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# Inhibition and possible induction of rat CYP2D after short- and long-term treatment with antidepressants

Władysława A. Daniel, A. Haduch and J. Wójcikowski

**Abstract**

The aim of this study was to investigate the influence of tricyclic antidepressants (imipramine, amitriptyline, clomipramine, desipramine), selective serotonin reuptake inhibitors (SSRIs: fluoxetine, sertraline) and novel antidepressant drugs (mirtazapine, nefazodone) on the activity of CYP2D, measured as a rate of ethylmorphine *O*-deethylation. The reaction was studied in control liver microsomes in the presence of the antidepressants, as well as in microsomes of rats treated intraperitoneally for one day or two weeks (twice a day) with pharmacological doses of the drugs (imipramine, amitriptyline, clomipramine, nefazodone 10 mg $kg^{-1}$ i.p.; desipramine, fluoxetine, sertraline 5 mg $kg^{-1}$ i.p.; mirtazapine 3 mg $kg^{-1}$ i.p.), in the absence of the antidepressants in-vitro. Antidepressants decreased the activity of the rat CYP2D by competitive inhibition of the enzyme, the potency of their inhibitory effect being as follows: clomipramine ($K_i$ = 14 $\mu$M) > sertraline ≈ fluoxetine ($K_i$ = 17 and 16 $\mu$M, respectively) > imipramine ≈ amitriptyline ($K_i$ = 26 and 25 $\mu$M, respectively) > desipramine ($K_i$ = 44 $\mu$M) > nefazodone ($K_i$ = 55 $\mu$M) > mirtazapine ($K_i$ = 107 $\mu$M). A one-day treatment with antidepressants caused a significant decrease in the CYP2D activity after imipramine, fluoxetine and sertraline. After prolonged administration of antidepressants, the decreased CYP2D activity produced by imipramine, fluoxetine and sertraline was still maintained. Moreover, amitriptyline and nefazodone significantly decreased, while mirtazapine increased the activity of the enzyme. Desipramine and clomipramine did not produce any effect when administered in-vivo. The obtained results indicate three different mechanisms of the antidepressants–CYP2D interaction: firstly, competitive inhibition of CYP2D shown in-vitro, the inhibitory effects of tricyclic antidepressants and SSRIs being stronger than those of novel drugs; secondly, in-vivo inhibition of CYP2D produced by both one-day and chronic treatment with tricyclic antidepressants (except for desipramine and clomipramine) and SSRIs, which suggests inactivation of the enzyme apoprotein by reactive metabolites; and thirdly, in-vivo inhibition by nefazodone and induction by mirtazapine of CYP2D produced only by chronic treatment with the drugs, which suggests their influence on the enzyme regulation.

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

Władysława A. Daniel, A. Haduch, J. Wójcikowski

**Correspondence:** W. A. Daniel, Polish Academy of Sciences, Institute of Pharmacology, Smętna 12, 31-343 Kraków, Poland. E-mail: nfdaniel@cyf-kr.edu.pl

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## Introduction

The CYP2D subfamily contributes to the metabolism of many drugs, including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, typical and atypical neuroleptics, $\beta$-blockers, antiarrhythmic agents, drugs of abuse such as codeine and amphetamines and carcinogens and neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1,2,3,4-tetrahydroisoquinoline (Fonne-Pfister et al 1987; Ohta et al 1990). The physiological role of CYP2D isoforms is not well known, though recent studies have shown that they are involved in the metabolism of endogenous neurochemical substrates such as tyramine and tryptamine (Martinez et al 1997; Hiroi et al 1998). The isoenzymes of human CYP2D subfamily are encoded by one active *CYP2D6* gene and two pseudogenes, while in the rat five genes, *CYP2D1–5*, and *CYP2D18* (probably a variant of *CYP2D4*) have been identified (Gonzalez et al 1988; Kimura et al 1989; Matsunaga et al 1990). The CYP2D proteins are present not only in liver microsomes, but also in the mitochondrial and microsomal membranes of the brain, where they are highly concentrated in specific cell types (Miksys et al 2000). The cerebellum is the region in which CYP2D is most abundant,

where its immunoreactivity is confined to the Purkinje and molecular layers, with very little staining in the cells of the granular layer. The medulla oblongata shows a much lower overall CYP2D level than the cerebellum, yet there are some areas, such as neurons in the vestibulocochlear ganglion and ventral cochlear nucleus, which stain very intensely. The hippocampus shows moderate staining confined to the neuronal and glial elements of the molecular and polymorphic layers of the hippocampus and dentate gyrus, being notably absent in the granule and pyramidal cell layers. The amygdaloid complex is highly abundant in CYP2D. The striatum shows moderate glial and some neuronal staining in the caudate-putamen and globus pallidus, but no CYP2D-positive cells have been found in the corpus callosum. There is a multitude of CYP2D in neurons in the internal granular layer of the olfactory bulbs. In the pons, there is a strong staining of neurons of the substantia nigra pars compacta and pars reticulata, and light-to-moderate staining of cell bodies of the red nucleus and interpenduncular nucleus. In the spinal cord, an ample amount of CYP2D has been observed in the motor neurons in the dorsal and ventral horns. The studies of Siegle et al (2001) indicate that in the human brain areas, CYP2D6 mRNA is more widely distributed compared with its protein. Neuronal cells, as well as glial cells in such structures as the neocortex, caudate nucleus, putamen, globus pallidus, hippocampus, hypothalamus, thalamus, substantia nigra and cerebellum, contain CYP2D mRNA. In contrast, CYP2D6 protein is primarily localized in large principal neurons, such as pyramidal cells of the cortex, pyramidal cells of the hippocampus and Purkinje cells of the cerebellum. In glial cells, CYP2D6 is absent.

The mentioned isoenzymes exhibit similar substrate/reaction specificity, but they differ in the kinetics of enzymatic 1′-hydroxylation of bufuralol (Boobis et al 1986; Wan et al 1997; Chow et al 1999b), 4-hydroxylation of debrisoquine (Schulz-Utermoehl et al 1999), 4-hydroxylation of bunitrolol (Yamamoto et al 1998), *O*-demethylation of dextromethorphan (Miksys et al 2000), as well as 8-hydroxylation and *N*-demethylation of mianserin (Chow et al 1999a). Moreover, the CYP2D subfamily members differ in their susceptibility to the CYP2D inhibitors, quinine and quinidine (Steiner et al 1988; Kobayashi et al 1989; Boobis et al 1990; Tyndale et al 1999). CYP2D subfamily polymorphism has been found in humans (Gonzalez et al 1988) and rats (Matsunaga et al 1989). The studies of Matsunaga et al (1989) have suggested that CYP2D1 is not expressed in the Dark Agouti rat (a model of the CYP2D6 poor metabolizer phenotype in humans), but recent data have shown that CYP2D2, and not CYP2D1, is absent in the liver of that rat strain (Yamamoto et al 1998; Schulz-Utermoehl et al 1999).

As previously mentioned, antidepressant drugs are substrates of the CYP2D subfamily isoenzymes. It has been shown in man that CYP2D6 catalyses 2- and 10-hydroxylation of tricyclic antidepressants (Brøsen et al 1991; Coutts et al 1994), *N*-demethylation of SSRIs (Kobayashi et al 1999; Margolis et al 2000; Ring et al 2001), 8-hydroxylation of mirtazapine (Störmer et al 2000) and hydroxylation of the trazodone and nefazodone metabolite, meta-chlorophenylpiperazine (Barbhaiya et al 1996). Moreover, it has been shown that in man some tricyclic antidepressants and SSRIs exert a potent inhibitory effect on CYP2D6 in-vitro and in-vivo (Crewe et al 1992; Jeppesen et al 1996), while nefazodone and mirtazapine are rather weak inhibitors of CYP2D6 in-vitro (Schmider et al 1996; Holm & Markham 1999; Störmer et al 2000). The potency of the effect of these antidepressants on the activity of CYP2D in laboratory animals has not been studied.

The effect of chronic treatment with antidepressants on CYP2D activity is less known. Our earlier study showed that two-week treatment of rats with pharmacological doses of imipramine (10 mg $kg^{-1}$ i.p., twice a day) increased the total amount of cytochrome P-450 in the liver, but it inhibited aromatic hydroxylation of the parent compound and its metabolite, desipramine (Daniel et al 1992). Imipramine given repeatedly at high daily doses to rats (100 mg $kg^{-1}$ p.o. once daily for four days) also increased the total content of cytochrome P-450, but inhibited the rates of the CYP2D specific reactions, such as 4-hydroxylation of debrisoquine and bunitrolol, 3-hydroxylation of lidocaine (lignocaine) and 4-, 5- and 7-hydroxylation of propranolol (Masubuchi et al 1995). The effect of chronic treatment with other antidepressants on CYP2D activity is not known.

The aim of this work was to study the effect of 24-h-exposure and chronic treatment with pharmacological doses of antidepressant drugs with different chemical structures and mechanisms of pharmacological action on CYP2D activity, which has not been elucidated so far (either in rats or in man), but which may be significant during long-term antidepressant therapy. Although the literature data described direct interactions of antidepressants with CYP2D6 in human liver (binding with cytochrome protein), we included in our study a direct interaction of antidepressant with rat CYP2D to show all possible effects of antidepressants on rat CYP2D (direct effect, 24-h-exposure and chronic treatment) and to show a difference in their direct effects on human CYP2D6 and rat CYP2D. Such an approach allowed us to distinguish between a direct effect of antidepressants on the enzyme and the changes produced by their short- or long-term administration. The obtained results indicate complex, drug- and time-dependent changes in the activity of rat CYP2D produced by antidepressants.

## Materials and Methods

### Drugs and chemicals

Imipramine hydrochloride was provided by Polfa (Jelenia Góra, Poland) and amitriptyline by H. Lundbeck A/S (Copenhagen, Denmark); clomipramine was from RBI (Natick, MA) and desipramine from Ciba-Geigy (Wehr, Germany). Fluoxetine hydrochloride was purchased from Eli Lilly (Indianapolis, USA) and sertraline hydrochloride from Pfizer Corp. (Brussels, Belgium). Mirtazapine hydrochloride was donated by Organon (Netherlands) and nefazodone by Bristol-Meyers Squibb International,

**Table 1** The influence of antidepressant drugs added in-vitro to rat liver microsomes on the rate of ethylmorphine *O*-deethylation (model I).

| Antidepressants (inhibitors) | Inhibition of ethylmorphine *O*-deethylation $K_i$ ($\mu$M) |
|---|---|
| Tricyclic antidepressants | |
| Imipramine | 26 |
| Amitriptyline | 25 |
| Clomipramine | 14 |
| Desipramine | 44 |
| Selective serotonin reuptake inhibitors (SSRIs) | |
| Fluoxetine | 17 |
| Sertraline | 16 |
| Novel antidepressants | |
| Mirtazapine | 107 |
| Nefazodone | 55 |

The inhibition constants ($K_i$) for competitive inhibition were calculated using Dixon analysis (Figure 1).

Ltd (Uxbridge, UK). Ethylmorphine, NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma (St Louis, MO) and morphine from Polfa S.A. (Kutno, Poland). All organic solvents were of HPLC purity and were supplied by Merck (Darmstadt, Germany).

### Animal procedures

All the experiments with animals were performed in accordance with the Polish governmental regulations (decree on animal protection DZ.U. 97.111.724, 1997). Male Wistar rats (230–260 g) were used in the experiments and were kept under standard laboratory conditions. The investigated antidepressant drugs were administered intraperitoneally, twice a day for one day or two weeks at the following pharmacological doses (mg $kg^{-1}$ i.p.): imipramine, amitriptyline, clomipramine and nefazodone 10; desipramine, fluoxetine and sertraline 5; mirtazapine 3. The rats were sacrificed at 12 h (one-day treatment) or 24 h (two-week treatment) after the drug withdrawal, and liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH 7.4), including washing with 0.15 M KCl according to a conventional method. The above procedure deprives microsomes of the presence of drugs administered in-vivo, which was confirmed in our experiment by the HPLC method described below.

### In-vitro studies into CYP2D activity – measurement of the rate of ethylmorphine *O*-deethylation in liver microsomes

The activity of the CYP2D subfamily isoenzymes was studied by measurement of the rate of CYP2D specific reaction, ethylmorphine *O*-deethylation, in liver microsomes. After optimizing the in-vitro conditions of the reaction, the drug effects were investigated at linear dependence of the product formation on time and protein and substrate concentrations.

To distinguish between a direct effect of antidepressants on the activity of CYP2D and the changes produced by their one-day or two-week administration, three experimental models were used. In model I, pooled liver microsomes from seven control rats were used. The rate of ethylmorphine *O*-deethylation (ethylmorphine concentration, 100–500 $\mu$M) was assessed in the absence and presence of one of the antidepressants added in-vitro (antidepressant concentration, 100–500 $\mu$M). Each sample was prepared in triplicate. In model II, liver microsomes from one-day antidepressant-treated rats were used. Ethylmorphine was added to the incubation mixture in-vitro at a concentration of 250 $\mu$M. Ethylmorphine *O*-deethylation was studied in the absence of antidepressants. In model III, liver microsomes from two-week antidepressant-treated rats were used. Ethylmorphine was added to the incubation mixture in-vitro at a concentration of 250 $\mu$M. The reaction was studied in the absence of antidepressants.

Incubations (models I, II and III) were carried out in a system containing liver microsomes (1 mg of protein in 1 mL), Tris/KCl buffer (20 mM, pH 7.4), $MgCl_2$ (2.5 mM), NADP (0.1 mM), glucose 6-phosphate (1.2 mM) and glucose-6-phosphate dehydrogenase (0.3 U in 1 mL). The final incubation volume was 1 mL. After a 10-min incubation, the reaction was stopped by adding 200 $\mu$L of methanol and then cooling it down in ice.

### Determination of the concentration of ethylmorphine and morphine in liver microsomes

Ethylmorphine and morphine were extracted from the microsomal suspension (pH 8.5) with chloroform containing 10% of 1-butanol (1 mL of microsomal suspension + 2 mL of 1 M $NHCO_3$ + 6 mL of the organic phase) (Kupfenberg et al 1964). Concentrations of ethylmorphine and morphine formed from ethylmorphine in liver microsomes were assessed by the HPLC method based on that used by Glare et al (1991). The residue obtained after evaporation of the extracts was dissolved in 1000 $\mu$L of the mobile phase described below. A sample (20 $\mu$L) was injected into the HPLC system (LaChrom, Merck-Hitachi), equipped with L-7480 fluorescence detector, L-7100 pump and D-7000 System Manager. The analytical column (Econosphere C18 5 $\mu$m, 4.6 × 250 mm) was purchased from Alltech (Carnforth, UK). The mobile phase consisted of 0.05 M $KH_2PO_4$–acetonitrile, 75:25. The flow rate was 1.0 mL $min^{-1}$ (1–8 min) and 2 mL $min^{-1}$ (8.1–22 min). The column temperature was 25°C. The fluorescence was measured at a wavelength of 210 nm (excitation) and 340 nm (emission). The compounds were eluted in the following order: morphine 6.4 min, ethylmorphine 14.55 min. The observed intra-day precision, inter-day reproducibility and accuracy were within commonly accepted values.

### Calculations and statistics

$K_i$ values were estimated from Dixon's plots. Statistical significance (model II and model III) was assessed using an analysis of variance followed by Dunnett's test. All values are means ± s.e.m. from 7–8 rats.

## Results

### Model I

The investigated antidepressant drugs added to liver microsomes of control rats competitively inhibited the rate of ethylmorphine *O*-deethylation (Table 1). Figure 1 shows examples of the Dixon plots obtained in our studies, which served as a basis for calculation of $K_i$ constants. The competitive character of inhibition was confirmed by the Lineweaver–Burk analysis (data not shown). The obtained $K_i$ values indicated that clomipramine and the SSRIs sertraline and fluoxetine were the most potent inhibitors of the studied reaction, while mirtazapine was the weakest in this respect (Table 1). The potency of the antidepressants in inhibiting the reaction was as follows: clomipramine > sertraline ≈ fluoxetine > imipramine ≈ amitriptyline > desipramine > nefazodone > mirtazapine.

### Model II

A one-day (i.e. 24-h) exposure to imipramine, fluoxetine or sertraline resulted in a significant decrease in the rate of ethylmorphine *O*-deethylation in rat liver microsomes (Figure 2). The other antidepressants studied did not significantly affect the rate of the CYP2D specific reaction.

### Model III

Two-week treatment with the investigated antidepressants produced more changes in the rate of ethylmorphine *O*-deethylation in liver microsomes than one-day exposure (Figure 3). After prolonged administration of antidepressants, the decreased CYP2D activity after imipramine, fluoxetine and sertraline was still maintained. Moreover, amitriptyline and nefazodone significantly decreased the activity of the enzyme, which was not the case after one-day exposure to the drugs. Unlike after one-day exposure, and in contrast to the other antidepressants tested, mirtazapine significantly increased the rate of ethylmorphine *O*-deethylation after chronic treatment. Desipramine and

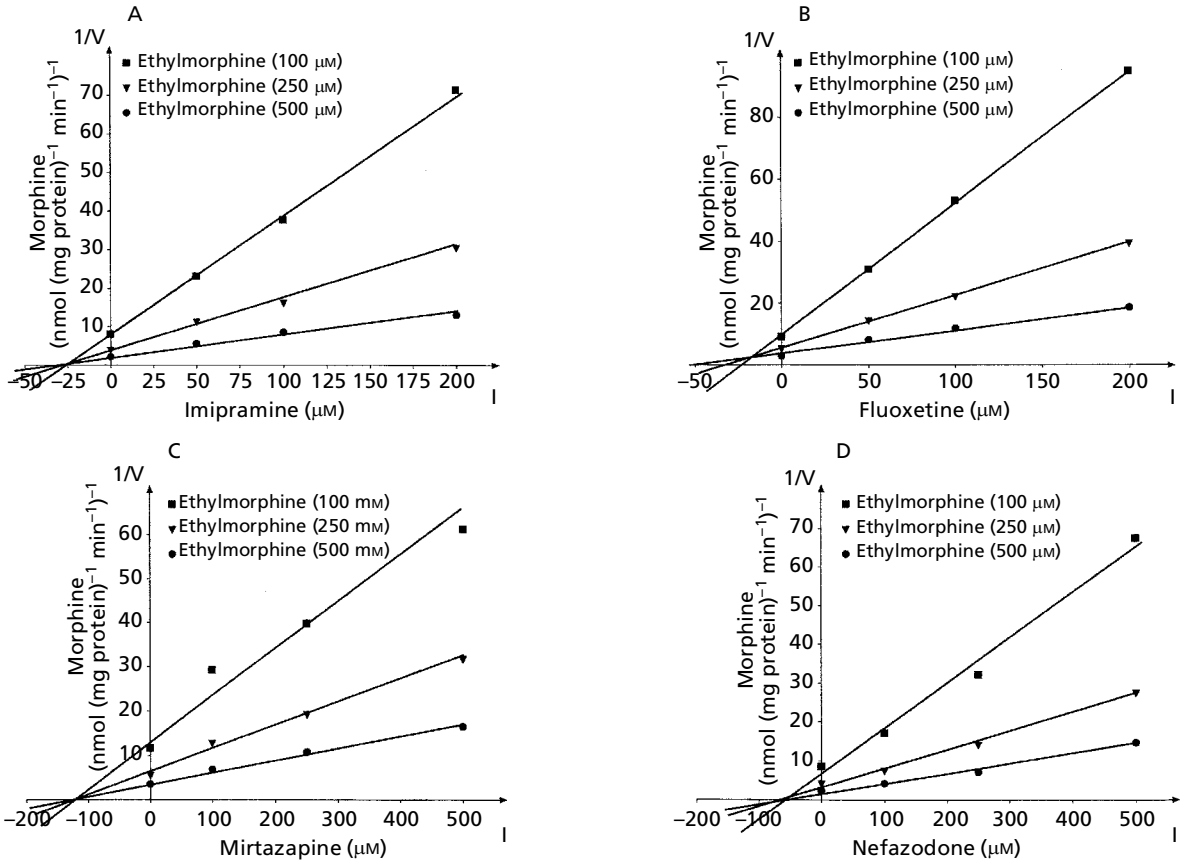


**Figure 1** Kinetics of the inhibition of ethylmorphine *O*-deethylation by antidepressant drugs (imipramine, A; fluoxetine, B; mirtazapine, C; nefazodone, D) in rat liver microsomes (Dixon plots). V = velocity of the reaction (nmol of morphine (mg of protein)$^{-1}$ min$^{-1}$); I = concn of inhibitor ($\mu$M). $K_i$ values are presented in Table 1.

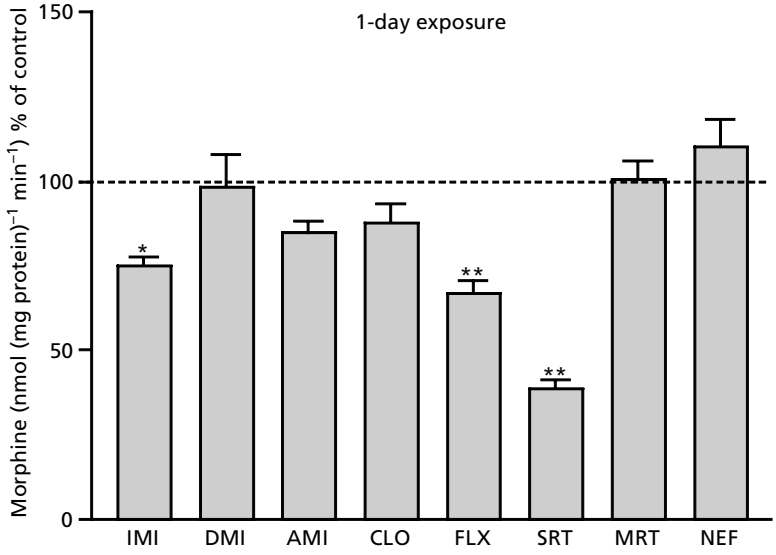


**Figure 2** The influence of one-day exposure to antidepressant drugs on the CYP2D activity measured as the rate of ethylmorphine *O*-deethylation in rat liver microsomes (model II). All values are means±s.e.m. from 7 or 8 rats (analysis of variance: tricyclic antidepressants – $P < 0.023$, F = 3.244; SSRIs+novel drugs – $P < 0.0001$, F = 25.57); $^{*}P < 0.05$, $^{**}P < 0.01$ (Dunnett's test), compared with control (0.103±0.011 nmol of morphine (mg protein)$^{-1}$ min$^{-1}$). IMI, imipramine; DMI, desipramine; AMI, amitriptyline; CLO, clomipramine; FLX, fluoxetine; SRT, sertraline; MRT, mirtazapine; NEF, nefazodone.

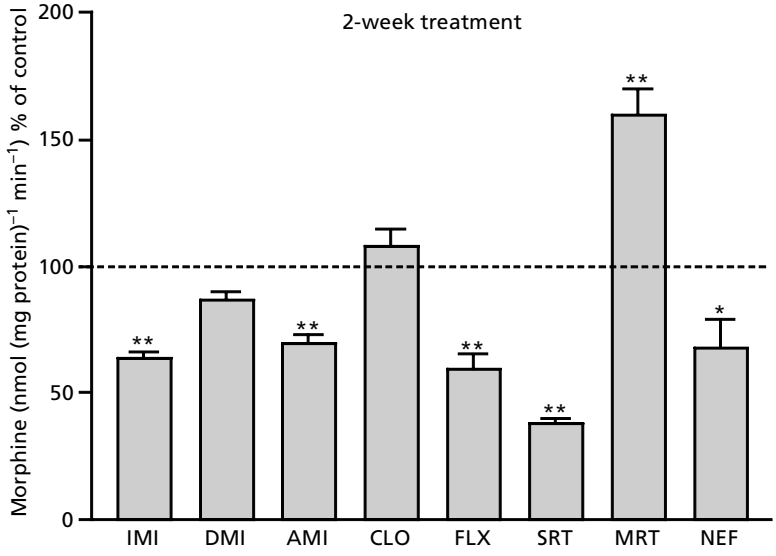


**Figure 3** The influence of two-week treatment with antidepressant drugs on the CYP2D activity measured as the rate of ethylmorphine *O*-deethylation in rat liver microsomes (model III). All values are means±s.e.m. from 7 or 8 rats (analysis of variance: tricyclic antidepressants – $P < 0.0001$, F = 17.72; SSRIs+novel drugs – $P < 0.0001$, F = 32.19); $^{*}P < 0.05$, $^{**}P < 0.01$ (Dunnett's test), compared with control (0.117±0.017 nmol of morphine (mg protein)$^{-1}$ min$^{-1}$). IMI, imipramine; DMI, desipramine; AMI, amitriptyline; CLO, clomipramine; FLX, fluoxetine; SRT, sertraline; MRT, mirtazapine; NEF, nefazodone.

clomipramine did not produce any significant effect when administered in-vivo.

## Discussion

The obtained results show complex interactions of the antidepressant drugs with CYP2D, leading, in most cases, to a decrease in its activity. The investigated antidepressants directly inhibit CYP2D activity in rats, shown as a competitive inhibition of the rate of the CYP2D specific reaction (i.e. *O*-deethylation of ethylmorphine) by the antidepressants added in-vitro to the control liver microsomes (model I). The tricyclic antidepressant clomipramine and the SSRIs fluoxetine and sertraline were the most potent inhibitors of the rat CYP2D among the studied drugs

($K_i$ = 14, 16 and 17 $\mu$M, respectively), which agreed with the results of Crewe et al (1992), who found these antidepressants to have a strong inhibitory effect on CYP2D6 activity in human liver microsomes, reflected as an inhibition of sparteine oxidation. However, the $K_i$ values obtained for these antidepressants in human samples were significantly lower ($K_i$ = 2.2, 0.6 and 0.7 $\mu$M, respectively). Other tricyclics tested (i.e. amitriptyline, imipramine and desipramine) exhibited a somewhat weaker inhibitory effect on the rat CYP2D ($K_i$ = 25, 26 and 44 $\mu$M, respectively) than clomipramine ($K_i$ = 14 $\mu$M), and their $K_i$ values were considerably higher than those obtained by Crewe et al (1992) in human liver microsomes ($K_i$ = 4 and 2.3 $\mu$M for amitriptyline and desipramine, respectively). Nefazodone, with a $K_i$ value of 55 $\mu$M, and mirtazapine, with its $K_i$ value over 100 $\mu$M, were weak inhibitors of the rat CYP2D, which agrees with the data obtained with human liver microsomes ($K_i$ = 18 and 41 $\mu$M, respectively), showing that the two antidepressants do not substantially inhibit CYP2D6 (Schmider et al 1996; Holm & Markham 1999).

The results obtained in our experiment (model I) indicate that the investigated antidepressants are less effective inhibitors of rat CYP2D than of human CYP2D6 in-vitro, though the enzymes share 97% similarity of amino-acid sequences. Those species differences in the efficacy of antidepressants as CYP2D inhibitors probably result from different structures of the catalytic sites of the rat and human counterpart enzymes. The above observation is consistent with the results of other authors concerning specific inhibitors of CYP2D isoforms, showing that quinine is a much stronger inhibitor of human CYP2D6 than of rat CYP2D, while its diastereoisomer quinine is a more potent inhibitor of rat CYP2D than of human CYP2D6 (Steiner et al 1988; Kobayashi et al 1989).

However, the investigated antidepressant drugs may also exert an inhibitory effect on CYP2D via other mechanisms, when they are administered to rats in-vivo. Thus, one-day exposure of rats to imipramine or the SSRIs decreased the activity of CYP2D in rat liver microsomes (model II). Chronic treatment with the investigated antidepressants produced more changes in the CYP2D activity than one-day exposure of rats to the drugs (model III). After prolonged administration of antidepressants, the decreased CYP2D activity after imipramine, fluoxetine and sertraline was still maintained. Moreover, amitriptyline and nefazodone significantly decreased, while mirtazapine increased, the activity of the enzyme (model III). The long half-lives of fluoxetine and its metabolite norfluoxetine allow for a more stable drug concentration between consecutive doses and may favour development of some adaptive changes in the regulation of CYP. However, the results concerning CYP2D after one-day exposure and two-week treatment with fluoxetine are similar and comparable with those obtained for the antidepressants with shorter half-lives.

The above data agree with the results of other authors who observed a decrease in the rat CYP2D activity after repeated administration of high doses of imipramine for a few days (which was not accompanied by a decrease in protein content), indicating binding of reactive metabolites of the antidepressant to the cytochrome P-450 apoprotein, causing inactivation of the enzyme (Kappus & Remmer 1975; Masubuchi et al 1995, 1996). Besides, tricyclic antidepressants and fluoxetine were shown to affect in this way CYP2A1, CYP2C11 and CYP3A2 in-vitro (Murray & Field 1992; Bensoussan et al 1995; McNeil & Murray 1996). This mechanism seems to apply to our in-vivo experiment with some of the tricyclic antidepressants and SSRIs, when their effect on CYP2D was observed after both one-day and two-week treatment of rats with antidepressants. However, in the case of nefazodone and mirtazapine, which affected CYP2D activity only when given chronically, an influence on regulatory mechanisms of the enzyme seems to be a more appropriate explanation.

It may seem surprising that CYP2D activity was increased by prolonged administration of mirtazapine. Inducers of CYP2D have not been found so far and mechanisms of regulation of the activity of the CYP2D subfamily isoenzymes are not well known. Recent studies indicate that CYP2D subfamily may be regulated by hepatic nuclear factor HNF-4, an orphan receptor, for which no physiologically relevant activator or ligand is yet known (Honkakoski & Negishi 2000). However, the state of pregnancy can induce CYP2D, which suggests certain regulatory influence of steroids. Bergh & Strobel (1996) and Baum & Strobel (1997) demonstrated regulation of expression of CYP2D mRNA in the rat brain with steroid hormones (an increase by testosterone and a decrease by progesterone). Therefore, a decrease in the level of cortisol by mirtazapine may have a connection with the observed increase in CYP2D activity in rats after chronic treatment with mirtazapine. Thus, final effects of antidepressants on CYP activity in-vivo may be a result of many different mechanisms (involving a parent compound or its metabolites), such as direct effect on enzyme protein, influence on the metabolism of endogenous ligands or acting as endogenous ligands of CYP regulatory receptors, time-dependent effect on CNS receptors and the level of hormones, interaction with neurotransmitter metabolism, etc. Therefore, finding mechanisms of the observed alterations in CYP2D activity in-vivo produced by administration of antidepressants requires further, more detailed, studies involving molecular processes influencing the enzyme function, such as regulation, synthesis and degradation.

The results of this study indicate that the investigated antidepressants may interfere with the metabolism of CYP2D substrates, both endobiotics (catecholamines) and xenobiotics such as medications used to treat psychiatric disorders, hypertension, cardiac arrhythmias and pain. Tricyclic antidepressants (Syrek et al 1997) and fluoxetine (Daniel et al 1999) administered at pharmacological doses (10 and 5 mg $kg^{-1}$ twice daily) for two weeks increased the concentrations of phenothiazine neuroleptics, the elimination of which is CYP2D dependent.

In summary, the obtained results indicate a complex (direct and indirect), drug- and time-dependent interaction between the investigated antidepressants and the rat cytochrome P-450 subfamily CYP2D. Three different mechanisms of the antidepressant–CYP2D interaction are postulated: firstly, competitive inhibition of CYP2D shown in-vitro, the inhibitory effects of tricyclic antidepressants and

SSRIs being stronger than those of nefazodone or mirtazapine, but weaker than the effects of the respective drugs on human CYP2D6; secondly, in-vivo inhibition of CYP2D produced by both one-day and chronic treatment with tricyclic antidepressants (except for desipramine and clomipramine) and SSRIs, which suggests inactivation of the enzyme apoprotein by reactive metabolites; and thirdly, in-vivo inhibition by nefazodone and induction by mirtazapine of CYP2D produced only by chronic treatment with the drugs, which suggests their influence on the enzyme regulation.

In conclusion, our study conducted on rats shows that, apart from a direct effect on CYP2D activity (via binding with enzyme protein), the investigated antidepressants can exert also indirect effects produced by longer exposure to the drugs, which are drug and time dependent. Therefore, further clinical studies in this direction are advisable to find out whether similar indirect effects of antidepressants on CYP2D6 occur in man during long-term therapy. It seems very interesting to find out whether the changes in CYP2D activity observed in the liver take place also in the brain, which might have some impact on the catecholamine level and psychotropic profiles of these drugs. Moreover, the obtained results also show that animal liver samples do not reflect well the potency of direct interactions of drugs with human CYP2D6 and that in-vitro studies are not sufficient for estimation of an influence of a drug on cytochrome P-450 in-vivo and for exploration of many possibilities of pharmacokinetic interactions. In particular, drugs affecting the CNS and hormone levels (which in turn affect enzyme regulation) should be tested in-vivo.

## References


Barbhaiya, R. H., Buch, A. B., Greene, D. S. (1996) Single and multiple dose pharmacokinetics of nefazodone in subjects classified as extensive and poor metabolizers of dextromethorphan. *Br. J. Pharmacol.* **42**: 573–581

Baum, L. O., Strobel, H. W. (1997) Regulation of expression of cytochrome P-450 2D mRNA in rat brain with steroid hormones. *Brain Res.* **765**: 67–73

Bensoussan, C., Delaforge, M., Mansuy, D. (1995) Particular ability of cytochromes P450 3A to form inhibitory P450-iron-metabolite complexes upon metabolic oxidation of aminodrugs. *Biochem. Pharmacol.* **49**: 591–602

Bergh, A. F., Strobel, H. W. (1996) Anatomical distribution of NADPH-cytochrome P450 reductase and cytochrome P4502D forms in rat brain: effects of xenobiotics and sex steroids. *Mol. Cell. Biochem.* **162**: 31–41

Boobis, A. R., Seddon, C. E., Davies, D. S. (1986) Bufuralol 1′-hydroxylase activity of the rat. *Biochem. Pharmacol.* **35**: 2961–2965

Boobis, A. R., Sesardic, D., Murray, B. P., Edwards, R. J., Singleton, A. M., Rich, K. J., Murray, S., de la Torres, R., Seguras, J., Pelkonen, O., Pasanen, M., Kobayashi, S., Zhi-Guang, T., Davies, D. S. (1990) Species variation in the response of the cytochrome P-450-dependent monooxygenase system to inducers and inhibitors. *Xenobiotica* **20**: 1139–1161

Brøsen, K., Zeugin, T., Meyer, U. A. (1991) Role of P4502D6, the target of the sparteine-debrisoquine oxidation polymorphism, in the metabolism of imipramine. *Clin. Pharmacol. Ther.* **49**: 609–617

Chow, T., Hiroi, T., Imaoka, S., Chiba, K., Funae, Y. (1999a) Isoform-selective metabolism of mianserin by cytochrome P-450 2D. *Drug Metab. Dispos.* **27**: 1200–1204

Chow, T., Imaoka, S., Hiroi, T., Funae, Y. (1999b) Developmental changes in the catalytic activity and expression of CYP2D isoforms in the rat liver. *Drug Metab. Dispos.* **27**: 188–192

Coutts, R. T., Su, P., Baker, G. B. (1994) Involvement of CYP2D6, CYP3A4, and other cytochromes P450 isoenzymes in N-dealkylation reactions. *J. Pharmacol. Toxicol. Methods* **31**: 177–186

Crewe, H. K., Lennard, M. S., Tucker, G. T., Woods, F. R., Haddock, R. E. (1992) The effect of selective re-uptake inhibitors on cytochrome P4502D6 (CYP2D6) activity in human liver microsomes. *Br. J. Clin. Pharmacol.* **34**: 262–265

Daniel, W., Netter, K. J. (1992) Alteration of cytochrome P-450 by prolonged administration of imipramine and/or lithium to rats. *Naunyn Schmiedebergs Arch. Pharmacol.* **342**: 234–240

Daniel, W. A., Syrek, M., Haduch, A., Wójcikowski, J. (1999) The influence of selective serotonin reuptake inhibitors (SSRIs) on the pharmacokinetics of thioridazine and its metabolites: in vivo and in vitro studies. *Exp. Toxicol. Pathol.* **51**: 309–314

Fonne-Pfister, R., Bargetzi, M. J., Meyer, U. A. (1987) MPTP, the neurotoxin inducing Parkinson's disease, is a potent competitive inhibitor of human and rat cytochrome P450 isoenzymes (P450buf1, P450db1) catalyzing debrisoquine 4-hydroxylation. *Biochem. Biophys. Res. Commun.* **148**: 1144–1150

Glare, P. A., Walsh, T. D., Pippenger, C. E. (1991) A simple, rapid method for the simultaneous determination of morphine and its principal metabolites in plasma using high-performance liquid chromatography and fluorometric detection. *Ther. Drug Monit.* **13**: 226–232

Gonzalez, F. J., Skoda, R. C., Kimura, S., Umeno, M., Zanger, U. M., Nebert, D. W., Gelboin, H. V., Hardwick, J. P., Meyer, U. A. (1988) Characterization of the common genetic effect in humans deficient in debrisoquine metabolism. *Nature* **331**: 442–446

Hiroi, T., Imaoka, S., Funae, Y. (1998) Dopamine formation from tyramine by CYP2D6. *Biochem. Biophys. Res. Commun.* **249**: 838–843

Holm, K. J., Markham, A. (1999) Mirtazapine. A review of its use in major depression. *Drugs* **57**: 607–631

Honkakoski, P., Negishi, M. (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem. J.* **347**: 321–337

Jeppesen, U., Gram, L. F., Vistisen, K., Loft, S., Poulsen, H. E., Brøsen, K. (1996) Dose-dependent inhibition of CYP1A2, CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine. *Eur. J. Clin. Pharmacol.* **51**: 73–78

Kappus, H., Remmer, H. (1975) Irreversible protein binding of [14C]imipramine with rat and human liver microsomes. *Biochem. Pharmacol.* **24**: 1079–1084

Kimura, S., Umeno, M., Skoda, R. C., Meyer, U. A., Gonzalez, F. J. (1989) The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. *Am. J. Hum. Genet.* **45**: 889–904

Kobayashi, S., Murray, S., Watson, D., Sesardic, D., Davies, D. S., Boobis, A. R. (1989) The specificity of inhibition of debrisoquine 4-hydroxylase activity by quinidine and quinine in the rat is the inverse of that in man. *Biochem. Pharmacol.* **38**: 2795–2799

Kobayashi, K., Ishizuka, T., Shimada, N., Yoshimura, Y., Kamijma, K., Chiba, K. (1999) Sertraline N-demethylation is catalyzed by multiple isoforms of human cytochrome P-450 in vitro. *Drug Metab. Dispos.* **27**: 763–766

Kupfenberg, H., Burkhalter, A., Way, E. L. (1964) A sensitive fluorometric assay for morphine in plasma and brain. *J. Pharmacol. Exp. Ther.* **145**: 247–251

Margolis, J. M., O'Donnell, J. P., Mankowski, D. C., Ekins, S., Obach, R. S. (2000) (R)-, (S)-, and racemic fluoxetine N-demethyl-

ation by human cytochrome P450 enzymes. *Drug Metab. Dispos.* **28**: 1187–1191

Martinez, C., Agundez, J. A. G., Gervasini, G., Martin, R., Benitez, J. (1997) Tryptamine: a possible endogenous substrate for CYP2D6. *Pharmacogenetics* **7**: 85–93

Masubuchi, Y., Takahashii, C., Fujio, N., Horie, T., Suzuki, T., Imaoka, S., Funae, Y., Narimatsu, S. (1995) Inhibition and induction of cytochrome P450 isoenzymes after repetitive administration of imipramine in rats. *Drug Metab. Dispos.* **23**: 999–1003

Masubuchi, Y., Igarashi, S., Suzuki, T., Horie, T., Narimatsu, S. (1996) Imipramine-induced inactivation of a cytochrome P450 2D enzyme in rat liver microsomes: in relation to covalent binding of its reactive intermediate. *J. Pharmacol. Exp. Ther.* **279**: 724–731

Matsunaga, E., Zanger, U. M., Hardwick, J. P., Gelboin, H. V., Meyer, U. A., Gonzales, F. J. (1989) The CYP2D gene subfamily: analysis of the molecular basis of the debrisoquine 4-hydroxylase deficiency in DA rats. *Biochemistry* **28**: 7349–7355

Matsunaga, E., Umeno, M., Gonzalez, F. J. (1990) The rat debrisoquine 4-hydroxylase CYP2D gene subfamily: complete sequences of four closely linked genes and evidence that gene conversions maintained sequence homogeneity at the heme binding region of the cytochrome P450 active site. *J. Mol. Evol.* **30**: 155–169

McNeil, C. M., Murray, M. (1996) Inhibition of microsomal cytochromes P450 in rat liver by the tricycle antidepressant drug desipramine and its primary oxidized metabolites. *Biochem. Pharmacol.* **51**: 15–20

Miksys, S., Rao, Y., Sellers, E. M., Kwan, M., Mendis, D., Tyndale, R. F. (2000) Regional and cellular distribution of CYP2D subfamily members in rat brain. *Xenobiotica* **30**: 547–564

Murray, M., Field, S. L. (1992) Inhibition and metabolite complexation of rat hepatic microsomal cytochrome P450 by tricyclic antidepressants. *Biochem. Pharmacol.* **43**: 2065–2071

Ohta, S., Tachikava, Y., Makino, Y., Tasaki, Y., Hirobe, M. (1990) Metabolism and brain accumulation of tetrahydroisoquinoline (TIQ), a possible parkinsonian inducing substance, in an animal model of a poor debrisoquine metabolizer. *Life Sci.* **46**: 599–605

Ring, B. J., Eckstein, J. A., Gillespie, J. S., Binkley, S. N., Vandenbranden, M., Wrighton, S. A. (2001) Identification of the human cytochromes P450 responsible for in vitro formation of R- and S-norfluoxetine. *J. Pharmacol. Exp. Ther.* **297**: 1044–1050

Schmider, J., Greenblatt, D. J., von Moltke, L. L., Harmatz, J. S., Shader, R. I. (1996) Inhibition of cytochrome P450 by nefazodone in vitro: studies of dextromethorphan O- and N-demethylation. *Br. J. Clin. Pharmacol.* **41**: 339–343

Schulz-Utermoehl, T., Bennett, A. J., Ellis, S. W., Tucker, G. T., Boobis, A. R., Edwards, R. J. (1999) Polymorphic debrisoquine 4-hydroxylase activity in the rat is due to differences in CYP2D2 expression. *Pharmacogenetics* **9**: 357–366

Siegle, I., Fritz, R., Eckhardt, K., Zanger, U. M., Eichelbaum, M. (2001) Cellular localization and regional distribution of CYP2D6 mRNA and protein expression in human brain. *Pharmacogenetics* **11**: 237–245

Steiner, E., Dumond, E., Spina, E., Dahlqvist, R. (1988) Inhibition of desipramine 2-hydroxylation by quinidine and quinine. *Clin. Pharmacol. Ther.* **43**: 577–581

Störmer, E., von Moltke, L. L., Schader, R. I., Greenblatt, D. J. (2000) Metabolism of the antidepressant mirtazapine in vitro: contribution of cytochromes P-450 1A2, 2D6, and 3A4. *Drug Metab. Dispos.* **28**: 1168–1175

Syrek, M., Wójcikowski, J., Daniel, W. A. (1997) Promazine pharmacokinetics during concurrent treatment with tricyclic antidepressants. *Pol. J. Pharmacol.* **51**: 453–462

Tyndale, R. F., Li, Y., Li, N.-Y., Messina, E., Miksys, S., Sellers, E. M. (1999) Characterization of cytochrome P-450 2D1 activity in rat brain: high-affinity kinetics for dextromethorphan. *Drug Metab. Dispos.* **27**: 924–930

Wan, J., Imaoka, S., Chow, T., Hiroi, T., Yabusaki, Y., Funae, Y. (1997) Expression of four rat CYP2D isoforms in *Saccharomyces cerevisiae* and their catalytic specificity. *Arch. Biochem. Biophys.* **348**: 383–390

Yamamoto, Y., Tasaki, T., Nakamura, A., Iawata, H., Kazusaka, A., Gonzalez, F. J., Fujita, S. (1998) Molecular basis of the Dark Agouti rat drug oxidation polymorphism: importance of CYP2D1 and CYP2D2. *Pharmacogenetics* **8**: 73–82