

ORIGINAL ARTICLE

Enhancement of psychostimulant effect and development of behavioural sensitisation to methamphetamine in mice by combined treatment with piracetam

Alena MACHALOVA^{1,2}, Karel SLAIS^{1,2}, Dagmar VRSKOVA^{1,2}, Alexandra SULCOVA^{1,2}

¹Central European Institute of Technology (CEITEC), Masaryk University, Brno; ²Faculty of Medicine, Department of Pharmacology, Masaryk University, Brno, Czech Republic.

Correspondence to: Alena Machalova, MD., Department of Pharmacology, Faculty of Medicine, Masaryk University Brno, Kamenice 5, 625 00 Brno, Czech Republic; TEL: +420 549 494 421; FAX: +420 549 492 364; E-MAIL: amachal@med.muni.cz

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Abstract

The present study evaluated in mice the possible modification of psychostimulant methamphetamine (MET) effects by co-administration of piracetam (PIR) and the development of behavioural sensitisation to the psychostimulant in the case of combined treatment.

On the Day 1 mice were randomly allocated into 4 groups and control open field test was performed. On the Day 7, after 5 days without treatment, mice were intraperitoneally administered: a) saline; b) MET 2.5 mg/kg/day; c) PIR 300 mg/kg/day; d) MET+PIR. These doses were repeated for 6 following days. On the Day 14 the acute “challenge” dose of MET 2.5 mg/kg was given to all groups and locomotor activity was measured. On the Day 21, after 6 days of withdrawal, MET challenge was repeated. Data were analysed using two-way ANOVA and Bonferroni post-test.

Significant increase in locomotion was recorded in mice treated with acute doses of MET+PIR compared with animals treated with MET ($p < 0.05$). The increased locomotor responses to MET challenge doses (a sign of behavioural sensitisation) after the repeated treatment were more pronounced in animals pre-treated with MET+PIR than in those pre-treated with just PIR or MET ($p < 0.01$ and $p < 0.001$).

Piracetam in our experiment enhanced acute effects of methamphetamine as well as development of sensitisation to its stimulatory effects. Background of these pharmacological drug-drug interactions remains unclear. Although clinically piracetam itself shows minimal adverse effects and toxicity, its combination with methamphetamine could worsen the negative drug abuse consequences.

INTRODUCTION

Piracetam (PIR) is a nootropic drug chemically related to neurotransmitter γ -aminobutyric acid (GABA). It was the first drug acting on cognition without causing sedation (Giurgea 1972). In spite of structural similarity of piracetam with GABA and its ability to modify functions of many neurotransmitter systems,

its actions are currently considered not to be based on direct interactions with any major postsynaptic receptor.

Its efficacy in the nervous tissue includes indirect modulation of several neurotransmitter systems, neuroprotective and anticonvulsant effects and positive influence on neuronal plasticity. It enhances glucose and oxygen metabolism in hypoxic brain tissue (Noble

& Benfield 1998). For its varied actions piracetam is used for treatment of many neurological conditions such as dementia and cognitive disorders, dyslexia or impaired electrical activity in sensorimotor cortex (treatment of cortical myoclonus).

Although large number of published works attends piracetam actions, some areas of interest remain to be clarified. There are reports that human abusers tend to combine piracetam with illicit psychostimulant drug methamphetamine (MET). They describe intensified efficacy of psychostimulant and suppressed its adverse effects. However, it is possible that those references are spread and supported by drug dealers, because then they can decrease the amount of MET in the drug cadget unit pack and replace it with piracetam (and decrease their expenses). In an effort to assess the possibility of such pharmacological interaction, the first aim of our study was to evaluate modification of methamphetamine stimulatory effects by piracetam co-administration.

The repeated administration of different drugs of abuse may result in so called behavioural sensitisation. It is a behavioural phenomenon, which plays an important role in development of the drug addiction and may be responsible for relapse to drug-seeking behaviour during the therapy. Sensitisation is defined as an increased response to the same dose of drug after previous long-term intermittent administration of this drug (Ohmori *et al* 2000; Robinson & Berridge 1993). Behavioural sensitisation is usually manifested after both repeated doses and application of a „challenge dose” administered after a certain period of withdrawal. Behavioural sensitisation is believed to be caused by persistent adaptations in striatal neurotransmission associated with repeated exposure to drugs of abuse (Vanderschuren *et al* 2001). There is evidence indicating that behavioural sensitisation can be divided into two temporally defined domains, called development (or initiation) and expression (Kalivas *et al* 1993). The term „development” of behavioural sensitisation refers to the progressive alterations in the ventral tegmental area and substantia nigra. These changes are temporary and may not be detected after a few weeks of withdrawal (Kalivas *et al* 1993). The term „expression” of behavioural sensitisation is defined as the enduring neural changes among several limbic nuclei centred on the nucleus accumbens (Pierce & Kalivas 1997).

Because of a high importance of behavioural sensitisation phenomenon in the methamphetamine dependence neurobiological mechanisms, the second aim of our study was to investigate the effects of piracetam and methamphetamine co-administration on the development and expression of behavioural sensitisation to psychostimulatory effects in mice. Because the most common symptom of behavioural sensitisation in laboratory rodents is an increase in locomotor/exploratory activities, the open field test was used for assessment of the effects of drugs.

MATERIAL AND METHODS

Animals

Adult male mice of the SPF ICR outbred strain (18–20 g) were used for experiments. Animals were housed in cages with free access to food and water and maintained at a temperature of 22 °C on a 12:12 h light–dark cycle (lights on at 08:00). Experimental sessions were always performed in the same light period (8:00–11:00 a.m.) in order to minimise possible variability due to circadian rhythms. The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the local Institutional Animal Ethics Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs

(+) Methamphetamine (d-N,a-Dimethylphenylethylamine; d-Desoxyephedrine), Sigma Chemical Co., dissolved in saline. Piracetam (2-oxo-1-pyrrolidine acetamide), Geratam inj. sol., UCB Pharma S.p.A., Italy, dissolved in saline. Vehicle and diluted drugs were always administered in a volume of 10 ml/kg.

Experimental procedure

Effects of drugs on the horizontal locomotor activity were tested in the open field test (Actitrack, Panlab S.L., Spain). On the Day 1, mice were randomly allocated into 4 groups and the first measurement was made. There were no applications in following 5 days. On the Day 7, four groups of mice were administered intraperitoneally a) saline; b) MET 2.5 mg/kg/day, 15 minutes prior to measurement; c) PIR 300 mg/kg/day, 60 minutes prior to measurement; d) MET+PIR. These applications were repeated for 6 following days. On the Day 14 acute “challenge” dose of MET 2.5 mg/kg was given to all groups and locomotor activity was measured. On the Day 21, after 6 days of withdrawal, mice were re-challenged again with MET 2.5 mg/kg and the fourth measurement of locomotion was made. This dosing regime had been used and confirmed to be efficient in previous experiments performed in our department (Kucerova *et al* 2006; Kucerova & Sulcova 2008). Data were analysed using two-way ANOVA and Bonferroni post-test. Level of statistical significance was determined to $p < 0.05$.

RESULTS

The first measurement (Day 1) showed no difference in all groups of animals and confirmed homogeneity of the groups (**Fig. 1**). In the following session (Day 7), when acute effects of drugs were recorded, MET given alone significantly ($p < 0.05$) increased locomotion. PIR given alone caused no significant changes in the mouse behaviour. Stimulatory influence of PIR+MET administered in combination was significantly higher in com-

parison with either MET or PIR alone ($p < 0.01$ and $p < 0.001$) (Fig. 2). On the Day 14 stimulatory effect of MET in mice was significantly increased after repeated pre-treatment with MET+PIR compared to only MET or PIR pre-treated animals ($p < 0.01$ and $p < 0.001$) (Fig. 3). On the Day 21 enhancement of locomotion in groups pre-treated with MET and MET+PIR was registered, however, this increase was not significant when compared to groups pre-treated with saline or PIR alone (Fig. 4).

DISCUSSION

In our experiment in mice we have confirmed potentiating effects of piracetam on MET stimulatory actions. While in acute dose used PIR itself had no stimulatory effect, it enhanced stimulatory effect of MET. The

development of sensitisation was recorded in measurement taken on the Day 14 (after 7 days of applications). The group of animals pre-treated with combination MET+PIR showed pronounced stimulation of locomotion compared to animals pre-treated with MET. However, this enhancing effect was not apparent after the wash-out period (Day 21). Interestingly in this paradigm MET alone had not been proved to cause development and expression of behavioural sensitisation as was expected with reference to previous experiments (Lorrain *et al* 2000; Mendrek *et al* 1998; Segal & Mandell 1974).

Sensitisation to drugs of abuse reported to be accompanied by plastic changes in mesocorticolimbic system (Thomas *et al* 2008; Wolf 1998), however, exact background of its development has not been fully clarified yet. More molecular mechanisms are considered to be

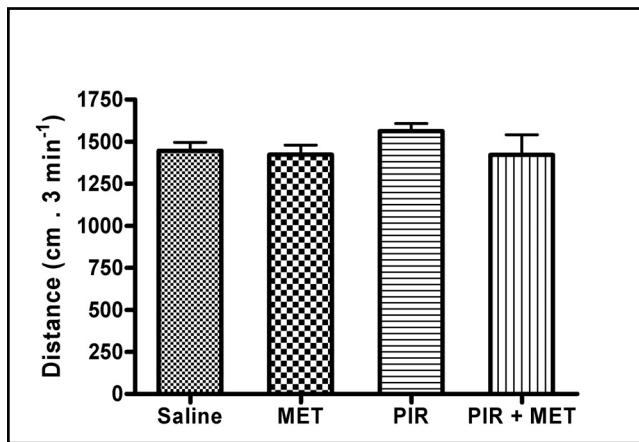


Fig. 1. Day 1 – the control measurement without application of drugs confirmed homogeneity of the groups of mice (mean ± SEM shown).

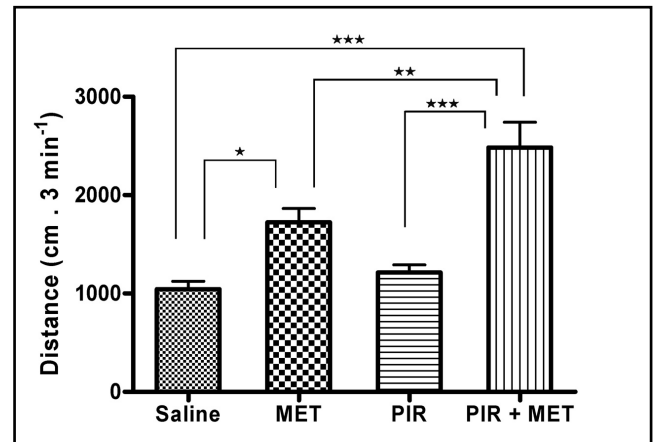


Fig. 2. Day 7 – Acute effects of drugs on horizontal locomotor activity. Piracetam enhanced methamphetamine stimulatory effect. MET: methamphetamine 2.5 mg/kg; PIR: piracetam 300 mg/kg. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; mean ± SEM shown).

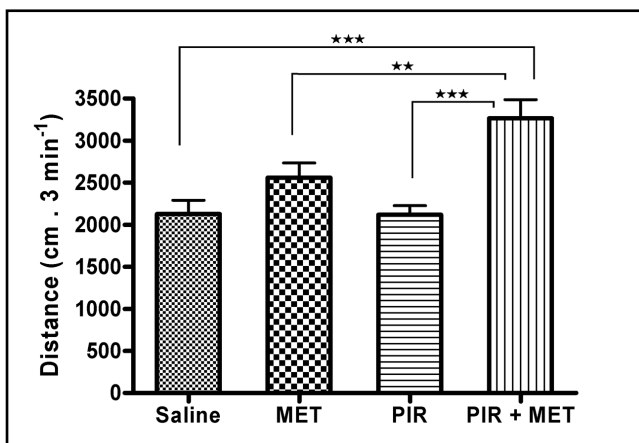


Fig. 3. Day 14 – Horizontal locomotor activity after challenge dose of MET 2.5 mg/kg. Animals pre-treated for 7 days with combination of MET+PIR showed development of sensitisation. MET: pre-treatment with methamphetamine 2.5 mg/kg; PIR: pre-treatment with piracetam 300 mg/kg. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; mean ± SEM shown).

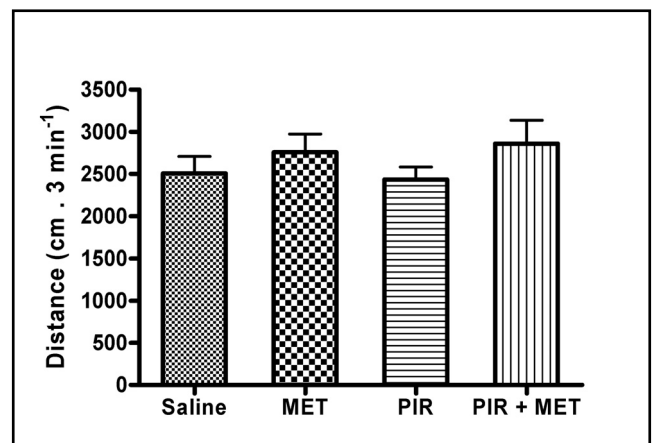


Fig. 4. Day 21 – Horizontal locomotor activity of animals after the second challenge dose of MET 2.5 mg/kg. Expression of sensitisation after 6 days of withdrawal was not recorded in any group of animals. MET: pre-treatment with methamphetamine 2.5 mg/kg; PIR: pre-treatment with piracetam 300 mg/kg. (mean ± SEM shown).

involved. One of the most probable theories is alteration of dopamine turnover, which is the mechanism shared by the most of psychostimulant drugs.

In previous studies piracetam indirectly influenced a number of neurotransmitter systems including dopaminergic and glutamatergic ones (Gouliaev & Senning 1994). It was shown that piracetam interferes with level of dopamine in the perfused isolated rat brain by increasing the K⁺-stimulated dopamine release (Budygin *et al* 1996). Others publications describe changed levels of dopamine or its metabolite homovanillic acid in the brain after administration of high doses of piracetam (Pavlik *et al* 1987; Petkov *et al* 1984). However, studies where lower doses of piracetam were used showed no significant influence on dopamine system (Bhattacharya *et al* 1989). The dose which we had used should be with respect to listed studies high enough to influence dopaminergic system, setting up a plausible explanation of our results.

During the last two decades the significance of excitatory amino acid transmission in addiction has been widely discussed (Gass & Olive 2008; Sulcova & Jezova 2006). Glutamate is the major excitatory neurotransmitter of the brain and the reward circuit consisting of dopamine releasing neurons receives multiple glutamatergic input (Ikemoto 2010). Many studies have already shown that amphetamines as well as other psychostimulants elevate extracellular levels of glutamate (Del Arco *et al* 1998; Gass & Olive 2008). Piracetam is known to interact with glutamatergic system in a yet not fully understood way (Gouliaev & Senning 1994). Because glutamatergic system impair was described in cognitive deficits (Ernst *et al* 2010; Segovia *et al* 2001), it is possible that this mechanism contributes to piracetams positive impact on cognition. Recent work showed that piracetam allosterically modulates glutamatergic AMPA receptors (Ahmed & Oswald 2010), structure involved in positive reinforcement in addiction (Bowers *et al* 2010).

While mechanism of increasing level of dopamine, noradrenalin and serotonin in the synaptic cleft by methamphetamine is well described (Sulzer *et al* 2005), background of interactions of piracetam with methamphetamine remains unclear. Piracetam seems to be able to modulate many monoaminergic pathways in unknown way and it is highly probable that its ability to interfere with changes in the brain depends on all of these (Gouliaev & Senning 1994). Certainly more research on this topic will be needed.

Possible efficacy of piracetam for the treatment of cocaine dependence in human abusers was tested in double-blind placebo-controlled trial (Kampman *et al* 2003). The authors pronounced hypothesis, that piracetam may improve cognitive functions in abstaining abusers and allow them better adherence to therapy, what might result in lower rate of relapse and less drug consumed. Interestingly piracetam showed in such measures as self-reported cocaine use, craving and anxi-

ety symptoms worse results than placebo in this study. Authors, however, admitted inadequacies in design of the study. At this point of our knowledge it is difficult to estimate possible influence of piracetam on development and sustantation of addiction. Excitatory amino acid system remains to be another possible joint structure where effects of psychostimulants and piracetam meet.

Piracetam in our experiment enhanced acute effects of methamphetamine as well as development of sensitisation to its stimulatory effects. Our results suggest that usage of MET+PIR combination by human abusers may not be coincidental. In consequence such usage of piracetam may contribute to higher risk of development of dependence on methamphetamine and increase susceptibility to relapse in abstaining individuals. Although piracetam itself shows minimal adverse effects and toxicity, its combination with psychostimulants may potentially lead to severe consequences for the user.

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