochromes CYP2B1, CYP2B2, CYP2C6, and CYP3A1. *Mol. Pharmacol.* **38**:462–470 (1990).
18. Omiecinski, C. J., C. A. Redlich, and P. Costa. Induction and developmental expression of cytochrome P4501A1 messenger RNA in rat and human tissues: detection by the polymerase chain reaction. *Cancer Res.* **50**:4315–4321 (1990).
19. Glovinski, J., and L. L. Iversen. Regional studies of catecholamines in the rat brain-I: the disposition of [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. *J. Neurochem.* **13**:655–669 (1966).
20. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. *Anal. Biochem.* **162**:156–159 (1987).
21. Buck, K. J., R. A. Harris, and J. M. Sikela. A general method for quantitative PCR analysis of mRNA levels for members of gene families: application to $GABA_A$ receptor subunits. *Biotechniques* **11**:636–641 (1991).
22. Farin, F. M., and C. J. Omiecinski. Regiospecific expression of cytochrome P450s and microsomal epoxide hydrolase in human brain tissue. *J. Toxicol. Environ. Health* **40**:323–341 (1993).
23. Dhawan, A., D. Pamar, M. Das, and P. K. Seth. Characterization of cerebral 7-ethoxycoumarin-O-deethylase: evidence for multiplicity of cytochrome P450 in brain. *Biochem. Med. Methods Biol.* **41**:184–192 (1989).
24. Pasco, D. S., K. W. Boyum, S. N. Merchant, S. C. Chalberg, and J. B. Fagan. Transcriptional and post-transcriptional regulation of the genes encoding cytochromes P450c and P450d *in vivo* and in primary hepatocytes cultures. *J. Biol. Chem.* **263**:8671–8676 (1988).
25. Christou, M., Wilson, N. M., and C. R. Jefcoate. Expression and function of three cytochrome P450 isoenzymes in rat extrahepatic tissues. *Arch. Biochem. Biophys.* **258**:519–534 (1987).
26. Omiecinski, C. J. Tissue-specific expression of rat mRNAs homologous to cytochromes P-450b and P-450e. *Nucleic Acids Res.* **14**:1525–1539 (1986).
27. Traber, P. G., J. Chianale, R. Florence, K. Kim, E. Wojcik, and J. J. Gumucio. Expression of cytochrome P450b and P450e genes in small intestinal mucosa of rat following treatment with phenobarbital, polyhalogenated biphenyls, and organochlorine pesticides. *J. Biol. Chem.* **263**:9449–9455 (1988).
28. Waxman, D. J., and L. Azaroff. Phenobarbital induction of cytochrome P-450 gene expression. *Biochem. J.* **281**:577–592 (1992).
29. Stroebel, H. W., E. Cattaneo, M. Adesnik, and A. Maggi. Brain cytochromes P-450 are responsive to phenobarbital and tricyclic amines. *Pharmacol. Res.* **21**:169–175 (1989).
30. Anandatheerthavarada, H. K., M. R. Boyd, and V. Ravindranath. Characterisation of a phenobarbital-inducible cytochrome P450, NADPH-cytochrome P450 reductase and reconstituted cytochrome P450 mono-oxygenase system from rat brain. Evidence for constitutive presence in rat and human brain. *Biochem. J.* **288**:483–488 (1992).
31. Shahi, G. S., N. P. Das, and S. M. Moochhala. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity: partial protection against striato-nigral dopamine depletion in C57BL/6J mice by cigarette smoke exposure and β-naphthoflavone-pretreatment. *Neurosci. Lett.* **127**:247–230 (1991).
32. Godwin-Austen, R. B., P. N. Lee, M. G. Marmot, and G. M. Stern. Smoking and Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **45**:577–581 (1982).

Send reprint requests to: C. J. Omiecinski, PhD, Department of Environmental Health, SC-34, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195.