ORIGINAL ARTICLE

Systemic administration of D2 antagonist raclopride inhibits CYP1A2 in the rat model of isolated perfused liver

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Introduction

Raclopride (CAS 98185-20-7) is a synthetic benzamide related to sulpiride eliciting selective antagonism on dopamine D2 receptors (Ericson *et al* 1996). It was investigated for the treatment of psychoses (Seeman 2002) and serves frequently in experimental pharmacology as a representative of selective D2 receptor blocking drugs (Seeman 2002; Eltayb *et al* 2005; Ginovart *et al* 2009). Up to date, in the literature available there is no data on possible influence of raclopride on the metabolic activity of cytochrome P450 enzymes. Recently it was published, that brain mesolimbic and tuberoinfundibular dopaminergic pathways influence the activity of hepatal cytochrome oxidase system (Wojcikowski *et al* 2007; Wojcikowski *et al* 2008). One of possible explanation considered could be changes in hormone and cytokine levels influencing some of receptor (e.g. glucocorticoid receptor, pregnane X receptor or constitutive androstane receptor) regulating P450 proteins (Wojcikowski *et al* 2007; 2008).

Our study addressed whether systemic administration of raclopride (D2 receptor antagonist) may influence the metabolic activity of rat CYP1A2, CYP2C6/11 or CYP2D2 in the model of isolated perfused liver. It was taken into consideration that CYP2C6/11 is a rat orthologue of human CYP2C9 (Matuskova *et al* 2009), CYP2D1 is rat orthologue of human CYP2D6 (Soucek & Gut 1992). The most used marker of CYP2D6 dextromethorphan is metabolized by CYP2D2 in rats (Kobayashi *et al* 2002). The model of isolated perfused rat liver was selected because it reflects most of physiological and biochemical linkages in the liver-mediated metabolism of xenobiotics.

Methods and materials

Animals

The experiment was carried out *on male Wistar* albino rats. After 10 days of adaptation to controlled laboratory conditions (21–22 °C; humidity 50–60%; light from 6:00 to 18:00, diet and water *ad libitum*), rats were randomly divided in 3 groups (9 animals each). The first group (R) was intraperitoneally administered raclopride at the dose of 0.1 mg/kg/day for 7 days. The drug was administered as the 0.1 mg/mL solution in saline. The second, control group (C) was administered proportional volume of saline. The third group (F) was administered comparative CYP enzyme inhibitor fluoxetine at the dose of 5 mg/kg/day (5 mg/mL solution in saline). Metabolic activities of CYP1A2, CYP2C6/11 and CYD2D2 were compared within groups.

Metabolic activity assessment

The model of isolated perfused rat liver was used (Zendulka *et al* 2009). Briefly: rats were anesthetized with the combination of xylasine 16 mg/kg (Rometar, Bioveta, Ivanovice na Hané, CZ) and ketamine 100 mg/kg (Narketan 10%, Vetoquinol, Nymburk, CZ). After laparotomy, a plastic cannula was introduced into the portal vein and the liver was briefly perfused with

tempered (38 °C) saline, which was then replaced by the perfusion medium (120 mL of Williams medium E, Sigma Chemical Co., St. Louis, USA) which was equilibrated with 95% O_2 and 5% CO_2. Liver was then placed into the modified Miller's perfusion apparatus (Miller *et al* 1951) and was tempered at 38 °C. Marker substances phenacetin (PHE)-CYP1A2; diclofenac (DCF) – CYP2C6/11 and dextromethorphan (DEM) – CYP2D2 were added as a bolus into perfusion medium after 20 minutes of pre-perfusion. Samples of perfusate were collected in the 30th, 60th and 120th minute of perfusion and were frozen at –75 °C until analysis.

HPLC measurement and statistical analysis

The rate of metabolism was assessed as a concentration ratio (metabolic ratio, MR): marker/metabolite, in the 30th, 60th and 120th minute of liver perfusion. The concentrations of PHE, DCF, and their CYP-specific metabolites (paracetamol-PAR; 4-OH diclofenac-4-DCF) were assessed after one-step liquid-liquid extraction procedure using validated RP-HPLC methods with diode array detection. DEM and its CYP2D2 specific metabolite dextrorphan (DOR) was assessed by the method described elsewhere (Zimova *et al* 2000), with only slight modifications. All experimental procedures were approved by the Czech Central Commission for Animal Welfare according to the Czech Act No. 246/1992. For statistical analysis, concentration ratios (metabolic ratios, MR) were calculated using following equations: $MR_{PHE/PAR} = c_{PHE}/c_{PAR}$, $MR_{DCF/4\text{-}DCF} = c_{DCF}/c_{4\text{-}DCF}$, $MR_{DEM/DOR} = c_{DEM}/c_{DOR}$. Repeated measure ANOVA with Fisher post-hoc test for multiple comparisons was used for the data analysis using software Statistica 8 for Windows. Data are expressed as means ± S.E.M. Values of $p<0.05$ were considered to be significant.

Results

The systemic 7-day treatment with fluoxetine significantly increased $MR_{PHE/PAR}$ in the 30th and 60th min ($p<0.01$). The Increase of $MR_{PHE/PAR}$ in the 120th min was insignificant, but trend for CYP1A2 inhibition was observed ($p=0.11$). Also the significant increase in $MR_{DCF/4\text{-}DCF}$ compared to control group was observed only in the 30th min of perfusion ($p<0.01$). The Increase in $MR_{DCF/4\text{-}DCF}$ in the 60th and 120th min was insignificant ($p=0.09$ and 0.48, respectively). The rate of *O*-demethylation of dextromethorphan was also decreased, $MR_{DEM/DOR}$ in the 30th min was significantly increased in fluoxetine-treated group compared to control group ($p<0.01$). In the 60th and 120th minute, there was observed only a trend for inhibition (increase of $MR_{DEM/DOR}$, $p=0.12$ and $p=0.6$).

The systemic 7-day raclopride tretament significantly increased $MR_{PHE/PAR}$ in perfusion medium in the 30th min of perfusion ($p=0.04$) compared to the control group. Differences in $MR_{PHE/PAR}$ between groups R and C in the 60th and 120th min were not statistically significant, but there was observed a trend ($p=0.08$ in 60th, $p=0.4$ in 120th min) for inhibition of metabolic activity of CYP1A2 (***Fig. 1***). The rate of hydroxylation of diclofenac ($MR_{DCF/4\text{-}DCF}$) as well as the rate of *O*-demethylation of dextromethorphan ($MR_{DEM/DOR}$) was not affected by 7 days of treatment with raclopride when compared to the control group (***Fig. 2*** and ***Fig. 3***) ($p>0.4$).

Discussion, conclusions

In this study, we have confirmed the inhibitory effect of fluoxetine on multiple CYP isoenzymes by means of the increased MR (compared to control group). The drug is known as an inhibitor of some human CYP enzymes, namely CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6 but the measure of such inhibition depends on distinct isoenzyme, and may be affected by the selection of specific substrates used by us (Hemeryck & Belpaire 2002).

Up to date, the effect of raclopride on the biotransformation processes was not described in the literature available. This study presents that at least 7 days of treatment with raclopride at the dose of 0.1 mg/kg decreases liver CYP1A2-mediated biotransformation. The question arises whether raclopride effect presented in this paper was caused by enzyme inhibition or raclopride-mediated D2 receptor antagonism in the central nervous system (mesolimbic or tuberoinfundibular dopaminergic pathways) as suggested elsewhere (Wojcikowski *et al* 2008) or due to the inhibition of synthesis of mRNA and CYP1A2 protein. To answer these questions, further studies are needed with e.g. *in vivo* intracerebral administration of raclopride into ventral tegmental area or nucleus accumbens or *in vitro* stud-

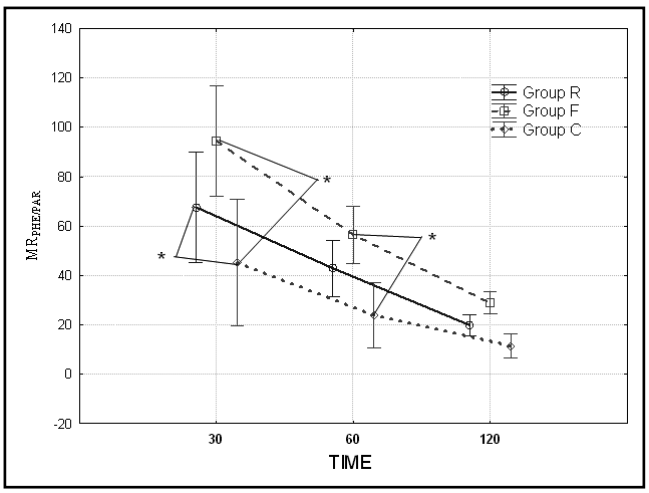

Fig. 1. $MR_{PHE/PAR}$ comparison between groups R (raclopride-treated), C (control) and F (fluoxetine-treated) in the 30th, 60th and 120th minute of perfusion *$p\leq0.05$.

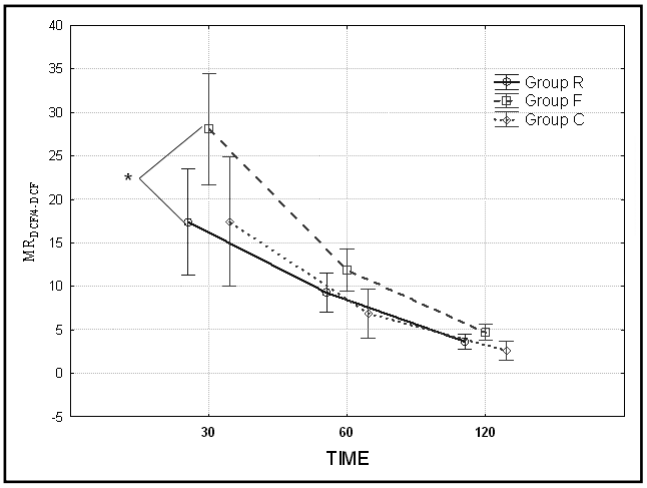

Fig. 2. $MR_{DCF/4\text{-}DCF}$ comparison between groups R (raclopride-treated), C (control) and F (fluoxetine-treated) in the 30th, 60th and 120th minute of perfusion.

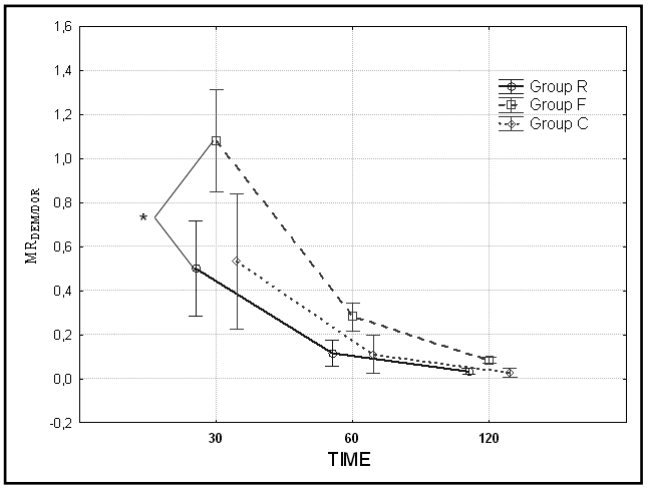

Fig. 3. $MR_{DEM/DOR}$ comparison between groups R (raclopride-treated), C (control) and F (fluoxetine-treated) in the 30th, 60th and 120th minute of perfusion.

ies for evaluation of the direct effect of raclopride on CYP1A2. With regard to quite high homology between human and rat CYP isoenzymes studied (Soucek & Gut 1992), our findings may be relevant also to clinical practice. Most of antipsychotic drugs elicit at least some D2 receptor antagonism and the considered influence on the liver biotransforming enzymes could have a great impact on the metabolism of them and a number of other drugs which undergo the same way of metabolism. The question remains, whether therapeutically used doses of antipsychotic medication can influence metabolic activity in a clinically significant manner.

Acknowledgement

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SHORT COMMUNICATION

Effects of ondansetron on social behaviour in male mice

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Background

The selective serotonin 5-HT3 receptor antagonist ondansetron is clinically used mainly to treat nausea and vomiting induced by chemotherapy. There are indications that it might be beneficial also in management of schizophrenia and alcoholism (Bennett & Vila 2010; Johnson 2010), however further studies are needed to elucidate the mechanisms involved and reveal other possible indications of ondansetron in clinics. Serotonergic neurotransmitter system plays a role in many central nervous functions, including those related to social behaviours such anxiety, fear and depression (Harmer *et al* 2006). In the present study, the aim was to assess the effect of repeated administration of ondansetron on behavioural profiles of singly-housed mice exposed to dyadic social interactions with non-aggressive group-housed male counterparts. The conspecific social conflict between a pair of adult male mice can be used as an ethological model for screening drugs for their behavioural effects (Krsiak 1975). In singly-housed male mice (isolates) during their interactions with non-aggressive group-housed partners the naturally occurring activities that can be characterised as sociable, defensive-escape (timid) or aggressive can be identified by ethological analysis, as well as the non-social activities such as ambulatory (locomotor) behaviours and rearing.

Methods

We used adult male mice of the albino ICR outbred strain (VELAZ s.r.o., Prague, Czech Republic). Animals were housed under constant light-dark cycle with lights on at 6.00 a.m. and off at 6:00 p.m. The animals (29–35 g) were randomly divided into two groups according to housing conditions. The mice housed in groups of 15–17 in standard plastic cages (38×22×14 cm) received no drug treatment. The other group (mouse isolates, n=62) were housed individually in self-cleaning cages (8×6×13 cm) for 21 days prior to behavioural testing that was performed during the light phase in the same room. On 22nd day each mouse isolate was administered with water orally and was transferred into the observational Plexiglas neutral cage (20×20×30 cm) with clean wooden shavings for 30 min adaptation period. Then the animal received a non-aggressive group-housed partner and their social interaction (control interaction) was video-recorded for 4 min. The frequencies of occurrence of the following behavioural elements were scored in the mouse isolates: sociable (following the partner, sniffing, climbing over the partner), timid (defence, escape, alert posture), aggressive (tail rattling, aggressive unrest, attack) agonistic activities and the locomotor parameters (walking, rearing). The behavioural data obtained from the singly-housed animals were subjected to the software system OBSERVER 3.1 (Noldus Information Technology b.v., Holland) used for further ethological and statistical analysis. The behavioural acts mentioned above were scored in the singly-housed individuals, while the non-aggressive group-housed partners served only as social stimuli for the mouse isolates. According to behavioural profiles during the initial 4-min agonistic interactions after water administration, we distinguished three behavioural types of

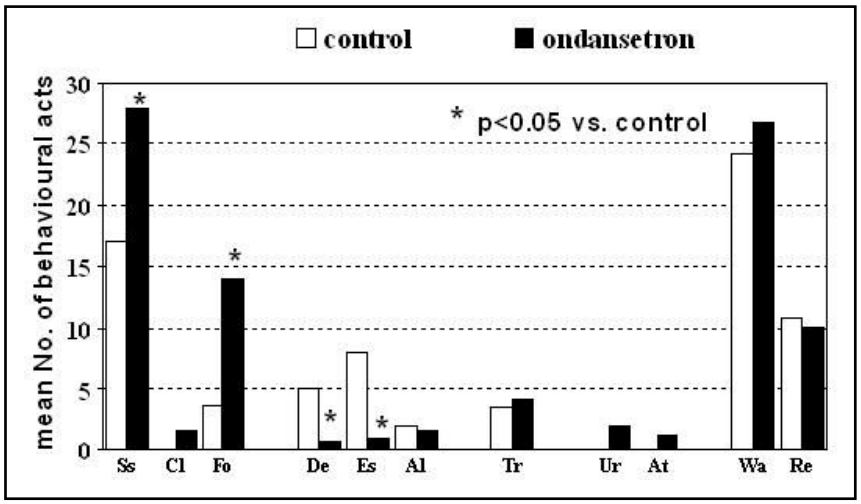

Fig. 1. Effect of ondansetron (1 µg/kg/day, orally for 21 days) on sociable (Ss, Cl, Fo), timid (De, Es, Al) and aggressive (Tr, Ur, At) behavioural elements and locomotor activities (Wa, Re) in singly-housed timid mice exposed to social interactions with non-aggressive group-housed partners. Ss – social sniffing, Cl – climbing, Fo – following the partner, De – defense, Es – escape, Al – alert posture, Tr – tail rattling, Ur – aggressive unrest, At – attack, Wa – walking, Re – rearing. Values represent mean frequencies. * $p<0.05$.

the subjects from individual housing. They were classified as a) aggressive (n=17), when at least one attack towards the non-aggressive partner occurred; b) timid (n=28), with pronounced defensive-escape behavioural elements and c) sociable (n=17), without attacks and with no defensive-escape activities (Pistovcakova & Sulcova 2002). They were randomly divided into two treatment groups with water administered as a control (10 ml/kg/day, orally), or ondansetron administered at the dose of 1 microgram/kg/day, orally in the same volume for three weeks. 24 hours after the last water/ondansetron administration the 4-min agonistic interaction of the singly-housed mouse with a non-aggressive group-housed mouse (the same partner as was in the previous behavioural testing) was performed using the same experimental conditions as described above (see the control interaction) and video-recorded for successive ethological analysis. Behavioural data subjected to the nonparametric Mann-Whitney statistical test were analysed separately for the timid, sociable and aggressive mice. The level of statistical significance was set at $p<0.05$. The study protocol was approved by the Animal Care Committee of the Masaryk University Brno, Faculty of Medicine, Czech Republic and carried out under the European Community guidelines for the use of experimental animals.

Results

In the singly-housed mice, which were in the control agonistic interaction classified as timid, ondansetron (1 microgram/kg/day, orally for 21 days) produced a significant ($p<0.05$) increase in the sociable behavioural acts such as sniffing and following the partner. Moreover, ondansetron significantly inhibited the frequencies of defences and escapes in the timid mice (***Fig. 1***). There were no significant antiaggressive effects induced by ondansetron in the aggressive group of isolates and neither there was any marked impact on behavioural profiles of the sociable group of mice (data not shown).

Conclusions

The behavioural data obtained indicate anxiolytic effect of ondansetron after its repeated administration. The explanation for this finding could be based on the fact, that 5-hydroxytryptamine (5-HT3) receptors are thought to participate in the stress-induced release of cortisol and adrenocorticotropin hormones (Patel *et al* 2011). The antagonistic action of ondansetron at 5-HT3 receptors could possibly reduce response to stress in timid mice. Present data add to ondansetron antidepressant-like effects described earlier in the model of depression induced in rats by bilateral olfactory bulbectomy (Pistovcakova *et al* 2010; Ramamoorthy *et al* 2008). Both, the anxiolytic and antidepressant potentials of ondansetron, that is often used in cancer patients following chemotherapy as the antiemetic agent, is a promising finding with regard to its potential psychotropic implications.

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