Effect of Acute and Repeated Administration of Methamphetamine on the Activity of CYP2D in Isolated Perfused Rat Liver

M. DOSTÁLEK[1], J. PISTOVČÁKOVÁ[1], V. MINAŘÍKOVÁ[1], J. JUŘICA[2], J. TOMANDL[2], E. HADAŠOVÁ[1]
[1]Masaryk University, Faculty of Medicine, Department of Pharmacology, Brno, Czech Republic

[2]Masaryk University, Faculty of Medicine, Department of Biochemistry, Brno, Czech Republic

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Abstract

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The aim of this study was to ascertain the influence of acute and repeated methamphetamine administration on metabolic activity of CYP2D2. The study was performed in the model of isolated perfused rat liver using dextromethorphan as a specific marker of CYP2D2 activity. Acute administration of methamphetamine (10, 20 or 40 mg/kg, i.p., once daily for two days) significantly increased dextrorphan O-formation in the isolated liver mediated by CYP2D2 at all investigated doses in the 120th min of liver perfusion. Repeated administration of methamphetamine (10, 20 or 40 mg/kg, i.p., once daily for six days) significantly stimulated dextromethorphan O-demethylation via CYP2D2 dose-dependently. Thus, an induction of CYP2D2 metabolic activity was proved in these experiments. With regard to a high level of homology between human and rat CYP isoform studied, the results may have clinical impact in pharmacotherapy of methamphetamine abusers.

Methamphetamine, Dextromethorphan, CYP2D, liver, isolated perfused, rat

The liver has been recognised for many decades as the major site of drug metabolism. By the 1960s, the drug metabolizing enzymes in the liver were characterized as heme proteins, now known as a cytochrome P450 microsomal enzymes. Adverse drug reactions are possible if compounds that act as inducers or inhibitors of these enzymes alter enzyme function. Enzyme induction results in accelerated enzyme synthesis, faster drug metabolism and sub-therapeutic drug concentration. Enzyme inhibition leads to accumulation of the drug, and if the drug has a narrow therapeutic window, serious toxicity may develop in a short time. Enzyme induction usually represents an absolute increase in enzyme synthesis, but phenobarbital, well-known enzyme inducer, increases blood flow to the liver, and its use results in increased liver size up to 50%. Currently, there are fewer than a dozen P450 inducer drugs of clinical importance. These include an phenobarbital (Levine et al. 1998; Valoti et al. 1998; Tang et al. 1999; Reid et al. 2002), glucocorticoids (Hoen et al. 2000; Tamasi et al. 2001; Eeckhoudt et al. 2002), rifampicin (Farombi et al. 1999; Branch et al. 2000; Niemi et al. 2003) and St. John's wort (Nebel et al. 1999; Barone et al. 2000; Breidenbach et al. 2000; Karliova et al. 2000; Piscitelli et al. 2000; Dostálek et al. 2005).

Methamphetamine has recently been reemerged as a significant drug abuse problem in the Czech Republic. Early study in humans (Caldwell et al. 1972) has shown that the urinary excretion products of this compound include the 4-hydroxy derivative, 4-hydroxy-methamphetamine and the N-demethylation product, amphetamine. These two dominant metabolites represent almost 50% of all metabolites excreted (Caldwell et al. 1972). The

Address for correspondence:
M. Dostálek
[1]Masaryk University, Faculty of Medicine
Department of Pharmacology
Tomešova 12, 602 00 Brno, Czech Republic

Tel.: +420-5-49493793
Fax.: +420-5-49493070
Email: mdostal@med.muni.cz (M. Dostalek),
hadasova@med.muni.cz (E. Hadasova)
http://www.vfu.cz/acta-vet/actavet.htm

results of Wu et al. (1997) have shown that 4-hydroxylation as well as N-demethylation of methamphetamine in human is catalyzed by CYP2D6.

The aim of our study was to ascertain the influence of acute and repeated methamphetamine pretreatment on metabolic activity of clinically most important isoenzyme of cytochrome P-450 in the model of isolated perfused rat liver. CYP enzymes comprise a large family of hemoprotein (Nelson et al. 1996), and enzymes from three families (CYP1, CYP2 and CYP3) are mainly involved in the biotransforation of xenobiotics in both human and rats (Nedelcheva and Gut 1994). Cytochrome P-450s from rats were one of the first isolated and characterized. Sequencial homology betwewn rat CYP2D2 and CYP2D6 is 71% (Souček and Gut 1992). The comparison make it clear when that similarities are high 70% (Souček and Gut 1992). Rat is a good model of metabolism depend on human CYP2D (Guengerich 1997). Dextromethorphan was used as a marker of CYP2D2 isozyme activity (Kobayashi et al. 2002). The dose of dextromethorphane was elicited from our previous pharmacokinetic experiments (Dostálek et al. 2003).

Materials and Methods

Animals and treatments

The experiment was carried out on male Wistar rats (weighing 220 ± 25 g, BioTest, Konárovice, Czech Republic) with free access to food and water under controlled environmental conditions (lights on from 6 : 00 a.m. to 6 : 00 p.m., temperature 21 – 22 °C, relative humidity 50 - 60%). After at least 10 days of adaptation to standard laboratory conditions, rats were randomly allocated and treated intraperitoneally according to the following design:

Experiment I – acute administration

Group 1, saline solution, 2ml/kg, once daily for 2 days; Group 2, Methamphetamine, 10 mg/2ml of saline solution/kg, once daily for 2 days; Group 3, Methamphetamine 20 mg/2ml of saline solution/kg, once daily for 2 days; Group 4, Methamphetamine 40 mg/2ml of saline solution/kg, once daily for 2 days.

Experiment II – repeated administration

Group 1, saline solution, 2ml/kg, once daily for 6 days; Group 2, Methamphetamine, 10 mg/2ml of saline solution/kg, once daily for 6 days; Group 3, Methamphetamine 20 mg/2ml of saline solution kg, once daily for 6 days; Group 4, Methamphetamine 40 mg/2ml of saline solution/kg, once daily for 6 days.

Chemicals

Methamphetamine hydrochloride, dextromethorphan hydrobromide, β-glucuronidase (from Escherichia coli) and Williams' Medium E from Sigma Chemical Co. (St. Louis, USA), all other chemicals and solvents used for HPLC assays were of HPLC or analytical grade and were obtained from Merck (Darmstadt, Germany) or Fluka Chemie AG (Buchs, Switzerland).

Liver preparation

The model of isolated perfused rat liver was used in our experiments. The liver was isolated from rat liver donors by a standard surgical technique under combined ketamine (Narkamon 5% 2ml/kg i.p.) and xylasine (Rometar 2% 0.8 ml/kg i.p.) anaesthesia. Glass canula was introduced into the portal vein, liver was shortly washed out by a tempered (38 °C) saline which in a short time was changed for the perfusion medium (120 ml of Williams medium E) equilibrated with 95% O_2 and 5% CO_2. The re-circulating perfusion apparatus was manufactured according to the principle originated by Miller et al. (1951). After 20 min pre-perfusion, specific marker - dextromethorphan (10.0 mg/l) was added as a bolus into the perfusion solution. Perfusion flow was maintained at a constant rate 25 ml/min. Samples of perfusate (1.0 ml) were withdrawn at the 30^{th}, 60^{th} and 120^{th} min of perfusion and stored at –30 °C until analysis. Each perfusion lasted 120 min. Liver viability was monitored by constant perfusion pressure and rate, pH of perfusate, oxygen consumption, liver weight and visual examination after finishing the perfusion.

Substrate assays

To determine dextromethorphan O-demethylase activity, 0.2 ml aliquot of the perfusion medium and beta-glucuronidase in sodium acetate buffer were added into the centrifuge tube (0.2 mol.l-1, pH = 5.0). The sample was gently mixed with a hand vortex mixer, the tubes were capped and incubated in a water bath at 37 °C for 18 h to ensure complete hydrolysis of conjugated metabolites. Internal standard (laudanosine 2.5 $mg \cdot l^{-1}$, in distilled water), 2.5 ml Na_2CO_3 (0.5 $mol \cdot l^{-1}$) and hexane: n-butanol (9 : 1, v/v) were added to this sample. All samples were mixed for 10 min and centrifuged for 5 min at 2,500 *g*. The layers were separated, the organic phase was vortexed gently with 0.3 ml of acetate buffer and centrifuged. The aliquots of the aqueous layer containing deconjugated compounds were injected into the HPLC. The HPLC system consisted of the phenyl column (C18), the mobile phase was acetonitrile – KH_2PO_4 (50 : 50, v/v) - 100 μl triethanolamine/100 ml mobile phase, pH 3.8. The chromatograph

consisted of a solvent delivery pump (Knauer HPLC pump 64), Rheodyne injection valve, Sepharon SGX Phenyl column (150 × 3 mm, 5 μm) and precolumn Sepharon SGX (30 × 3.7 μm), fluorescence detector (Shimadzu, model RX 535), Single Channel Recorder (Pharmacia) and Hewlett Packard Integrator (model 3390A). The volume of each sample injected into column was 20 μl. The flow rate was maintained at 0.6 ml/min. The fluorescence at 320 nm was measured on excitation 280 nm. Duration of analysis was 11 min.

Statistical analysis

Dextromethorphan and dextrorphan perfusate concentrations vs. time data were analyzed according to a one-compartment pharmacokinetic model. The data was analysed by Kinetica version 4.1 (InnaPhase Corporation). The statistical significance of the effects of drugs on the perfusate concentration of markers was determined by one-way analysis of variance (ANOVA), followed by Fisher's protected least significant difference test (PLSD; SigmaStat). Data is expressed as means ± SEM. Values of $p < 0.05$ were considered to be significant.

Results

Methamphetamine was well-tolerated by all animals, body and liver weights rats treated with methamphetamine (246.0 ± 20.0 g and 10.5 ± 1.5 g, respectively) were similar to those in control rats (250.0 ± 15.0 g and 10.0 ± 2.0 g, respectively).

Acute pre-treatment with methamphetamine

The effect of acute administration of methamphetamine (10, 20 or 40 mg/kg, i.p., once daily for two days) on the pharmacokinetics of dextromethorphan is demonstrated in Fig. 1. Columns in the graphs demonstrate concentrations of dextromethorphan (A) and dextrorphan - (B) in the 30th, 60th and 120th minute of liver perfusion. The kinetic characteristics of dextromethorphan O-demethylation to dextrorphan via CYP2D2 in the livers are given in the Table 1.

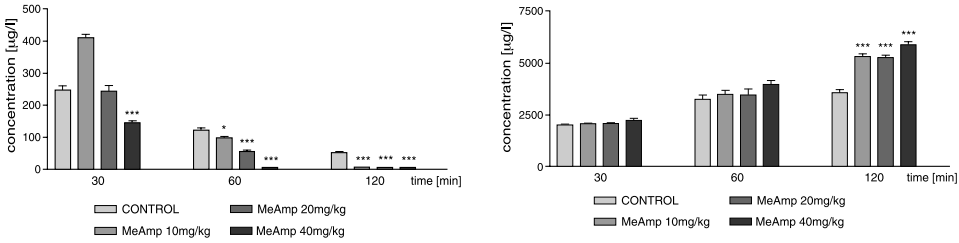

Fig. 1. Perfusate dextromethorphan (A) and dextrorphan (B) concentration after acute pretreatment with various doses of methamphetamine. Data are mean ±SEM (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

Table 1. Pharmacokinetic parameters of dextromethorphan (DEM) and dextrorphan (DOR) in the isolated perfused rat liver after acute administration of methamphetamine (10 mg/kg, 20 mg/kg, 40 mg/kg, i.p., once daily for two days). Data are expressed as means ± SEM

Group	N	$C_{max\ DOR}$ (μg/l)	$t_{1/2\ DEM}$ (min)	Cl_{DEM} (l/h)	AUC_{DEM} (μg/l/h)	AUC_{DOR} (μg/l/h)
Control	7	3692 ± 158	19 ± 3	3.02 ± 0.05	324 ± 8	5964 ± 166
MeAmp 10 mg/kg	8	5323 ± 140 ***	12 ± 1 ***	3.54 ± 0.03 ***	246 ± 9 ***	6878 ± 81 ***
MeAmp 20 mg/kg	8	5000 ± 137 ***	10 ± 1 ***	4.23 ± 0.02 ***	226 ± 5 ***	6812 ± 55 ***
MeAmp 40 mg/kg	9	5952 ± 153 ***	6 ± 1 ***	9.75 ±0.9 ***	76 ± 2 ***	7709 ± 76 ***

$C_{max\ DOR}$ – maximum dextrorphane perfusate concentration, $t_{1/2\ DEM}$ – perfusate elimination half life, Cl_{DEM} – clearance, AUC_{DEM} – area under the entire perfusate concentration of dextromethorphan, AUC_{DOR} – area under the entire perfusate concentration of dextrorphan (*** $p < 0.001$)

Repeated pre-treatment with methamphetamine

The effect of repeated administration of methamphetamine (10, 20 or 40 mg/kg, i.p., once daily for six days) on the pharmacokinetics of DEM is demonstrated in Fig. 2. Columns in the graphs represent concentrations of dextromethorphan (A) and dextrorphan (B) in the 30th, 60th and 120th minute of liver perfusion. The kinetic

characteristics of dextromethorphan O-demethylation to dextrorphan via CYP2D2 in the livers are given in the Table 2.

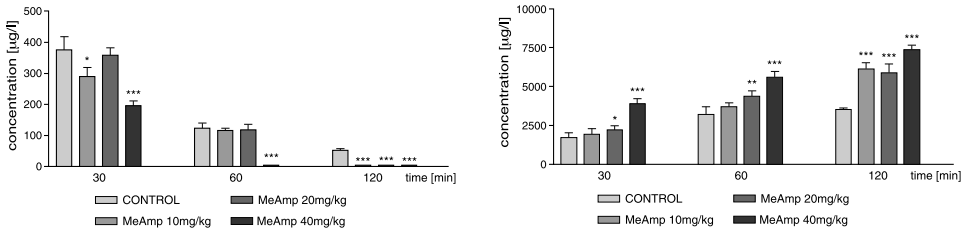

Fig. 2. Perfusate dextromethorphan (A) and dextrorphan (B) concentration after repeated pretreatment with various doses of methamphetamine. Data are mean ± SEM (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$)

Table 2. Pharmacokinetic parameters of dextromethorphan (DEM) and dextrorphan (DOR) in the isolated perfused rat liver after repeated administration of methamphetamine (10 mg/kg, 20 mg/kg, 40 mg/kg, i.p., once daily for six days). Data are expressed as means ± SEM

Group	N	$C_{max\ DOR}$ (µg/l)	$t_{1/2\ DEM}$ (min)	Cl_{DEM} (l/h)	AUC_{DEM} (µg/l/h)	AUC_{DOR} (µg/l/h)
Control	7	3780 ± 87	19 ± 6	3.01 ± 0.06	309 ± 12	5922 ± 93
MeAmp 10 mg/kg	8	6105 ± 162 ***	10 ± 1 **	4.04 ± 0.07 **	206 ± 7 ***	7321 ± 43 ***
MeAmp 20 mg/kg	8	5906 ± 249 ***	8 ± 1 ***	4.62 ± 0.08 ***	179 ± 4 ***	8095 ± 65 ***
MeAmp 40 mg/kg	9	7643 ± 130 ***	5 ± 1 ***	10.5 ± 0.14 ***	60 ± 2 ***	10963 ± 670 ***

$C_{max\ DOR}$ – maximum dextrorphane perfusate concentration, $t_{1/2\ DEM}$ – perfusate elimination half life, Cl_{DEM} – clearance, AUC_{DEM} – area under the entire perfusate concentration of dextromethorphan, AUC_{DOR} – area under the entire perfusate concentration of dextrorphan (*** $p < 0.001$; ** $p < 0.01$)

Discussion

This study provides the first direct evidence that methamphetamine, a potent and highly abusable psychomotor stimulant with a wide range of behavioral actions, is an inducer of CYP2D isozymes in the isolated perfused rat liver. Perfused liver systems simulate conditions *in vivo* more than any of the above techniques. Though, *in vivo* studies using whole animals are the most physiological, but the multitude of variables introduced by changes in the hormonal, circulatory, and neural systems make interpretation of the data difficult or imposible. In the perfused liver studies, hepatic architecture, microcirculation, and bile production are maintained, and studies of the role of different cell types in specific events, imposible with isolated hepatocytes preparations, can be undertaken easily. It is possible to monitor several biochemical variables continuously in the perfused liver, and thus it is an ideal model for study of intermediary metabolism and biotransformation of xenobiotics and their interactions. Disadvantages of the perfused liver system include the loss of neural and hormonal signals present *in vivo* and the absence of nutrients provided from the diet and peripheral tissues. However, studies comparing drug metabolism in the perfused liver and *in vivo* show good correlation between the *in vivo* and perfused liver models (Bickel and Minder 1970; Von Bahr et al. 1970).

Systemic acute administration of methamphetamine to rats resulted in a significant and dose dependent decrease in AUC of dextromethorphan, shortened half-life of dextromethorphan and increase in dextromethorphan clearance. Concurrently, AUC of major metabolite dextrorphan was significantly decreased, namely, after the dose of 40 mg/kg of methamphetamine. Thus, the results of the acute administration of methamphetamine suggest its stimulatory effect on dextromethorphan to dextrorphan

metabolic conversion, i.e. reaction catalyzed by CYP2D2 isozyme. This effect intensifies with time, reaching a maximum in the 120th minute for all doses of methamphetamine. The results allowed us to conclude that the stimulation of dextrorphan formation in the CYP2D2 mediated reaction did not closely depend on the dose of methamphetamine. This statement was well founded, supported by the statistically non-significant differences in the concentration of dextrorphan in the perfusion medium after the administration of different doses of methamphetamine, particularly when the effect of 10 mg/kg was compared with that of 40 mg/kg of methamphetamine.

Repeated administration of methamphetamine resulted in a significant decrease in AUC and half-life of dextromethorphan, increase in Cl of dextromethorphan and above all, in a marked enlargement of AUC of dextrorphan. The results of the repeated administration of methamphetamine at the above-mentioned doses resembled the results described in the acute experiment to some extent. Here, the differences between the methamphetamine-treated groups and the control group were statistically significant again. The level of the statistical significance increased with time and reached its maximum at the 120th minute of the perfusion. There was certain similarity of changes observed after the administration of 10, 20 and 40 mg/kg of methamphetamine. One could thus conclude from the results obtained after the repeated administration of methamphetamine that the stimulation of O-demethylation was dose-dependent.

Induction of CYP enzymes can result in clinical drug interactions whereby the systemic exposure to one drug that is cleared primarily via CYP-mediated biotransformation is elevated when coadministered with a second drug that induces this activity. During the past decade, it has become increasingly facile to be able to conduct *in vitro* experiments with human/ aminal CYP isozymes or human/ aminal tissue preparations to measure the induction of CYP activity. Such data can be used to predict whether the potential exist for a drug interaction *in vivo*. For example, methamphetamine biotransformation is inhibited by fluoxetine co-administration; which could be used in methamphetamine dependence treatment and enables the reduction of methamphetamine dose without decreasing methamphetamine psychotropic effect (Dostálek et al. 2003).

This preclinical study did not support observations of W u et al. (1997) regarding plasma concentrations of various drugs metabolized by CYP450 following methamphetamine ingestion. They reported from their study *in vitro* that all the amphetamine analogs sharing the phenylisopropylamine structure acted as inhibitors of CYP2D6 activity in human microsomes. Their apparent Ki values ranged over three orders of magnitude – from 0.17 to 340 μM (amphetamine and methamphetamine had Ki values 26.5 and 25 μM, respectively). The polar metabolite of amphetamine, (+)-4-hydroxyamphetamine, was 7-fold less potent inhibitor than (+)-amphetamine itself, and the metabolite of methamphetamine, 4-hydroxymethamphetamine, was 2.5 fold less potent than methamphetamine.

In contrast, the present results are in a full agreement with results of our previous pharmacokinetic experiments (Dostálek et al. 2002); significant reduction of plasma concentrations of dextromethorphan was found when methamphetamine had been given intraperitoneally at the dose of 10 mg/kg/day for six days; the mentioned study *in vivo* provides the first direct evidence that methamphetamine is an inducer of CYP2D2 metabolic activity (Dostálek et al. 2002).

Finally, the present findings might help to explain and predict possible methamphetamine interactions with co-administered drugs - other substrates of CYP2D like neuroleptics, antidepressants, lipophylic beta-blockers or opioids on the pharmacokinetic level. Cytochrome P-450s from rats were one of the first isolated and characterized. With regard to a high level of homology between human and rat CYP isoforms studied, the results may have clinical impact in pharmacotherapy of methamphetamine abusers.

Vliv akutního a opakovaného podávání metamfetaminu na metabolickou aktivitu CYP2D na modelu izolovaných perfundovaných jater

Cílem studie bylo popsat vliv akutního a opakovaného podávání metamfetaminu na metabolickou aktivitu izoenzymu CYP2D2. Studie byla provedena na modelu izolovaných perfundovaných jater, jako specifický marker metabolické aktivity izoenzymu CYP2D2 byl použit dextrometorfan. Na modelu izolovaných perfundovaných jater akutní podání metamfetaminu (10, 20 or 40 mg/kg, i.p., jednou denně po dobu dvou dnů) statisticky významně zvýšilo O-demetylaci dextrometorfanu na dextrorfan, reakci katalyzovanou CYP2D2. Zvýšení bylo popsáno ve 120. minutě jaterní perfuze po všech sledovaných dávkách, u všech sledovaných dávek. Opakované podávání metamfetaminu (10, 20 nebo 40 mg/kg, i.p., jednou denně po dobu šesti dnů) též statisticky významně stimulovalo dextrometorfanu. V provedeném experimentu byl popsán stimulační vliv metamfetaminu na metabolickou aktivitu CYP2D2. S ohledem na vysoký stupeň podobnosti studované lidské a potkaní izoformy CYP mohou mít získané výsledky klinický význam ve farmakoterapii jedinců závislých na metamfetamin.

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