Pharmacological Reports
2013, 65, 1519–1528
ISSN 1734-1140

Review

Role of brain cytochrome P450 (CYP2D) in the metabolism of monoaminergic neurotransmitters

Anna Haduch, Ewa Bromek, Władysława A. Daniel

Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Władysława A. Daniel, e-mail: nfdaniel@cyf-kr.edu.pl

Abstract:
This article focuses on recent research on the cytochrome P450 2D (CYP2D) catalyzed synthesis of the monoaminergic neurotransmitters dopamine and serotonin in the brain and on the influence of psychotropic drugs on the activity of brain CYP2D. Recent *in vitro* and *in vivo* studies performed in rodents indicate that dopamine and serotonin may be formed in the brain *via* alternative CYP2D-mediated pathways, i.e., tyramine hydroxylation and 5-methoxytryptamine *O*-demethylation, respectively. The contribution of these alternative pathways to the total synthesis of brain neurotransmitters may be higher in humans and may be significantly increased under specific conditions, such as tyrosine hydroxylase and amino acid decarboxylase or tryptophan hydroxylase deficiency. These alternative pathways of neurotransmitter synthesis may also become more efficient when the CYP2D enzyme is mutated or activated by inducers (e.g., alcohol, nicotine, psychotropics), which may be of importance in some neurodegenerative or psychiatric diseases.
In addition to the previously observed influence of antidepressants and neuroleptics on CYP2D in the liver, the investigated drugs also produce an effect on CYP2D in the brain. However, their effect on brain CYP2D is different than that in the liver and is structure-dependent. The observed psychotropic drug-brain CYP2D interactions may be important for the metabolism of endogenous neuroactive substrates (e.g., monoaminergic neurotransmitters, neurosteroids) and for the local biotransformation of drugs. The results are discussed with regard to the contribution of CYP2D to the total synthesis of neurotransmitters in the brain *in vivo* as well as the possible significance of these alternative pathways in specific physiological and pathological conditions and in the pharmacological actions of psychotropic drugs.

Key words:
brain, cytochrome P450, dopamine, serotonin, antidepressants, neuroleptics

Introduction

Cytochrome P450 (CYP) is a hemoprotein enzyme that is present in the liver and extrahepatic tissues. The distribution of CYP isoforms in the body is isoform- and tissue-specific. In the brain it catalyzes local metabolism of drugs and endogenous substrates, such as steroids. The present state of knowledge on the biological significance and drug metabolism of brain CYP has recently been well described and discussed in a few extensive reviews [16, 30, 36]. The present article focuses on recent research on the CYP2D-catalyzed synthesis of the monoaminergic neurotransmitters dopamine and serotonin in the brain and on the influence of psychotropic drugs on the activity of brain CYP2D. The results are discussed regarding the contribution of CYP2D to the total synthesis of neurotransmitters in the brain *in vivo* as well as the possible significance of alternative pathways in specific physiological and pathological conditions and in the pharmacological actions of psychotropic drugs.

The functional role of the CYP2D subfamily in the brain

The CYP2D subfamily of cytochrome P450 enzymes consists of six isoforms in rats (CYP2D1–5 and CYP2D18) but has only one representative isoform, CYP2D6, in humans. The amount of CYP2D protein in rat hepatic microsomes increases with development until 14 weeks of age. However, in contrast to other rat CYP isoforms, the observed increase in CYP2D isoforms is not sex dependent [10].

CYP2D4 is regarded as the main CYP2D isoform in the rat brain, whereas CYP2D1 and CYP2D2 are the most abundant CYP2D isoforms in the liver [16]. The presence of CYP2D mRNA, protein, and activity in the rodent brain is predominantly observed in the basal ganglia (substantia nigra) and cerebellum [5, 26, 29]. Brain CYP2D isoforms, similar to their hepatic homologs, exhibit enzymatic competence toward endo- and xenobiotics, including psychotropic drugs [40]. The metabolism of codeine, amitriptyline, imipramine, and desipramine by rat brain CYP2D has been described [8, 21, 36]. Moreover, testosterone [1], clozapine and nefazodone [18, 22], resveratrol [35], nicotine [27, 43], and alcohol [28, 41] induce CYP2D in the brains of humans, monkeys, and rats, while MPTP attenuates the expression of mouse CYP2D22 [34].

It has been reported that CYP2D6 polymorphism may influence personality traits, as poor metabolizers of debrisoquine are more anxiety prone and less successfully socialized than extensive metabolizers of debrisoquine. Studies on the effect of the CYP2D6 genotype on resting brain perfusion support the hypothesis of a functional role of the enzyme in the human brain [25], suggesting the importance of CYP2D in the metabolism of endogenous neuroactive substrates in the brain [4, 17].

Brain CYP2D isoforms play an important role in the local metabolism of neurosteroids, and rat cytochromes CYP2D1 and CYP2D4 and the human protein CYP2D6 are reportedly involved in the metabolism (e.g., steroid 21-hydroxylation) of progesterone and allopregnanolone [24, 26]. Additionally, rat CYP2D18 mediates the ω-hydroxylation and epoxidation of arachidonic acid and may support the oxidation of dopamine to aminochrome, which is likely involved in the destruction of dopaminergic neurons in Parkinson's disease [38]. Several studies suggest that CYP2D may be involved in the metabolism of monoaminergic neurotransmitters in the brain. Hiroi et al. [23] showed that human CYP2D6 was capable of catalyzing the aromatic hydroxylation of tyramine to dopamine, and Yu et al. [42] demonstrated the ability of CYP2D6 to catalyze the *O*-demethylation of 5-methoxytryptamine to serotonin. Recent research on that subject was aimed at determining whether these reactions in fact occur in the brain.

Dopamine

The largest pathway in the brain dopaminergic system is the nigro-striatal pathway, combining the substantia nigra (neuronal group A9) with the striatum and controlling voluntary motor movement. Anatomically associated with this route is the mesolimbic pathway, which starts mainly from dopaminergic neurons in the ventral tegmental area (neuronal group A10) and connects them to the structures of the limbic system, which are involved in emotional behavior. Another route starting in the ventral tegmental area is the mesocortical pathway, which ends in different cortical areas and is engaged in intellectual processes. Directly related to the regulation of the endocrine system is the tuberoinfundibular pathway, which begins in the arcuate nucleus (neuronal group A12) and the perivenricular area of the hypothalamus and continues to the median eminence, releasing dopamine into the pituitary portal circulation.

In the classical pathway, dopamine is synthesized from phenylalanine, which is transformed into tyrosine *via* phenylalanine hydroxylase and then oxidized by tyrosine hydroxylase to dihydroxyphenylalanine (L-DOPA); the latter is further metabolized by aromatic amino acid decarboxylase into dopamine. The alternative way of dopamine formation in the brain may involve the hydroxylation of tyramine *via* CYP2D, which was first demonstrated for human cDNA-expressed CYP2D6 and liver microsomes [23]. Although tyramine derived from food does not cross the blood-brain barrier, it can be formed in the brain from phenylethylamine [3]. Hence, this pathway may constitute an alternative to tyrosine hydroxylase-mediated dopamine synthesis (Fig. 1).

In vitro studies

Tyramine is an endogenous compound that occurs in the brain as a trace amine in the form of *m*- and *p*-ty-

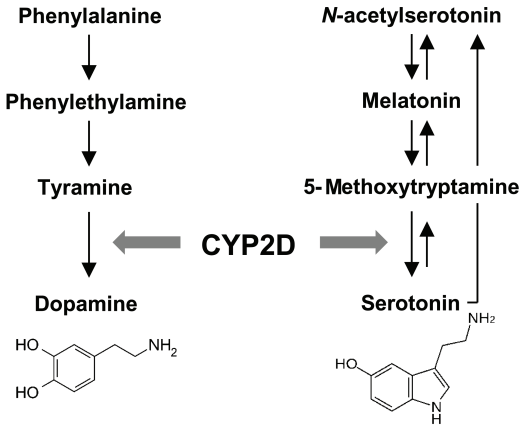

Fig. 1. Alternative pathways of the synthesis of the monoaminergic neurotransmitters dopamine and serotonin with the contribution of cytochrome P450 2D (CYP2D)

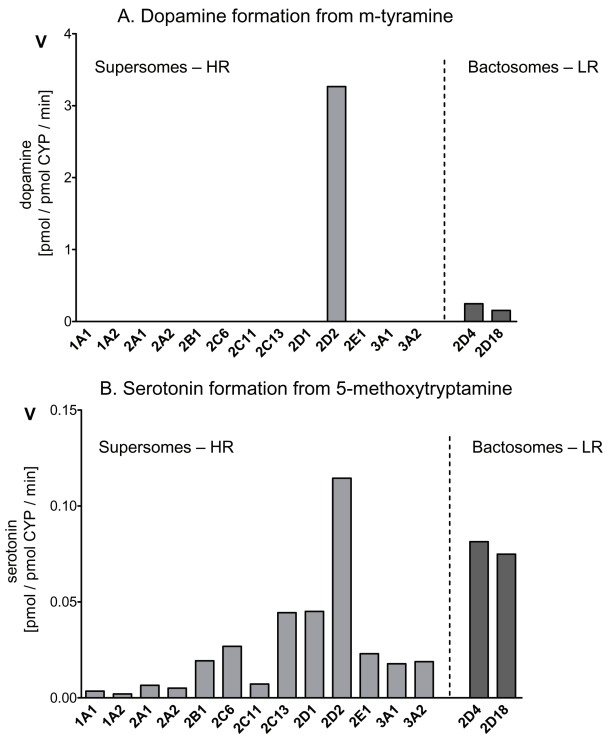

Fig. 2. The rate of the tyramine hydroxylation (**A**) and 5-methoxytryptamine *O*-demethylation (**B**) for thirteen rat cDNA-expressed CYPs: Supersomes-HR (CYP1A1/2, 2A1/2, 2B1, 2C6/ 11/13, 2D1/2, 2E1, and 3A2) and Bactosomes-LR (CYP2D4/18). Recombinant rat cytochromes P450 were co-expressed with a 'high' CYP reductase in Baculovirus-infected insect cells (Supersomes-HR, Gentest) or a 'low' CYP reductase in *Escherichia coli* (Bactosomes-LR, Cypex). Tyramine (250 μM) or 5-methoxytryptamine (500 μM) was added to the incubation mixture containing Supersomes-HR or Bactosomes-LR. Each bar represents the mean value of two independent analyses. HR or LR – 'high' or 'low' CYP reductase (i.e., the high or low co-expression of CYP reductase, respectively); V – reaction velocity (pmol of serotonin/pmol CYP/min). According to Bromek et al. [5] (**A**) and Haduch et al. [19] (**B**)

ramine [3]. Bromek et al. [5] were the first to demonstrate that the hydroxylation of tyramine to dopamine may occur in rat brain microsomes, with *m*-tyramine as the preferred substrate over *p*-tyramine. Quinine, a CYP2D inhibitor, and anti-CYP2D4 antibodies decrease the aromatic hydroxylation of tyramine to dopamine, indicating that CYP2D plays an important role in dopamine formation.

Among the rat CYP isoforms, only CYP2D2, CYP2D4 – the main brain isoforms – and CYP2D18 (but not CYP2D1) are capable of forming dopamine from tyramine (Fig. 2A). The rat CYP2D isoforms involved in dopamine formation (CYP2D2, 2D4, and 2D18) were less efficient than human CYP2D6, and the efficiency of both the human and rat enzymes was higher for *m*-tyramine than for *p*-tyramine. Therefore, it may be assumed that the CYP2D-mediated biosynthesis of dopamine in the brain is more pronounced in humans than in rodents.

Our study of rat brain CYP2D activity [5], which was measured as the rate of bufuralol 1'-hydroxylation in several cerebral regions, indicated a relatively high level of CYP2D activity in the substantia nigra, cerebellum, nucleus accumbens, and brain stem (containing neurons initiating the mesolimbic and mesocortical pathways). Therefore, it appears that this alternative pathway of dopamine formation in the brain may have some implications for Parkinson's disease and addiction in view of the relatively high level of CYP2D activity in the substantia nigra and nucleus accumbens, respectively.

In vivo studies

In the brain, tyramine may be formed *via* the aromatic hydroxylation of phenylethylamine or *via* the decarboxylation of tyrosine. Both tyramine [3] and CYP2D protein/activity are present in the same structures and cell types of the brain, particularly in the basal ganglia. CYP2D is present in the cellular membranes of neurons [28], whereas tyramine is only partly sequestered into synaptic vesicles [3]. Because CYP2D isoforms and tyramine are both present in the catecholaminergic structures of the brain, it is possible that the CYP2D-mediated pathway of dopamine synthesis may occur in these structures *in vivo*.

In our study performed on reserpinized rats with a blockage of the classical pathway of dopamine synthesis using two complementary experimental *in vivo* models, we showed that the hydroxylation of tyramine to dopamine may occur in the brain [6]. In an experiment in which do-

pamine was synthesized from endogenous tyramine, quinine decreased the level of dopamine in the striatum and nucleus accumbens and showed a similar tendency in the substantia nigra and frontal cortex. The different effects of quinine on brain structures appears to be a result of the pharmacological mechanism of action of reserpine, which prevents dopamine accumulation in synaptic vesicles. For this reason, the effect of reserpine was stronger in those structures abundant in dopaminergic nerve terminals and rich in synaptic vesicles (e.g., the striatum, nucleus accumbens, frontal cortex) than in those containing the bodies of neurons (e.g., the substantia nigra, brain stem).

In an *in vivo* microdialysis model, reserpinized rats with a blockage of the classical dopamine synthesis pathway were administered exogenous tyramine into the striatum (in the absence or presence of the CYP2D inhibitor quinine). A large increase in the extracellular dopamine level and its decrease in the presence of quinine provide good *in vivo* evidence for possible dopamine synthesis in the brain and indicate that this pathway is catalyzed by brain CYP2D.

Hence, both *in vitro* [5] and *in vivo* [6] experiments indicate the existence of a functional, alternative CYP2D-mediated pathway of dopamine synthesis in the rat brain. This pathway appears to be quantitatively modest under normal physiological conditions in rats but may be more efficient in the human brain and become important under specific conditions, such as tyrosine hydroxylase or aromatic amino acid decarboxylase deficiency, which may occur as a result of neurodegeneration or mutagenesis. Moreover, the synthesis of dopamine *via* an alternative CYP2D6-mediated pathway may be relatively significant in individuals expressing more than one *CYP2D6* gene (e.g., in Mediterranean populations). In such cases, the tyramine pathway of dopamine synthesis may alleviate symptoms of neurotransmitter deficiency in the brain, particularly under conditions of enzyme induction.

The physiological function of the brain CYP2D forms has not been fully recognized to date. As mentioned elsewhere, it has been observed that CYP2D6 polymorphism may affect personality traits [4, 17], and some studies have indicated an increased risk of Parkinson's disease in CYP2D6-deficient individuals. However, the available data are contradictory, as negative (lack of) associations between CYP2D6 polymorphisms and personality traits or Parkinson's disease have also been published (reviewed in [30]). The protective effect of nicotine in smokers may stem from not only its known receptor action but also from the increased dopamine synthesis and neurotoxin detoxification *via* nicotine induction of brain CYP2D6. Furthermore, the relatively high CYP2D activity and tyramine content in the substantia nigra may be of importance in Parkinson's disease. The CYP2D-mediated synthesis of dopamine may attenuate the decrease in dopamine synthesis in the substantia nigra and VTA, thereby supporting compensatory processes in the brain (discussed in [6]).

CYP2D-mediated dopamine synthesis and nicotine-evoked CYP2D induction in the mesolimbic and nigrostriatal pathways [27, 43] may also be involved in the addiction process. Addictive drugs, including nicotine, preferentially increase extracellular dopamine levels in the nucleus accumbens and dorsal striatum [14, 15], i.e., brain structures that are involved in the development and maintenance of addiction.

Thus, the CYP2D-mediated synthesis of dopamine from tyramine, shown *in vitro* for human recombinant CYP2D6 [23] and later for rat CYP2D forms (CYP2D2/4/18) and brain microsomes [5], has recently been *in vivo* demonstrated to function in the brain [6]. This pathway may be important under specific conditions and may also be considered as a target for the pharmacological action of drugs in the brain in the course of treatment of neurodegenerative diseases and some psychiatric diseases.

Serotonin

Serotonin pathways in the brain originate from 5-HT-containing groups (B1–B9) of neurons of the raphe nuclei in the brain stem. Serotonergic neurons from these cell groups project to almost all parts of the CNS; the dorsal and median raphe nuclei (B5–B8) and B9 project to distal forebrain structures and account for approximately 80% of the forebrain serotonergic terminals. The concentration of serotonin in the brain depends on the free plasma tryptophan levels, the activity of the system that transports tryptophan to the brain, or the activity of tryptophan hydroxylase 2, which is considered to be the concentration-limiting step in the synthesis of serotonin. Serotonin is involved in some basic elements of behavioral and physiological control (e.g., food intake, impulsivity, aggression) and, subsequently, in the pathophysiology of various psychiatric syndromes.

Using *CYP2D6*-transgenic mice, liver microsomes, and recombinant enzymes, Yu et al. [42] demonstrated that the human CYP2D6 isoform was capable of catalyzing the *O*-demethylation of 5-methoxytryptamine to serotonin. The functional importance of that reaction was shown in peripheral tissues by measuring the plasma concentration of serotonin after *iv* injection of 5-methoxytryptamine into wild-type and *CYP2D6*-transgenic mice. The reaction of serotonin regeneration from 5-methoxytryptamine closes the cycle of melatonin-serotonin biochemical transformations [42], and the chemistry of the *O*-demethylation reaction of 5-methoxytryptamine to serotonin by CYP2D6 was demonstrated by Schyman et al. [33]. 5-Methoxytryptamine is an endogenous trace amine that is formed mainly by the deacetylation of melatonin in the pineal gland [2]. However, 5-methoxytryptamine has also been found in the raphe nuclei in the brain stem [31], with the latter containing the cell bodies of serotonin neurons.

Because 5-methoxytryptamine and CYP2D are in close proximity in the brain, the *O*-demethylation of 5-methoxytryptamine to serotonin may occur to prevent the deprivation of the biological activity of indolethylamines by monoamine oxidase (MAO). This may constitute an alternative pathway (to tryptophan hydroxylation) of serotonin formation in the brain.

In vitro studies

A recent *in vitro* study of ours provided new evidence for the hypothesis that the *O*-demethylation of 5-methoxytryptamine to serotonin may occur in the brain [19]. Serotonin was found to be synthesized from 5-methoxytryptamine in different rat brain structures, with the highest rate being observed in cerebellum-derived microsomes. The two selective CYP2D inhibitors used in our study, quinine and fluoxetine, decreased the rate of this reaction, with quinine being an approximately fourfold more potent inhibitor of 5-methoxytryptamine *O*-demethylation to serotonin than of tyramine hydroxylation to dopamine [5, 19].

Essentially, the isoforms of the CYP2D subfamily, CYP2D1/2 and CYP2D4/18 (the major brain CYP2D isoforms), are capable of catalyzing the *O*-demethylation of 5-methoxytryptamine to serotonin (Fig. 2B). Of the other rat CYP isoforms studied (1A1/2, 2A1/2, 2B1, 2C6/11/13, 2E1, and 3A1/2), isoform CYP2C13 showed an activity of approximately one-third of that of CYP2D2, whereas the other CYP isoforms (2B, 2C6, 2E1, and 3A) displayed low activity in this respect (one-fifth of that of CYP2D2). Thus, the reaction of 5-methoxytryptamine *O*-demethylation to serotonin in rats is less specifically catalyzed by CYP2D than is the reaction of tyramine hydroxylation to dopamine [5, 19].

An experiment with human liver microsomes derived from individuals possessing the allelic variant *CYP2D6*4*4* indicated that a genetic defect in *CYP2D* influences serotonin metabolism. The results of our study demonstrate that rat CYP2D isoforms are less efficient at catalyzing 5-methoxytryptamine *O*-demethylation to serotonin than is human CYP2D6 and suggest that this reaction proceeds in the human brain at a higher rate than in the rat brain [19]. Thus, brain CYP2D is likely to positively influence the normal levels of the indolamines serotonin and melatonin, with both being of antidepressive importance.

In vivo studies

As mentioned above, the CYP2D-catalyzed *O*-demethylation of 5-methoxytryptamine has been shown *in vitro* using rat brain microsomes [19]. The follow-up study on rat brain demonstrated the *in vivo* formation of serotonin from 5-methoxytryptamine *via* cytochrome P450 in a brain microdialysis model [7]. The microdialysis probe was implanted into the striatum, and an increase in the extracellular serotonin level was observed after 5-methoxytryptamine given intrastructurally. The CYP2D inhibitor quinine decreased this 5-methoxytryptamine-elevated concentration of serotonin in the striatum, and the effect was more pronounced after the inhibition of the classical pathway of serotonin synthesis. The results indicate that the *in vivo* CYP2D-catalyzed synthesis of serotonin from 5-methoxytryptamine may occur in the rat brain.

The involvement of CYP2D in melatonin-serotonin transformation in the brain was investigated in another *in vivo* study in rats [20]. Melatonin (*ip*) raised the concentration of serotonin in different brain structures, whereas quinine, a CYP2D inhibitor, administered prior to melatonin prevented this elevation in serotonin concentration. The obtained results provide further *in vivo* evidence that the CYP2D-mediated synthesis of serotonin occurs in the rat brain.

Recently, Cheng et al. [9] observed that the level of serotonin and its metabolite 5-HIAA in the brain of *CYP2D6*-transgenic mice was higher than in wild-type mice and that the *CYP2D6*-transgenic mice were less susceptible to anxiety. Thus, parallel studies per-

formed on rats and mice provide convincing evidence to indicate that serotonin may be synthesized from 5-methoxytryptamine *via* the alternative CYP2D-mediated pathway in rodents. It may be assumed that the same pathway also functions in the human brain.

Brain serotonin has been implicated in the pathophysiology of different psychiatric disorders (such as depression, anxiety, schizophrenia) and in the mechanism of action of psychotropic drugs (e.g., antidepressants, anxiolytics, antipsychotics). The *in vivo* studies indicate that this alternative pathway of serotonin synthesis may contribute to the overall serotonin content in different regions of the brain, and this situation may particularly occur when the classical pathway *via* tryptophan hydroxylase is deficient and/or when CYP2D is activated by inducers. Therefore, the alternative pathway of serotonin synthesis may be considered as a target for the pharmacological action of drugs in the course of treatment of psychiatric diseases.

The effect of psychotropic drugs on brain CYP2D

The interactions between psychotropics and liver cytochrome P450 have been extensively reviewed [11]. It has been emphasized that, in addition to the direct action of psychotropic drugs on cytochrome P450 (i.e., the binding of the parent drug or its metabolites to the enzyme), indirect mechanisms of CYP-psychotropic interactions, namely their influence on enzyme regulation, are also very important. Furthermore, the described interactions, which are time-, drug-, and CYP isoform-dependent, may overlap during long-term treatment. However, the effect of psychotropics on brain CYP2D appears to be different than that found in the liver and is cerebral structure dependent [18].

Direct inhibition of CYP2D activity by psychotropics *in vitro*

Certain antidepressants and phenothiazine neuroleptics (e.g., imipramine, fluoxetine, nefazodone, thioridazine, perazine) added *in vitro* to brain microsomes inhibited CYP2D activity to a lower extent (K_i = 255–485 μM) than when added to liver microsomes (K_i = 1–45 μM). This finding may be due to the stronger affinity of these drugs for liver CYP2D2 (K_i = 2.7 and 1.25 μM for imipramine and fluoxetine, respectively) than for brain CYP2D4 (K_i = 25 and 10 μM for imipramine and fluoxetine, respectively) [18]. Imipramine, fluoxetine, and thioridazine inhibited the activity of the cDNA-expressed CYP2D2 isoenzyme (hepatic) several times more potently than the activity of CYP2D4 (cerebral). However, perazine diminished the activities of both isoforms to a similar extent, whereas nefazodone only inhibited the activity of CYP2D4, without affecting that of CYP2D2. The observed differences in the inhibitory effect of psychotropics in the brain and liver may also be due to different levels of non-specific binding and the relatively low CYP level in the brain and, subsequently, higher protein concentration used for experiments with brain microsomes [18]. However, the low K_i values for recombinant CYP2D4 suggest that the mentioned antidepressants (except for mirtazapine) and phenothiazines may directly affect CYP2D4 at pharmacological concentrations *in vivo*.

Effects of chronic antidepressant treatment on brain CYP2D

A differential effect of psychotropic drugs on CYP2D activity in the brain and liver is also observed after chronic treatment (Tab. 1). Unlike in the liver [12], the prolonged administration of imipramine or mirtazapine did not affect the activity of the enzyme in the brain [18]. Moreover, nefazodone, which decreased CYP2D activity in the liver after chronic treatment [12], increased the activity of the enzyme in the brain stem, though no change in the CYP2D4 protein level was found in that structure. This result may suggest post-translational enzyme regulation. As in the liver, fluoxetine significantly decreased CYP2D activity in the striatum and nucleus accumbens but increased it in the cerebellum. Fluoxetine-induced changes in CYP2D activity were found to correlate positively with the CYP2D4 protein level in the brain,

Tab. 1. The effect of chronic treatment with psychotropic drugs on the activity and protein levels of CYP2D in different brain structures (a comparison to the liver). ↑ – increase or ↓ – decrease in enzyme activity; ⇑ – increase or ⇓ – decrease in enzyme protein level; "–" no change in activity or protein level. According to Haduch et al. [18] and Hedlund et al. [22][a]

Drug (chronic)	Liver	Brain structures							
		Nucleus accumb.	Frontal cortex	Striatum	Subst. nigra	Cerebellum	Brain stem	Bulbus olfact.	Rest of brain
IMI	↓	–	–	–	–	–	–	–	–
FLU	↓	↓⇓	–	↓⇓	–	↑⇑	–	–	–
MRT	↑	–	–	–	–	–	–	–	–
NEF	↓	–	–	–	–	–	↑–	–	–
TIOR	↓	↓⇓	–	↑⇑	↓⇑	↑⇑	–	–	–
CLOZ	–[a]	↓⇓	–	–	⇓	⇑	↑⇑[a]	⇑	↑⇑

IMI – imipramine, FLU – fluoxetine, MRT – mirtazapine, NEF – nefazodone, TIOR – thioridazine, CLOZ – clozapine

suggesting an effect of the drug on the expression of the gene coding for this CYP isoform.

It may be assumed that the investigated antidepressants (except for mirtazapine) may exert an inhibitory effect on CYP2D in the brain, which stems from a direct interaction with the enzyme (binding), and – in the case of fluoxetine – also from a down-regulation in enzyme expression. Consequently, the inhibition of CYP2D activity by antidepressants may enhance the neurosteroid concentration in the brain (discussed in [18]). Clinical studies have shown a significantly lower level of allopregnanolone in the plasma and cerebrospinal fluid of depressed patients, with the low level being normalized by treatment with selective serotonin reuptake inhibitors (SSRIs: fluoxetine and fluvoxamine) or tricyclic antidepressant drugs (TADs) [32, 37, 39].

In addition, by increasing CYP2D activity in the brain stem (containing dopaminergic neurons of the VTA and serotonergic neurons of the raphe nuclei), nefazodone may accelerate the CYP2D-mediated tyramine hydroxylation to dopamine and *O*-demethylation of 5-methoxytryptamine to serotonin. As both of these neurotransmitters are important for the pathophysiology and pharmacotherapy of depression, the inhibition of neurosteroid metabolism by SSRIs and TADs (*via* CYP2D inhibition) or the enhancement of dopamine/serotonin synthesis by nefazodone (*via* an increase in CYP2D activity) may be of pharmacological and clinical importance.

Effects of chronic neuroleptic treatment on brain CYP2D

The influence of neuroleptics on brain CYP2D after chronic treatment is also different than in the liver and is structure dependent (Tab. 1). Similar to the liver [11], thioridazine decreased CYP2D activity in the substantia nigra and nucleus accumbens but significantly increased the enzyme activity in the striatum and cerebellum [18]. Clozapine, which was previously shown to induce brain CYP2D4 in a post-transcriptional phase [22], significantly increased CYP2D activity in the brain stem and the remainder of the brain though decreased the enzyme activity in the nucleus accumbens. An opposite change in the activity (decrease) and protein level (increase) of CYP2D in the substantia nigra after thioridazine treatment may have resulted from the formation of thioridazine-reactive metabolites [13] by cytochromes other than CYP2D (CYP2B/CYP2E1/CYP3A) present in this brain area.

The weak-to-moderate induction of CYP2D in the striatum or cerebellum after chronic thioridazine treatment is likely to be abolished *in vivo* as a result of direct CYP2D inhibition by the neuroleptic (*via* binding). In contrast, the ability of clozapine to increase CYP2D activity in the brain stem (a structure comprising the VTA) may lead to increased dopamine synthesis *via* a CYP2D-catalyzed pathway [5, 23],

which – in addition to the receptor profile of the drug – may become an extra add-on component of the atypical neuroleptic action of the drug.

A possible explanation for the diverse effect of chronic psychotropics on the level of CYP2D in the liver and brain are the differences in the mechanisms regulating the expression of these enzymes in the two organs and in particular brain structures [30].

The results of recent research indicate that the psychotropics examined (except for mirtazapine) may directly inhibit CYP2D activity in the brain, yet less potently than in the liver. Their effect on brain CYP2D after chronic treatment is different than in the liver and is cerebral structure-dependent. The interactions of psychotropic drugs with cytochrome P450 in the brain may contribute to their pharmacological effect and clinical outcome.

Conclusions

Recent *in vitro* and *in vivo* studies performed in rodents indicate that dopamine and serotonin may be formed in the brain *via* alternative CYP2D-mediated pathways, tyramine hydroxylation and 5-methoxytryptamine *O*-demethylation, respectively. These alternative pathways modestly contribute to the total concentration of the neurotransmitter in the rodent brain under normal physiological conditions. However, their contribution to the total synthesis of brain neurotransmitters may be higher in humans and may significantly increase under specific conditions, such as deficiencies in tyrosine hydroxylase and amino acid decarboxylase (catalyzing the classic pathway of dopamine synthesis) or tryptophan hydroxylase (catalyzing the classic pathway of serotonin synthesis). These alternative pathways of neurotransmitter synthesis may also become more efficient when the *CYP2D* gene is mutated (duplicated or amplified) or activated by such inducers as alcohol, nicotine, or some psychotropic drugs, which may be of importance in some neurodegenerative or psychiatric diseases.

In addition to the previously observed influence of antidepressants and neuroleptics on CYP2D in the liver, the investigated drugs also produce an effect on CYP2D in the brain, though their effect on brain CYP2D is different than that in the liver and is brain structure-dependent. The observed psychotropics-brain CYP2D interactions may be important for the metabolism of endogenous neuroactive substrates (e.g., monoaminergic neurotransmitters, neurosteroids) and for the local biotransformation of drugs, and such interactions may modify their pharmacological action. The inhibition of CYP2D activity by classic antidepressants may contribute to the enhancement of the neurosteroid concentration in patients during the pharmacotherapy of depression, whereas the ability of nefazodone and clozapine to induce CYP2D (and CYP2D-mediated synthesis of monoaminergic neurotransmitters) in the brain stem (in the VTA) may be an add-on component of the pharmacological action of these drugs. It should therefore to be ascertained *in vivo* whether the long-term exposure to therapeutic concentrations of psychotropics affects CYP2D6 activity in the human brain (as has been shown for rat brain CYP2D4) and whether this effect is important for the clinical outcome of these drugs. If so, genetic variations in the enzyme may have some impact on the therapeutic action of psychotropics in the brain and not merely on their metabolism in the liver. Moreover, the interference of psychotropics with brain CYP2D may contribute to the differences in clinical effects among drugs acting *via* similar neuronal mechanisms.

Acknowledgments:
This article was supported by grant DeMeTer no. POIG.01.01.02-12-004/09 (European Union and the Ministry of Science and Higher Education, Warsaw, Poland) and by statutory funds from the Institute of Pharmacology, Polish Academy of Sciences (Kraków, Poland).

References:

1. Baum LO, Strobel HW: Regulation of expression of cytochrome P-450 2D mRNA in rat brain with steroid hormones. Brain Res, 1997, 765, 67–73.
2. Beck O, Jonsson G: In vivo formation of 5-methoxytryptamine from melatonin in rat. J Neurochem, 1981, 36, 2013–2018.
3. Berry MD: Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. J Neurochem, 2004, 90, 257–271.
4. Bertilsson L, Alm C, De Las Carreras C, Widen J, Edman G, Schalling D: Debrisoquine hydroxylation polymorphism and personality. Lancet, 1989, 1, 555.
5. Bromek E, Haduch A, Daniel WA: The ability of cytochrome P450 2D forms to synthesize dopamine in the

brain: An in vitro study. Eur J Pharmacol, 2010, 626, 171–178.
6. Bromek E, Haduch A, Gołembiowska K, Daniel WA: Cytochrome P450-mediates dopamine formation in the brain *in vivo*. J Neurochem, 2011, 118, 806–815.
7. Bromek E, Haduch A, Gołembiowska K, Daniel WA: The formation of serotonin from 5-methoxytryptamine via cytochrome P450 in the brain in vivo – a microdialysis study. Eur Neuropsychopharmacol, 2013, 23, Suppl 2, S240–S241.
8. Chen AR, Irvine RJ, Bochner F, Somogyi AA: Morphine formation from codeine in rat brain: possible mechanism of codeine analgesia. Life Sci, 1990, 46, 1067–1074.
9. Cheng J, Zhen Y, Miksys S, Beyoglu D, Krausz KW, Tyndale RF, Yu A et al.: Potential role of CYP2D6 in the central nervous system. Xenobiotica, 2013, 43, 973–984.
10. Chow T, Imaoka S, Hiroi T, Funae Y: Developmental changes in the catalytic activity and expression of CYP2D isoforms in the rat liver. Drug Metab Dispos, 1999, 27, 188–192.
11. Daniel WA: The influence of long-term treatment with psychotropic drugs on cytochrome P450: the involvement of different mechanisms. Expert Opin Drug Metab Toxicol, 2005, 1, 203–217.
12. Daniel WA, Haduch A, Wójcikowski J: Inhibition and possibile induction of rat CYP2D after short- and long-term treatment with antidepressants. J Pharm Pharmacol, 2002, 54, 1545–1552.
13. Daniel WA, Haduch A, Wójcikowski J: Inhibition of rat liver CYP2D *in vitro* and after 1-day and long-term exposure to neuroleptics *in vivo* – possible involvement of different mechanisms. Eur Neuropsychopharmacol, 2005, 15, 103–110.
14. Di Chiara G, Imperato A: Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA, 1988, 85, 5274–5278.
15. Ferrari R, Le Novère N, Picciotto MR, Changeux JP, Zoll M: Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. Eur J Neurosci, 2001, 15, 1810–1818.
16. Funae Y, Kishimoto W, Cho T, Niwa T, Hiroi T: CYP2D in the brain. Drug Metab Pharmacokin, 2003, 18, 337–349.
17. Gonzáles I, Peñas-Lledo, EM, Pérez B, Dorado P, Alvarez M, Llerena A: Relation between CYP2D6 phenotype and genotype and personality in healthy volunteers. Pharmacogenomics, 2008, 9, 833–840.
18. Haduch A, Bromek E, Daniel WA: The effect of psychotropic drugs on cytochrome P450 2D (CYP2D) in rat brain. Eur J Pharmacol, 2011, 651, 51–58.
19. Haduch A, Bromek E, Sadakierska-Chudy A, Wójcikowski J, Daniel WA: The catalytic competence of cytochrome P450 in the synthesis of serotonin from 5-methoxytryptamine in the brain: an in vitro study. Pharmacol Res, 2013, 67, 53–59.
20. Haduch A, Bromek E**,** Wójcikowski J**,** Gołembiowska K, Daniel WA: Involvement of cytochrome P450 (CYP) in melatonin-serotonin transformation in the brain. The 11th World Congress of Biological Psychiatry, 23–27 June, Kyoto, Japan, 2013, P–18–013.
21. Hansson T, von Bahr C, Marklund M, Svensson JO, Ingelman-Sundberg M, Lundström J: Different regiospecificity in the hydroxylation of the antidepressant desmethylimipramine between rat brain and liver. Pharmacol Toxicol, 1992, 71, 416–419.
22. Hedlund E, Wyss A, Kainu T, Backlund M, Köhler C, Pelto-Huikko M, Gustafsson JÅ, Warner M: Cytochrome P4502D4 in the brain: Specific neuronal regulation by clozapine and toluene. Mol Pharmacol, 1996, 50, 342–350.
23. Hiroi T, Imaoka S, Funae Y: Dopamine formation from tyramine by CYP2D6. Biochem Biophys Res Commun, 1998, 249, 838–843.
24. Hiroi T, Kishimoto W, Chow T, Imaoka S, Igarashi T, Funae Y: Progesterone oxidation by cytochrome P450 2D isoforms in the brain. Endocrinology, 2001, 142, 3901–3908.
25. Kirchheiner J, Seeringer A, Godoy A, Ohmle B, Maier C, Beschoner P, Sim EJ, Viviani R: CYP2D6 in the brain: genotype effects on resting brain perfusion. Mol Psychiatr, 2011, 16, 333–341.
26. Kishimoto W, Hiroi T, Shiraishi M, Osada M, Imaoka S, Kominami S, Igarashi T, Funae Y: Cytochrome P450 2D catalyze steroid 21-hydroxylation in the brain. Endocrinology, 2004, 145, 699–705.
27. Mann A, Miksys S, Lee A, Mash DC, Tyndale RF: Induction of the drug metabolizing enzyme CYP2D in monkey brain by chronic nicotine treatment. Neuropharmacology, 2008, 55, 1147–1155.
28. Miksys S, Rao Y, Hoffmann E, Mash DC, Tyndale RF: Regional and cellular expression of CYP2D6 in human brain: higher levels in alcoholics. J Neurochem, 2002, 82, 1376–1387.
29. Miksys S, Rao Y, Sellers EM, Kwan M, Mendis D, Tyndale RF: Regional and cellular distribution of CYP2D subfamily members in rat brain. Xenobiotica, 2000, 30, 547–564.
30. Miksys S, Tyndale RF: Cytochrome P450-mediated drug metabolism in the brain. J Psychiatry Neurosci, 2013, 38, 152–163.
31. Patel S, Dulluc J, Geffard M: Comparison of serotonin and 5-methoxytryptamine immunoreactivity in rat raphe nuclei. Histochemistry, 1986, 85, 259–263.
32. Pinna G, Costa E, Guidotti A: Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteriod content at doses that are inactive on 5-HT reuptake. Psychopharmacology, 2006, 186, 362–372.
33. Schyman P, Usharani D, Wang Y, Shaik S: Brain chemistry: how does P450 catalyze the O-demethylation reaction of 5-methoxytryptamine to yield serotonin? J Phys Chem B, 2010, 114, 7078–7089.
34. Singh S, Singh K, Patel DK, Singh C, Nath C, Singh VK, Singh RK, Singh MP: The expression of CYP2D22, an ortholog of human CYP2D6, in mouse striatum and its modulation in 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease phenotype and nicotine-mediated neuroprotection. Rejuvenation Res, 2009, 12, 185–197.
35. Srivastava G, Dixit A, Yadav S, Patel DK, Prakash O, Singh MP: Resveratrol potentiates cytochrome P450 2 d22-mediated neuroprotection in maneb- and paraquat-

induced parkinsonism in the mouse. Free Radic Biol Med, 2012, 52, 1294–1306.
36. Strobel HW, Thompson CM, Antonovic L. Cytochromes P450 in brain: function and significance. Curr Drug Metab, 2001, 2, 199–214.
37. Ströhle A, Pasini A, Romeo E, Hermann B, Spalletta G, di Michele F, Holsboer F, Rupprecht R: Fluoxetine decreases concentrations of 3 α, 5 α-tetrahydrodeoxy corticosterone (THDOC) in major depression. J Psychiatr Res, 2000, 34, 183–186.
38. Thompson CM, Capdevila JH, Strobel HW: Recombinant cytochrome P450 2D18 metabolism of dopamine and arachidonic acid. J Pharmacol Exp Ther, 2000, 294, 1120–1130.
39. Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A: Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci USA, 1998, 95, 3239–3244.
40. Voirol P, Jonzier-Perey M, Porchet F, Reymond MJ, Janzer RC, Bouras C, Strobel HW et al.: Cytochrome P-450 activities in human and rat brain microsomes. Brain Res, 2000, 855, 235–243.
41. Warner M, Gustafsson JĹ: Effect of ethanol on cytochrome P450 in the rat brain. Proc Natl Acad Sci USA, 1994, 91, 1019–1023.
42. Yu AM, Idle JR, Byrd LG, Krausz KW, Küpfer A, Gonzalez FJ: Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. Pharmacogenetics, 2003, 13, 173–181.
43. Yue J, Miksys S, Hoffmann E, Tyndale RF: Chronic nicotine treatment induces rat CYP2D in the brain but not in the liver: an investigation of induction and time course. J Psychiatry Neurosci, 2008, 33, 54–63.

Received: July 31,2013; **in the revised form:** September 23, 2013; **accepted:** September 24, 2013.