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PRELIMINARY COMMUNICATION

EFFECTS OF CHRONIC TREATMENT WITH CLASSIC AND NEWER ANTIDEPRESSANTS AND NEUROLEPTICS ON THE ACTIVITY AND LEVEL OF CYP2D IN THE RAT BRAIN

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Effects of chronic treatment with classic and newer antidepressants and neuroleptics on the activity and level of CYP2D in the rat brain. A. HADUCH, J. WÓJCIKOWSKI, W.A. DANIEL. Pol. J. Pharmacol., 2004, 56, 857–862.

The aim of the present work was to study the effect of chronic treatment with pharmacological doses of selected antidepressants (imipramine, mirtazapine) and neuroleptics (thioridazine, risperidone) on the activity and level of CYP2D in the rat brain. Our previous studies carried out on the liver showed that after chronic treatment with psychotropics, the activity of CYP2D was significantly decreased by imipramine, thioridazine and risperidone, but increased by mirtazapine. Our preliminary results suggest that the same may happen in the brain, where similar tendencies in changes in CYP2D activity were observed. Imipramine, thioridazine and risperidone diminished, while mirtazapine tended to accelerate the rate of ethylmorphine *O*-deethylation, a specific reaction for measurement of CYP2D activity. In the case of thioridazine, the observed decrease in the enzyme activity was the most pronounced and statistically significant. The level of brain CYP2D4 was not substantially changed by the prolonged administration of the investigated drugs (imipramine 136.3 ± 14.9%, thioridazine 121.9 ± 3.5%, risperidone 113.5 ± 7.8%, mirtazapine 80.3 ± 1.5% of the control), and did not correspond positively with the measured CYP2D activity. This may imply that the observed changes in the CYP2D activity were not caused by the involvement of those psychotropics in the regulation of CYP2D4. In conclusion, our preliminary results suggest that the effects of prolonged treatment with antidepressants and neuroleptics on the activity of CYP2D found in our previous study in the liver also occur in the brain, which may have an impact on the pharmacological and clinical profile of those drugs.

Key words: *CYP2D, brain, imipramine, mirtazapine, thioridazine, risperidone*

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INTRODUCTION

Our previous studies showed that most of the investigated antidepressants (tricyclic antidepressant drugs – TADs, selective serotonin reuptake inhibitors – SSRIs, mirtazapine, nefazodone) and neuroleptics (phenothiazines, haloperidol, risperidone, sertindole) produced significant changes in the activity of CYP2D in the rat liver. Apart from direct effects of the parent drugs on CYP2D activity (*via* binding with enzyme protein), the investigated psychotropics exerted also substantial indirect effects, produced by longer exposure to the drugs, which were drug- and time-dependent [2, 3].

Three different mechanisms of the antidepressants-CYP2D interaction were postulated: 1) competitive inhibition of CYP2D shown *in vitro,* the inhibitory effects of TADs and SSRIs being stronger than those of nefazodone or mirtazapine, but weaker than the effects of the respective drugs on human CYP2D6; 2) *in vivo* inhibition of CYP2D appearing already after 24-hour exposure and developing during chronic treatment with TADs and SSRIs, which suggests inactivation of the enzyme by reactive nitroso-alkane metabolites of antidepressants; 3) *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 [2].

Similar mechanisms seemed to be involved in the interaction between CYP2D and the investigated neuroleptics: 1) competitive or mixed inhibition of CYP2D by the parent drugs shown *in vitro,* the inhibitory effects of phenothiazines being stronger than those of haloperidol or atypical neuroleptics, but weaker than the effects of the respective drugs on human CYP2D6; 2) *in vivo* inhibition of CYP2D, produced by one-day and maintained during chronic treatment with phenothiazines, which suggests inactivation of enzyme by radical cations of phenothiazines; 3) *in vivo* inhibition of CYP2D by risperidone, produced only by chronic treatment with the drug, which suggests its influence on the enzyme regulation [3].

The CYP2D subfamily isoforms are also present in the brain, where they take part in the metabolism of dopamine [9, 16] serotonin [21], steroids [10, 11], arachidonic acid [16] and local biotransformation of drugs [8, 15, 17, 18], Therefore, the aim of the present work was to study the effect of chronic treatment with pharmacological doses of selected antidepressants (imipramine, mirtazapine) and neuroleptics (thioridazine, risperidone) on the activity of CYP2D in the rat brain. We also examined the effects of those drugs on the protein level of CYP2D4, the latter being the main CYP2D isoform in the rat brain. It was interesting to find out whether the drug-induced changes in the CYP2D activity observed in the liver, simultaneously occur in the brain.

MATERIALS and METHODS

Drugs and chemicals

Imipramine hydrochloride was provided by Polfa (Jelenia Góra, Poland), mirtazapine hydrochloride was donated by Organon (The Netherlands). Thioridazine (hydrochloride) was obtained from Jelfa (Jelenia Góra, Poland), risperidone was from Janssen Pharmaceutica (Beerse, Belgium). Ethylmorphine, NADP, glucose-6-phosphate and glucose-6-phosphate-dehydrogenase were purchased from Sigma (St. Louis, USA) and morphine from Polfa S.A. (Kutno, Poland). Polyclonal antibody, anti-rat CYP2D4 rabbit antiserum was a gift from Prof. Y. Funae from the Osaka City University Medical School, Japan. Secondary antibody, horseradish peroxidase-conjugated Goat Anti-Rabbit IgG was obtained from Sigma (St. Louis, USA). All organic solvents of HPLC purity were supplied by Merck (Darmstadt, Germany).

Animals

All the experiments on animals were performed in accordance with the Polish governmental regulations (Decree on Animals Protection 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 psychotropics were administered intraperitoneally (*ip*), twice a day for two weeks at the following pharmacological doses (mg kg^{-1}): imipramine and thioridazine 10, mirtazapine 3, risperidone 0.1. The doses of the investigated drugs were of pharmacological magnitude which produced adequate behavioral or biochemical effects in animals. The control animals were injected with saline. The rats were sacrificed at 24 h after the drug withdrawal. The whole brains were removed, immediately homogenized at 4^{o}C, and microsomes were prepared by differential centrifugation, according to Hiroi et

al. [10]. The final concentration of protein in a sample was about 10 mg/ml.

In vitro studies into CYP2D activity: measurement of the rate of ethylmorphine *O*-deethylation in brain microsomes

The activity of the CYP2D subfamily isoenzymes was studied by measurement of the rate of CYP2D specific reaction, ethylmorphine *O*-deethylation [20] in brain microsomes. Incubations were carried out in a system containing brain microsomes (5 mg of protein in 1 ml), Tris/KCl buffer (20 mM, pH = 7.4), $MgCl_2$ (2.5 mM), NADP (0.3 mM), glucose 6-phosphate (3.6 mM) and glucose-6-phosphate-dehydrogenase (0.9 U in 1 ml). Ethylmorphine was added to the incubation mixture *in vitro* at a concentration of 600 μM. The reaction was studied in the absence of psychotropics, which were washed out during the procedure of microsome preparation. The final incubation volume was 1 ml. After a 20-min incubation, the reaction was stopped by adding 200 μl of methanol and then by cooling the reaction mixture down in ice.

Determination of the concentration of ethylmorphine and morphine in brain microsomes

Ethylmorhine and morphine were extracted from the microsomal suspension (pH = 8.5) with chloroform containing 10% of 1-butanol. Concentrations of ethylmorphine and morphine formed from ethylmorphine in brain microsomes were assessed by the high performance liquid chromatography method (HPLC) based on Glare et al. [4]. The residue obtained after evaporation of the extracts was dissolved in 500 μl of the mobile phase. An aliquot (20 μl) was injected into the HPLC system (LaChrom, Merck-Hitachi), equipped with L-7480 fluorescence detector, L-7100 pump, D-7000 System Manager and the analytical column (Econosphere C18 5 M, 4.6 × 250 mm; Carnforth, England). The mobile phase consisted of 0.05 M KH_2PO_4 and acetonitrile in proportion 75 : 25. The flow rate was 1.0 ml min^{-1} (18 min) and 2 ml min^{-1} (8.122 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 14.5 min. The sensitivity of the method allowed for quantification of morphine and ethylmorphine levels as low as 0.005 nmol in 1 ml of microsomal suspension. The accuracy of the method was ca. 2%. The intra- and inter-assay coefficients of variance were below 4% and 7%, respectively.

Western blots

SDS-PAGE and immunoblot assay were performed using a methodology provided by Gentest, USA. Briefly, 76 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). Protein was electroblotted onto a nitrocellulose membrane and blocked overnight with 5% dried nonfat milk in PBS (phosphate-buffered saline, pH = 7). After incubation with primary antibody – polyclonal rabbit anti-rat antibody raised against CYP2D4 [11] (1 h, 20°C), the blots were incubated with secondary antibody, i.e. the appropriate species-specific horseradish peroxidase-conjugated anti-IgG (1 h, 20°C). Immunoreactivity was assessed using enhanced chemiluminescence detection system (ECL, kit, Amersham, UK). 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). The CYP2D4 protein bands of 50 kDa [19] were compared with protein standards (Precision Plus Protein Standards, Unstained, Bio-Rad).

Calculations and statistics

Statistical significance was assessed using an analysis of variance followed by Dunnett's test. All values are means ± SEM from four samples, each containing three pooled brains.

RESULTS and DISCUSSION

The CYP2D proteins are present not only in liver microsomes, but also in the microsomal and mitochondrial membranes of the brain [14], where they take part in the metabolism of important endogenous neuroactive substrates [9–11, 16, 21] and local biotransformation of drugs [8, 15, 17, 18]. The cerebellum, amygdaloid complex, olfactory bulbs, substantia nigra pars compacta and pars reticulata are the most abundant regions in CYP2D [14]. The hippocampus and striatum show moder-

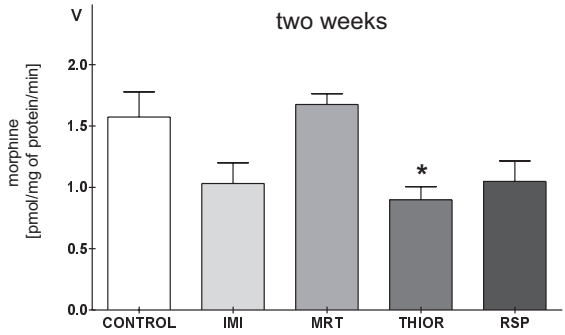

Fig. 1. The influence of two-week treatment with antidepressants and neuroleptics on the activity of CYP2D in brain microsomes. The presented results are the means ± SEM of four samples (each sample consists of three pooled brains). * $p < 0.05$ (Dunnett's test), compared to the control (1.573 ± 0.411 pmol of morphine/mg of protein/min). IMI = imipramine, MRT = mirtazapine, THIOR = thioridazine, RSP = risperidone

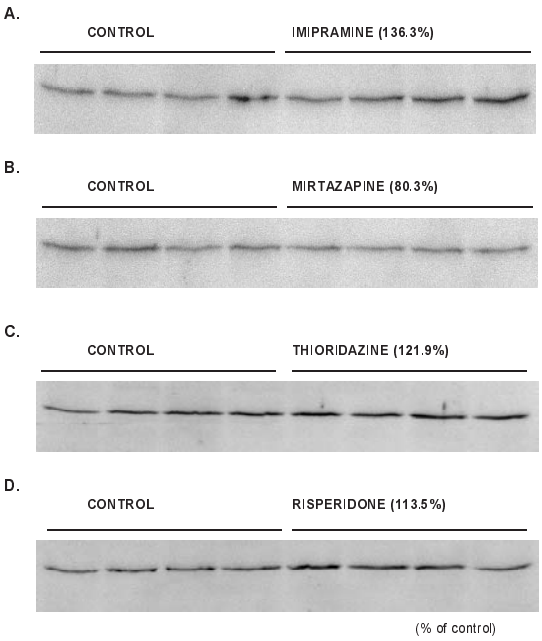

Fig. 2. The influence of two-week treatment with imipramine (A), mirtazapine (B), thioridazine (C) and risperidone (D) on the level of CYP2D4 in rat brain microsomes (n = 4). Each sample consists of three pooled brains; 76 μg of microsomal protein was subjected to Western blot analysis, and the immunoblot was probed with polyclonal rabbit anti-rat antibody raised against CYP2D4

ate amount of CYP2D. Out of six rat CYP2D isoforms (CYP2D1-5 and CYP2D18), CYP2D4 is the most abundant in the brain and, therefore, was the main object of our preliminary study, which was carried out on the whole brain.

Our previous studies carried out on liver microsomes showed that after chronic treatment with psychotropics, the activity of CYP2D was significantly decreased by imipramine, thioridazine and risperidone, but increased by mirtazapine [2, 3]. Our preliminary results suggest that the same may happen in the brain, where similar tendencies in the changes in CYP2D activity were observed (Fig. 1). Imipramine, thioridazine and risperidone diminished, while mirtazapine tended to accelerate the rate of ethylmorphine *O*-deethylation, which is a specific reaction for measurement of CYP2D activity. In the case of thioridazine, the observed decrease in the enzyme activity was the most pronounced and statistically significant.

The level of brain CYP2D4 was not substantially changed by the prolonged administration of the investigated drugs (imipramine 136.3 ± 14.9%, thioridazine 121.9 ± 3.5%, risperidone 113.5 ± 7.8%, mirtazapine 80.3 ± 1.5% of the control) (Fig. 2), and did not correspond positively with the measured CYP2D activity (Fig. 1). And this may imply that the observed changes in the CYP2D activity were not caused by the involvement of those psychotropics in the regulation of CYP2D4.

Since the decrease in the CYP2D activity in the liver appeared already after 24 hour-exposure to imipramine [2] or thioridazine [3] and was maintained during chronic treatment, we concluded that it was produced by inactivation of the cytochrome by reactive metabolites of those drugs. Longer time is necessary to influence the activity of an enzyme *via* its regulation, while forming reactive nitrosoalkane metabolites of imipramine [1, 12, 13] and radical cations of phenothiazines [57], inactivating enzyme proteins, has been shown *in vitro*. The same mechanism decreasing enzyme activity by imipramine and thioridazine may apply to the brain CYP2D.

On the other hand, changes in CYP2D activity, produced by mirtazapine (an increase) and risperidone (a decrease) in the liver, were gentle and appeared only after chronic treatment [2, 3], suggesting an influence of those new drugs on the enzyme regulation. However, such an explanation does not seem to apply to the brain CYP2D4, since we did not observe a positive correlation between changes in the enzyme activity and protein level in the present study. However, one has to consider different isoform composition of CYP2D in the liver and brain, the regulation of which has not been well

known. It also cannot be excluded that alterations in other components of the mixed-function oxidase system (NADPH cytochrome P-450 reductase or cytochrome b-5) may be involved in the observed changes in the activity of CYP2D after chronic treatment.

Therefore, further studies with greater number of samples and including different CYP2D isoforms are necessary to confirm our present results and to clarify mechanisms of the observed alterations in the activity of CYP2D by the investigated psychotropics. Moreover, it is of particular interest to find out how the activity of CYP2D is changed in the brain structures, where the enzyme catalyzes the synthesis of neurotransmitters, i.e. dopamine from tyramine [9] and regeneration of serotonin from 5-methoxytryptamine [21].

In conclusion, our preliminary results suggest that the effects of prolonged treatment with antidepressants and neuroleptics on the activity of CYP2D found in our previous study in the liver also occur in the brain, which may have an impact on the pharmacological and clinical profile of those drugs.

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