Methamphetamine does not influence the metabolic activity of CYP 1A2, 2C6 and 2D2 isoenzymes in the animal study

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Introduction

Methamphetamine (MET) belongs to the amphetamine group of sympathomimetic drugs. It is approved by the FDA for the treatment of ADHD and exogenous obesity, but it is frequently illegally used for its psychostimulatory, euphorizing and anorectic activities. The abuse as well as the pharmacotherapy with MET brings out the problem of drug-drug interactions, when MET is co-administered/abused with other medications. On the pharmacokinetic level the interactions can be based on biotransformation of MET by CYP450 enzymes (Lin et al 1995). MET abusers, who are extensive metabolizers are more sensitive to neurocognitive impairment caused by MET's metabolites (Cherner et al 2010). The only study (Dostalek et al 2007) available according to the literature aimed on the influence of MET on the rat CYP enzymes indicates that MET acts as an inducer. Thus the knowledge whether MET modulates the activity of CYP is important for safe pharmacotherapy with MET, and perhaps also for the prediction of cognitive disorders in abusers. The aim of the present study was to investigate MET influence on the activity of selected CYP isoenzymes in the model of isolated perfused rat liver.

Methods and materials

Animals

Male Wistar albino rats (Biotest, Konarovice, CZ) weighing 220 ± 20g were divided into 2 groups of 10 animals. For 10 days the Group I was administered intraperitoneally with MET (Sigma Chemical Co., St. Louis, USA) dissolved in saline at increasing doses in bolus injections as follows: 1st day – 2.5 mg/kg/day; 2nd day – 5 mg/kg/day; 3rd day – 7.5 mg/kg/day; 4th–10th days – 10 mg/kg/day. The group II (control animals) was administered in the same manner with saline. All experimental procedures were approved by the Czech Central Commission for Animal Welfare.

Model of isolated perfused rat liver

Model of perfused rat liver was used for the determination of CYP 1A2, 2C6 and 2D2 isoenzyme activity. Rats were anesthetized with the combination of ketamine (100 mg/kg) and xylasine (16 mg/kg). The liver isolation was performed as described elsewhere (Zendulka et al 2009). Liver was then placed into the modified Miller's (Miller et al 1951) perfusion apparatus and was perfused with Williams' medium E (Sigma Chemical Co., St. Louis, USA).

Marker substances phenacetin (PHE)-CYP 1A2, diclofenac (DCL)-2C6 and dextromethorphan (DEM)-2D2 (all Sigma Chemical Co., St. Louis, USA) were added as a bolus into perfusion medium after 20 minutes of pre-perfusion. Perfusion medium samples were collected in the 30th; 60th and 120th minute of perfusion.

Quantitative analysis of markers and metabolites

Levels of markers and their specific metabolites paracetamol (PAR)-1A2; 4-hydroxydiclofenac (HDCL)-2C6 and dextrorphan (DEX)-2D2 were analyzed after extraction from perfusion medium using validated HPLC method.

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Tab. 1: Levels of phenacetin (PHE), paracetamol (PAR) and their metabolic ratio ($MR_{PHE/PAR}$) in the 30th, 60th and 120th minute of perfusion as the measure of the CYP 1A2 metabolic activity. Data are expressed as means ± S.E.M.

	Control	Methamphetamine
PHE [mg/L]		
30	7.8 ± 0.7	7.6 ± 0.2
60	7.1 ± 0.8	6.9 ± 0.6
120	6.3 ± 0.7	6.4 ± 0.7
PAR [mg/L]		
30	0.21 ± 0.10	0.16 ± 0.07
60	0.39 ± 0.21	0.26 ± 0.15
120	0.69 ± 0.34	0.53 ± 0.30
$MR_{PHE/PAR}$		
30	45.2 ± 22.2	58.0 ± 21.5
60	23.8 ± 13.2	32.9 ± 12.2
120	11.5 ± 6.0	14.8 ± 8.5

Tab. 2: Levels of diclophenac (DCL), 4-hydroxydiclophenac (HDCL) and their metabolic ratio ($MR_{DCL/HDCL}$) in the 30th, 60th and 120th minute of perfusion as the measure of the CYP 2C6 metabolic activity. Data are expressed as means ± S.E.M.

	Control	Methamphetamine
DCL [mg/L]		
30	1.29 ± 0.32	1.26 ± 0.32
60	0.83 ± 0.19	0.96 ± 0.17
120	0.49 ± 0.18	0.82 ± 0.49
HDCL [mg/L]		
30	0.07 ± 0.01	0.06 ± 0.04
60	0.12 ± 0.03	0.11 ± 0.07
120	0.19 ± 0.03	0.22 ± 0.10
$MR_{DIC/4-OHDIC}$		
30	17.4 ± 2.9	24.1 ± 8.8
60	6.9 ± 1.2	10.0 ± 3.1
120	2.6 ± 0.9	4.1 ± 1.3

Tab. 3: Levels of dextrometorphan (DEM), dextrorphan (DEX) and their metabolic ratio ($MR_{DEM/DEX}$) in the 30th, 60th and 120th minute of perfusion as the measure of the CYP 2D2 metabolic activity. Data are expressed as means ± S.E.M. *$p \leq 0.05$; **$p \leq 0.01$

	Control	Methamphetamine
DEM [µg/L]		
30	42.8 ± 12.1	70.0 ± 22.0**
60	16.5 ± 5.0	45.3 ± 21.6**
120	5.3 ± 3.1	22.6 ± 13.6**
DEX [µg/L]		
30	81.8 ± 44.6	104.5 ± 54.0
60	154.1 ± 76.2	249.5 ± 80.1*
120	237.5 ± 149.9	391.3 ± 98.1*
$MR_{DEM/DEX}$		
30	0.64 ± 0.31	0.64 ± 0.15
60	0.13 ± 0.07	0.17 ± 0.08
120	0.03 ± 0.02	0.05 ± 0.02

Analysis of both, markers and metabolites, was performed on Shimadzu HPLC chromatograph using described methods (Zendulka et al 2008; Zimova et al 2001). Metabolic ratios (MRs) were calculated by equation MR = conc. marker / conc. metabolite.

Statistical analyses

Values of DEM and DEX concentration were tested for outliners with the Dixon's Q-test and outliners were rejected (95% confidence level). Remaining values were statistically analyzed with the F-test and Student's t-test (Microsoft Excel 2000). The level $p \leq 0.05$ was considered to be a statistically significant difference.

Results

Concentrations of PHE and DCL in the perfusion medium as well as amounts of their metabolites PAR and HDCL did not differ between MET administered and control animals (Tab.1 and Tab. 2). The metabolic activities of CYP 1A2 and 2C6 isoenzymes were similar between MET and control animals, as MRs were not different between monitored groups.

The levels of DEM were elevated significantly ($p \leq 0.01$) in MET administered animals during all sampling intervals and similarly, levels of DEX were higher in MET group than in controls except the 30th minute of perfusion (Tab. 3). DEM/DEX metabolic ratios of MET animals were similar to that of control rats, due to the elevation of both DEM and DEX and therefore MRs was not affected.

Conclusions

Our present results documented that administration of MET at graduating doses for 10 days does not influence the hepatic metabolic activity of 1A2, 2C6 or 2D2 iso-enzymes in rat. This finding is different from the other published results (Dostalek *et al* 2007) describing the induction of all three isoenzymes in a dose dependent manner when 4 doses of MET from 10mg/kg/day up to 40 mg/kg/day were applied for 6 days. The dissimilarity in dosing regimen used in these two studies could be a reason of differential results received. No significant changes of the CYP 1A, 2A, 3A, 2B or 2D activity except the inhibition of isoenzyme 2E were found in the *in vitro* model of rat liver microsomal fraction when MET was given *in vivo* orally at the dose of 10 mg/kg/day for 5 days (Minarikova et al 2006). Despite the relatively low dose and the oral route of administration (which could substantially influence results obtained) results obtained by Minarikova correlate with our presented results. Thus we conclude that methamphetamine at the dose of 10 mg/kg/day does not influence the activity of CYP but the induction after high doses can not be excluded.

Acknowledgement

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