



The expression and function of P450 enzymes at the neurovascular unit have a key role in drug resistance and brain cell viability.



Pathophysiological implications of neurovascular P450 in brain disorders

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Over the past decades, the significance of cytochrome P450 (CYP) enzymes has expanded beyond their role as peripheral drug metabolizers in the liver and gut. CYP enzymes are also functionally active at the neurovascular interface. CYP expression is modulated by disease states, impacting cellular functions, detoxification, and reactivity to toxic stimuli and brain drug biotransformation. Unveiling the physiological and molecular complexity of brain P450 enzymes will improve our understanding of the mechanisms underlying brain drug availability, pharmacological efficacy, and neurotoxic adverse effects from pharmacotherapy targeting brain disorders.

Introduction

The blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier regulate the composition of the brain milieu. Through structural modifications, including the expression of tight junctions, these central nervous system (CNS) barriers restrict the diffusion of numerous drugs and xenobiotics into the brain [1]. It is well established that the cells of the BBB and blood–CSF barriers express ATP-dependent, membrane-bound efflux transporters, which control drug penetration into the brain. It has been extensively documented that lipophilic compounds aimed at CNS therapy are substrates for drug efflux transporters that are expressed at the luminal side of the cerebrovasculature. This is considered one of the mechanisms contributing to pharmacoresistance in CNS disorders [2–4]. New evidence has shown that brain P450 enzymes also have a significant role in pharmacotherapy. CYP enzymes are heme-containing proteins that, alongside their role of drug metabolism in the liver, are functionally active at the neurovascular unit [5–8], and are also expressed in other brain cells [9–11]. In contrast to drug transporters, whose functions impact the pharmacokinetic properties of a drug, CYP enzymes can also have a

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pharmacodynamic role in biotransforming a parent drug into its metabolite, which changes the affinity and downstream efficacy on a specific target [7,8]. Furthermore, the identification of xenobiotic-metabolizing enzymes in the human brain raises the question of whether these enzymes have a direct impact on disease physiology or progression [9].

Approximately 15 CYP isotypes, subdivided into three families, are known to be involved in drug metabolism [12,13]. Intracellular CYPs were initially described in hepatocytes [14]. CYP localization at the cellular and subcellular levels was subsequently demonstrated in the brain [7,8,11,15,16]. In the human liver, CYP2C9, CYP2C19, CYP2D6, CYP1A2, and CYP3A4 are responsible for the oxidative metabolism of most xenobiotic substrates. CYP3A4 is one of the most abundant enzymes in the liver and intestine, and is responsible for the metabolism of not only a large number of drugs, but also endogenous compounds, such as prostaglandins, steroid hormones, and fatty acids [12]. Similarly, in the brain, CYPs are responsible for the oxidative metabolism of exogenous substrates (e.g., xenobiotics, dietary components, and pollutants) and endogenous substrates (e.g., steroids, cholesterol, and bile acids), impacting neurological disorders (Fig. 1).

Cytochrome P450 exerts the following activities within a catalytic cycle (Fig. 2) [13]: (i) the first catalytic activity of CYP enzymes, acting as mono-oxygenases, is to activate molecular oxygen with electrons from NADPH via NADPH-CYP450 reductase, and insert one atom of molecular oxygen into the substrate; (ii) the second catalytic activity, commonly referred to as the oxidase activity, involves electron transfer from reduced CYP450 to molecular oxygen with the formation of a superoxide anion radical and H_2O_2 ; and (iii) the third catalytic activity of the

P450 system, known as reductase activity, involves direct electron transfer to reducible substrates, such as quinones, and proceeds under anaerobic conditions.

Earlier studies were conducted to demonstrate the presence and metabolic functions of CYP450 and CYP450-dependent monooxygenases in rat olfactory mucosa (OM) compared with the liver [17]. Interestingly, results showed specific activities toward phenacetin, chlorzoxazone, and dextromethorphan to be higher in OM compared with in the liver. However, lauric acid and testosterone metabolic activity was similar in both tissues, and tolbutamide was lower in OM [17]. Considerable differences were observed with regard to mRNA expression of P450 isoforms, including CYP1A1, 2G1, 2B21, and 4B1; these isoforms were expressed in OM, whereas CYP2C6, 2C11, 2D2, 3A1, 3A2, and 4A1 mRNA was found only in the hepatic tissue [17]. Although the hepatic and brain CYP isoforms are broadly identical, functional differences can occur [18]. It was suggested that local brain metabolism of drugs at their site of action influences the therapeutic efficacy independently from liver metabolism, and that differences in brain CYP enzymes contribute to variability in brain-drug responses [9,19,20].

Neurovascular expression of CYP450 enzymes

To elucidate the distribution of CYPs at the neurovascular juncture, initial studies were performed by Ghersi-Egea and colleagues to explore the fundamental aspects of drug-metabolizing enzymes in human and rat brain tissue or microvessels [6,21]. Total CYP was found to be at least nine times higher in the mitochondrial fraction than in the microsomal subcellular fractions when six regions of the human brain were analyzed post autopsy [6]. Another report suggested the activities of drug-metabolizing enzymes to be significant

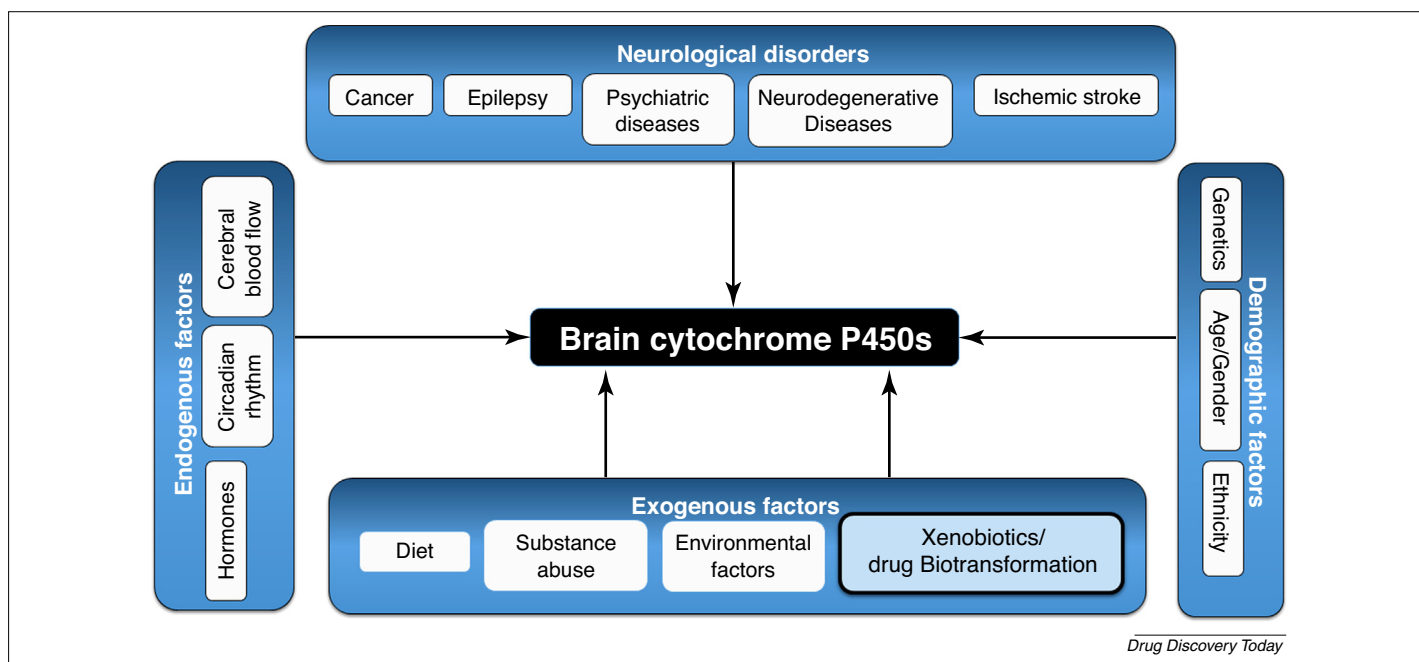
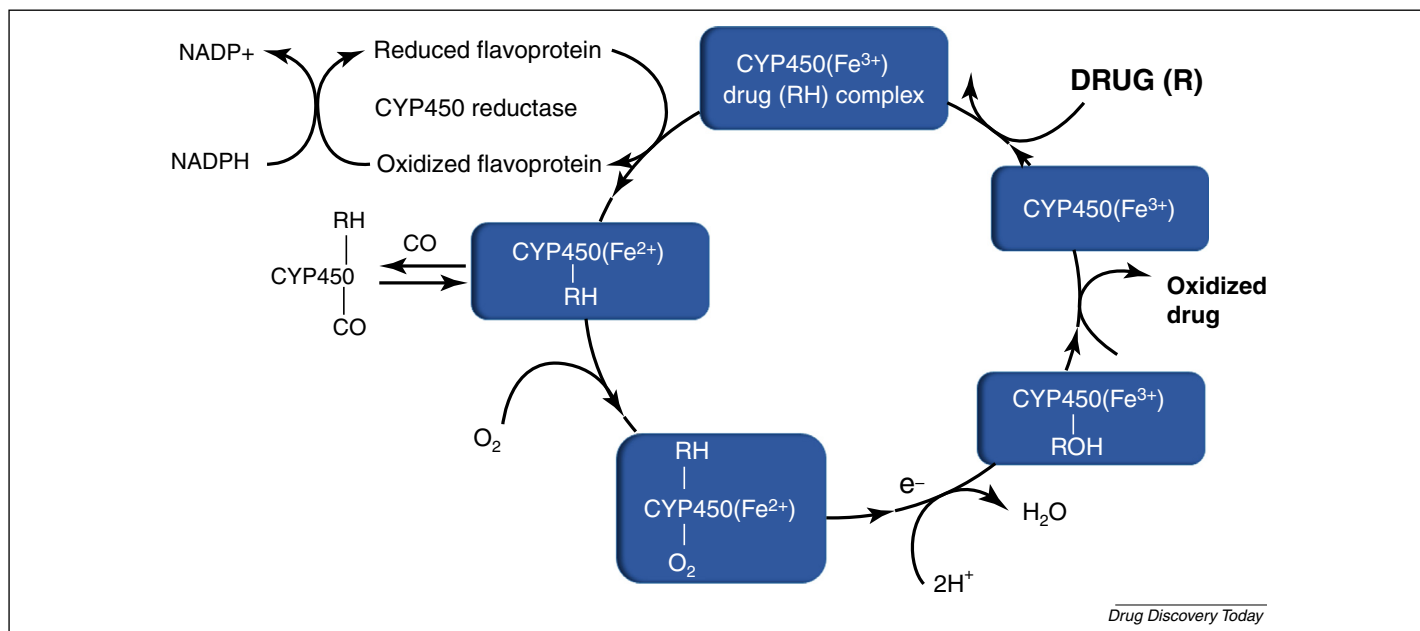


FIGURE 1

Factors regulating cytochrome P450 (CYP450) enzymes in the brain. Brain bioavailability of systemically administered drugs may be P450 dependent. Drug response is not only linked to brain P450 enzymes, but also indirectly influenced by factors that independently or synergistically affect CYP enzymatic activity: (i) endogenous factors or regulators (e.g., hormones, neurotransmitters, biological clock, or cerebral blood flow); (ii) exogenous factors (e.g., xenobiotics, dietary constituents, substances of abuse, or pesticides); (iii) demographics (e.g., genetics, age, gender, ethnicity or race); and (iv) disease-specific factors (e.g., cancer, epilepsy, psychiatric diseases, neurodegenerative disease, or stroke).

**FIGURE 2**

The cytochrome P450 (CYP450) catalytic cycle. The P450 cytochrome enzymes chemically oxidize or reduce drugs using a reactive heme ring, with an iron atom as the ultimate electron acceptor or donor and NADPH as a necessary co-factor [13]. These enzymes are also called mixed function oxidases, and are similar to other enzymes involved in electron transport.

Adapted from [124].

in the three circumventricular organs (i.e., the neural lobe of the hypophysis, pineal gland, and median eminence) and in cerebral microvessels [10]. These findings also supported the hypothesis that drug-metabolizing enzymes are located at blood–brain interfaces, where they form an ‘enzymatic and metabolic barrier’ [6,7,10].

In contrast to peripheral organs of metabolic competence, the brain is not ‘homogeneous’ because each region differs in cellular composition, cell density, and function. As a result, the expression of CYP varies (Table 1) [10,11,18]. Dutheil *et al.* showed selective P450 expression in neuronal and glial cells in several cerebral regions [9,22]. The enzymes studied were CYP1B1, CYP2D6, CYP2E1, CYP2J2, CYP2U1, and CYP46A1, with pronounced heterogeneous distributions in different brain areas [9,22]. Expression of P450s in astrocytes has also been reported [5,6]. The latter is relevant to the notion of a metabolic barrier, because astrocytic end-feet envelop the cerebrovasculature. The circumventricular organs also express CYP1A, CYP1B, and CYP2D [10,16,23,24]. These findings are suggestive of evolved protection against harmful xenobiotics [18].

CYP distribution patterns across various brain regions are documented in both humans and rodents (Table 1). For example, evidence exists suggesting that, in rodents, CYP1A1 is mainly expressed at the BBB but is also detected in other brain parenchymal regions [24,25]. CYP1A1 has also been identified in human brain and localized to the cortical regions, midbrain, basal ganglia, and cerebellum [26]. According to reports, CYP1A2 has been found in most brain regions examined across both humans and rats [9,27,28]. CYP1B1 was also shown to be expressed in various brain regions and at the BBB interface [7,24,25].

The existence of these enzymes in BBB endothelial cells, together with the observation that serotonin and noradrenergic transporters are also present on the surface of ECs in the brain [29],

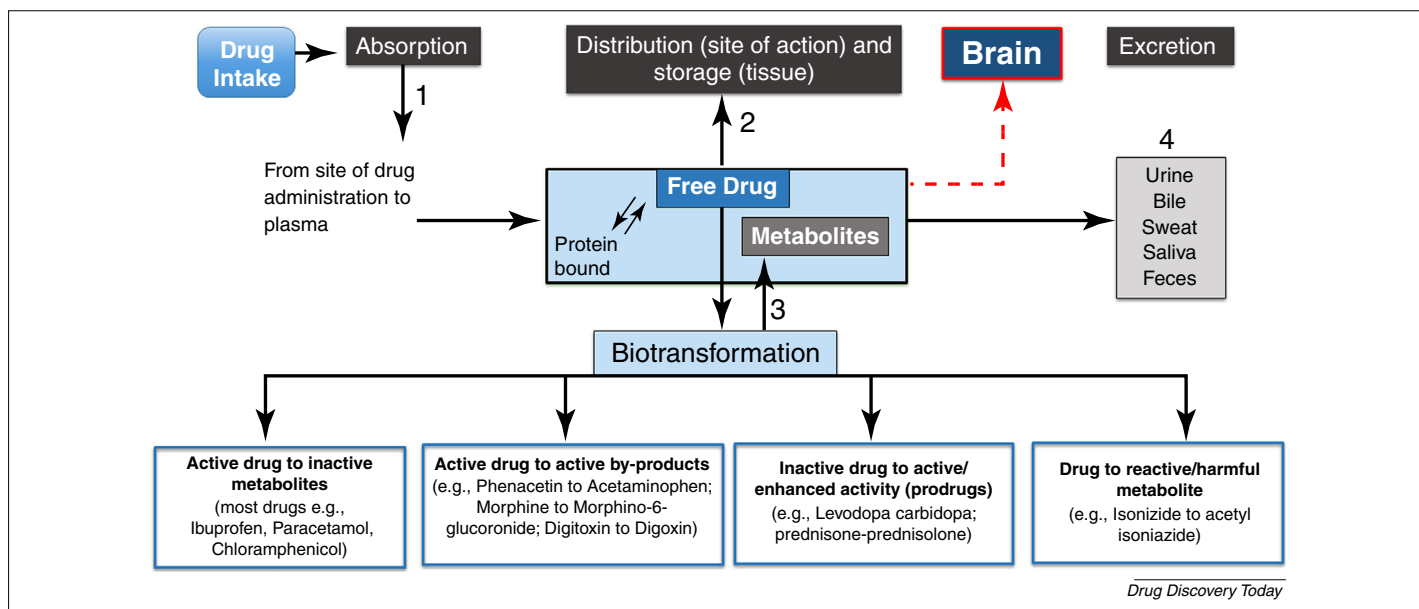
alludes to the possible role of serotonin and norepinephrine in regulating vascular flow along with a xenobiotic role. A role for astrocytes in the control of cerebral microcirculation mediated by P450 2C11-catalyzed conversion of arachidonic acid (AA) to epoxyeicosatrienoic acids (EETs) has also been identified [30]. The P450 metabolites of AA are of particular interest because of their vasoactive properties and their involvement in vascular control and cerebrovascular disease, such as subarachnoid hemorrhage (SAH) and ischemic stroke [30,31]. The relative role of these CYP enzymes in relation to changes that can occur as part of the neurodegenerative process and subsequent effects on the BBB could be further explored. Additionally, these CYP enzymes might have an important role in regulating the levels of endogenous GABA, which is thought to participate in brain cholesterol homeostasis as well as in the elimination of retinoids [32].

Routes of drug biotransformation

Pharmacokinetic processes involve drug absorption, distribution, metabolism (biotransformation), and excretion. The pharmacodynamic processes involve target site of action, and pharmacological and toxicological effects. Overall, these processes determine serum drug levels, the onset, peak, and duration of drug actions, half-life, and therapeutic and adverse drug effects (Fig. 3). Biotransformation is mediated by drug-metabolizing enzymes, leading to the conversion of (i) the active drug into inactive metabolite; (ii) the active drug into active by-products; (iii) the inactive drug into active drug (prodrug); or (iv) the drug into a harmful metabolite. Drug biotransformation occurring in the brain is proposed to locally impact pharmacological responses and contribute to adverse effects. Although CYP levels in the brain are generally lower than in the liver, changes in CYP brain biotransformation were proposed to contribute to CNS drug failure or toxicity [33].

TABLE 1
Expression of CYP450 drug-metabolizing enzymes and their specific localization in the brain

Gene product	Species	Brain region	Expression	Refs
Endothelial cells				
CYP1B1	Mouse, human	BBB	Protein	[5,25,101,107]
CYP2J2	Human	Cortex, microvessels	mRNA	[5,101]
CYP2E1	Mouse, human	BBB	Protein	[15]
CYP3A4	Human, rat	Cortex, frontal lobe, thalamus	mRNA, protein	[7,8,26,108–110]
CYP3A11	Mouse	Hypothalamus, hippocampus, olfactory bulb, cerebellum	Protein	[111]
CYP3A13	Mouse	Hippocampus, hypothalamus, olfactory bulb	Protein	[111]
Astrocytes				
CYP1A1	Mouse, rat, human	Multiple brain regions	Protein, mRNA	[112]
CYP1A2	Rat, human	Cortex, cerebellum, brain stem, thalamus, hippocampus, striatum	Protein, mRNA	[9,27,28]
CYP2B6	Human	Cortex, cerebellum, hippocampus	Protein	[18,113]
CYP2C9	Human		mRNA	[108]
CYP2C11	Rat	Multiple brain regions		[114]
CYP2C29	Mouse	Astrocytes	Protein	[115]
CYP2E1	Mouse, human	BBB	Protein	[15]
CYP3A11	Mouse	Hypothalamus, hippocampus, olfactory bulb, cerebellum	Protein	[111]
CYP3A13	Mouse	Hippocampus, hypothalamus, olfactory bulb	Protein	[111]
Neurons				
CYP1A1	Mouse, rat, human	Multiple brain regions	Protein, mRNA	[112]
CYP1A2	Rat, human	Cortex, cerebellum, brain stem, thalamus, hippocampus, striatum	Protein, mRNA	[9,27]
CYP2B1	Rat	Frontal cortex, striatum, olfactory tubercle	Protein, mRNA	[16]
CYP2B6	Human	Cortex, cerebellum, hippocampus	Protein	[113]
CYP2B10	Mouse	Hippocampus	Protein	[116]
CYP2C19	Mouse	Hippocampus of fetal brain	mRNA	[117]
CYP2D1	Rat	Multiple brain regions	Protein, mRNA	[11]
CYP2D4	Rat	Substantia nigra, olfactory bulb, cerebellum		[32]
CYP2D6	Human	Multiple regions, especially hippocampus, cortex, cerebellum	mRNA, protein	[26,40]
CYP2E1	Mouse, rat, human	Cortex, cerebellum, basal ganglia, hippocampus, medulla oblongata, pons	mRNA, protein	[5,15,84,85]
CYP2G	Rat	Olfactory bulb		[118]
CYP2J9	Mouse	Cerebellum, hippocampus, cerebral cortex, brain stem	mRNA	[119]
CYP3A4	Human, rat	Cortex, frontal lobe, thalamus	mRNA, protein	[7,8,26,108–110]
CYP3A9	Rat	Cerebellum	mRNA	[120]
CYP3A11	Mouse	Hypothalamus, hippocampus, olfactory bulb, cerebellum	Protein	[111]
CYP3A13	Mouse	Hippocampus, hypothalamus, olfactory bulb	Protein	[111]
CYP4A	Rat	Cortex, cerebellum, brain stem, hypothalamus	mRNA	[121]



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FIGURE 3

Potential routes to drug biotransformation. Specific pharmacokinetic processes trigger subsequent pharmacodynamics processes that determine many important aspects of drug therapy. Biotransformation events are not limited to the liver and gut, because they are also mediated by drug-metabolizing enzymes expressed in regions of the brain.

Evidence suggests that CYP isoforms in the brain are also involved in the metabolism of many endogenous substances, such as neurosteroids, monoaminergic neurotransmitters, vitamins, and AA [9,11,18,34]. CYP2D mediates the hydroxylation of tyramine to dopamine in the brain, which has been demonstrated in two experimental rat models by using reserpinized rats and blocking the classical pathway of dopamine synthesis from tyrosine [35]. It has been further documented that CYP2D in rat brain has the ability to metabolize drugs and can be inhibited or induced *in vivo*, independently of hepatic CYP2D involvement [36]. Given that CYP2D metabolically activates codeine to morphine, which is required for codeine analgesia, the authors showed that nicotine treatment increased codeine analgesia through the induction of brain CYP2D in rats during activation of codeine to morphine [37]. Similarly, this has been demonstrated to be the case with CYP2B. Manipulating rat CYP2B metabolic activity in the brain (and not liver) altered the sleep timing (circadian rhythm) induced by the anesthetic propofol, which is metabolically inactivated by CYP2B [38]. The neurotoxicity from chlorpyrifos (an insecticide), which is metabolically activated by CYP2B, has also been reported [39]. Therefore, these examples suggest a role of CYPs across brain regions, potentially affecting the drug response independently from hepatic mechanisms [34,36,38,39].

The relevance of brain P450 expression might become even greater in the setting of disease states, including psychiatric diseases (e.g., depression or schizophrenia), vascular alteration (e.g., ischemic stroke), neurodegenerative diseases (e.g., Alzheimer's and Parkinson's diseases; AD and PD), and epilepsy. Here, we provide an overview of the available data.

Psychiatric diseases

Constitutive expression of CYP2D in human brain neurons indicates its possible role in the metabolism of psychoactive drugs [40]. CYP2D mRNA was found to be expressed in neurons of the cerebral cortex, Purkinje and granule cell layers of the cerebellum, reticular neurons of the midbrain, and pyramidal neurons of the hippocampus. Additional studies demonstrated the presence of the CYP2D protein in the cortex, cerebellum, midbrain, striatum, and thalamus of human brain and its association with psychoactive drug metabolism [40]. Neuroimaging studies have recently shown an effect of CYP2D6 polymorphism on brain functions, vulnerability to mental disorder [41], and extrapyramidal adverse effects of neuroleptic and antidepressants [42]. Other CYPs, such as the CYP3A4, are becoming increasingly important in psychopharmacology as a result of their involvement in the metabolism of a range of antidepressants and benzodiazepines [41,43].

Pharmacogenomics studies conducted on patients with schizophrenia showed antipsychotic drug metabolism via CYP1A2, where a putative gene–gene interaction between dopamine D-3 receptors and CYP1A2 was documented [44]. Neuropsychiatric differences have been reported among individuals with genetic variations in CYP2D6, which metabolizes the endocannabinoid anandamide and influences brain function [45]. Another study suggested that the abundant presence of CYPs in selective cell populations has a role in maintaining brain function in psychiatric disorders [9].

Ischemic stroke

Ischemic strokes occur as a result of an obstruction within a blood vessel supplying blood to the brain. Such sudden loss of blood circulation to an area of the brain results in a corresponding loss of neurologic function [46]. Cerebral blood flow (CBF) is a crucial component of nutrient supply to the brain; this is achieved by adapting regional CBF to local neural activity (autoregulation) [46]. The basis of CBF functionality lies in the hyperemic response, which occurs because of an increase in blood flow to tissues mediated by vasoactive ions and metabolites, which are themselves released from astrocytes and neurons in response to neural activity [46]. Another possible mechanism contributing to autoregulation involves the upregulation of astrocytic CYP2C11 epoxigenases, which mediate the conversion of AA into the active vasodilator epoxyeicosatrienoic acid; the latter appeared to reduce experimental stroke damage in a rodent model [47]. Evidence suggests that ω -hydroxylases (e.g., CYP4A) use AA to generate the vasoconstrictor 20-hydroxyeicosatetraenoic acid (HETE), which is implicated in the regulation of myogenic tone and inflammation [48]. CYP1B1 might also regulate CBF [25,49], because it metabolizes prostanoids and eicosanoids [9]. In summary, P450 epoxigenase and AA metabolism, acting via the P450-dependent pathway, could contribute to CBF regulation [47,50].

Neurodegenerative diseases

PD is a progressive neurodegenerative disease characterized by loss of dopamine neurons in the substantia nigra [51]. Exposure to environmental toxins, including pesticides, is a risk factor for PD [51]. A case report suggested that decreased levels of the *CYP2D6* gene translates into a higher risk of developing PD [52], while another report indicated that risk is further increased with pesticide exposure [51]. In rat models, it has been proven that brain CYP2D catalyzes the conversion of tyramine into dopamine [35]. Therefore, the loss of dopamine because of decreased CYP2D6-activated gene expression in the brain could be a potential factor in the onset of PD [35]. Another study investigated the link between single nucleotide polymorphisms (SNPs) in CYP17 and CYP19 genes and cumulative incidence of AD in subjects with Down syndrome [53]. The authors suggested genetic variations in specific CYPs to be associated with increased susceptibility to AD affecting cognition in these subjects [53]. Furthermore, carrying high-risk alleles or mutation in both *CYP17* and *CYP19* genes was associated with a fourfold increased risk for AD, as well as the binding of elevated sex hormones (as endogenous CYP substrates) to globulin in postmenopausal women. This suggests that genes contributing to estrogen bioavailability influence the risk of AD in women with Down syndrome. Another study reported that the APP23 mouse model of AD presented an abundance of amyloid- β peptides following inhibition of CYP46A1 expression, which resulted in brain cholesterol accumulation and neuronal death, suggesting a neuroprotective role of brain CYP [54].

Cancer

CYP3A4/5 metabolizes antineoplastic molecules (e.g., ifosfamide, vinblastine, etoposide, and doxorubicin) and contributes to drug resistance in patients with cancer [55]. The presence of P450s in tumor cells might be part of a pleiotropic response to tumor development, because certain P450 enzymes provide an essential

cellular function by inactivating antitumor compounds, such as 2-methoxyestradiol, or by activating tumor-promoting compounds, such as 4-hydroxyestradiol [56]. This underscores the role of CYPs in controlling the balance between cytotoxicity and protection. It was shown that the levels of ellipticine required to kill a glioblastoma cell line are dependent on CYP1A1-, CYP1B1-, and CYP3A4-mediated biotransformation, implying an association of P450s with anticancer activity [57].

The cancer-preventative agent resveratrol (a natural constituent of red wine) undergoes metabolism via the CYP450 enzyme CYP1B1 to form a metabolite (piceatannol) with antileukemic properties, suggesting that CYP1B1 in tumors functions as a growth-suppressor enzyme [58]. Tumor cells are exposed to hypoxic conditions, with cells alternating between hypoxic and aerobic conditions [59]. In such cases, CYPs are capable of supporting the oxidative, peroxidative, and reductive metabolism of a range of xenobiotic and endogenous compounds, including the generation of active AA metabolites [56]. Therefore, in a hypoxic environment, P450 enzymes might function as reductive enzymes [56,60].

Epilepsy and multiple drug resistance

Patients with epilepsy whose seizures do not respond to antiepileptic drug (AED) therapy are considered drug resistant (DRE) [61,62]. Several factors regulate the efficacy of AEDs, including metabolism, transport of drugs to the epileptic focus, and target sensitivity. The mechanisms underlying the development of DRE are complex and not fully understood. Drug resistance depends on several clinical aspects, including etiology, seizure onset at an early age, type of epileptic syndrome and seizure, and structural brain abnormalities or lesions [4,61,62]. Reports suggest that DRE is a progressive disorder, which, if controlled early, could be prevented from developing into a full syndrome (refractory epilepsy or DRE) [61,63]. There have also been several hypotheses proposed for multiple drug resistance [61,64]. The difficulty lies in its identification at an early stage in patients who are likely to progress to intractability. Furthermore, reports indicate that drug resistance might arise during the course of epilepsy regimen, even after an initially positive response to AEDs was seen, suggesting that epilepsy-related acquired changes affecting AED efficacy or progression of the disease are involved in intractability [61,63].

Recent findings suggest that the BBB significantly contributes to the phenomenon of multiple drug resistance [3,7,20,62,65]. BBB changes have been observed in brain tissue of patients with epilepsy and in experimental models [3,4,7,62,66,67]. It has been illustrated that multiple drug transporter proteins contribute to the phenomenon of drug resistance in epilepsy [3,4,8,62,66,67]. The role of neurovascular P450s in the epileptic brain was also recently proposed [7,8]. AEDs could be locally metabolized, resulting in the loss of therapeutic efficacy or production of neurotoxic molecules [20]. AEDs, such as oxcarbazepine, carbamazepine, and phenytoin, are associated with CYP3A4 in human hippocampal pyramidal neurons [68]. As a result, induction of CYP3A4 metabolism in the brain can alter brain function. An example of CYP3A4 localization in the microvessels and neurons of human drug-resistant epileptic brain is shown in Fig. 4a,b by immunohistochemistry. Remarkably, autopsy brain tissues (Fig. 4c,d) from two cardiomyopathic subjects (non-neurological case) displayed

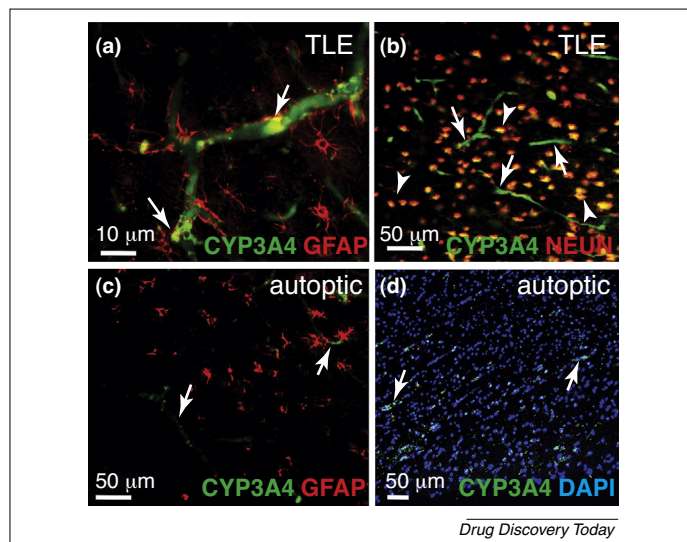


FIGURE 4

Cytochrome P 3A4 (CYP3A4) localization in human temporal lobe epileptic (TLE) brain. Representative images showing CYP3A4 localized in the cerebrovasculature (a) and neurons (b) of a human drug-resistant epileptic brain evaluated by immunohistochemistry. The autoptotic brain tissues obtained from cardiomyopathic subjects (non-neurological case) showed lower levels of CYP3A4 compared with drug-resistant epileptic brains (c,d). Colocalization was evaluated with a neuronal marker staining the neuronal nuclei [NEUN (b)] and glial marker [Glial fibrillary acidic protein, GFAP (a,c)]. DAPI [(4',6-diamidino-2-phenylindole (d))] was used as nuclear counterstain. Data from [7,8].

lower levels of CYP3A4 relative to DRE brains. In addition, earlier studies showed CYP3A4 expression (in protein and mRNA) to be significantly elevated in epileptic brain endothelial cells, isolated from brain resections of DRE, when compared with commercially procured, control brain endothelial cells [7,8]. Patients treated with AED have higher expression of both androgen receptors and CYP3A4 in the hippocampus compared with patients with untreated epilepsy [68]. Similarly, administration of phenytoin to mice leads to the induction of androgen receptor expression in the hippocampus [69]. Expression and function of CYP2E1 in human drug-resistant epileptic brain and rodent models of seizure were also observed [15].

Drug interactions and P450 enzymes

The effect of P450 metabolism becomes even more significant in polytherapies. This is relevant when a drug modulating P450 expression and activity is co-administered with a compound that undergoes P450 biotransformation. The results are a deviation from the expected pharmacokinetic or pharmacodynamic drug profiles [70]. For instance, a recent study performed on primary brain cells (endothelial and neuronal) showed neurotoxic interactions between carbamazepine and sertraline occurring at sub-therapeutic concentrations, and possibly linked to an increased CYP3A4-mediated production of reactive carbamazepine metabolites [33]. Other reports suggested that children with refractory epilepsy treated with clobazam (CLB) and cannabidiol (CBD) experience drug–drug interactions owing to the fact that CLB and CBD metabolism involves a P450 pathway [71]. The reduction in CLB dosage alleviated the adverse effects because subjects were found to have tolerated CBD.

The possible role for CYPs in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice has also been investigated. A neurotoxic metabolite 1-methyl-4-phenylpyridinium ion (MPP⁺) derived from MPTP was increased in brain slices from phenobarbital-treated mice (a known P450 inducer) and decreased in brain slices pretreated with a CYP inhibitor [72]. Predicting the pharmacokinetic properties of a drug might become more complex when co-administering drugs that inhibit P-glycoprotein, including clarithromycin, erythromycin, ritonavir, or verapamil, as well as P-glycoprotein inducers, such as rifampicin and St. John's Wort. In addition, P-glycoprotein substrates overlap with those of CYP3A4 and most P-glycoprotein inhibitors are also inhibitors of CYP3A4. As a result, drug interactions could result from the influence on transporter and metabolic mechanisms in the liver or brain [70,73]. This indicates that CNS drugs should be selected with more caution while considering the brain CYPs. Examples are provided in Table 2. The antiepileptic drug phenytoin induces CYP2B and CYP3A enzymes in the mouse brain, which results in altered brain testosterone metabolism [74]. Some drugs can also induce or repress their own local metabolism in the brain, which is P450 mediated. For example, clozapine is inactivated by CYP2D enzymes. Given that drugs are co-administered with clozapine, induction of brain CYP2D by clozapine could result in faster CYP2D-mediated metabolic inactivation of other substrates, including antidepressants, or increased activation of the oral opiate codeine to morphine. In addition, some of these centrally acting drugs could modify

CYP-mediated brain metabolism of endogenous neurochemicals, including tyramine to dopamine by CYP2D6 [35] and the metabolism of neurosteroids by CYP3A [75], which in turn can modify therapeutic outcome.

Additional factors influencing P450 function in the brain

Hemodynamic and rheological factors as regulators of CYP expression and function

Laminar shear stress has been proven to have a key role in modulating CYP expression *in vitro* [7,20]; this supports the hypothesis that turbulent flow (e.g., aneurysm) or transient loss of flow (e.g., stroke or ischemia) can influence CYP expression. Microarray studies demonstrated that the gene expression of CYP1A1, CYP1B1, and CYP3A4 is upregulated by shear stress in cultured human brain endothelial cells [7]. Han *et al.* showed that the aryl hydrocarbon receptor regulates CYP1A1 expression and cell cycle in vascular endothelial cells under laminar shear stress conditions [76]. Furthermore, the duration of shear stress influences CYP1A1 and CYP1B1 expressions in human endothelial cells [49]. Reports suggest that, in rat brain slices and the isolated retina, the fluctuation of intracellular calcium concentrations in astrocytes affects vessel constriction. This event is mediated by CYP4A and occurs during the conversion of AA to 20-hydroxy-eicosatetraenoic acid [77]. These reports suggest that cerebral blood flow directly or indirectly affects brain CYP expression and activity.

TABLE 2

CYP isoenzymes and CNS-acting drugs as substrates (exogenous or endogenous)

CYP enzymes	Exogenous substrates (drugs, xenobiotics, and other compounds)	Endogenous substrates	Refs
CYP1A1	7-Ethoxyresorufin, nicotine, 7,12-dimethylbenz(a)anthracene (DMBA), rifampicin	Melatonin, estradiol, AA, progesterone	[23–25]
CYP1A2	Caffeine, phenacetin, diazepam, haloperidol, aspartame, phenytoin, primidone, 7-ethoxyresorufin	AA, estradiol and other steroids, fatty acids	[9,18,23,24]
CYP1B1	Arachidonic acid, stilbene, β -naphthoflavone, 7,12-dimethylbenz(a)anthracene (DMBA), nicotine	Melatonin, estradiol	[9,16,18]
CYP2B6	Methadone, meperidine, ethanol, nicotine, pentobarbital, phencyclidine, propofol, sertraline, phenytoin, chlorpyrifos, cyclophosphamide, <i>N,N</i> -diethyl- <i>m</i> -toluamide (DEET), efavirenz, ifosfamide, malathion, paraquat, parathion	17 β -Estradiol, anandamide, AA, estrone, serotonin, testosterone	[19,38,39,74,83,122]
CYP2C	Felbamate, fluoxetine, phenobarbital, (S)- warfarin, phenytoin, valproic acid, topiramate, carvedilol, celecoxib, carbamazepine, primidone, rifampicin	Testosterone, progesterone, AA, serotonin, harmaline, harmine, linoleic acid, melatonin	[29,30,117,123]
CYP2D	Amitriptyline, brofaromine, clomipramine, codeine, opiate, citalopram, clozapine, desipramine, dextromethorphan, morphine, fluoxetine, fluvoxamine, haloperidol, imipramine, mianserin, mirtazapine, nicotine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), parathion	5-Methoxytryptamine, anandamide, progesterone, tyramine	[9,11,18,32,34–37,45,98,122]
CYP2E1	Isoflurane, phenytoin, phenobarbital, acetaminophen, acetone, aniline, benzene, carbon tetrachloride, chloroform, chlorzoxazone, ethanol, nicotine, aspartame	17 β -Estradiol, arachidonic acid, linoleic acid, oleic acid, prostaglandin	[15,19,84,85,90]
CYP3A	Carbamazepine, oxcarbamazepine, phenytoin, caffeine, ketoconazole, nifedipine, dexamethasone, felbamate, pesticides	Steroids, progesterone, testosterone, estradiol	[20,33,43,69,73–75,77,110,120]

Genetic polymorphisms of CYPs

Hepatic P450 mutations profoundly alter systemic drug metabolism and distribution. However, the possibility that mutated or polymorphic variants of P450 exist in the brain is understudied. The latter is clinically relevant because it potentially alters brain drug metabolism. Indirect evidence derives from the association between CYP17 and CYP19 variants and onset of AD [53]. Another example is the genetic variation of CYP2D6 that was linked to neuropsychiatric phenotype differences among patients. The latter could be related to dissimilar interactions of CYP2D6 with endogenous substrates (e.g., anandamide) [45].

Evidence also exists supporting a role for the CYP2D6 genotype in brain function during cognitive tests performed on individuals and measured using fMRI [78]. A range of CYP2D6 activity is observed among different individuals because the gene encoding CYP2D6 is highly polymorphic. Approximately 7% of Caucasians have gene variants leading to a lack of the functional enzyme and, therefore, are referred to as CYP2D-poor metabolizers [79]. Both hepatic and brain levels of CYP2D6 are reduced in genetic CYP2D6-poor metabolizers [11]. CYP2D6-poor metabolizers might have observable differences in brain function and behavior that could result from the altered production of endogenous signaling molecules [34]. Increased anxiety and impulsivity have been associated with a CYP2D6-poor metabolizer [80]. By contrast, CYP2D6-extensive metabolizers have increased cerebral activity in the thalamus and hippocampus, two regions that have high expression of CYP2D6 protein and mRNA [81]. Along the same lines of evidence, polymorphic CYP2C19, which metabolizes sex hormones and 5-hydroxytryptamine, was associated with specific personality traits (such as temperament and character assessment) within 487 Japanese healthy volunteers (male and female) [82]. Another report suggested that genetic polymorphism affecting the activity of primarily CYP2B6 and CYP3A4 is associated with the neurotoxicity of efavirenz during antiretroviral drug therapy in patients with HIV [83].

Substance abuse

A link between alcohol consumption, smoking, and increased levels of CYP2B6, CYP2E1, and CYP2D6 in specific brain regions has been observed [16,19,84,85]. This is a significant adaptive response to chronic exposure to ethanol or nicotine. Addicted subjects can respond differently to drugs and endogenous compounds because of the elevated levels of selected CYPs in the brain [16,19,84,85].

Another example is fluoxetine, a selective serotonin reuptake inhibitor (SSRI), that was found to inhibit CYP enzymes and that interferes with the metabolism of cocaine in rats [86]. Another study performed in rodents showed the interaction of cocaine and nifedipine (a 1,4-dihydropyridine calcium channel blocker) affecting total P450 levels exclusively in the liver; however, no significant alteration in P450 levels was identified in the brain. This study suggested that the combination of drugs and dosages impacts the total P450 levels in the brain [87]. Evidence also suggests that transient exposure to fluoxetine in early postnatal days leads to decreased brain AA metabolism and CYP4A levels in adult mice, possibility related to long-term behavioral alterations in brain development [88,89].

Dietary factors

Aspartame consumption and insulin treatment in a juvenile diabetic rat model were shown to upregulate CYP2E1 and CYP3A2 isozyme levels in the brain [90]. Activity of CYP2E1 can induce the production of oxygen radicals, and both CYP2E1 and CYP3A2 metabolize endogenous or exogenous substances, increasing brain susceptibility to neurotoxins [90].

Hyperlipidemia affects cerebral blood flow by the formation of plaque leading to atherosclerosis and coronary heart disease [91]. Hyperlipidemia was also linked to brain CYP modifications. It has been shown that induction of CYP2E1 in the brain occurs in hyperlipidemia subjects, or after associated ischemia-reperfusion (IR) injury [92]. Furthermore, CYP2E1 induction can contribute to the production of tissue-damaging radicals, inflammatory response, and neuronal apoptosis. Therefore, the occurrence of hyperlipidemia in patients with cerebral ischemia presents a higher threat to neuronal degeneration, because of CYP2E1 expression [92].

Environmental factors

CYP enzyme levels in brain can impact the distribution of environmental molecules, modifying their pharmacological and/or toxicological effects [93]. CYPs and other enzymes, such as carboxylesterases and paraoxonase 1, have the capacity to metabolize pesticides, which could interfere with the metabolic changes documented in pregnant mice exposed to such environmental contaminants, further impacting the developing brain in the offspring [93,94]. In another study, exposure to uranium in rats was linked to molecular changes in the regulation of cerebral cholesterol metabolism, with an involvement of the cholesterol-catabolizing enzyme, CYP46A1 [95]. Along with CYPs, the role of P-glycoprotein is also associated with environmental toxins, such as heavy metals and pesticides [96]. Chlorpyrifos, a commonly used insecticide that can be activated by CYP2B into the acetylcholinesterase inhibitor chlorpyrifos-oxon, caused cholinergic overstimulation and neurotoxicity in rats [39]. Furthermore, one study reported that, in rats, acute exposure to chlorpyrifos resulted in neurochemical-behavioral symptoms indicating possible neurotoxicity [97]. As described above, subjects who are CYP2D6-poor metabolizers are at higher risk of developing PD [52], and this risk is further increased when individuals are exposed to pesticides [98]. Therefore, CYP2D6 metabolism could have a neuroprotective effect against PD risk factors, including pesticide exposure.

CYP regulation in the brain

The expression of P450 enzymes in the liver and intestine is regulated by the pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AHR), and glucocorticoid receptor (GR). These nuclear receptors (NRs) respond to xenobiotics or endogenous compounds entering the cell by modulating downstream enzyme targets [99]. In the CNS, NRs also control the regulation of CYPs as well as drug transporters [5,100]. However, the NR mechanism of CYP regulation in the brain could be different from that in the liver and needs to be better understood. In the human brain, several NRs have been detected, including AHR, PXR, farnesoid X receptor (FXR), CAR, retinoid-x-receptors (RXR α and β), and peroxisome proliferator-activated

receptor (PPAR- α , - δ , and - γ) [22]. Dauchy *et al.* showed that the AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increased mRNA expression of CYP1A1 and CYP1B1 in the human brain cell line hCMEC/D3 [5,101]. In a recent study, Jacob *et al.* showed the differential modulation of CYP1B1 and ABCB1/ABCG2 expression in hCMEC/D3 cells following exposure to environmental pollutants, such as TCDD, suggesting AhR involvement in the TCDD-mediated increase in CYP1B1 expression [100]. Other evidence also indicates the expression of PXR and CAR in the mammalian cerebrovasculature [2,102].

Reports of varied NR distribution among brain regions are documented. CAR, for example, was detected only in the human caudate nucleus [103]; PXR was expressed at highest levels in the human thalamus, pons, and medulla [104]; PPAR and RXR were expressed highly in the rat brainstem, but at low levels in the substantia nigra [105]; AhR was expressed highly in the rat olfactory cortex but at low levels in the amygdala [106]. Much remains to be understood in terms of the expression of NR across the brain regions, their cellular specificity, and their role in regulating brain CYPs. Furthermore, whether the PXR/CAR system regulates CYP expression in neurological diseases and its contribution to CNS drug resistance remains to be elucidated.

Relevance of brain P450 to drug discovery

Drug discovery and the development of next-generation medication revolve around several critical elements and factors that regulate brain pharmacokinetic and drug metabolism. Understanding how and where brain CYPs function could be exploited for drug development (e.g., prodrugs transformed into active

compounds) might in turn facilitate the development of novel therapeutics. Knowledge of CYP and transporter function in the neurovascular interface could increase our ability to deliver active forms of drugs specifically to the brain and minimize drug interactions. Although there have been substantial advances in our understanding of brain CYPs and distribution, much remains to be explored, particularly the interindividual variability in response to CNS drugs, and risk factors controlling neurotoxicity and brain disorders.

Concluding remarks

In conclusion, the role of cytochrome P450 enzymes in the brain is becoming increasingly evident. In addition, a role in promoting or hampering neuronal survival has been demonstrated, suggesting that these enzymes have been unjustly neglected in favor of other mechanisms, such as drug extrusion transporters. Further understanding of this complex molecular network that interfaces the xenobiotic world with neuronal activity could help to reduce undesirable adverse effects of current and future drugs.

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