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Effect of neuroleptics on cytochrome P450 2C11 (CYP2C11) in rat liver

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Abstract:

The aim of the present study was to investigate the influence of classic and atypical neuroleptics on the activity of cytochrome P450 2C11 (CYP2C11), measured as a rate of testosterone 2α- and 16α-hydroxylation. The reaction was studied in control liver microsomes in the presence of neuroleptics, as well as in the microsomes of rats treated intraperitoneally (*ip*) with pharmacological doses of the drugs (promazine, levomepromazine, thioridazine and perazine 10 mg/kg; chlorpromazine 3 mg/kg; haloperidol 0.3 mg/kg; risperidone 0.1 mg/kg; sertindole 0.05 mg/kg) for one day or two weeks (twice a day), in the absence of the neuroleptics *in vitro*.

The investigated neuroleptics added to control liver microsomes produced some inhibitory effects on CYP2C11 activity, which were moderate (thioridazine: K_i = 55), modest (sertindole and perazine: K_i = 76 and 94 µM, respectively) or week (promazine, levomepromazine, haloperidol and chlorpromazine: K_i = 285, 280, 223 and 157 µM, respectively). Risperidone had the weakest inhibitory effect on the CYP2C11 activity (K_i = 641 µM).

One-day exposure of rats to the neuroleptics did not significantly change the activity of CYP2C11 in liver microsomes. Of the neuroleptics studied, only chronic treatment with levomepromazine, perazine and thioridazine diminished CYP2C11 activity; those effects were positively correlated with the observed decreases in the protein level of the enzyme. The *in vivo* inhibition of CYP2C11 by chronic treatment with the three phenothiazines suggests their influence on the enzyme regulation. A possible mechanism of CYP2C11 regulation by the neuroleptics and its pharmacological significance are discussed.

Key words:
neuroleptics, rat, CYP2C11, direct effect, one-day treatment, chronic treatment

Introduction

Cytochrome P450 (CYP) subfamily CYP2C constitute the main pool of CYP in rat liver. Cytochrome P450 2C11 (CYP2C11) is the most abundant male-specific isoform of CYP, comprising approximately 50% of the total hepatic CYP in the adult male rat. On the other hand, CYP2C12 as a constitutive female-specific isoform represents about 40% of the total hepatic CYP in female rats. The gender differences in CYP expression are caused by the sexually dimorphic growth hormone (GH) secretion pattern in adult animals. It has been shown that the expression of "male" CYP2C11 depends on pulsatile GH secretion (of the proper frequency, duration and amplitude of the pulse), while continuous secretion of GH stimulates the expression of "female" CYP2C12 in the adult liver [1, 22, 27, 30, 40].

CYP2C11 is involved in the metabolism of benzphetamine, aminopyrine, benzo(a)pyrene, antipyrine, aflatoxin B_1, R-mephenytoin and S-warfarin [6, 21, 29, 39]. Moreover, CYP2C11 mediates hydroxylation of some endogenous steroids such as, e.g., testoster-

one and androstenedione, the epoxygenation of arachidonic acid and the hydroxylation of vitamin D [4, 24, 32, 36]. The 2α- and 16α-hydroxylation of testosterone is used as a marker reaction for studying CYP2C11 activities in rats [37, 44]. The CYP2C11 isoform is inhibited by cimetidine, diclofenac, ethanol and inflammatory mediators [3, 23, 25, 42]. On the other hand, the influence of glucocorticoids on the expression of CYP2C11 may be dual: at low levels, they induce enzyme activity, but suppress it at high (stress-induced) concentrations [19]. At a molecular level CYP2C11 is regulated by GH *via* the Janus kinase 2 (JAK2)-signal transducer and activator of the transcription 5b (STAT5b) pathway [7, 45, 46]. It has been reported that phenobarbital stimulates the transcription of *CYP2C11* gene in the rat [1].

Previous studies showed that rat CYP2C11 exhibited a 77% homology of the amino acid sequence, some substrate preference and functional analogies to human CYP2C9, which catalyzes the metabolism of such clinically important drugs as S-warfarin, phenytoin, ibuprofen, diclofenac, tolbutamide and antidepressant drugs, as well as steroids and arachidonic acids [2, 4, 29, 34, 39, 43]. However, the CYP2C11-specific reactions in rats, i.e., the 2α- and 16α-hydroxylation of testosterone, are catalyzed by CYP3A4/5 (2α-hydroxylation) and by CYP2B6, CYP2C8/9 and CYP3A4 (16α-hydroxylation) in humans [26]. Human CYP2C9 is also differently regulated compared to rat CYP2C11 [1, 5, 22, 31].

Our recent studies have established the brain dopaminergic system as an important center regulating the expression of liver CYP. They have demonstrated that a lesion or activation of the tuberoinfundibular or the mesolimbic pathway of the brain dopaminergic system affects liver CYP activity and protein level (CYP1A, CYP2B, CYP2C11 and CYP3A), as well as blood plasma concentration of the respective pituitary hormones (GH, T_3 and corticosterone) in the rat. Thus the neuroleptic drugs that block dopaminergic D_2 receptors may affect CYP expression *via* their action on the brain dopaminergic system, having an impact on the endocrine and immune systems [49–51, 53, 54].

Some literature data indicate that neuroleptics affect CYP activity. Promazine and chlorpromazine administered in high doses (80 and 89 mg/kg, *ip*, respectively) for 3 days induced CYP2B1; furthermore, chlorpromazine simultaneously down-regulated CYP2C11 [28, 33]. Two-week treatment with clozapine in a dose substantially exceeding the pharmacological/therapeutic one (114 mg/kg/day) increased the level and activity of CYP1A2, CYP2B1 and CYP3A1 in rats, whereas sulpiride (137 mg/kg/day) and remoxipride (31 mg/kg/day) produced a decrease in the level of CYP2B1, CYP2C11 and CYP3A1 and CYP1A2 (in the case of the latter enzyme – only remoxipride), having also dimished CYP2C11 mRNA. Another study conducted by Tateishi et al. [41], who administered chlorpromazine and thioridazine in a relatively high dose of 20 mg/kg *ip* for 4 days, showed that chlorpromazine did not change the total level of CYP, but induced CYP2B and CYP3A, whereas thioridazine reduced the total level of CYP, as well as the level and activity of CYP2C11, CYP2E1 and CYP3A. Moreover, our previous studies demonstrated that phenothiazine neuroleptics administered in pharmacological doses (promazine, levomepromazine, thioridazine, perazine 10 mg/kg, *ip*, chlorpromazine 3 mg/kg, *ip*) for two weeks to rats, were able to inhibit some CYP isoforms by a different mechanism, i.e., by binding the parent compound to the cytochrome (CYP2A, CYP2B, CYP2C6 and CYP2D), or by forming inhibitory CYP-radical cation complexes (CYP2D). In contrast, haloperidol (0.3 mg/kg, *ip*) and the atypical neuroleptics (risperidone 0.1 mg/kg, *ip*, sertindole 0.05 mg/kg, *ip*) were usually weak or inactive in this respect [9, 10, 15–17].

To date, there have been no complete data on the interaction of classic and novel neuroleptics with rat liver CYP2C11. Moreover, earlier studies into the influence of neuroleptics on CYP isoenzymes were conducted using very high, non-pharmacological/non-clinical doses, administered for a short period of the time, hence not reflecting the clinical mode of treatment. For this reason, the present study was aimed at investigating the effect of short- and long-term treatment with pharmacological doses of classic and atypical neuroleptics on the level and activity of CYP2C11.

Materials and Methods

Drugs and chemicals

Promazine and chlorpromazine (hydrochlorides) were provided by Polfa (Jelenia Góra, Poland), thioridazine (hydrochloride) was obtained from Jelfa (Jelenia Góra, Poland), perazine (dimaleate) from Labor

(Wrocław, Poland), while levomepromazine (maleate) was purchased from Egyt (Budapest, Hungary) and haloperidol from RBI (Natick, USA). Risperidone was donated by Janssen Pharmaceutica (Beerse, Belgium), sertindole by Lundbeck (Copenhagen, Denmark). Testosterone and its metabolites, 2α- and 16α-hydroxytestosterone were from Steraloids (Newport, USA). NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were purchased from Sigma (St. Louis, USA). Polyclonal antibody, anti-rat CYP2C11 goat serum and phenobarbital-treated rat liver microsomes were obtained from Gentest Corp. (Woburn, USA). A LumiGLO chemiluminescent substrate was provided by KPL (Gaithersburg, USA). All organic solvents of HPLC purity were supplied by Merck (Darmstadt, Germany).

Animal procedures

All the experiments with 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 neuroleptics were administered intraperitoneally, twice a day for one day or two weeks at the following pharmacological doses (mg/kg, *ip*): promazine, levomepromazine, thioridazine, perazine 10; chlorpromazine 3; haloperidol 0.3; risperidone 0.1; sertindole 0.05. 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 parent drugs administered *in vivo*, which was confirmed in our experiment by the HPLC method [11, 12].

In vitro studies into CYP2C11 activity – measurement of the rate of 2α- and 16α-hydroxylation of testosterone in liver microsomes

The activity of the CYP2C11 was studied by measurement of the rate of CYP2C11-specific reactions, i.e., 2α- and 16α-hydroxylation of testosterone in liver microsomes. After optimizing of *in vitro* conditions of the reactions, 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 neuroleptics on the activity of CYP2C11 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 2α- and 16α-hydroxylation of testosterone (testosterone concentration between 50–300 μM) was assessed in the absence and presence of one of the neuroleptics added *in vitro* (neuroleptic concentration between 50–200 μM). Each sample was prepared in duplicate.

Model II

The experiment was carried out on liver microsomes from rats treated with an neuroleptic for one day. Testosterone was added to the incubation mixture *in vitro* at a concentration of 100 μM. The 2α- and 16α-hydroxylation of testosterone was studied in the absence of neuroleptics.

Model III

The experiment investigated liver microsomes from rats subjected to two-week neuroleptic treatment. Testosterone was added to the incubation mixture *in vitro* at a concentration of 100 μM. The reaction was studied in the absence of neuroleptics.

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 down in ice.

Determination of the concentration of testosterone and its metabolites (2α- and 16α-hydroxytestosterone) in liver microsomes

Testosterone and its metabolites, 2α- and 16α-hydroxytestosterone, were extracted from the microsomal suspension with dichloromethane (1 ml of microsomal sus-

pension + 6 ml of the organic phase). Concentrations of testosterone, 2α- and 16α-hydroxytestosterone formed in liver microsomes were assessed by the high performance liquid chromatography (HPLC) method based on Sonderfan et al. [38]. 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 UV detector, L-7100 pump and D-7000 System Manager. The analytical column (Supelcosil 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: 16α-hydroxytestosterone 8.7 min, 2α-hydroxytestosterone 11.1 min and testosterone 15.6 min. The sensitivity of the method allowed for quantification of 16α-hydroxytestosterone as low as 0.005 nmol and 2α-hydroxytestosterone as low as 0.004 nmol in one sample. The accuracy of the method amounted to 1.3% (16α-hydroxytestosterone) and 1.2% (2α-hydroxytestosterone). The inter- and intra-assay coefficients of variance were about 7% for both metabolites.

Western blot analysis

The level of CYP2C11 protein in liver microsomes of rats treated chronically with neuroleptics (Model III) was estimated by western analysis. SDS-PAGE and immunoblot assay were performed using a methodology provided by Gentest, USA. Briefly, 5 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 CYP2C11), the blots were incubated with secondary antibody, i.e., the appropriate species-specific horseradish peroxidase-conjugated anti-IgG. Supersomes CYP2C11 (cDNA-expressed rat isoform) 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 Luminescent Image analyzer LAS-1000 using Image Reader LAS-1000 and Image Gauge 3.11 programs (Fuji Film, Japan).

Calculations and Statistics

The presented inhibition constants (K_i) for the inhibition of a specific metabolic pathway were obtained using a non-linear regression analysis (Program Sigma Plot 8.0, Enzyme Kinetics). 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 showed that the investigated neuroleptics directly inhibited CYP2C11 activity in rats, shown as inhibition of the rate of CYP2C11-specific

Tab. 1. The influence of neuroleptics given *in vitro* to rat liver microsomes on the CYP2C11 activity measured as the rate of 2α- and 16α-hydroxylation of testosterone (*Model I*)

Neuroleptics (inhibitors)	Inhibition of CYP2C11 activity		
	2α-OH-T K_i [µM]	16α-OH-T K_i [µM]	$\bar{X}$ K_i [µM]
I. Phenothiazines			
Promazine	178	392	285
Chlorpromazine	115	200	157
Levomepromazine	262	299	280
Perazine	96	93	94
Thioridazine	56	55	55
II. Butyrophenones			
Haloperidol	216	231	223
III. Atypical neuroleptics			
Risperidone	758	524	641
Sertindole	73	79	76

The presented inhibition constants (K_i) for the inhibition of a specific metabolic pathway were obtained using a non-linear regression analysis (Program Sigma Plot 8.0, Enzyme Kinetics)

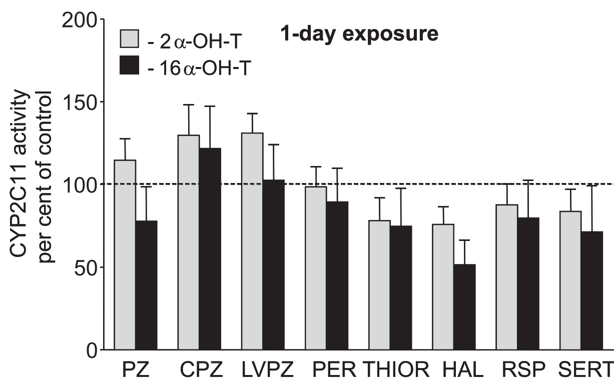

Fig. 1. The influence of 1-day exposure to neuroleptics on the CYP2C11 activity measured as the rate of 2α- and 16α-hydroxylation of testosterone in rat liver microsomes (*Model II*). All values are the means ± SEM from 7–8 animals; (Dunnett's test), compared with control (0.579 ± 0.057 nmol of 2α-hydroxytestosterone or 0.407 ± 0.054 nmol of 16α-hydroxytestosterone (mg protein)$^{-1}$ min^{-1}). PZ = promazine, CPZ = chlorpromazine, LVPZ = levomepromazine, PER = perazine, THIOR = thioridazine, HAL = haloperidol, RSP = risperidone, SERT = sertindole

reactions, i.e., the 2α- and the 16α-hydroxylation of testosterone by the drug added to control liver microsomes *in vitro* (*Model I*). Thioridazine was a more potent inhibitor of the reactions studied, while perazine and sertindole were weaker in this respect (Tab. 1). The inhibitory effects of the tested neuroleptics were moderate (thioridazine: K_i = 55), modest (sertindole and perazine: K_i = 76 and 94 μM, respectively) or weak (promazine, levomepromazine, haloperidol and chlorpromazine: K_i = 285, 280, 223 and 157 μM, respectively) (Tab. 1). Risperidone produced the weakest inhibitory effect on CYP2C11 activity (K_i = 641 μM) (Tab. 1).

Our study demonstrated that the investigated neuroleptics exerted no significant effect on CYP2C11 activity when they were given to rats for one day (i.e., for 24 h; *Model II*) (Fig. 1).

After two-week treatment with the tested neuroleptics (*Model III*), perazine, levomepromazine and especially thioridazine significantly decreased the activity of CYP2C11 (Fig. 2). The other neuroleptics studied did not produce any significant effect when administered *in vivo* for two weeks. As shown in Figs. 3A–C, the changes observed in CYP2C11 protein level after

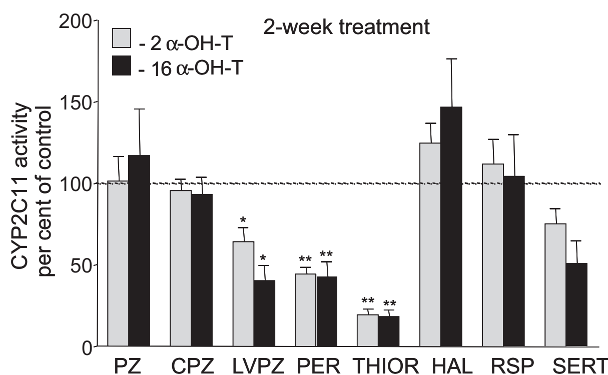

Fig. 2. The influence of two-week treatment with neuroleptics on the CYP2C11 activity measured as the rate of 2α- and 16α-hydroxylation of testosterone in rat liver microsomes (*Model III*). All values are the means ± SEM from 7–8 animals; * $p < 0.05$, ** $p < 0.01$ (Dunnett's test), compared with control (0.639 ± 0.083 nmol of 2α-hydroxytestosterone or 0.381 ± 0.084 nmol of 16α-hydroxytestosterone (mg protein)$^{-1}$ min^{-1}). PZ = promazine, CPZ = chlorpromazine, LVPZ = levomepromazine, PER = perazine, THIOR = thioridazine, HAL = haloperidol, RSP = risperidone, SERT = sertindole

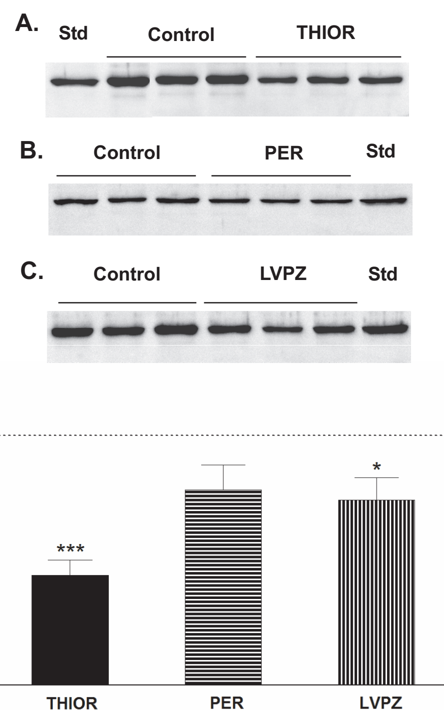

Fig. 3. The effect of two-week treatment (Model III) with thioridazine (**A**), perazine (**B**) and levomepromazine (**C**) on the level of CYP2C11 protein in rat liver microsomes. 5 μg of microsomal protein was subjected to western analysis, and the immunoblot was probed with polyclonal goat anti-rat antibody raised against CYP2C11. Supersomes CYP2C11 (cDNA-expressed rat isoform) were used as a standard. The presented results are typical of three separate animals per treatment. The histogram is the quantification of the corresponding band intensities from the tested isoforms. All values are the mean ± SEM (n = 5). Statistical significance was assessed by Dunnett's test and indicated with * $p < 0.05$, *** $p < 0.01$ compared to the control. THIOR = thioridazine, PER = perazine, LVPZ = levomepromazine

chronic treatment with phenothiazine neuroleptics corresponded well with those related to the enzyme activity. Thioridazine, perazine and levomepromazine visibly decreased CYP2C11 protein level to 44, 78 and 74% of the control, respectively.

Discussion

Our present data show that neuroleptics can affect CYP2C11 *via* a modest direct interaction with the enzyme and a significant indirect mechanism (enzyme regulation) produced by chronic treatment. The obtained results revealed interactions of the neuroleptics (added *in vitro* to control liver microsomes, *Model I*) with rat CYP2C11, which led to a decrease in the enzyme activity. Thioridazine (K_i = 55 μM), perazine (K_i = 94 μM) and sertindole (K_i = 76 μM) were the most potent inhibitors of rat CYP2C11 of the drugs studied, whose effect was more pronounced than that of the other neuroleptics tested. The K_i values obtained for thioridazine and perazine may be of importance *in vivo* regarding the dosage and the pharmacokinetics of these drugs. Phenothiazine neuroleptics are administered in relatively high doses compared to other neuroleptics, and being taken up by the tissue, reach concentrations that are 10–15 times higher in the liver than in blood plasma [13, 48, 52]. Therefore, phenothiazine neuroleptics whose K_i values are below 100 μM, in particular thioridazine, may reach the hepatic level close to the respective K_i values and are expected to decrease CYP2C11 activity *in vivo*. These findings may be of physiological, pharmacological or toxicological significance, considering the catalytic competence of this enzyme (the metabolism of steroids, drugs and toxins). As to the other investigated drugs, the calculated K_i values are above their pharmacological/therapeutic concentrations. Sertindole (K_i = 76 μM) is not likely to directly inhibit the activity of CYP2C11 when administered *in vivo*, considering its low pharmacological/therapeutic doses and concentrations [47].

The observed inhibitory potency of the investigated neuroleptics was weaker compared to that of the previously studied CYP isoforms, i.e., CYP2D, CYP2A and CYP2B (the K_i values for all the phenothiazines being between 15–23, 11–83 and 26–190 μM, respectively), but similar to those of CYP2C6 (except for levomepromazine and sertindole whose K_i values were 31 and 25 μM, respectively) [10, 15–17].

None of the investigated neuroleptics produced any significant effect on CYP2C11 activity when administered *in vivo* for one day (i.e., for 24 h; *Model II*); however, after 2-week exposure to the neuroleptics (*Model III*), decreases in CYP2C11 activity and protein level were observed after levomepromazine, perazine and thioridazine. The effect observed in *Model III* could not be ascribed to the binding of the parent drug to the enzyme protein, found after a short incubation period of the neuroleptics with control liver microsomes in *Model I*. For liver microsomes obtained from neuroleptic-treated animals, they were prepared including the procedure of washing, which deprived microsomes of the parent drugs administered *in vivo.*

As mentioned in the introduction, our recent studies provided direct evidence for the important role of the brain dopaminergic system in the regulation of CYP expression in rat liver [49–51, 53, 54]. Accordingly, the neuroleptic drugs that block dopaminergic D_2 receptors may influence the secretion of pituitary hormones (e.g., GH, ACTH, TSH) which directly or indirectly activate nuclear/cytosolic receptors controlling *CYP* genes, including *CYP2C11*. Therefore, a possible cause of the inhibitory effects on CYP2C11 expression observed in the case of levomepromazine, perazine and thioridazine given *in vivo* (*Model III*) may be their influence on *CYP2C11* gene regulation *via* blockade of dopaminergic D_2 receptors in the pituitary and, in consequence, the inhibition of GH secretion. The above-mentioned hormone plays a crucial role in the positive regulation of male rat CYP2C11 [1, 22, 30, 40].

The results obtained in the present study are also in line with other observations indicating down-regulation of CYP2C11 in rats after 2-week treatment with the selective antagonists of dopaminergic D_2 receptors, sulpiride and remoxipride and after a 4-day treatment with the less specific dopaminergic D_2 receptor-blocking neuroleptic thioridazine [33, 41]. However, the other phenothiazines tested, i.e., promazine and chlorpromazine, do not produce any significant effect on CYP2C11 activity when administered *in vivo* for two weeks. The lack of effect of promazine on CYP2C11 may steam from the fact that of the phenothiazines tested, promazine is the weakest blocker of the dopaminergic D_2 receptor [8, 35]. Accordingly, the effect of promazine on the secretion of

pituitary hormones regulating the *CYP2C11* gene (mainly GH) may be negligible. In addition, this neuroleptic shows the ability to induce cytochrome P450 [28], which may mask its inhibitory effect on CYP2C11 regulation *via* the neuroendocrine system. On the other hand, chronic treatment with chlorpromazine, which is a potent antagonist of the dopaminergic D_2 receptor [8, 35], does not affect CYP2C11 activity, either. Like in the case of promazine, the D_2 receptor-mediated effect of chlorpromazine on the hormonal regulation of CYP2C11 (down-regulation) may be masked by the inducing effect of the neuroleptic on cytochrome P450 at a the level of the liver. It was shown previously that chlorpromazine induced CYP2B and CYP3A in the rat [28, 41]. Our results with chlorpromazine are in contrast to those of Murray [28], who reported that chlorpromazine inhibited CYP2C11 activity. However, the latter author used a very high dose of the neuroleptic (89 mg/kg, *ip*) for 3 days. At such a dosage schedule, the process of enzyme induction might not have fully developed in the liver to mask the inhibitory properties of chlorpromazine *via* neuroendocrine regulation. Moreover, the high dose of chlorpromazine may have yielded a great number of reactive phenothiazine metabolites, such as radical cations, which are known to interact with proteins [14, 20]. Therefore, the decreased activity of CYP2C11, observed by Murray [28], may result from the summing up of the two mechanisms mentioned above (neuroendocrine down-regulation and the enzyme inhibition by reactive chlorpromazine metabolites).

In contrast to the phenothiazines studied, haloperidol (another typical neuroleptic) administered in the pharmacological/therapeutic dose of 0.3 mg/kg, *ip* did not produce significant changes in CYP2C11 activity in our experiments. However, haloperidol given to rats in a very high/toxic dose of 40 mg/kg, *ip* (the dose close to LC_{50}) visibly increased the mRNA level of CYP2C11, CYP1A2 and CYP3A2 in normal liver, but not in fatty liver [18].

In conclusion, our study conducted on rats shows that neuroleptics may exert both a direct inhibitory effect and an indirect one – evoked by chronic treatment with some neuroleptics (levomepromazine, perazine and thioridazine), which may be of importance *in vivo*. The present data may be useful for the interpretation of the results of pharmacological experiments, obtained after administration of neuroleptics to rats. However, we do not know yet to what extent the obtained results may be transposed to humans, since rat CYP2C11 and human CYP2C9 differ in their regulation. Thus the obtained results may trigger further studies into clinical aspect.

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