The activity of cytochrome P450 CYP2B in rat liver during neuroleptic treatment

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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 rat CYP2B measured as a rate of 16β-hydroxylation of testosterone. The reaction was studied in control liver microsomes in the presence of neuroleptics, 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 (promazine, levomepromazine, thioridazine and perazine, 10 each; chlorpromazine 3; haloperidol 0.3; risperidone 0.05), in the absence of the neuroleptics in vitro. Some of the neuroleptics added in vitro to control liver microsomes decreased the activity of CYP2B. The obtained K_i values indicated that thioridazine was the most potent inhibitor of the studied reaction (K_i = 26 μM). The inhibitory effects of chlorpromazine, perazine and sertindole were moderate (K_i = 45–75 μM), while promazine, haloperidol, levomepromazine and risperidone were rather weak inhibitors of CYP2B activity (K_i = 125–225 μM, respectively). After a one-day (i.e. 24 h) exposure of rats to the investigated neuroleptics, the decreased CYP2B activity was observed after haloperidol, risperidone and sertindole. All the investigated neuroleptics did not produce any significant effect on CYP2B activity when administered in vivo for two weeks. Considering relatively high pharmacological/therapeutic doses and liver concentrations of phenothiazines, it seems that the direct inhibitory effect of those neuroleptics with K_i values below 100 μM found in vitro (thioridazine, chlorpromazine, perazine), as well as indirect effects produced by one-day treatment with haloperidol, risperidone or sertindole may be of some physiological, pharmacological or toxicological importance in vivo.

Key words:
phenothiazines, haloperidol, risperidone, sertindole, CYP2B, testosterone 16β-hydroxylation, liver microsomes, rat, in vitro study, one-day treatment, chronic treatment

Introduction

The CYP2B gene subfamily in the rat consists of eight genes, of which three CYP2B1, CYP2B2 and CYP2B3 produce detectable transcripts in the liver. The isoforms CYP2B1 and CYP2B2 show 97% homology of amino acid sequence, while CYP2B3 displays 77% sequence identity to CYP2B2. On the other hand, the rat CYP2B1 isoform shows 75% identity to the human CYP2B6. The constitutive level of CYP2B expression in the liver is low and does not exceed 5% of the total CYP contents. The presence of CYP2B activity was shown not only in the liver, but also in the brain, ileum, adrenal glands, kidney and lungs [1, 7, 9].

In general, catalytic competence of CYP2B1 and CYP2B2 is similar, but each of them has some different substrate/reaction preferences [11]. The N-demethylation of benzphetamine and 16β-hydroxylation of testosterone are used as CYP2B1-specific reactions,
though the reactions are also catalyzed (but at a lower rate) by CYP2B2. The rat CYP2B1/2 enzymes and human CYP2B6 show partially similar substrate specificity as indicated by their ability to hydroxylate testosterone and lidocaine [2, 10, 14, 18, 22, 28]. Moreover, CYP2B6 was shown to metabolize many drugs, such as S-mephenytoin (N-demethylation), benzodiazepines, bupropion, mianserin, tamoxifen and to activate cyclophosphamide and ifosfamide [8, 15, 23, 27]. Both rat and human CYP2B isoforms are of high toxicological significance, since they activate natural and synthetic procarcinogens, such as aflatoxin B1, 6-aminochrysene, benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene or dibenz[a,h]anthracene [27].

The subfamily CYP2B isoforms are inhibited by metyrapone (CYP2B1/2) and orphenadrine (CYP2B1/2/6) [23, 27]. CYP2B isoforms (except CYP2B3) are induced by phenobarbital and CYP2B6 also by classical CYP3A inducers dexamethasone and rifampicin [9, 14, 27, 30]. CYP2B genes are regulated physiologically by thyroid hormones, glucocorticoids and growth hormone [16, 24]. At the molecular level, nuclear receptors CAR (constitutive androstane receptor), PXR (pregnane X receptor) and GR (glucocorticoid receptor) participate in their regulation [16, 17, 19–21, 31].

Our previous studies showed that some neuroleptic drugs affect cytochrome P450, in particular isoforms of the subfamilies CYP2C, CYP2D and CYP3A [4, 5]. Since CYP2B isoenzymes are of considerable pharmacological and toxicological importance, the aim of the present study was to investigate the effect of short- and long-term treatment with classical and atypical neuroleptics on the activity of rat CYP2B1/2 in the liver.

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) was from Labor (Wroclaw, Poland), while levomepromazine (maleate) was purchased from Égypt (Budapest, Hungary) and haloperidol from RBI (Natick MA, USA). Risperidone was donated by Janssen Pharmaceutica (Beerse, Belgium), sertindole by Lundbeck (Copenhagen, Denmark). Testosterone and its metabolite, 16β-hydroxytestosterone were from Steraloids (Newport, USA). NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were purchased from Sigma (St. Louis, USA). All organic solvents of HPLC purity were supplied by Merck (Darmstadt, Germany).

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 neuroleptics were administered intraperitoneally (ip), twice a day for one day or two weeks at the following pharmacological doses (mg/kg): promazine, levomepromazine, thioridazine and perazine 10, chlorpromazine 3, haloperidol 0.3, risperidone 0.1, sertindole 0.05. The control animals were injected with saline. 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.

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

The activity of the CYP2B was studied by measurement of the rate of CYP2B-specific reaction, i.e. 16β-hydroxylation of testosterone in the liver microsomes. To distinguish between a direct effect of neuroleptics 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 16β-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 exposed to a neuroleptic 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 neuroleptics. Model III: Liver microsomes from rats subjected to two-week neuroleptic 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 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), MgCl2 (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. [29]. 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) to solvent B (70% methanol:water:acetonitrile, 80:18:2) 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 as low as 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%.

Calculations and 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 and Discussion

The obtained results show that some of the investigated neuroleptics exert a direct inhibitory effect on CYP2B in vitro. The neuroleptics added to liver microsomes of control rats directly inhibited the activity of CYP2B, which was shown as an inhibition of the rate of CYP2B-specific reaction, i.e. 16β-hydroxylation of testosterone (Tab. 1). Figures 1A–D show examples of the Dixon plots obtained in our studies, which served as a basis for calculation of K_i constants. The obtained K_i values indicated that thioridazine was the most potent inhibitor of the studied reaction (K_i = 26 µM). The inhibitory effects of chlorpromazine, perazine and sertindole were moderate (K_i = 45–75 µM), while promazine, haloperidol, levomepromazine and risperidone were rather weak in-

<table>
<thead>
<tr>
<th>Neuroleptics (inhibitors)</th>
<th>Inhibition of CYP2B activity K_i(µM)</th>
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<tr>
<td>I. Phenothiazines</td>
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<tr>
<td>Promazine</td>
<td>125</td>
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<tr>
<td>Chlorpromazine</td>
<td>45</td>
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<td>Levomepromazine</td>
<td>190</td>
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<tr>
<td>Perazine</td>
<td>45</td>
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<tr>
<td>Thioridazine</td>
<td>26</td>
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<tr>
<td>II. Butyrophenones</td>
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<tr>
<td>Haloperidol</td>
<td>140</td>
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<tr>
<td>III. Atypical neuroleptics</td>
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<tr>
<td>Risperidone</td>
<td>225</td>
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<tr>
<td>Sertindole</td>
<td>75</td>
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</table>
Inhibitors of CYP2B activity ($K_i = 125–225 \mu M$, respectively) (Tab. 1). The potency of the neuroleptics to inhibit the CYP2B-specific reaction was as follows (according to the $K_i$ values): thioridazine > chlorpromazine = perazine > sertindole > promazine > haloperidol > levomepromazine > risperidone.

The $K_i$ values obtained for thioridazine, chlorpromazine and perazine may be of importance in vivo considering the dosage and pharmacokinetics of these drugs. Phenothiazine neuroleptics are administered at relatively high doses compared to other neuroleptics and are taken up by tissues [3] reaching in the liver concentrations which are 10–15 times higher than those in blood plasma [33, 34]. Moreover, they accumulate in tissues when given repeatedly [6]. Therefore, some phenothiazine neuroleptics (with $K_i$ values below 100 $\mu M$), in particular thioridazine, may reach hepatic levels close to the respective $K_i$ values and are expected to decrease the activity of CYP2B in vivo. And this may be of physiological, pharmacological or toxicological significance, considering catalytic competence of that enzyme (the metabolism of steroids, drugs and toxins).

As for other investigated drugs, the calculated $K_i$ values are above their pharmacological/therapeutic concentrations. Sertindole ($K_i = 7.5 \mu M$) should not directly inhibit the activity of CYP2B when administered in vivo, considering low pharmacological/therapeutic doses and concentrations of the neuroleptic [32].

The observed inhibiting potency of the investigated neuroleptics was weaker compared to that obtained for earlier studied CYP isoforms: CYP2D (with $K_i$ values for all phenothiazines between 15–23 $\mu M$) [5] and even CYP2A (except for thioridazine and sertindole with $K_i$ values of 50 and 78 $\mu M$, respectively) [13], but a little more pronounced than for CYP2C6 (except for levomepromazine and sertindole with $K_i$ values of 31 and 25 $\mu M$, respectively) [12].

After a one-day (i.e. 24 h) exposure to the investigated neuroleptics, the decreased CYP2B activity was
observed after haloperidol, risperidone and sertindole (Fig. 2A). Other studied neuroleptics did not produce any significant changes in the rate of 16β-hydroxylation of testosterone after one-day treatment. All the investigated neuroleptics did not produce any significant effect on CYP2B activity when administered in vivo for two weeks (Fig. 2B). It seems, therefore, that the direct inhibitory effects of the three phenothiazines observed in vitro (a competitive inhibition) will be the only mechanism capable of gentle and moderate decreasing the activity of CYP2B after chronic treatment. The decreases in enzyme activity observed after one-day treatment with haloperidol, risperidone or sertindole were transient and did not persist or develop during continuous treatment.

The presented results concerning prolonged administration of promazine and chlorpromazine in vivo differ from those obtained by Murray [25], who observed the induction of the enzyme by those phenothiazines. However, the author used very high (non-pharmacological/non clinical) doses of promazine and chlorpromazine (80 and 89 mg/kg ip, respectively), compared to our study (10 and 3 mg/kg ip, respectively). It means that the regulation of CYP2B isoforms by these neuroleptics is concentration-dependent. Moreover, it seems also feasible that at pharmacological/therapeutic concentrations used in our experiment the inducing effect of phenothiazines resulting from their chemical structure was masked by their pharmacological effect on hormonal regulation of CYP (down-regulation), which is mediated by the brain D2-dopaminergic receptors [26, 35]. Recent studies of Wójcikowski et al. showed that a lesion of the tuberoinfundibular pathway of the brain dopaminergic system decreased the level and activity of CYP2B in the liver [35].

In summary, it seems that the direct inhibitory effect of the investigated neuroleptics with Kᵢ values below 100 μM found in vitro (thioridazine, chlorpromazine, perazine) or indirect effects produced by one-day treatment with haloperidol, risperidone or sertindole may be of some physiological, pharmacological or toxicological importance in vivo. However, no serious pharmacokinetic interaction with CYP2B-catalyzed drug metabolism or no significant changes in CYP2B-mediated activation of procarcinogens evoked by the chronic treatment with pharmacological/therapeutic doses of neuroleptics are expected. However, we do not know yet how far the obtained results may be transferred to humans, since as mentioned in the introduction, the rat and human CYP2B isoforms differ in their structure, catalytic competence and regulation.

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References:


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