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Effect of selected antidepressant drugs on cytochrome P450 2B (CYP2B) in rat liver. An *in vitro* and *in vivo* study

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Abstract:
The aim of the present study was to investigate the influence of antidepressants with different chemical structures and mechanisms of action affecting serotonergic and/or noradrenergic systems – tricyclic antidepressant drugs (TAD), selective serotonin reuptake inhibitors (SSRIs) and novel antidepressants (mirtazapine, nefazodone) – on the activity of rat CYP2B measured as the rate of 16β-hydroxylation of testosterone. The reaction was studied in control liver microsomes in the presence of antidepressants, as well as in microsomes of rats treated intraperitoneally for one day or two weeks (twice a day) with pharmacological doses (mg/kg) of the drugs (imipramine, amitriptyline, clomipramine, nefazodone 10; desipramine, fluoxetine, sertraline 5; mirtazapine 3). The obtained K_i values indicated that nefazodone and the SSRIs sertraline and fluoxetine were the most potent inhibitors of the studied reaction (K_i = 10–20 μM). The inhibitory effects of TADs were modest (K_i = 62–85 μM), while mirtazapine was a very weak inhibitor of CYP2B activity (K_i = 286 μM). After a one-day exposure of rats to the investigated antidepressants, a significant increase in CYP2B activity was only observed after sertraline exposure (300% of the control). Chronic treatment with the antidepressants led to a significant enhancement of CYP2B activity after sertraline, fluoxetine and desipramine (580, 200 and 150% of the control, respectively) treatment, which positively correlated with the observed elevation in CYP2B protein levels. In summary, two different mechanisms of the antidepressant-CYP2B interaction are postulated: 1) a direct inhibition of CYP2B shown *in vitro* by nefazodone, SSRIs and TADs; 2) *in vivo* induction of CYP2B produced by prolonged administration of SSRIs and desipramine, which suggests their influence on enzyme regulation. The marked CYP2B-induction produced by SSRIs corresponds with their selective serotonin reuptake inhibition, while the effect of desipramine corresponds with its selective inhibition of noradrenaline reuptake.

Key words:
antidepressants, CYP2B, rat liver microsomes, *in vitro* study, one-day treatment, chronic treatment, enzyme inhibition, enzyme induction

Introduction

Cytochrome P450 2B (CYP2B) belongs to the important CYP subfamilies, which are mainly involved in the metabolism of xenobiotics. The *CYP2B* gene subfamily in the rat consists of eight genes, of which three *CYP2B1, CYP2B2,* and *CYP2B3,* produce detectible transcripts in the liver. The isoforms CYP2B1 and CYP2B2 share 97% amino acid sequence homology, and CYP2B1 has about 75% identity with the human CYP2B6. The constitutive level of CYP2B expression in the liver is low and does not exceed 5% of the total CYP content. The presence of CYP2B activ-

ity was shown not only in the liver, but also in the ileum, adrenal glands, kidneys, lungs and brain [2, 16, 20, 57]. Significant regional differences exist in the expression of CYP2B1 and CYP2B2 isoforms in rat brain, which may be attributed to the differences in the cell type expressing these CYP isoforms [21, 39, 51, 59]. Accordingly, recent studies revealed that neuronal cells exhibited two-fold higher activity of CYP2B than glial cells [34]. The activities of the constitutive and induced form of CYP2B1 have recently been demonstrated in rat brain *in vivo* [42].

The catalytic competence of rat isoforms CYP2B1 and CYP2B2 is similar, and in many cases overlaps that of the human isoform CYP2B6. However, each of the CYP2B isoforms exhibits different substrate/reaction preferences [24]. The N-demethylation of benzphetamine and 16β-hydroxylation of testosterone are CYP2B1-specific reactions, though the reactions are also catalyzed (but at a lower rate) by CYP2B2. The rat CYP2B1/2 enzymes and human CYP2B6 demonstrate partially similar substrate specificity as indicated by their ability to hydroxylate testosterone and lidocaine [3, 22, 27, 33, 38, 49]. Moreover, CYP2B6 was shown to metabolize many drugs, such as S-mephenytoin (N-demethylation), benzodiazepines, bupropion, mianserin, and tamoxifen, and to activate cyclophosphamide and ifosfamide [17, 29, 44, 48]. Both rat and human CYP2B isoforms are of high toxicological significance, since they activate natural and synthetic procarcinogens, such as aflatoxin B_1, 6-aminochrysene, benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, and dibenz[a,h]anthracene [44]. The subfamily of CYP2B isoforms is inhibited by metyrapone (CYP2B1/2) and orphenadrine (CYP2B1/2/6) [44, 48].

CYP2B genes are regulated physiologically by thyroid hormones, glucocorticoids and growth hormone [31, 45], which all remain under the control of the central nervous system [18, 65, 64]. At a molecular level, the nuclear receptors – constitutive androstane receptor (CAR), pregnane X receptor (PXR), and glucocorticoid receptor (GR) participate in their regulation [31, 32, 35, 36]. The CAR regulation of *CYP2B* genes is species-specific [61]. CYP2B isoforms (except CYP2B3) are induced by phenobarbital, and CYP2B6 is also induced by the classical CYP3A inducers dexamethasone and rifampicin [20, 28, 46, 47, 48, 55]. CYP2B1 is highly inducible, while CYP2B2 is only moderately inducible by phenobarbital in the liver. Phenobarbital also induces the expression of extrahepatic CYP2B isoforms. Lee et al. [37] observed phenobarbital CYP2B6 induction in monkey brain. Recently Kapoor et al. [34] observed that cultured rat brain glial cells exhibited greater magnitude of phenobarbital induction than neuronal cells; moreover, a greater magnitude of induction of CYP2B2 than CYP2B1 was seen in the brain cells. On the other hand, ethanol induces CYP2B1/2 in rat liver but not in rat brain [52]. Interestingly, CYP2B1 is inducible by nicotine in the brain, but not in the liver [39], which may have some relevance to Parkinson's disease [42].

Antidepressant drugs that increase noradrenergic and serotonergic transmission in the brain may affect the secretion of anterior pituitary hormones by regulating the hypothalamic secretion of the respective releasing and inhibiting factors [53], which, in turn, may stimulate the expression of some CYP isoforms. Accordingly, we have shown that some antidepressants stimulate the expression of rat proteins CYP2C and CYP3A [8, 10, 27]. Since CYP2B isoenzymes are of considerable pharmacological and toxicological importance, the aim of the present study was to investigate the effect of antidepressants with different chemical structures and mechanisms of action affecting serotonergic and/or noradrenergic systems (tricyclic antidepressant drugs – TAD, selective serotonin reuptake inhibitors – SSRIs, and the novel antidepressants nefazodone and mirtazapine) on the activity and protein levels of CYP2B1/2 in liver microsomes (*ex vivo* study). We also examined the direct interactions of antidepressants with rat CYP2B *in vitro* (binding with cytochrome protein). The obtained results indicate drug- and time-dependent changes in the activity of rat CYP2B1/2 produced by antidepressants.

Materials and Methods

Drugs and chemicals

Imipramine hydrochloride was provided by Polfa (Jelenia Góra, Poland), amitriptyline by H. Lundbeck A/S (Copenhagen, Denmark), while clomipramine was from RBI (Natick, MA, USA) 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 (The Netherlands) and nefazodone by Bristol-Myers Squibb International, Ltd. (Uxbridge, UK).

Testosterone and its metabolite, 16β-hydroxytestosterone, were from Steraloids (Newport, USA). NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase, as well as anti-goat IgG (secondary antibody) and nitrocellulose membranes, were purchased from Sigma (St. Louis, USA). All organic solvents were supplied by Merck (Darmstadt, Germany). A polyclonal antibody, anti rat CYP2B1 goat serum and rat Supersomes CYP2B1 were obtained from Gentest Corp. (Woburn, USA). A LumiGLO chemiluminescent substrate was provided by KPL (Gaithersburg, USA).

Animal procedures

All the experiments on animals were performed in accordance with the Polish governmental regulations (Animals Protection Act, DZ.U. 97.111.724, 1997). The experiments were carried out on male Wistar rats (230–260 g) kept under standard laboratory conditions. The investigated antidepressant drugs were administered intraperitoneally (*ip*), twice a day for one day or two weeks at the following pharmacological doses (mg/kg): imipramine, amitriptyline, clomipramine, and nefazodone: 10, desipramine, fluoxetine and sertraline: 5, mirtazapine: 3. The control animals were injected with saline. The rats were sacrificed at 15 h (one-day treatment) or 24 h (two-week treatment) after 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*.

In vitro studies into CYP2B activity – measurement of the rate of 16β-hydroxylation of testosterone in liver microsomes

The activity of CYP2B was studied by measurement of the rate of the CYP2B-specific reaction, testosterone 16β-hydroxylation in the liver microsomes, as described previously [26]. To distinguish between a direct effect of antidepressants on the activity of CYP2B and the changes produced by their one-day or two-week administration, three experimental models were used. *Model I:* The experiment was conducted on pooled liver microsomes from three control rats. The rate of testosterone 16β-hydroxylation (testosterone concentration between 50–200 μM) was assessed in the absence and presence of one of the antidepressants added *in vitro* (antidepressants concentration between 50–200 μM). Each sample was prepared in duplicate. *Model II:* The experiment was carried out on liver microsomes from rats exposed to an antidepressant for 24 h. Testosterone was added to the incubation mixture *in vitro* at a concentration of 200 μM. The 16β-hydroxylation of testosterone was studied in the absence of antidepressants. *Model III:* Liver microsomes from rats subjected to two-week antidepressant treatment were investigated. Testosterone was added to the incubation mixture *in vitro* at a concentration of 200 μM. 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 (50 mM, pH = 7.4), $MgCl_2$ (3.0 mM), EDTA (1 mM), NADP (1.0 mM), glucose 6-phosphate (5 mM), and glucose-6-phosphate-dehydrogenase (1.7 U in 1 ml). The final incubation volume was 1 ml. After a 15 min incubation, the reaction was stopped by adding 200 μl of methanol, and then by cooling it down in ice.

Determination of the concentration of testosterone and its metabolite 16β-hydroxytestosterone, in liver microsomes

Testosterone and 16β-hydroxytestosterone were extracted from the microsomal suspension with dichloromethane, and their concentrations were assessed by the high performance liquid chromatography (HPLC) method based on Sonderfan et al. [54]. The residue obtained after evaporation of the extracts was dissolved in 100 μl of 50% methanol. An aliquot (20 μl) was injected into the HPLC system (LaChrom, Merck-Hitachi), equipped with a UV detector, L-7100 pump, and D-7000 System Manager. The analytical column (Supelcosil^{TM} LC-18, 5 μM, 4.6 × 150 mm) was purchased from Supelco (Bellefonte PA, USA). The mobile phase was applied as a gradient from solvent A (100% methanol:water:acetonitrile, 39:60:1 v/v/v) to solvent B (70% methanol:water:acetonitrile, 80:18:2 v/v/v) over 22 min at a flow rate of 1.5 ml/min. The column temperature was 40°C. The absorbance was measured at a wavelength of 254 nm. The compounds were eluted in the following order: 16β-hydroxytestosterone, 9.6 min and testosterone, 15.6 min. The sensitivity of the method allowed for quantification of 16β-hydroxytestosterone down to 0.005 nmol in one sample. The accuracy of the method amounted to 4.7% (16β-hydroxytestosterone). The inter- and intra-assay coefficients of variance were below 6%.

Western blot analyses

The level of CYP2B in liver microsomes of rats treated chronically with antidepressants (*Model III*) was estimated by Western blot analysis. SDS-PAGE and immunoblot assays were performed using a methodology provided by Gentest, USA. Briefly, 10 μg of microsomal protein was separated on a 0.75 mm-thick sodium dodecyl sulfate-polyacrylamide 4% (w/v) stacking gel and a 12% (w/v) resolving gel employing a MINIPROTEAN II electrophoresis system (Bio-Rad, Hemmel Hempstead, UK; 130 V, 65 min). Protein was electroblotted onto a nitrocellulose membrane (100 V, 100 min) and blocked overnight with 5% dried nonfat milk in PBS (phosphate-buffered saline, pH = 7). After incubation with primary antibody (polyclonal goat anti-rat antibody raised against CYP2B1, which recognized also the CYP2B2 form), the blots were incubated with secondary antibody, i.e., the appropriate species-specific horseradish peroxidase-conjugated anti-IgG. Rat CYP2B1 Supersomes (cDNA-expressed rat isoenzyme) were used as a standard. Immunoreactivity was assessed using an enhanced LumiGLO chemiluminescent substrate. The intensities of the bands corresponding to the enzyme protein on the nitrocellulose membrane were measured with the Luminescent Image analyzer LAS-1000 using the Image Reader LAS-1000 and the Image Gauge 3.11 program (Fuji Film, Japan).

Statistical analysis

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 the means ± SEM from 5–8 animals.

Results

The obtained results show that some of the investigated antidepressant drugs exert a direct inhibitory ef-

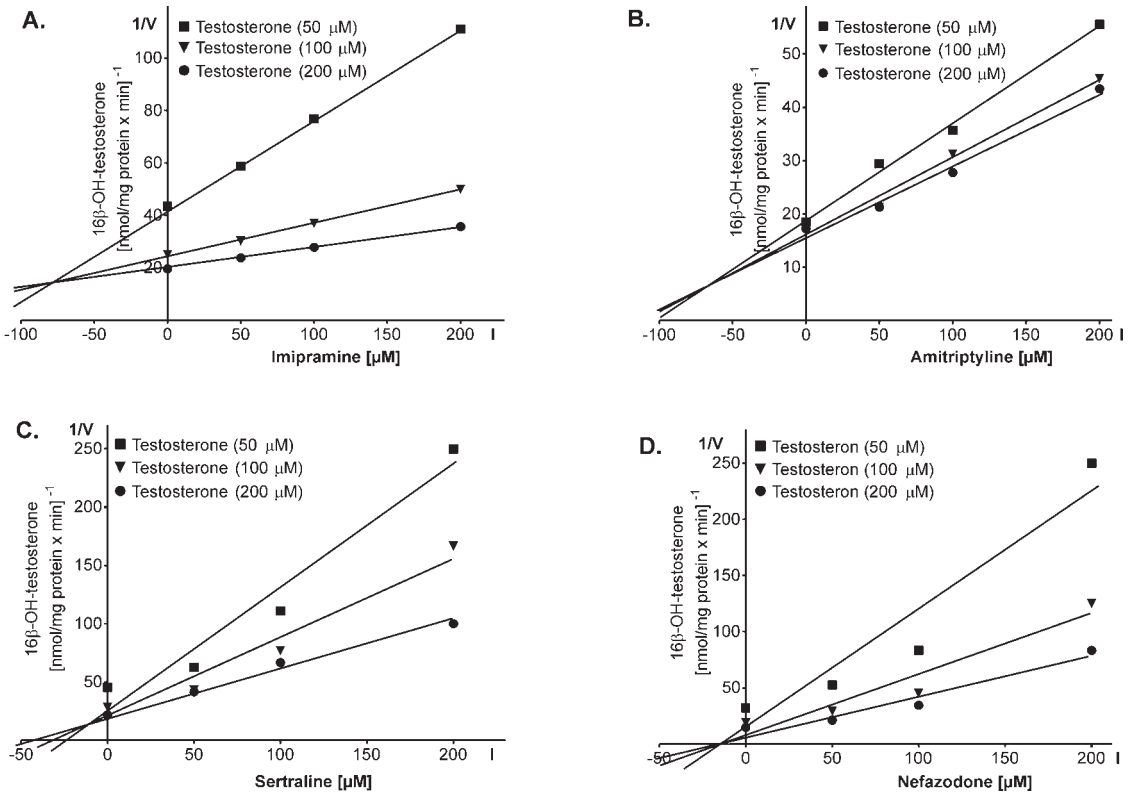

Fig. 1. Kinetics of the inhibition of 16β-hydroxylation of testosterone by imipramine **(A)**, amitriptyline **(B)**, sertraline **(C)** and nefazodone **(D)** (Dixon plots). V – velocity of the reaction (nmol of 16β-OH-testosterone/mg of protein/min); I – inhibitor concentration (μM). K_i values are presented in Table 1

Tab. 1. The influence of antidepressants added to rat liver microsomes *in vitro* on CYP2B activity measured as the rate of testosterone 16β-hydroxylation (*Model I*). The presented inhibition constants (K_i) were calculated using Dixon analysis (Fig. 1A–1D)

Antidepressants (inhibitors)		Inhibition of CYP2B activity K_i (μM)
I.	Tricyclic antidepressants (TADs)	
	Imipramine	78
	Desipramine	85
	Clomipramine	62
	Amitriptyline	70
II.	Selective serotonin reuptake inhibitors (SSRIs)	
	Fluoxetine	20
	Sertraline	10
III.	Novel antidepressants	
	Mirtazapine	286
	Nefazodone	13

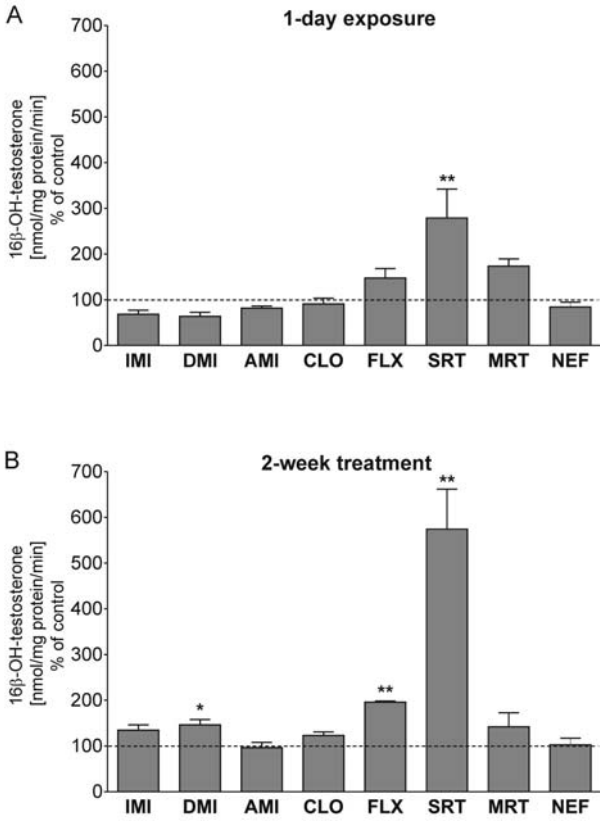

Fig. 2. The activity of CYP2B after one-day **(A)** and two-week treatment **(B)** with antidepressants (*Model II* and *III*, respectively). The activity was measured as the rate of testosterone 16β-hydroxylation in rat liver microsomes. All values are the means ± SEM of 7–8 animals; * $p < 0.05$, ** $p < 0.01$ (Dunnett's test) compared to the control (A: 0.022 ± 0.003 nmol of 16β-OH-testosterone/mg of protein/min; B: 0.026 ± 0.003 nmol of 16β-OH-testosterone/mg of protein/min). IMI – imipramine, DMI – desipramine, AMI – amitriptyline, CLO – clomipramine, FLX – fluoxetine, SRT – sertraline, MRT – mirtazapine, NEF – nefazodone

fect on CYP2B *in vitro*. The antidepressants, added to liver microsomes of control rats, directly inhibited the activity of CYP2B, specifically, testosterone 16β-hydroxylation (Tab. 1). Figures 1A–D show examples of the Dixon plots obtained in our studies, which served as the basis for calculation of the K_i constants. The obtained K_i values indicated that nefazodone and the SSRIs sertraline and fluoxetine were the most potent inhibitors of the studied reaction (K_i = 10, 13, and 20 μM, respectively), while mirtazapine was the weakest inhibitor in this respect (K_i = 286 μM) (Tab. 1). The potency of the antidepressants to inhibit the CYP2B-specific reaction was as follows (according to the K_i values): sertraline ≈ nefazodone > fluoxetine > clomipramine ≈ amitriptyline ≈ imipramine ≈ desipramine > mirtazapine.

After a one-day (i.e., 24 h) exposure to sertraline, increased CYP2B activity was observed (up to 300% of the control) (Fig. 2A). Other studied antidepressants did not produce any significant changes in the rate of the CYP2B-specific reaction.

The two week-treatment with the investigated antidepressants caused more changes in the rate of testosterone 16β-hydroxylation than one-day treatment (Fig. 2B). The increased CYP2B activity observed after chronic treatment with sertraline (580% of the control) was almost two times higher than after one-day exposure to this antidepressant. Moreover, desipramine and fluoxetine produced a significant increase in CYP2B activity (to 150% and 200% of the control, respectively), which was not observed after 24 h exposure to desipramine, and in the case of fluoxetine, only a slight increase was observed. Other studied antidepressants did not produce any significant effects on CYP2B activity when administered *in vivo* for two weeks, though a tendency of enhancement of enzyme activity was seen with imipramine and mirtazapine (Fig. 2B).

As shown in Figure 3, the antidepressants chosen for further molecular studies increased the CYP2B protein level in the liver microsomes of rats treated chronically with antidepressants: fluoxetine to 165.7 ± 17.26% and sertraline to 137.7 ± 11.02% of the control (the mean ± SEM; n = 5).

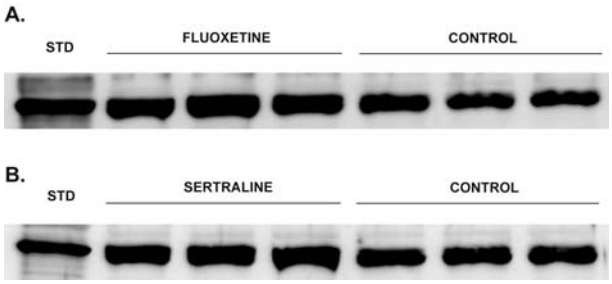

Fig. 3. The effect of two-week treatment (*Model III*) with sertraline **(A)** and fluoxetine **(B)** on the level of CYP2B protein in rat liver microsomes (n = 5). 10 μg of microsomal protein was subjected to Western analysis, and the immunoblot was probed with polyclonal goat anti-rat antibody raised against CYP2B. Supersomes CYP2B1 were used as a standard. Results are typical of three separate animals per treatment

Discussion

The obtained results showed two mechanisms of interactions of the antidepressant drugs with the CYP2B subfamily, leading to opposite effects on CYP2B activity. Thus most of the investigated drugs directly inhibited CYP2B activity in rats, which was demonstrated by an inhibition of the rate of the CYP2B specific reaction, testosterone 16β-hydroxylation by the antidepressants added to the control liver microsomes *in vitro* (*Model I*). On the other hand, prolonged administration of some antidepressants to rats *in vivo* increased the activity of the enzyme (*Model III mainly*), and the effects correlated positively with the observed enhancement of CYP2B protein level. Of the tested drugs only sertraline, fluoxetine (selective serotonin reuptake inhibitors) and desipramine (selective noradrenaline reuptake inhibitor) induced CYP2B. TADs, which, apart from the inhibition of noradrenaline and serotonin reuptake, also display either α_1-adrenergic receptor (imipramine, amitriptyline, clomipramine) or 5-HT_2-serotonergic receptor (amitriptyline) antagonistic activities [50], did not exert such an effect. Also, newer antidepressants, such as nefazodone (a 5-HT_2-receptor antagonist and a moderate inhibitor of serotonin and noradrenaline reuptake) and mirtazapine (an α_2-adrenergic receptor antagonist at presynaptic sites of noradrenergic and serotonergic neurons and 5-HT_2- and 5-HT_3-serotonergic receptor antagonist), were not active in this respect [30, 50]. This may imply that the observed effects of the tested antidepressants on CYP2B regulation *in vivo* depends on the different action of the drugs on the neuroendocrine regulation of the enzyme.

Analyzing the extent of changes in CYP2B activity and protein levels, it may seem surprising that the SSRI-produced increase in enzyme activity was much more pronounced than the elevation in protein levels; moreover, sertraline, which was more effective than fluoxetine in enhancing CYP2B activity (580 and 200% of the control, respectively), increased the enzyme protein levels to a less than fluoxetine (137 and 165% of the control, repectively). In order to explain these discrepancies, one has to take into account that liver CYP2B1 is an isoform with very low basal expression that is more inducible and more efficiently hydroxylates testosterone in the 16β-position, compared to CYP2B2 [24, 33]. In addition, the antibody used recognized both isoforms, CYP2B1 and CYP2B2, which migrated close to each other and were determined jointly. It is therefore conceivable that the CYP2B1 induction produced by SSRIs was masked in the presence of CYP2B2 (an isoform with a relatively high basal expression), and the CYP2B1/CYP2B2 induction ratio was higher for sertraline than fluoxetine.

Considering the two mechanisms of the antidepressant-CYP2B interaction it seems possible that the observed direct inhibitory effects on CYP2B may in some cases attenuate the indirect enzyme-inducing properties of antidepressants exhibiting a dual action on CYP2B activity, since *in vivo* direct and indirect effects of drugs overlap. However, the direct inhibitory effects of the investigated drugs were very weak (mirtazapine, K_i = 286 μM), modest (TADs, K_i = 62–85 μM), or moderate (nefazodone, sertraline and fluoxetine, K_i = 10–20 μM). Hence, mirtazapine should practically be inactive *in vivo* toward rat CYP2B, due to its pharmacological/therapeutic dosage and concentrations in the blood plasma and tissues, which are lower compared to its respective K_i value [1, 43, 56]. Furthermore, the direct inhibitory effect of tricyclic antidepressants observed *in vitro* should be of minor importance *in vivo*, since these drugs rarely reach liver concentrations that approximate their relatively high K_i values, as gets out of their plasma concentrations [9, 12, 23] and plasma/tissue distribution patterns [6, 7, 13, 14, 62, 63].

On the other hand, the K_i value observed for nefazodone (K_i = 13 μM) may be of pharmacological/toxicological importance, since the antidepressant may reach *in vivo* plasma concentrations close to its

K_i value [15]. Also, the K_i values obtained for the tested SSRIs sertraline and fluoxetine (K_i = 10 and 20 μM, respectively) reflect the presumed concentration range of the antidepressants in a lipophilic phase of the liver endoplasmic reticulum after chronic treatment *in vivo*, according to results from their plasma concentrations [4, 23, 60] and distribution characteristics [5, 7, 13, 14, 19, 58, 62, 63]. It seems, therefore, that the direct inhibitory effects of the investigated SSRIs on CYP2B activity may attenuate the enzyme induction produced by these drugs *in vivo*. However, the high inducing potency of sertraline should predominate *in vivo*. Moreover, as soon as the antidepressant concentrations fall below their respective K_i values in the liver after cessation of treatment, the enzyme induction produced by sertraline or fluoxetine will still continue not being attenuated by the direct inhibitory effect of the SSRIs.

The results of the present study indicate that some of the investigated antidepressants may interfere with the metabolism of CYP2B substrates, such as steroids and CYP2B-metabolized drugs, as well as natural and synthetic toxins and procarcinogens, as mentioned in the introduction. Therefore, the direct inhibitory effect of nefazodone and SSRIs on CYP2B activity and SSRI-evoked CYP2B induction deserve further consideration. It is still not known whether similar CYP2B-induction by the investigated SSRIs also takes place also in extrahepatic tissues. As mentioned before, brain P450 isoforms are not always regulated in the same way as their liver counterparts [39, 52]. The brain expression of some P450 isoforms (2B6, 2D6, 2E1), which can metabolize many neurotoxins is higher in smokers and the rat counterparts of these human P450 isoforms are induced by nicotine [39, 40]. Since smoking is known to be protective against Parkinson's disease, Miksys and Tyndale hypothesized that nicotine-induced elevation of brain P450s in smokers may contribute to neuroprotection against Parkinson's disease [39, 42]. Therefore, it would be interesting to investigate whether the CYP2B-induction found after sertraline treatment in the liver also occurs in the brain, and whether sertraline is neuroprotective.

In summary, two different mechanisms of the antidepressant-CYP2B interaction are postulated: 1) a direct inhibition of CYP2B shown *in vitro* by nefazodone, SSRIs, and TADs, the inhibitory effect of nefazodone and SSRIs being the strongest; 2) *in vivo* induction of CYP2B produced by prolonged administration of SSRIs and desipramine (the inducing effect of sertraline being the most potent), which suggests their influence on the enzyme regulation. The marked CYP2B-induction produced by SSRIs corresponds with their selective serotonin reuptake inhibition, while the effect of desipramine corresponds with its selective noradrenaline reuptake inhibition.

Thus, the influence of the investigated antidepressants on rat CYP2B reminds rather that on CYP2C6 [10] than on CYP2D or CYP3A [11, 25, 27]. In both cases, CYP2B and CYP2C6, TADs and SSRIs did not cause enzyme inactivation by reactive metabolites (observed for CYP2D and CYP3A after 24 h-exposure), but SSRIs and some TADs led to enzyme induction after prolonged administration. On the other hand, the strongest direct inhibitory effect of these antidepressants on P450 was attributed to CYP2D [11].

In conclusion, our study conducted on rats shows that the investigated antidepressants exert a weak-moderate direct effect on rat CYP2B, and some of them also lead to enzyme induction after longer exposure. However, we do not know yet how the results we obtained apply to humans, since the rat and human CYP2B isoforms differ slightly in their structure, catalytic competence, and regulation. Extrapolating from rat CYP2B to human CYP2B6 is not simple because of species differences concerning 'cross-talk' between PXR and CAR in the regulation of *CYP2B* genes in rodents and humans [18]. Therefore, further clinical studies in this direction are advisable to find out whether similar effects of antidepressants occur in human CYP2B6 during long-term therapy.

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