Proc. Indian natn Sci Acad. **B69** No. 6 pp 893-916 (2003)

Expression of Xenobiotic Metabolising Cytochrome P450s in Brain: Physiological, Pharmacological and Toxicological Consequences

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(Received on 4 September 2002; Accepted after revision 24 December 2002)

Brain is separated from rest of the body by the blood-brain barrier which restricts the entry and exit of certain endogenous and exogenous chemicals. Several of the chemicals being highly lipophilic readily cross the blood brain barrier and could be transformed within the brain. Cytochrome CYPs (CYPs) have been identified as the functional enzymes in the brain, enabling the central nervous system (CNS) to catalyze the oxidative metabolism of substrates present in or reaching the organ. Although many of the CYPs have been identified in the brain, this review focuses primarily on the xenobiotic metabolizing CYPs because of their role in metabolic activation and toxicity of xenobiotics and also in regulating various endogenous functions of the brain. Significant regional and cellular differences have been observed in the distribution of xenobiotic metabolizing CYPs within the brain with neurons in some specific brain areas and brain mitochondria exhibiting high local CYP activity. The activities of brain CYPs towards exogenous chemicals have generally been found to be higher in brain microsomes than in mitochondria and involvement of cerebral CYPs in chemical induced neurodegeneration has been demonstrated. In addition, overlapping substrate specificity of the brain CYPs with dopamine transporters, sigma and opiate receptor binding sites indicate that brain CYPs may not only be involved in complex metabolic pathways controlling neuronal function but also serve as a target where xenobiotics could act and affect brain physiological functions.

Key Words: Cytochrome P450, Brain, Xenobiotics, mRNA expression, Immunochemical studies

Introduction

Humans are exposed to a wide variety of xenobiotics, from food products to environmental toxins to pharmaceuticals. Many of these compounds show little relationship to previously encountered compounds or metabolites, and yet our bodies are capable of managing environmental exposures by detoxifying them by evolving complex system of detoxification enzymes. The phase I detoxification system, composed mainly of the cytochrome P450 (CYP) supergene family of enzymes, is generally the first defense against foreign compounds. Majority of the drugs and foreign chemicals are metabolized by the CYPs. In a typical phase I reaction, a CYP enzyme uses molecular oxygen and a cofactor, NADPH to add a reactive group to the substrate. As a consequence of this step, reactive molecules, which may be more toxic than the parent molecule, are produced. If these reactive intermediates are not further metabolized by phase II i.e. conjugation reactions, they may cause damage to proteins and other tissue macromolecules within the cell (Nebert 1991, Vermeulen 1996, Iyer & Sinz 1999).

Phase II i.e. conjugation reactions generally follow CYP dependent metabolic activation, resulting in a xenobiotic that has been transformed into a water-soluble compound and which can be excreted through urine or bile. Several types of conjugation reactions are present in the body, including glucuronidation, sulfation and glutathione and amino acid conjugation (Nebert 1991, Vermeulen 1996, Iyer & Sinz 1999). An additional mechanism by which cells are protected from chemicals, and which has only been recognized relatively recently, is described as phase III and commonly referred to as xenobiotic

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transporters (Klaassen 2002). These transporters are proteins on the cell membranes that can transport chemicals into and out of the cells. Because a chemical must attain a high enough concentration for a sufficient amount of time to produce toxicity, transport of the chemicals out of the cells and out of the body can have a protective effect. A number of families of xenobiotic transporters have been cloned during the last few years (Ling 1997, Klaassen 2002). This toxication and detoxification in humans appears to be very extensive, highly complex and influenced by myriad regulatory mechanisms and is the resultant effect of the interaction of phase I, II & III reactions. The present review will focus on the toxication - detoxication mechanisms regulated largely by CYPs with particular emphasis on the CYP dependent metabolism in the brain.

CYPs also play an important role in the metabolism of endogenous substrates such as steroids and fatty acids (Nebert 1991). Though, much of the CYP mediated endogenous metabolism is highly specific, there appears to be a great deal of overlapping substrate specificity in the metabolism of foreign chemicals. This extraordinarily broad substrate specificity of the enzyme results from the multiplicity of different molecular forms of the CYP, which have distinct but overlapping substrate specificities as evidenced by identification, purification and characterization of different molecular forms of mammalian liver CYPs. Based on their amino acid homologies, the CYP superfamily has been classified into several families and subfamilies (Nebert et al. 1989, 1991, Nelson et al. 1996, http://drnelson.utmem.edu/homepage.links.html; http://drnelson.utmem.edu/cytochromeCYP.html). The CYP proteins with 40% or greater sequence identity are included in the same family (designated by arabic number), and those with 55% or greater identity in the same subfamily (designated by a capital letter) where the individual genes are numbered arbitrarily.

Some of the CYPs are highly inducible; for example expression of CYP1A1 can be elevated 100 fold or more in liver and many extrahepatic tissues following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 3-methylcholanthrene (MC) or other polycyclic aromatic hydrocarbons (PAHs) (Whitelock 1989, Lewis 1996 a,b). Phenobarbital (PB), as well as a large number of structurally unrelated chemicals termed 'PB-like' inducers, induce the expression of CYP2A, 2B & 2C subfamilies both in the laboratory animals and in the humans (Waxman & Azaroff 1992). CYP2E1 expression in liver is increased several folds following exposure to ethanol, acetone and other drugs and chemicals while clofibrate and other peroxisome proliferators induce the expression of CYP4A in liver and kidney (Lewis et al. 1996 a,b). Likewise the 3A subfamily can be induced effectively by macrolide antibiotics and synthetic steroids such as dexamethasone or by PB (Waxman & Azaroff 1992). Because of the ability of drugs and environmental chemicals to modulate the expression of CYP and/ or stabilization of CYP, they can play a significant role in increasing the rate of metabolism of foreign compounds to detoxified products or in some cases to reactive intermediates.

Although liver is the major organ involved in the CYP mediated metabolism of endogenous compounds and xenobiotics, several extrahepatic tissues like skin, kidney, lung, intestine, adrenal, ovaries, testis, pancreas, brain, etc. have been shown to be equipped with CYP monooxygenases (Guengerich 1977, Fang & Strobel 1978, Gram 1980). Moreover, tissue-specific expression of certain CYPs isoenzymes is also reported. For example, CYP1A1 is expressed in most induced cells of hepatic and extrahepatic tissues whereas CYP1A2, a constitutive form is expressed only in liver of animals. Similarly, CYP2B1 in rat is constitutively present in lung and testes and not in liver, while CYP2B2 is constitutively expressed in liver and intestinal enterocytes only. Neither CYP2B1 nor 2B2 are inducible in most extrahepatic tissues (Waxman & Azaroff 1992).

Brain is the most important organ of the body as it controls behaviour and several important physiological functions. Structurally, brain is divided into distinct regions, which control specific function. For example, hippocampus controls learning and memory, cerebellum regulates muscular co-ordination, corpus striatum governs behavioral responses etc. The composition of brain regions is also different which provides the biochemical basis of their physiological control. Brain is separated from the rest of the body by blood brain barrier, which prevents entry of blood-borne chemicals into the brain and also restricts exit of chemicals from the

brain into the blood (Ghersi-Egea et al. 1995). However, several of the chemicals, being lipophilic, readily cross the blood brain barrier and reach the brain and are biotransformed within the brain. The metabolite(s) so formed are often polar and therefore, cannot move out of the blood-brain barrier. Bioactivation of xenobiotics *in situ* within the brain could result in metabolites that cause damage to macromolecules in the brain cells and/or bind at different receptor sites. Thus, cerebral CYPs could play an important role in i) altering the pharmacokinetic behaviour of neuroactive drugs; ii) activating some substrates to carcinogenic intermediates at the site of cancer formation; iii) the production of chemically-induced toxic reactions or behavioral disorders; and iv) the metabolism of endogenous substrates e.g. neurohormones, fatty acids and prostaglandins.

CYPs in Brain

Historical Background

Cooper and Brodie (1957) were the first to show that like liver, barbiturates are metabolised in the brain by the side chain oxidation though the rate of metabolism was several fold less in brain as compared to the liver. Thereafter, Milthers (1951) and Elison and Elliott (1963) demonstrated that hypothalamus, medial thalamus and corpus striatum in brain possesses the capacity to O- and N-demethylate drugs like morphine, codeine and meperidine thereby reflecting the presence of mixed function oxidase system in rat brain. The CYP mediated N-demethylation of aminopyrene was characterized by Mareitta et al. (1978) and they demonstrated that brain aminopyrene N-demethylase (APD) exhibit linear kinetics in contrast to biphasic kinetic behavior of liver enzyme (Pederson & Aust 1970, Poland & Nebert 1973, Matsubara et al. 1977).

Norman and Neal (1976) showed that the brain is capable of metabolizing neurotoxic insecticides such as parathion to its metabolites. Like in liver, both paraoxon and diethyl phosphoric acid are formed as products of desulphuration of parathion in rat brain microsomes (Neal 1967). The parathion metabolism in brain was stimulated by NADPH and inhibited by carbon monoxide, proadifen (SKF-525A) and piperonyl butoxide, typical inhibitors of CYP monooxygenases. The induction of CYP in brain was for the first time reported by Cohn et al. (1977) by demonstrating induction of CYP dependent aryl hydrocarbon hydroxylase (AHH) in rat brain. They also reported qualitative and quantitative differences in the metabolite formed as a result of this activity during perinatal development. Significant induction of brain CYP in adult rats following administration of PB and MC was reported by Guengerich and Mason (1979). Bergh and Strobel (1992) were the first to demonstrate the presence of functional mixed function oxidase system in rat brain. They solubilized brain microsomal membrane and purified its component, cytochrome P450 reductase and CYP. The purified cytochrome P450 reductase and partially purified CYP were able to reconstitute substrate hydroxylation activities. They found similar mixed function oxidase (MFO) turnover with purified brain and liver CYP and reductase in the reconstituted system.

Brain CYPs: CO-difference Spectrum

Sasame et al. (1972) measured the levels of CYP in rat brain and demonstrated that brain microsomes contain approximately 30 times less amount of CYP than found in liver. Cohn et al. (1977) also identified the CYPs by the CO difference spectrum in dithionite reduced microsomes of whole rat brain. Anandatheerthavarada et al. (1992) reported that reduced carbon monoxide spectrum of CYP purified from microsomes of brain isolated from PB pretreated rats exhibited a single peak at 450 nm. Holtzman and Desautel (1970) demonstrated that microsomal CYP content varies as a function of age in different brain regions. Ghersi-Egea et al. (1987) identified CYP in brain microsomes and mitochondria by second derivative spectro-photometric method which allows better accuracy than by direct measurement of the CYP levels. Male rat brain exhibited higher levels of CYP as compared to the females and testosterone elevated the levels of total CYP contents in female rat brain to levels comparable with that of the males (Ravindranath & Anandatheerthavarada 1989, Anandatheerthavarada & Ravindranath 1991). Bhamre et al. (1992, 993) reported CYP levels in microsomes in human brain regions obtained from autopsy. Marked variation in the levels of CYP was observed with brain stem, comprising of the mid brain, pons & medulla exhibiting high concen-tration of CYPs. Geng and Strobel (1993, 1995,1998) have further reported that microsomes prepared from rat glioma C6 cells showed CYP spectra with absorption at 450 nm in untreated and benzo (a) anthracene (BA) treated glioma microsomes.

Cellular and Subcellular Distribution of Brain CYPs

CYPs in brain were found to be concentrated mainly in the mitochondria and exhibited dual localization, being associated with synaptic and non-synaptic mitochondria. Only a small quantity of the enzyme was found in the microsomal fraction. Peeling of the outer membrane of mitochondria showed that CYP was retained in the inner membrane fraction. Significantly higher activity of NADH-dependent CYP- mediated AHH activity in rat brain mitochondria as compared to brain microsomes was reported by Das et al. (1981 a,b, 1982, 1985 a,b). NADH-dependent rat brain mitochondrial AHH exhibited a Km value of 1.18 μM with benzo(a)pyrene (BP) as a substrate which was five to six times lower than the Km of NADPH-dependent microsomal AHH indicating that higher metabolism of BP is likely to take place in mitochondria than in brain microsomes. Presence of NADH-dependent AHH activity in brain mitochondria has assumed significance as mitochondrial DNA has been shown to be the major cellular target for the dihydriodiol epoxide derivative of BP. Perrin et al. (1990) also showed that CYP content of rat brain mitochondrial fractions was at least five times higher than in microsomes in almost all of the brain regions. However, the activities of brain CYP towards exogenous substrates were reported to be higher in microsomes than in mitochondria. Our studies have further demonstrated comparatively higher activity of xenobiotic metabolizing CYPs in brain microsomes (Dhawan et al. 1989, Parmar et al. 1998, 1999).

CYP activity has been shown in endothelial cells of brain capillaries forming the blood-brain-barrier and isolated microvessels (Walther et al. 1986, Perrin et al. 1990, Ghersi-Egea et al. 1988, 1994, 1995). Also several CYP enzymes involved in hepatic drug metabolism have been found in the choroid plexuses, the leptomeninges as well as in some circumventricular organs (CVOs) which lack a typical blood brain barrier (table 1). Interestingly, most of the CYPs in microvessels and CVOs are mitochondrial and their endogenous substrates have been identified. These CYPs are inducible and may act at various interfaces as enzymatic barriers to influx of the xenobiotics. The activity of CYP enzymes in choroidal tissue and CVOs is so high that the choroidal plexuses can well be the major site of drug metabolism in the brain (Ghersi-Egea et al. 1994, 1995).

Table 1. *Distribution of CYPs in Blood Brain Interfaces*

Blood Brain Barrier (BBB)	Capillary endothelial cells
Meningis	Arachnoid, dura matter
Circumventricular organs	Choroid plexus, pineal gland, posterior pituitary, median eminence

Perrin et al. 1990 *Biochem. Pharmacol.* **40** 2145-51
Ghersi-Egea et al. 1992 *Prog. Brain Res.* **91** 373-78
Ghersi-Egea et al. 1994 *J. Neurochem.* **62** 1089-96

Distinct cellular distribution of CYP dependent enzymes has been demonstrated in rat brain in our laboratory. The neuronal cells exhibited 2-3 fold higher activity of CYP dependent AHH, ECOD and EROD activity than the glial cells (table 2). Pretreatment with PB significantly increased the activity of ECOD (60-85%) in neuronal and glial cells, while a 140% increase was observed in neuronal AHH activity. Exposure to MC resulted in a significant induction of the activity of AHH, ECOD and EROD in the neuronal and glial cell preparations. The neurons, in general, exhibited greater sensitivity towards PB and MC induction indicating qualitative as well as quantitative differences in the CYPs of the neuronal and glial cells (Dhawan et al. 1990).

Multiplicity of Brain CYPs

(i) mRNA expression and cloning of brain CYPs

Expression of multiple forms of CYPs has been shown in the mammalian brain (table 3). Strobel et al. (1989) were the first to report in 1989 that

Table 2. *Distribution of CYP dependent monooxygenases in rat brain cells*

	Neurons	Glia	Neurons/ Glia
AHH[a]	56.1±4.2	14.1±2.0	3.3
ECOD[b]	12.9±1.8	4.8±0.9	2.7
EROD[c]	10.5±1.5	3.3±0.7	3.1

a - pmole 3-OH benzo(a)pyrine/min/mg protein
b - pmole resorufin/min/mg protein
c - pmole 7-OH Coumarin/min/mg protein

Dhawan et al. 1990 *Biochem. Biophys. Res. Commun.* **170** 441-447

the treatment of rats with PB and tricyclic antidepressants markedly increase the brain RNA hybridising with CYP2B1 cDNA probe. Using Reverse transcription-polymerase chain reaction (RT-PCR), Hodgson et al. (1993) demonstrated expression of CYP1A1, 2B2, 2D and 2E1 in rat brain. Cytochrome P450 reductase expression was also detected in the brain samples, giving evidence that the brain contains a competent mixed function oxidase system. Several of these isoforms (1A, 2B, 2D and 2E) and reductase were found to be expressed even in the brains of untreated animals and it appears that at least two forms of CYP (1A1 &2B2) are under various levels of control by different CYP inducers (Strobel et al. 2001).

Schilter and Omiecinski (1993) reported that expression of CYP1A1, 1A2, 2B1, 2B2 and 3A1 mRNA in rat brain was region specific. Highly heterogeneous inter-regional profile of CYP1A1 mRNA expression observed in control brain was altered after β-naphthoflavone (β-NF) treatment into a much more homogeneous pattern whereas CYP1A2 mRNA increased in region specific manner. However, mRNA expression of CYP2B1 and 2B2 observed in control animals exhibited a complex pattern of distribution after pretreatment with single dose of PB (80 mg/kg). Increase in the expression of CYP2B1 mRNA was observed in medulla oblongata, mid brain and cortex but decrease in content was noted in other brain regions whereas CYP2B2 mRNA content in each region of PB-treated rat brain was sharply reduced in comparison with the corresponding regions of the control brain. CYP3A1 mRNA levels varied only slightly across the respective regions in treated and untreated rat brain. Among the different CYP isoforms, CYP1A1 was the most abundant form in the CNS.

Warner et al. (1997) reported induction in rat CYP1A1 & 1A2 mRNA in the brain after partial hepatectomy. Though the mechanism of this induction remains to be understood, 60% partial hepatectomy resulted in rapid transient rise in the mRNA for CYP1A1 & 1A2. Interestingly, olfactory lobes which show the highest constitutive level of CYP1A2 exhibited the maximum induction after partial hepatectomy. CYP1A1 expressed all over the brain in control rats, was also induced in olfactory lobes after hepatectomy. CYP1A1, 1A2, 2E1 and 3A mRNA has also been demonstrated to be constitutively expressed in human brain with distinct pattern of expression in different brain regions (Farin &

Table 3. *mRNA of expression metabolising CYPs found in brain*

mRNA	Source	Reference
CYP1A1, 1A2	Rat glioma C6 cell line, human	Hodgson et al. 1993, Farin & Omiecinski 1993, Schilter & Omiecinski 1993, Geng & Strobel 1993, 1995, Strobel et al. 1995, 2001, Warner et al. 1997
CYP1B1	Human	Sutter et al. 1994, Shimada et al. 1996, Reider et al. 1998, 2000, Muskhelishvili et al. 2001
CYP 2A1	Rat, rat glioma C6 cell line	Geng & Strobel 1993, 1995, Strobel et al. 1995
CYP 2B1/2B2	Rat, rat glioma C6 cell line	Strobel et al. 1989, 1995, 2001, Geng & Strobel 1993, 1995, Hodgson et al. 1993, Schilter & Omiecinski 1993, Hedlund et al. 1998, Tirumalai et al. 1990
CYP 2C7, 2C11	Rat, rat glioma C6 cell line	Geng & Strobel 1993, 1995, Zaphiropoulos & Wood 1993, Strobel et al. 1995, 2001
CYP 2D1, 2D2,	Rat, canine, human	Komori 1993, Wyss et al. 1995, Bergh & Strobel 1996
2D3, 2D4		Gelham et al. 1997
CYP2E1	Rat, rat glioma C6 cell line, rat hippocampal & glial cell cultures, gerbil astrocytes, human	Hodgson et al. 1993, Geng & Strobel 1993, 1995, Farin & Omiecinski 1993, Strobel et al. 1995, Tindberg & Ingelman-Sundberg 1996, Tindberg et al. 1996, Tirumalai et al. 1998, Upadhya et al. 2000
CYP 3A	Rat, human	Schilter & Omiecinski 1993, Farin & Omiecinski 1993
CYP 4A2, 4A3, 4A8	Rat	Stromstedt et al. 1994

Omiecinski 1993). Rosenbrock et al. 2001 provided evidence for the constitutive expression of CYP2B1 and CYP2B2 mRNAs in different rat brain regions. *In situ* hybridization studies revealed similar expression throughout the brain, predominantly in the neuronal populations, but to some extent in astrocytes of corpus callosum and olfactory bulb.

Tindberg and Ingelman-Sundberg (1996) demonstrated the expression of CYP2E1 mRNA in rat hippocampus and cortical glial cell cultures and the CYP2E1 dependent chlorzoxazone hydroxylase, was found to increase after ethanol treatment. Studies on the different forms of CYP and cytochrome P450 reductase in brain tumors have shown the expression of CYP1A1, 1A2, 2B1, 2B2, 2C7, 2E1 and cytochrome P450 reductase in rat glioma C6 cell lines (Geng & Strobel 1993, 1995). Using RT-PCR & restriction digestion of the PCR products, the induction of CYP1A1 and 2B was quantified. Ten- and five- fold induction of CYP1A and 2B mRNA after BA or PB treatments respectively, were detected by competitive PCR in the rat glioma C6 cell line. Similar results were observed with BA or PB treated rat brain. Control rat glioma C6 cells possess CYP1A1, 2B1 & 2B2 levels similar to that of control rat brain. mRNAs of CYP1A1 and 2Bl/2B2 in rat glioma C6 cells were found to have short poly (A) tails and 1/10th and 1/3rd half lives of that of corresponding liver mRNA, which may be due to the low level of expression in glial cells. The induction of CYPs by BA or PB did not change their mRNA half-lives, indicating transcriptional regulation of these CYPs (Strobel et al. 1995).

Presence of constitutive CYPs belonging to 2C subfamily was demonstrated using RT-PCR in brain of female rats and olfactory lobes of ethanol-treated male rats (Zaphiropoulos & Wood 1993). It has also been shown that mRNA of CYP1B1 capable of activating chemically diverse human procarcinogens and of 4-hydroxylation of estradiol, is expressed in human brain tissue (Sutter et al. 1994, Shimada et al. 1996, Rieder et al. 1998). *In situ* hybridization analysis revealed that in human brain cortex CYP1B1 mRNA is expressed mainly in the neurons (Muskhelishvile et al. 2001).

To investigate the possible relationship between CYP in brain and degenerative diseases of the CNS, the expression of the CYP2D subfamily has been studied in rat brain (Wyss et al. 1995). The mRNA for CYP2D4 was much more abundant in rat brain than those for CYP2D1 & 2D5, which are the major hepatic forms. CYP2D2 & 2D3 mRNA which are also abundant forms in liver, were not detectable in brain. To evaluate the quantitative significance of CYP2D4 in brain, the full length CYP 2D4 cDNA was cloned from brain mRNA by RT-PCR amplification and was translated in a reticulocyte lysate system, into a protein of approximately 50 kDA. A [^{35}S] methionine-labelled protein of 50 kDa could be immunoprecipitated from *in vitro* translated 2D4 mRNA. There was no detectable developmental regulation of CYP2D4 mRNA and no change was observed during pregnancy, Flactation or after treatment with ethanol, conditions under which the CYP content of brain increases. These studies led Wyss et al. (1995) to conclude that CYP2D4 is expressed as a stable protein in the brain of untreated rats, where it represent < 5% of total CYPs. Using northern and southern blots and RT-PCR analysis of rat brain mRNA, Komori (1993) also reported expression of only CYP2D4 amongst the 2D CYPs in the rat brain. Using immunoblotting, southern blotting and radioligand binding, CYP2D1 (debrisoquine/sparteine monooxygenase) was demonstrated in canine and rat brain (Fonne-Pfister et al. 1987, Lee & Moochhala 1989, Niznik et al. 1990, Tyndale et al. 1991) and was reported to be widely and constitutively expressed in neuronal and some glial population of the different rat brain region (Norris et al. 1996). Presence of CYP2D6 mRNA was demonstrated in pigmented neurons of substantia nigra of human fetal and adult brain by *in situ* hybridization (Gilham et al. 1997). A novel CYP belonging to the 2D family has recently been cloned from the control and imipramine treated rat brain cDNA library (Kawashima & Strobel 1995a) which was not expressed in the liver.

Expression of CYP2D and cytochrome P450 reductase in the CNS and effect of a number of xenobiotics and hormones upon their levels in the brain has been studied (Bergh & Strobel 1996). mRNA expression for CYP2D was found to increase as one moves caudally from the olfactory bulbs to the brain stem with highest being in the tectum. Although xenobiotics such as PB & β-NF and tricyclic antidepressants e.g. imipramine and amitriptyline produced no significant effect on the CYP2D or cytochrome P450 reductase mRNA expression in brain, dramatic differences were

observed in the CYP2D levels on exposure to sex steroids. Testosterone when administered to ovariectomized rats resulted in high level of CYP2D expression in brain. The levels of CYP2D mRNA in brain were much higher when compared to non-ovariectomized animals treated with testosterone plus estrogen. These results suggest that estrogen reduces the stimulatory effect of testosterone upon the expression of CYP2D in brain. While further studies are needed to define the mechanism, the data indicate a positive influence of testosterone on CYP2D expression in rat brain. Apparently the levels of expression of CYP2D in the brain is different between the sex though the levels are comparable between male and female rat in the liver.

Cloning studies have led to the identification of novel CYPs expressed in mammalian brain (table 4). Full length cDNA (CYP3A9) from untreated male rat brain cDNA library having 68-76% homology with reported CYP3A sequences has been cloned (Wang et al. 1996). No significant induction of the CYP3A9 expression was observed in rat brain by dexamethasone. CYP3A9 cDNA was expressed in *E. coli* and preliminary catalytic studies of the solubilised *E. coli* membrane fractions showed that the expressed CYP3A9 is able to catalyze the demethylation of erythromycin as well as benzphetamine, the marker substrate of CYP3A subfamily (Wang et al. 1996, Wang & Strobel 1997). Region specific expression of members of CYP4A subfamily involved in the synthesis of metabolites of arachidonic acid was demonstrated in rat brain by PCR studies (Stromstedt et al. 1994) and mRNA for CYP4A2 & 4A3 were detectable in rat brain. A very weak signal for CYP4A8 was seen by PCR only in the cerebral cortex, while no signal was detected by northern blot analysis since brain content of CYP4A was 0.1% of that in liver as demonstrated by immunoblotting. N-terminal sequencing of protein in purified CYP fractions has also revealed the presence of CYP4A8 and 4A3 in brain.

Using CYP4A5 cDNA as a probe, Kawashinma and Strobel (1985b) isolated the cDNA clones belonging to CYP4F subfamily from untreated rat brain cDNA library. The expression levels of these forms of CYPs in the brain were somewhat low with relatively high level of expression of similar forms of subfamily 4F CYP in liver and kidney. All the three clones were *in vitro* translatable using a reticulocyte lysate system indicating that multiple forms of subfamily 4F CYP exist in the brain with the subfamily 4F CYP being one of the major forms. Recently, another novel CYP belonging to CYP4 family has been cloned with RT-PCR reaction from rat brain (Bylund et al. 2003). The CYP designated CYP4X1 revealed 41-51% similarity in amino acid sequence with that of the members of CYP4 subfamilies. Northern blot analysis showed that CYP4X1 is highly and specifically expressed in the brain. *In situ* hybridization experiments have indicated that CYP4X1 is mainly expressed in neurons in different brain regions as well in vascular endothelial cells suggesting that CYP4X1 might play a role in neurovascular function (Bylund et al. 2002).

Kainu et al. (1995) reported the localisation of aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) in the rat brain through *in situ* mRNA hybridization. While the distribution of these related transcription factors, involved in the regulation of expression of CYP1A1, were partly similar, differences in their localisation also existed, with ARNT being more abundantly and more widely expressed. Thus, ARNT may have a further role in the brain, in addition to, being the heterodimerization partner of AHR. A recent

Table 4. *cDNA cloning of novel rat brain* CYPs

cDNA	Source	Probe	Reference
2d-29, 2d-30 (P450 2D subfamily)	Control & imipramine treated rat brain cDNA libraries	PCR product of CYP 2D	Kawashima & Strobel 1995a
4f-8, 4f-44, 4f-41 (P450 4F4, 4F5, 4F-6)	Control rat brain cDNA library	CYP 4A5 cDNA	Kawashima & Strobel, 1995b
3aH15 (P450 3A9)	Control rat brain cDNA library	PCR generated cDNA fragment of CYP3A2	Wang et al. 1996
hct-1 (P450 7)	Control rat hippocampus cDNA library	Radiolabelled cDNA probes.	Stapleton et al. 1995

study reported the construction of AHR knock-out mice which showed no histological abnormalities in the brain (Fernandez- Salguero et al. 1995) and could serve as a good model for the study of the endogenous function of AHR in brain and chemical abnormalities which may occur in neurons of such mice.

(ii) *Immunochemical Studies*

Immunological studies have provided evidence for spatial distribution and neuronal or glial occurrence of CYP1A, 2B, 2C, 2E & 3A forms in mammalian brain. Widespread immunoreactivity with rat hepatic anti-CYPlA1/1A2 in neurons and glia of most brain regions was demonstrated by Kohler et al. (1988). Kapitulnik et al. (1987) also reported immunostaining in brain with antibody raised against rat liver CYP1Al/1A2. Immunoreactivity with anti-CYP1A1/1A2 was found to be localised in nerve fibre and cell bodies of basal ganglia, septum and hypothalamus, the brain areas shown to be involved in CYP mediated metabolism of morphine (Fishman et al. 1976) and catechol estrogens (Fishman & Norton 1975) suggesting that this CYP isoenzyme may have a role in endogenous and exogenous metabolism in brain. Using antipeptide derived antibody, Farin and Omeicinski (1993) also reported constitutive expression of CYP1A1 in human brain. Most of the neurons and some of the astrocytes from various brain regions displayed significant immunoreactivity with anti-CYP1A1. The presence of constitutive levels of CYP isoenzymes was also demonstrated in cultures derived from rat embryo mid-brain (Brown et al. 1989). Recent studies have revealed CYP1B1 immunoreactivity at the blood-brain interface areas of the microcirculation in human temporal lobe (Rieder et al. 2000). Immunohistochemical analysis with anti-CYP1B1 also showed that neurons express CYP1B1 protein and that it is localized in the nucleus of the cell. Most of the astrocytes were also found to express CYP1B1 protein and that like in the neurons, the enzyme is localized in the nucleus (Muskhelishvili et al. 2001).

Western blotting studies using whole brain microsomes have been inconclusive and failed to differentiate between the constitutive and inducible forms of CYP. While no immunoreactivity with anti-CYP1A1/1A2 and anti-CYP2B1/2B2 was observed by many investigators in CYPs extracted from brain microsomes of untreated or β-NF treated rat brain, Warner et al. (1988) reported immunoreactivity in partially purified preparations with these antisera only in some brain regions. Based on the studies, they concluded that most of the CYPs in the brain consist of forms other than CYP2B1/2B2 or 1A1/1A2 and remain to be characterised. Recently Morse et al. (1998) reported low levels of CYP1A2 immunoreactive protein in brain microsomes from the several brain regions isolated from control rats. They, however, could not detect CYP1A1 immunoreactive protein in regional brain microsomes or whole tissue homogenates of CVOs. CYP1A1 was further reported to be induced in the arachinoid, duramater, pineal gland, pituitary and medean iminence while CYP1A2 was undetectable following treatment with β-NF. In contrast, studies of Ravindranath and her group (Ravindranath et al. 1989, Ravindranath & Anandatheerthavarada et al. 1989 & 1990) showed that though the CYP levels in the brain were very low as compared to the liver, control brain microsomes isolated from rat, mouse and human brain exhibit significantly high immunoreactivity with antibodies raised against purified rat hepatic and brain CYP2B1/2B2 and 1A1/1A2 isoenzymes. Anandatheerthavarada et al. (1990) purified CYP and cytochrome P450 reductase to apparent homogeneity from the brain microsomes of PB treated rat. The activity of brain CYP was reconstituted *in vitro* and the immunological characterization of brain CYP was demonstrated in rat and human brain. The purified brain CYP cross reacted with antibodies to rat liver CYP2B1/2B2. Immunoblotting experiments with untreated rat and human brain microsomes using antisera to purified rat brain CYP indicated that these forms of CYP exist constitutively in rat and human brain.

Studies from our laboratory have demonstrated low, but significant immunore-activity of anti-CYP1A1/1A2 with MC-pretreated solubilised brain microsomes and anti-CYP2B1/2B2 with PB-pretreated microsomes. No immunoreactivity was observed with anti-CYP1A1/1A2 and 2B1/2B2 in untreated brain microsomes (Parmar et al. 1998) and could be attributed to extremely low level of expression of these isoenzymes in brain. These studies suggest that with PCR it might be possible to amplify the expression, up to billion fold, of the DNA within a single cell or with immunohisto-

chemical studies to detect individual forms of CYP in a single cell. However, the same sensitivity may not be observed with western blotting using whole brain untreated microsomes because of the dilution of particular form of CYP, which may be present only in limited number of cells, with other more abundant forms of the enzyme (Naslund et al. 1988). Our recent studies have shown that significant regional differences exist in the expression of constitutive and inducible CYP2B1/2B2 isoenzymes (Dayal 2000). PB pretreatment significantly increased the immunoreactivity in all these brain regions with maximum being in olfactory lobe followed by hippocampus and mid brain. Tirumalai et al. (1998) also reported distinct regionality in the expression of CYP2B1/2B2 isoenzymes in rat brain. Immunoblots from untreated brain regions showed immunological cross reactivity between certain forms of hepatic CYP and cerebral CYP, further suggesting that multiple forms of CYP are constitutively but differentially distributed in rat brain regions. Interestingly, the intensity of the immunoreactive bands from brain regions varied among the blots immunostained with antiserum to the rat liver CYP2B and the antiserum to the phenobarbital inducible form of rat brain CYP.

Anandatheerthavarada et al. (1993) also provided evidence for constitutive expression of CYP2E1 in rat brain and chronic ethanol ingestion was found to significantly induce these levels in rat brain microsomes. Immunocytochemical studies revealed the preferential localization of CYP2E1 in the neuronal cells of hippocampus, cortex, basal ganglia, hypothalamic nuclei and reticular nuclei in the brain stem. Bhagwat et al. (1995) also demonstrated the presence of CYP2E1 immunoreactivity in rat brain mitochondria. Warner & Gustafsson (1994) reported significant increase in the CYP contents in the brain regions after single dose of ethanol (0.8 ml/kg), of which 10 to 20 fold increase being in the olfactory lobes and hypothalamic preoptic area. On Western blot analysis and microsequencing, CYPs were identified as CYP2C7, 2C11, 2E1, 4A3, 4A8 and members of CYP2D family. Although, no CYP2E1 immunoreactivity was observed in purified control and ethanol pretreated brain microsomes, the increase in the content of CYP2E1, 2C & 4A in olfactory lobes of rats treated with ethanol was comparable to the liver levels. Region specific distribution of CYP2E1 was also shown by Hansson et al. (1990) in brain using polyclonal antibodies to CYP2E1 of rat liver. The presence of CYP2E1 and its induction by ethanol was also demonstrated in primary cultures of astrocytes using immunofluorescence technique, confocal microscopy and dot blot assay by Montoliu et al. (1995). Wyss et al. (1995) also reported low levels of CYP2D protein expression in partially purified brain microsomes isolated from ethanol treated rats.

(iii) *Evidences from Catalytic Activity*

Detailed studies undertaken in our laboratory and elsewhere on the various forms of CYP have provided evidence that brain is capable of handling a wide variety of CYP substrates (table 5). Although broadly, the brain enzymes were found to resemble the liver ones, some differences were observed in the regulation of cerebral enzymes as compared to the liver CYPs. Brain CYP enzymes exhibited lower apparent K_m and V_{max} than liver microsomal enzymes. Dhawan et al. (1989) reported significant differences in the regulation of ethoxycoumarin-O-deethylase (ECOD) activity in brain as compared to liver. Whereas both PB and 3-MC inducible forms of

Table 5. *Monooxygenase reactions catalyzed by cerebral* CYPs

Reactions	Representative substrates	Reference
N- and O-dealkylation	Aminopyrene, morphine, N-nitrosodimethyl amine, 7-ethoxy, pentoxy- and benzyloxy resorufin, 7-ethoxycoumarin	Ravindranath et al. 1989 Tirumalai et al. 1998 Parmar et al. 1998,1998a Dhawan et al. 1999
Arylhydrocarbon hydroxylation	Benzo[a]pyrene	Das et al. 1981a
Benzene ring hydroxylation	Amphetamine, para-chloroamphetamines, aniline, estrogens	Miller et al. 1986 Mensil, Testa & Jenner 1984 Ravindranath et al. 1989
Oxidative desulfuration	Parathion	Mensil, Testa & Jenner 1984

CYP catalyse the deethylation of ethoxycoumarin (EC) in the liver, ECOD was shown to be regulated by MC inducible forms in rat brain. Kinetic studies showed that unlike biphasic oxidation of EC in the liver, the brain enzyme oxidises EC monophasically . The differential induction of ECOD activity after MC treatment and differences in the inhibition of MC induced brain ECOD activity in rat, mice, guinea pigs and rabbits have further indicated differences in the expression of MC inducible forms of CYPs in different animal species. A marked variation in CYP dependent AHH activity was also found in brain of mammals and birds with rodents having higher activity than birds.

Anandatheerthavarada et al. (1990) reported that rat brain contains significant amounts of CYP and high concentration of certain CYP mediated monooxygenases. Prior treatment with PB was found to result in two fold induction in the activities of aminopyrine demethylase (APD) and morphine-N-demethylase (MND) while MC selectively induced the levels of ECOD and AHH. A distinct sex-related differences were observed in the activities of APD & MND. The female brain levels were 60% of those in the males. Administration of testosterone elevated the levels of total CYP, APD and MND in female rat brain to the levels comparable with that of the male rat brain (Ravindranath & Anandatheerthavarada 1989, Anandatheerthavarada & Ravindranath 1991). CYP and cytochrome P450 reductase were also purified from brain of PB pretreated rats and the purified CYP efficiently catalysed the N-demethylation of aminopyrine in a reconstituted system. Among the different substrates studied, the highest catalytic activity was observed for aminopyrene, followed by morphine. The deethylation of 7-ethoxycoumarin was catalysed to a lesser extent (Anandatheerthavarada et al. 1992). Regional variations in CYP mediated monooxygenase activities within the brain have also been reported. APD activity was significantly higher in the thalamus as compared to other brain regions and whole brain. ECOD activity was found to be significantly higher in cortex, brain stem and thalamus than in the whole brain. N-nitrosodimethylamineN-demethylase (NDMA-d) was higher in cortex and was reported to be comparable with whole brain (Tirumalai et al. 1998)). Ravindranath et al. (1989) also reported the presence of CYP associated monooxygenases in human brain regions and their selective enrichment in the brain stem. Significant amounts of the microsomal CYPs were reported in all regions of the human brain obtained at autopsy, from victims of traffic accidents with no known neurological disorders. All the regions of brain examined except cortex and cerebellum had higher capability to N-demethylate morphine as compared to the liver from the same individual. In fact, the specific activities of the CYPs were higher in the brain stem region as compared to the liver, in spite of the possible autolytic changes that would have taken place in both the organs during the interval between the death and collection of tissue at autopsy.

Tindberg and Ingelman-Sundberg (1996) demonstrated that rat brain hippocampal homogenates and cortical glial cell cultures hydroxylated chlorzoxazone (CZN), a CYP2E1 substrate. Exposure of cortical glial cell cultures to ethanol was found to produce fourfold to sixfold increase in the rate of 6-hydroxylation of CZN and chlormethiazole, a potent inhibitor of CYP2E1 significantly inhibited the ethanol dependent induction in the enzyme activity. A much higher activity of chlorzoxazone hydroxylase was reported in hippocampus and cortex of rats exposed chronically to ethanol (Upadhya et al. 2000) though the enzyme activity was found to be downregulated in brain stem and unchanged in cerebellum, striatum and thalamus. The presence of functionally active CYP2E1 dependent catalytic activity was also observed in human brain regions obtained at autopsy from traffic accident victims (Upadhya et al. 2000).

Recent studies from our laboratory have demonstrated specificity of the brain CYP enzymes in the O-dealkylation of alkoxyresorufin derivatives and found that substituted resorufin derivatives may serve as a useful probe to study the induction of CYP isoforms in the brain (Parmar et al. 1998, 1998a, Dhawan et al. 1999). Brain microsomes were found to catalyse the O-dealkylation of 7-pentoxyresorufin (PR), benzyloxyresorufin (BR) and 7-ethoxyresorufin (ER) and this dealkylation was found to be inducer selective. While pretreatment with PB resulted in significant induction in pentoxy-resorufin-O-dealkylase (PROD) and benzyloxy-resorufin (BROD) activities, MC had no effect on

the activity of PROD & only a slight effect on that of BROD. MC pretreatment significantly induced the activity of ethoxyresorufin-O-deethylase (EROD) while PB had no effect on it. Kinetic studies have shown that the increase in the activities following pretreatment with CYP inducers was associated with a significant increase in the velocity of the reaction (V*max*) of O-dealkylation. *In vitro* studies using organic inhibitors and antibodies have further provided evidence that the O-dealkylation of alkoxyresorufin is isoenzyme specific. While *in vitro* addition of a-naphthoflavone, an inhibitor of CYP1A1/1A2 catalyzed reactions and antibody for hepatic CYP 1A1/1A2 forms produced a concentration dependent inhibition of EROD activity, metyrapone, an inhibitor of CYP2B1/2B2 and antibody for CYP 2B1/2B2 significantly inhibited the activity of PROD *in vitro*. These studies have suggested that as in liver, dealkylation of alkoxyresorufins can be used as a biochemical tool to characterise the xenobiotic metabolising CYPs and substrate selectivity of CYP enzymes in brain microsomes (Parmar et al. 1998, 1998a, Dhawan et al. 1999).

Physiological Role Of Brain CYP

CYP dependent monooxygenases act on a wide variety of endogenous substrates in physiologically significant manner. In a tissue as heterogenous as brain, it is often difficult, if not impossible to differentiate the role this enzyme system might play in both endogenous metabolism as well as in the metabolism of foreign compounds. The cerebral effects of a xenobiotic might be interpreted as being either due to disruption of the normal physiological function of the CYP or by acting as a substrate of CYP thereby leading to the formation of a reactive species (Mensil et al. 1984). The most important role for cerebral CYP demonstrated to date is the transformation of androgens to estrogens by aromatization and of the latter to catechol estrogens by 2-hydroxylation. The physiological significance of catechol estrogens in brain is well established and their detection has provided evidence for the role of CYPs in the neuromodulation (Paul et al. 1977, 1980, Mensil et al 1984). The fact that brain has the highest estrogen 2-hydroxylase activity of any tissue other than liver, suggests that it is a target for catechol estrogens. Significant quantities of catechol estrogens formed in specific areas of brain, such as hypothalamus and pituitary and the fact that these compounds are formed under normal circumstances strongly suggests that they may have a physiological role within the brain. Although the exact role of the brain catechol estrogens remains to be characterized, the role for CYPs in the synthesis of catechol estrogens is well established and may represent one of the first examples of the involvement of this enzyme system in brain neuromodulation. Mensil et al. (1984) have shown that CYPs also functions in the synthesis of endogenous ligand for benzodiazepine receptors and exert a role in normal endogenous physiological transmission.

Cerebral dealkylation for a number of drugs with direct evidence for demethylation reactions occurring in brain was provided by Elison and Elliot as early as in 1963. Using rat brain slices morphine, codeine and meperidine were shown to be demethylated. The differences in the sensitivity of brain and liver demethylases to inhibitors suggest that different CYP forms mediate the demethylation reactions in the two organs. Induction studies have provided evidence that in brain N-demethylation is catalyzed by PB-inducible CYP forms (Chand & Clausen 1982). In brain, demethylation appeared to occur only in the hypothalamus, medial thalamus and corpus striatum. Interestingly, hypothalamus contains both N-demethylating and catechol forming CYPs. Fishman et al. (1976) provided evidence for an association in the sites of N-demethylation of morphine and presence of opiate receptor in brain indicating an involvement of receptor-mediated mechanism of opiate action in N-demethylation. Although the role of CYPs in the action of drugs/chemicals at the opiate sites is not clearly understood, any environmental factor which could alter the levels of brain CYPs and also influence the interaction of drugs/chemicals acting on the opiate receptors might thus exert an effect on opiate transmission and modulate brain functions.

The more direct evidence for the involvement of brain CYPs in the cerebral neurotransmission process has been provided by Niznik et al. (1990). A major binding protein for several inhibitors of dopamine transporter have been identified in striatum as CYP2D. Two [^{3}H] GBR-12935 (a ligand for dopamine transporter) binding proteins, identified as the dopamine transporter and CYP2D1, were solubilized from canine striatal membranes, and resolved

following wheat germ agglutinin (WGA)- lectin column chromatography. Protein adsorbed to and specifically eluted from WGA - lectin was indicative of the dopamine uptake site whereas protein not adsorbed to WGA - lectin was found to be insensitive to dopamine uptake blockers. However, binding to this protein was inhibited by various compounds with a pharmacological profile indicative of CYP2D1. Western blotting and immunoprecipitation studies have indicated identity of this binding site as CYP2D1. An intriguing possible functional association between the dopamine transporter and CYP2D1 could be related to the cellular processing of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin which causes an irreversible Parkinsonian syndrome in man and the selective degeneration of substantia nigral dopaminergic neurons in primates. Both MPTP and its neurotoxic metabolite, MPP^+ (1-methyl 4-phenylpyridine) are substrates and competitive inhibitors of the dopamine uptake site and CYP2D1 and microsomal flavin containing monooxygenases involved in detoxification of MPTP. Moreover, budipine, which is a potent inhibitor of both CYP2D1 activity and [^{3}H] GBR-12935 binding to neuronal CYP2D1 (but not of the dopamine transporter) protects against MPTP- and MPP^+ -induced neurotoxicity. Furthermore, the distinctly cellular localization of the transporter and CYP2D1 supports the hypothesis that dopamine transporter is involved in the uptake of certain signal components whereas the CYPs is involved in the metabolism and elimination following uptake or the transformation of substances to secondary signal mediating metabolites (Niznik et al. 1990, Tyndale et al. 1991).

The binding properties, affinities, and drug specificities of sigma receptor ligands were identical in membranes isolated from rat brain and liver microsomes, suggesting that the sigma receptor is, in fact, a CYP enzyme (Ross 1990, 1991). The high affinity of proadifen (SKF-525A), a potent inhibitor of some isoforms of CYP, for the sigma sites have indicated that the protein binding the sigma ligands is a proadifen-sensitive form of CYP. Similarities in the inhibition pattern of each of the ligands in brain and liver and heterogeneity of the sigma binding have suggested the presence of multiple forms of sigma binding sites, which maybe some isoforms of CYP. Experiments with [^{3}H]-GBR 12935 binding indicate that CYP2D1 is not involved in the binding of the sigma ligands. CYP3A enzymes have been shown to metabolise haloperidol to its neurotoxic pyridinium form in brain suggesting that sigma receptor may be CYP3A enzyme (Igarashi et al. 1995).

Members of CYP2C & 4A subfamily are key enzymes involved in the synthesis and degradation of metabolites of arachidonic acid, which are of physiological importance in the brain. Microsomal fraction from rat hypothalamus was found to catalyse CYP dependent metabolism of arachidonic acid to form several oxygenated products. The major metabolic products were identified as 5,6-epoxyeicosatrienoic acid (5,6-EET) and its hydration product, 5,6-dihydroxyeicosatrienoic acid (5,6-DHET). Both novel arachidonate metabolites, particularly 5,6-EET are potent *in vitro* stimuli for the release of neuropeptide, somatostatin (SRIF) from the hypothalamic median eminence. 5,6-EET was also shown to be capable of evoking lutenizing hormone-releasing hormone (LHRH) release (Capdevila 1983). Junier et al. (1990) also demonstrated that arachidonic acid is metabolised in the hypothalamus by an CYP dependent epoxygenase pathway to metabolites which appear to be involved in the transmembrane signalling mechanism by which activation of D2 dopamine receptors stimulates secretion of the neuropeptide SRIF. The remarkable effectiveness of EETs in stimulating SRIF release suggest that EETs maybe physiological components of the regulatory system that controls SRIF release and, hence, GH secretion *in vivo*. Warner and Gustafsson (1994) have shown that exposure to ethanol increases the levels of CYP4A in rat brain which may possibly result in the increased elimination of prostaglandins and other metabolites of arachidonic acid. In view of the above evidence that prostaglandins are mediators of some of the effects of ethanol in brain, increase in the pathway for the elimination of prostaglandins and other metabolites of arachidonic acid may influence the sensitivity of the brain to ethanol. Another pathway that could be influenced by an increase in the levels of CYP4A family in the brain is the release of peptides by dopamine (Snyder et al. 1983).

Steroid hormones act on the CNS to produce diverse neuroendocrine and behavioral effects (Stromstedt et al. 1993). The sedative and anesthetic effects of gonadal steroid, progesterone and the

mineralocorticoid- deoxycorticosterone, as well as several of their metabolites, is due in part to their ability to enhance the inhibitory action of GABA. The ring A metabolites of progesterone and deoxycorticosterone- namely, 3α-hydroxy-5α-dihydroprogesterone (3α-OH-DHP) and 3α,5α-tetrahydro-deoxycorticosterone (3α-THDOC), respectively-are potent modulators of the GABA-receptor complex and interact at a site close to or identical with that of the barbiturates. Changes in mood and sleep/ wakefulness pattern during the stress, pregnancy and the menstrual cycle are thought to be due to the fluctuations in the levels of 3α-OH-DHP and 5α-THDOC (Purdy et al. 1991). The levels of these steroids in the brain are determined both by their rate of synthesis and by their inactivation and/or elimination. Studies of Stromstedt et al. (1993) have shown that CYPs expressed in brain are involved in the elimination of these steroids and have indicated that this inactivation pathway in the brain may serve to regulate the levels of GABA receptor active steroids.

Interest in brain steroid metabolism has been further aroused by the finding that the brain derived steroids can modulate cognitive function and synaptic plasticity. Pregnenolone and the steroids derived from pregnenolone are reported to have memory- enhancing effects in mice. Investigation of CYP inhibitors and of steroid hormone effects on the metabolism of pregnenolone showed that different CYPs are involved in 7α- and 7β-hydroxylation of pregnenolone. CYP1A1 was found to catalyse part of 7β-hydroxylation of pregnenolone (Doostzadeh & Morfin 1997). A novel CYP, *hct*-1 was identified in rat and mouse brain hippocampus (Stapleton et al. 1995). Expression of hct-1 was particularly enriched in the hippocampus though was detected at low levels also in rat liver and kidney. Sequence analysis of rat and mouse hct-1 cDNAs revealed extensive homologies with CYP7 family subfamily and contains a postulated steroidogenic domain present in other steroid metabolizing CYPs. hct-1 is unusual in that, unlike all other CYPs described , the primary site of expression is in the brain. Similarity to CYP7 subfamily and other steroid metabolizing CYPs suggest that hct-1 might play a role in steroid metabolism in brain and control physiological functions in the CNS because of the documented ability of brain derived steroids to modulate cognitive functions *in vivo* (Stapleton et al. 1985).

Pharmacological and Toxicological Implication of CYP Mediated Metabolism

Expression of CYPs demonstrated in brain could play a significant role in the toxicity of the neurotoxins. Recent reports have indicated a role of CYP in the etiopathogenesis of several of the neurodegenerative disorders induced by drugs and environmental toxins. There is a growing evidence suggestive of an interaction between polymorphic CYP2Ds and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an agent which results in clinical syndromes indistinguishable from idiopathic Parkinson's disease (PD) in man. MPTP and the environmental toxins structurally similar to MPTP has been shown to be potent inhibitors of rat cerebral CYP2D forms catalysing debrisoquine 4-hydroxylation, which is one of the best studied example of genetically determined polymorphism of drug oxidation (Langston et al. 1983, Fonne Pfister et al. 1987, Ohta et al. 1990). The presence of CYP2D6 in humans and similar form 2D1 in rat brain indicate that this CYP form may be a factor responsible in susceptibility to MPTP neuronal toxicity (Fonne-Pfister et al. 1987, Gilham et al. 1990). CYP2D in brain has also been shown to detoxify tetrahydroisoquinolone, a naturally occurring compound causing Parkinsonism in monkeys (Ohta et al. 1990, Yoshida et al. 1990).

There has been several epidemiological studies investigating association of an increased risk for the development of PD with the defects in the CYP2D6 alleles. Some studies show an over-representation of poor metabolizers among patients with Parkinson's disease (Comella et al. 1987, Armstrong et al. 1992) and some do not (Kallio et al. 1991) Though the hypothesis is that differences in the ability of certain individuals to metabolize environment toxicns may increase their risk of developing PD, the association of the poor metabolizer genotype of CYP2D6 with risk of PD have been largely inconclusive. An initial meta-analysis demonstrated a significant association of the poor metabolizer genotype with PD (McCann et al. 1997). A subsequent meta-analysis also showed similar association through contributed by a single large study (Christensen et al. 1998). However, the third meta-analysis failed to reveal any significant association (Rostami-Hodjigan et al. 1998).

The presence of polymorphic enzymes i.e. CYP2D6 in brain, may however, affect the metabolism of neuroleptic and other psychoactive drugs, which are the substrates of these enzymes

in brain (Masimirembwa & Hasler 1997). Pai and Ravindranath (1991) using saggital slices of mouse brain, have shown that monoamine oxidase inhibitors, deprenyl and paragyline, which also inhibit CYPs, protected against the MPTP induced neurotoxicity. Piperonyl butoxide and SKF-525A, the other CYP inhibitors also offered protection against MPTP toxicity. Significant potentiation of MPTP neurotoxicity in brain slices prepared from PB pretreated rats suggest that CYPs may modulate the MPTP induced neurotoxicity by altering the uptake of the toxin in the brain.

Studies of Niznik et al. (1990) have indicated that there is an overlap of substrate specificities between the dopamine transporter and CYP2D1. Cocaine displayed a significantly higher K_i for canine striatal CYP2D6 than that for dopamine transporters, where it is believed to cause its primary pharmacological effects (Tyndale et al. 1991). Since CYP2D6 is a polymorphic protein in humans, there may be differences between phenotypes in the pharmacological actions of cocaine. D-amphetamine, another dopamine transporter ligand is known to bind to CYP2D6 and is polymorphically metabolized to p-hydroxyamphetamine in humans. The metabolism of amphetamine to p-hydroxyamphetamine is of interest because of the association between the formation of p-hydroxynorephedrine, a "false neurotransmitter" that is derived from p-hydroxyamphetamine and the development of tolerance to the drug (Smith 1986, Kuhn et al. 1978). The hallucinogen 4-methoxyamphetamine is also polymorphically O-demethylated by CYP2D6 in humans and it is speculated that poor metabolizers may be unable to demethylate O-methylated psychotoxins, perhaps contributing to the symptoms/etiology of schizophrenia (Kitchen et al. 1979). Codeine is O-demethylated to morphine by the polymorphic CYP2D6, which results in differences in pain thresholds between extensive and poor metabolizers (Sindrup et al. 1990). Experimental studies in laboratory animals have indicated that morphine formed in brain from codeine may be responsible for the analgesic effects of codeine (Chen et al. 1990). Some neuroleptics have also been demonstrated to be metabolized by CYP2D6 (Tyndale et al. 1991).

The role of CYPs in the metabolism of drugs such as amphetamine is well established (Cho et al. 1977, Kuhn et al. 1978). Para-chloroamphetamine (PCA) has been shown to be selectively toxic to serotonergic neurons in laboratory animals. There is evidence to suggest that PCA neurotoxicity may be mediated by a metabolite rather than by parent drug itself. Miller et al. (1986) studied the metabolic activation of PCA to chemically reactive intermediates by hepatic and brain microsomal preparations. The serotonergic neurotoxin was converted by rat hepatic and brain microsomal enzymes to chemically reactive species which covalently bind to the microsomal proteins. The requirement of oxygen and NADPH and inhibition by SKF-525A have indicated that CYP monooxygenases are involved in the metabolic activation of PCA. MC was found to significantly induce the metabolic activation of PCA. In contrast to rats, rabbits which do not exhibit PCA neurotoxicity, showed very little, almost negligible covalent binding with the hepatic and brain microsomal preparations, thus suggesting that oxidative metabolic activation of PCA to reactive and toxic intermediates is related to long term neurotoxicity of this agent.

Brain CYPs have also been shown to be involved in activation of precarcinogens to carcinogens and mutagens (Das et al. 1985). Rouet et al. (1981) reported the qualitative and quantitative differences in metabolites of benzo(a)pyrene (BP) formed in brain microsomes during perinatal development indicating the greater susceptibility of younger ones to these carcinogens. Das et al. (1985) have also shown that BP binds with cerebral DNA which may explain the process of malignant tumor formation in brain by polycyclic aromatic hydrocarbons, PAHs (Napalkov & Alexandrov 1974). It has also been shown that some of the hamster fetal brain cells became malignant when incubated with BP *in vitro*. Likewise, treatment of pregnant rats with polycyclic aromatic hydrocarbons yielded a high percentage of brain tumors in the newborns. Species differences in the extent of activity may also be of significance in this context since it appears that mouse fetal brain tissue is very reactive in metabolizing PAHs to mutagenic metabolites in comparison to rat tissue (Mensil et al. 1984). Since the brain CYPs are also inducible and altered by the administration of the other xenobiotics, there is some evidence to suggest that this may be a mechanism of importance in determining the carcinogenicity in brain. Although the mechanism of carcinogenesis is still to be understood in the humans, this potential

route for the formation of ultimate carcinogens could be of significance when investigating the mechanisms of quantification of the cerebral tumors. Demonstration of functional activity of CYPs in rat glioma cells and induction of CYP activity following exposure to PB and BA have further suggested the role of brain CYPs in the etiology of brain neoplasm (Geng & Strobel 1993 &1995). Moreover constitutive expression and wide distribution of CYP1B1 in normal human brain and its preferential localization in the neurons have indicated the possibility of its important regulational role and its further involvement in local metabolism of xenobiotics (Muskhelishvile et al. 2001). As human CYP1B1 is also involved in activation of chemically diverse procarcinogens (Shimada et al. 1996) and 4-hydroxylation of estradiol (Hayes et al. 1996), it is tempting to speculate that it may have some role in gender related differences in neurobiology.

Interestingly, CYP1A1 inducers, β-NF and cigarette smoke have been reported to protect mice against the toxicity of MPTP (Shahi et al. 1991). Furthermore, epidemiological studies have shown that Parkinson's disease occurs less frequently in smokers than in non-smokers (Godwin-Austen et al. 1982). In addition, allelic variants of human CYP1A1 exist with the homozygous CYP1A1 Val mutation conferring a six-fold relative risk to develop Parkinson's disease compared to the wild type (Takakubo et al. 1996). Sexual dimorphism in CYP expression has long been reported in rat liver and more recently in brain. Sex hormones have been reported to modulate the mRNA expression of CYP2D in rat brain. Estrogen has been implicated in modulating affective disorders and the effects of the tricyclic antidepressants. While more studies are needed to investigate the mechanism of sexual dimorphism of CYPs in brain, the differences in the expression of CYPs may have implications for differential susceptibility to environmental toxins and to differential responses to therapeutic agents.

Chronic exposure to phenytoin, an epileptic drug has been shown to cause cerebral dysfunction and in rare cases cerebellar degeneration. The pharmacological as well as toxicological effects of phenytoin are hypothesised to be based on its accumulation in the membrane system of neurons and glia influencing their excitation behaviour and function (Volk et al. 1995). It was found that the ratios of the phenytoin concentration in brain and liver and those of total organ weights are comparable. Phenytoin has been shown to act as a substrate and inducer of specific CYP isoforms and degradation of phenytoin is initiated by CYP catalysed reaction in the liver. Volk et al. (1988, 1991 & 1995) demonstrated that phenytoin induced the expression of CYP2B1 and 2C29 immunorelated forms in rat and mouse central and peripheral neurons system and primary cultures of cell types from the brain. *In situ* hybridization, RT-PCR and immunocytochemical analysis revealed that CYP2B1 related protein showed only a weak constitutive expression *in vivo* and *in vitro* whereas CYP2C29 related protein is expressed at higher constitutive levels and is highly inducible by phenytoin in the brain. CYPs in cultures prepared from mouse astrocytes and microglia were found to be induced to a much lesser extent than in those prepared from rat brain cells. Since the metabolic activity and clearance of phenytoin by CYP2B1 in rat liver is more effective than that by CYP2C29 in mouse liver, the lesser activity of phenytoin to induce the concentration of CYP in mouse brain tissue as well as in cultured cells could possibly account for the extrahepatic effects of phenytoin reported in the mice.

Ethanol has been shown to increase the CYP content of rat brain and specifically induce the levels of 2C, 2E & 4A CYPs in several brain regions (Warner & Gustafsson 1994). The induction of brain CYP2E1 by ethanol is also of toxicological interest for several reasons. It does indicate the production of the toxin, acetaldehyde in the brain. The other potential toxicolgoical consequence of the increase in the levels of CYP2E1 in the brain could be i) an increase in the release of active oxygen and increase in lipid peroxidation and destruction of membranes; ii) metabolism of many organic solvents such as n-hexane, benzene and toluene, which easily enter the brain, and are neurotoxins, and iii) the possibility that inhalation of other organic solvents (e.g. acetone, isopropanol or ethyl-acetate) that are common in workplace and induce CYP2E1 in the liver, may also induce this enzyme in the brain and increase the risk of neuronal damage.

Watts et al. (1998) also identified CYP2E1 and CYP2C13/2C6 expression in the rat substantia nigra. CYP2E1 was found to be selectively localized in the nigral dopamine containing cells. The expression of CYP2E1, a potent generator of the free radicals, has led them to conclude that CYP2E1 may have a role in neurodegeneration or in the detoxification of environmental

neurotoxin similar to the metabolism of MPTP carried out by CYP2D6 and other enzymes. Electron leakage from CYP redox reactions in the substantia nigra might be expected to induce oxidative stress under certain conditions. Such an increase in the formation of reactive species from CYP2E1 in nigral dopaminergic neurons could be a potential cause of the oxidative stress which has been detected in substantia nigra in Parkinson's disease. Alternatively CYP2E1 activity in the nigra might be of importance in the activation of pro-neurotoxins in a manner similar to the activation of MPTP by monoamine oxidase B. There is also increasing evidence that exposure to n-hexane, as a component of petroleum spirit and of other solvents is linked to the development of Parkinsonian syndrome. Since hexane is neurotoxic only following CYP2E1 induced activation to 2,5-hexanedione, the presence of CYP2E1 in the nigra may contribute in part to the neurotoxicity of hexane (Watts et al. 1998). Likewise, members of the CYP2C family, the expression of which is known to be induced by ethanol (Warner & Gustafsson 1994), inactivate steroids and activate several of the pro-carcinogens to carcinogens. In addition, CYP2C7, which is specifically induced by ethanol in the brain, metabolizes retinol and retinoic acid (Leo et al. 1989). Though the role of CYP2C7 has not been investigated in brain nor there is any information available on the levels of retinoic acid after ethanol administration, induction in the levels of CYP2C7 could be of significance as ethanol consumption has been shown to deplete Vitamin A in liver (Ryle et al. 1986). Another catalytic activity of some members of CYP2C family that may be relevant in brain is the arachidonic acid epoxygenase activity and suggests role of CYP2C in intracellular signalling.

Cerebral CYPs have been reported to be involved in manganese (Mn) neurotoxicity (Qato & Maines 1985, Liccione & Maines 1989). Moreover, Mn was found to increase CYP dependent hydroxylation activity in both the mitochondrial and microsomal fractions of rat striatum. The effects were more pronounced in the mitochondrial fraction where CYP hydroxylation activities were increased by 2-3 fold. Mn induced selective dopamine depletion in striatum was found to be associated with specific induction of CYP dependent metabolism of amphetamine to p-hydroxyamphetamine, an inhibitor of neural dopamine uptake, particularly in the mitochondrial fractions of striatum which may influence indirect dopaminergic action of amphetamine. In addition, the superoxide anion radicals produced by CYPs can lead to the formation of reactive electrophiles of dopamine and other catechols. Thus, the Mn mediated alterations in brain CYP dependent drug metabolism, along with possible concomitant enhanced formation of active oxygen species, might partially explain Mn neurotoxicity of dopamine pathways.

Studies from our laboratory have shown that the neurotoxicity of acrylamide, a monomer used in plastics & polymer industry could be attributed to its metabolite formed during CYP mediated metabolism (Srivastava et al. 1985). Pretreatment of rats with PB, trans-stilbene oxide or dichloro diphenyl trichloroethane (DDT) resulted in earlier onset and subsequent development of acrylamide induced hind limb paralysis that was observed only in animals treated with acrylamide. Pretreatment of cobalt(II)chloride ($CoCl_2$), an inhibitor of CYPs, caused a significant delay in the onset and development of hind limb paralysis. Our hypothesis that a "presumed" intermediate of acrylamide formed by the CYP system is responsible for the toxicity of acrylamide is supported by the earlier appearance of neurotoxicity in rats treated with the inducers of CYP. The delayed development of acrylamide toxicity in $CoCl_2$ pretreated animals further supports the concept that the observed toxicity of acrylamide is, at least in part, mediated by an intermediate formed by the CYP system (Srivastava et al. 1985).

Role of CYP mediated metabolism in the activation of certain neurotoxic pesticides like parathion has also been demonstrated in rat brain (de Lima et al. 1996). The oxidative desulfuration of parathion was one of the first CYP reactions extensively investigated in the brain (Neal 1967). Norman and Neal (1976) reported that brain is capable of activating parathion to paraoxon and other toxic products and that covalent binding of sulfur does occur with the microsomal proteins. The involvement of CYPs in the metabolic activation of parathion was demonstrated by *in vitro* studies indicating almost complete inhibition of the metabolism of parathion when incubated with CYP inhibitors such as SKF-525A or piperonyl butoxide. As found

with rat lung but in contrast to the liver, PB and MC were not found to induce the parathion metabolism in brain (Mensil et al. 1984).

Recent studies from our laboratory have shown the involvement of CYP mediated metabolism in the neurobehavioural toxicity of deltamethrin, a widely used type II pyrethroid insecticide (Dayal et al. 1999). Deltamethrin has been shown to act on multiple site in the CNS. Its marked neurotoxicity in laboratory animals following acute and chronic exposure has been attributed to its effect on nerve membrane sodium channels and interaction with γ-aminobutyric acid (GABA) receptor ionophore complex. Studies on the levels of insecticide in different tissues of rats have indicated that pharmacokinetics plays an important role in the neurotoxicity of deltamethrin (Rickard & Brodie 1985, Sheets et al. 1994). Our studies have shown that deltamethrin produces a marked dose- and time dependent increase in the expression and activity of CYP monooxygenases in rat brain and that these alterations in CYPs could be related to the amounts of pyrethroids and its metabolites reaching and accumulating in the brain. These studies have further shown that the increase in the activity of CYP monoxygenases in brain after deltamethrin exposure is due to the increase in the expression of CYP2B1/2B2 & 1A1/1A2 forms. Furthermore, region specific induction in the activity of CYP monooxygenases was reported after deltamethrin treatment which has indicated differences amongst the different brain regions to metabolize deltamethrin and could be involved in regulating the response of brain to the pyrethroid insecticides by modulating their concentration per se or their metabolites at the target site(s) (Dayal et al. 2001). Recent study from our laboratory has shown that the induction in the cerebral CYPs is associated with the neurobehavioral toxicity of deltamethrin (Dayal et al. 2003).

Conclusions

CYPs in mammalian brain have been identified as the functional enzymes enabling the CNS to metabolize a variety of substrates of both exogenous and endogenous origin. Though levels of CYPs are low in the brain (1-5% of the liver enzyme), they are of significance due to protective nature of the organ and its role as target for a variety of drugs and foreign chemicals. Using RT-PCR, immunocytochemistry and cloning approaches, multiple forms of xenobiotic metabolizing CYPs have been shown to be expressed in mammalian brain. Enzymatic studies have demonstrated the substrate specificity and selectivity of some of the xenobiotic metabolizing CYPs expressed in brain and have provided evidence that the substituted alkoxyresorufins may serve as a biochemical tool to characterize the brain CYP activities. Though overlapping substrate specificity of the xenobiotics with endogenous substrates have made it exceedingly difficult to distinguish the xenobiotic metabolizing activity and the endogenous functions of the CYPs in brain, co-purification of the brain CYPs with dopamine transporter and sigma receptors have indicated the involvement of CYPs in cerebral neurotransmission. Several of the neurotoxins including drugs and drug contaminants such as antidepressants, psychotoxins, neuroleptics, N-methyl-4-phenyl-2,5,6-tetrahydropyridine (MPTP) and environmental chemicals such as pesticides (synthetic pyrethroids), solvents etc. may interact with the brain CYPs as they are known to be metabolized by these enzymes in liver. By acting as specific inducers and inhibitors of the brain CYPs, these chemicals might interfere with the functioning and regulation of the cerebral processes or maybe activated by this system to produce tissue specific toxicity. Because of the low levels in brain, CYPs are unlikely to influence the overall pharmacokinetics of the neurotoxins, nevertheless the *in situ* bioactivation could lead to the damage of macromolecules at the target site and might play a critical role in activation/detoxication processes for environmental or endogenous toxins involved in the neurodegenerative disease. However, further studies are needed to distinguish the xenobiotic metabolizing and the endogenous functions of CYPs in brain to identify the precise role of CYPs in the physiology and toxicology of the brain.

Acknowledgement

Authors are grateful to Dr R C Srimal, Ex-Director, Industrial Toxicology Research Centre, Lucknow and Emeritus Scientist, CSIR for critically reviewing the manuscript. The financial assistance of Department of Biotechnology, New Delhi is also gratefully acknowledged. The secretarial assistance of Mr. Mohd. Aslam is also gratefully acknowledged. ITRC Communication Number: 2170.

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