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Memantine protects against amphetamine derivatives-induced neurotoxic damage in rodents

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ABSTRACT

We hypothesize that 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) interact with alpha-7 nicotinic receptors (nAChR). Here we examine whether memantine (MEM), an antagonist of NMDAR and alpha-7 nAChR, prevents MDMA and METH neurotoxicity.

MEM prevented both serotonergic injury induced by MDMA in rat and dopaminergic lesion by METH in mice. MEM has a better protective effect in front of MDMA- and METH-induced neurotoxicity than methyllycaconitine (MLA), a specific alpha-7 nAChR antagonist. The double antagonism that MEM exerts on NMDA receptor and on alpha-7 nAChR, probably contributes to its effectiveness.

MEM inhibited reactive oxygen species production induced by MDMA or METH in synaptosomes. This effect was not modified by NMDA receptor antagonists, but reversed by alpha-7 nAChR agonist (PNU 282987), demonstrating a preventive effect of MEM as a result of it blocking alpha-7 nAChR.

In synaptosomes, MDMA decreased 5-HT uptake by about 40%. This decrease was prevented by MEM and by MLA but enhanced by PNU 282987. A similar pattern was observed when we measured the dopamine transport inhibited by METH. The inhibition of both transporters by amphetamine derivatives seems to be regulated by the calcium incorporation after activation of alpha-7 nAChR.

MDMA competitively displaces [^{3}H]MLA from rat brain membranes. MEM and METH also displace [^{3}H]MLA with non-competitive displacement profiles that fit a two-site model.

We conclude that MEM prevents MDMA and METH effects in rodents. MEM may offer neuroprotection against neurotoxicity induced by MDMA and METH by preventing the deleterious effects of these amphetamine derivatives on their respective transporters.

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) are illicit amphetamine derivatives, which are increasingly used alone or together with other abuse substances. Their psychostimulant effects are associated with an increase in serotonin (5-HT) and dopamine (DA) concentrations in synaptic cleft. Such increases are thought to be mediated by reverse transport of these neurotransmitters from the cytosol to the extracellular space either through the serotonin transporter (SERT) or the dopamine transporter (DAT) (Leviel, 2001), or via inhibition of their presynaptic uptake. MDMA not only increases these monoamines release by reverse transport but also decreases extracellular GABA levels in rat striatum (Yamamoto et al., 1995) and glutamate efflux in nucleus accumbens (White et al., 1994).

MDMA, also known as "ecstasy", is especially popular at dance parties ("raves"). Single administration of MDMA produces a rapid and sustained hyperthermia. This is the most dangerous clinical symptom of amphetamine derivatives intoxication in humans (Orio et al., 2004) and is apparent at high ambient temperatures. Reduced SERT availability has been observed (using positron emission tomography) in ecstasy users and seems to be a transient effect of prolonged use (Thomasius et al., 2006).

Repeated MDMA administration at doses that are not thought to have any persistent effect in the dopaminergic system (Pubill et al., 2003) produces long-term deficits in neurochemical indices of serotonergic function in the rat brain (for a review see Green et al., 2003). Evidence of such deficits includes: a decrease in the density of SERTs labeled by [^{3}H]paroxetine (Battaglia et al., 1987) in cortex, striatum and hippocampus, which lasts for at least 7 days post-treatment. No glial activation was noticeable 3 and 7 days after the final MDMA administration (Pubill et al., 2003; Baumann et al., 2007; Straiko et al., 2007). Fluoxetine pre-treatment has been recommended as protection from MDMA-induced long-term neurotoxicity, but recently Upreti and Eddington (2008) found that fluoxetine decreases the elimination of MDMA and its metabolite

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methylenedioxyamphetamine and may lead to an increased risk of acute MDMA toxic effects.

Some differences have been observed in the susceptibility to MDMA neurotoxicity between different strains of rat. Dark Agouti rats (a strain devoid of some CYP isoforms) suffer a significant serotonergic lesion in response to a single dose of MDMA (Kovacs et al., 2007). In contrast, Sprague–Dawley and Wistar strains do not usually show a similar injury until they have received several doses (Battaglia et al., 1987). Conversely, in mice, it is generally agreed that MDMA is a dopaminergic neurotoxin, which produces a dopaminergic terminal injury and a sustained loss of dopamine (DA) in the striatum.

METH (known colloquially as "ice" or "speed") is another amphetamine derivative often consumed by adolescents. A METH regimen administered to rodents that mimics chronic human use induces a neurotoxic injury (characterized by a loss of DAT) 3 days after the last dose. A simultaneous increase in striatal peripheral-type benzodiazepine receptor density, suggests a glial reaction (Escubedo et al., 1998). Numerous studies have shown that both astrocytes and microglia express this receptor type (Park et al., 1996) and that the density of the receptor (measured by [^{3}H]PK 11195 binding) can increase under conditions that result from glial activation. This enables us to use the receptor as an indirect marker of neuronal damage (Benavides et al., 1987).

Oxidative stress appears to be one of the main factors involved in nerve terminal injury induced by METH and MDMA (Yamamoto and Zhu, 1998; Jayanthi et al., 1999). Therefore, rat or mouse striatal synaptosomes can be used as an in vitro model of dopaminergic neurotoxicity induced by METH, and mouse striatal synaptosomes as an in vitro model of dopaminergic neurotoxicity induced by MDMA (Kim et al., 2000; Pubill et al., 2005; Chipana et al., 2006). Inside the dopaminergic terminals, METH releases DA from synaptic vesicles and increases cytosolic DA concentration. This increased free cytosolic DA can be oxidized to reactive oxygen species (ROS) in striatal synaptosomes (Pubill et al., 2005), in a process that depends on calcium and calcium-dependent enzymes such as protein kinase C (PKC), or nitric oxide synthase (NOS). In this in vitro model, MDMA behaves in the same way as METH. However, no oxidative stress induced by MDMA is detected in rat or mouse hippocampal synaptosomes (mainly serotonergic terminals).

The same in vitro model also demonstrates that the alpha-7 nicotinic acetylcholine receptor (nAChR) is involved in the oxidative effect of METH and MDMA (Pubill et al., 2005; Chipana et al., 2006). Furthermore, methyllycaconitine (MLA), a specific antagonist of alpha-7 nAChR, significantly prevents in vivo dopaminergic neurotoxicity induced by METH and MDMA (Escubedo et al., 2005; Chipana et al., 2006). To our knowledge, alpha-7 nAChR has never been considered to be involved in the serotonergic neurotoxicity induced by MDMA.

Turner (2004) suggested that the alpha-7 nAChR-mediated pathway is tightly and specifically coupled to refilling of the readily releasable pool of vesicles in DA terminals. The nicotine-induced increase in the size of this readily releasable pool was blocked by alpha-bungarotoxin and by the calmodulin antagonist calmidazolium, suggesting that Ca^{2+} entry through alpha-7 nAChRs specifically enhances synaptic vesicle mobilization at dopamine terminals. Then, it can be speculated that alpha-7 antagonists can modulate some behavioral effects of MDMA or METH.

Memantine (MEM), a non-competitive antagonist of the NMDA receptor, is a drug used to treat moderate-to-severe Alzheimer's disease; it reduces tonic, but not synaptic, NMDA receptor activity (Reisberg et al., 2003). More recently, Aracava et al. (2005) demonstrated that, at clinically relevant concentrations, MEM non-competitively blocks alpha-7 nAChR more effectively that it does NMDA receptors. Unger et al. (2005) described how treatment with MEM significantly increases the number of alpha-7 nAChR binding sites in the frontal and retrosplenial cortex mice. It suggests the possibility of MEM interacting with these nicotinic receptors, as an up-regulation of nAChR is a characteristic effect induced by nicotinic ligands (agonists and antagonists). In a previous paper, we also described an up-regulation of alpha-7 and heteromeric nAChR induced by METH and MDMA (García-Ratés et al., 2007). We therefore expect these three substances to interact with alpha-7 nAChR.

The present paper aimed to study the usefulness of MEM (an alpha-7 nAChR antagonist with a better therapeutic profile than MLA) as a drug to prevent MDMA-induced serotonergic and METH-induced dopaminergic neurotoxicity. Prevention of serotonergic neurotoxicity induced by MDMA has not been previously described for any alpha-7 nAChR antagonists. We also study the effect of MEM on the inhibition of SERT and DAT induced by MDMA and METH, respectively, as these are the main targets for these drugs of abuse.

First, we study dopaminergic neurotoxicity (characteristic of METH and MDMA in mice) by examining whether MEM prevents ROS production induced by METH or by MDMA in in vitro mouse striatal synaptosomes. This in vitro model allows us to study the direct effect of amphetamines on DA terminals without interference from glutamate or glial mechanisms and thereby avoiding the effects of hyperthermia. Although this model cannot be used as an in vitro model of MDMA-induced serotonergic neurotoxicity (characteristic of rats), it does indicate MDMA-induced oxidative effect, which suggests MDMA neurotoxicity.

In the in vivo experiments, we administered METH to mice to study the effect of MEM on dopaminergic METH-induced neurotoxicity. We also administered MDMA to Dark Agouti rats, as a model of serotonergic neurotoxicity induced by MDMA. Finally, we used competition studies with [^{3}H]MLA to compare the affinity of MEM, METH and MDMA to rat alpha-7 nAChR.

MEM has shown promising results in the treatment of amphetamine addiction (Levi and Borne, 2002). No drugs are currently approved in the U.S.A. or Europe for the treatment of addictions to METH or MDMA. MEM could be used to treat these addictions or to prevent the effects of these amphetamine derivatives, and it may also have a beneficial effect on the memory impairment that abusers of these drugs usually manifest (Simon et al., 2002).

2. Methods

2.1. Drugs and reagents

Drugs and reagents were obtained from the following sources: Cocaine and 3,4-methylendioxy-methamphetamine were provided by the National Health Laboratory (Barcelona, Spain). alpha-Bungarotoxin, bupropion, clomipramine, EGTA, fluoxetine, GF-109203X, glutamic acid (GLU), glutathione, methamphetamine chlorhidrate, methyllycaconitine (MLA), 7-nitroindazole (7-NI), pargyline, phencyclidine (PCP), PK 11195, and superoxide dismutase (SOD) were purchased from Sigma–Aldrich. PNU 282987 was obtained from Tocris (Bristol, UK). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was obtained from Molecular Probes (Leiden, The Netherlands). MEM was a generous gift of Lundbeck laboratories (Denmark). [^{3}H]MLA was from American Radiolabelled Chem. (St. Louis, USA). [^{3}H]paroxetine, [^{3}H]PK 11195, [^{3}H]WIN 35428, [^{3}H]DA and [^{3}H]5-HT were from Perkin–Elmer Life Sci. (Boston, USA). All buffer reagents were of analytical grade. Drugs were dissolved in bi-distilled water and added in the in vitro experiments at a volume of 10 μl to each ml of synaptosomal preparation. DCFH-DA was added to synaptosomal preparation dissolved in DMSO. The final concentration of DMSO was less than 0.5%, a concentration that had no effect on MDMA- or METH-induced ROS generation.

2.2. Animals

The experimental protocols for the use of all animals in this study follow the guidelines of the European Communities Council (86/609/EEC). Male Swiss CD1 mice (25 ± 3 g), male Dark Agouti (185 ± 20 g) or male Sprague–Dawley (250 ± 25 g) rats obtained from Charles River (Barcelona, Spain) were used. Except for treatments for in vivo neurotoxicity assessment, animals were housed at 22 ± 1 °C under a 12-h light/dark cycle with free access to food and drinking water.

2.3. In vivo neurotoxicity assays

During the experiments, the animals were maintained in an ambient temperature of 26 ± 2 °C and were kept under these conditions until 1 h after treatment.

To investigate the neurotoxicity induced by MDMA, male Dark Agouti rats were used. The MDMA group received a dose of 18 mg/kg, s.c. The MEM + MDMA group received a dose of MEM (5 mg/kg, i.p.) 30 min before the corresponding dose of MDMA. There were also two more groups: one received saline (1 ml/kg) and the other received MEM alone at the same dose as above. Body temperature was measured at 1 h and 3 h after drug treatment using a lubricated, flexible rectal probe inserted into the rectum (for 40 s) and attached to a digital thermometer (0331 Panlab SL, Barcelona, Spain).

To investigate the neurotoxicity induced by METH, male Swiss CD1 mice were used. The METH group received a dose of 7.5 mg/kg s.c. every 2 h and for a total of four doses. MEM (also four doses of 5 mg/kg i.p.) was administered with the same schedule as above. Body temperature was measured at 1 h after the first and the third dose. Animals were killed by decapitation under isoflurane anesthesia (rats) or cervical dislocation (mice) or at 72 h (mice) and 7 days (rats) post-treatment for neurotoxicity determination.

2.3.1. Tissue sample preparation

Immediately after sacrifice the brains were rapidly removed from the skull. Hippocampus, frontal cortex or striata were quickly dissected out, frozen on dry ice, and stored at −80 °C until use. When required, tissue samples were thawed and homogenized at 4 °C in 10 volumes of buffer consisting of 5 mM Tris–HCl, 320 mM sucrose, and protease inhibitors (aprotinin 4.5 μg/μ1, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate), pH 7.4, with a Polytron homogenizer. The homogenates were centrifuged at 15,000 × *g* for 30 min at 4 °C and the resulting pellet were washed and re-centrifuged two more times. The final pellets were resuspended in the appropriate buffer and stored at −80 °C until use in radioligand binding experiments. Protein content was determined using the Bio-Rad protein reagent, according to the manufacturer's directions.

2.3.2. DAT/SERT density and glial activation

The density of DAT in mice striatal membranes was measured by [^{3}H]WIN 35428 binding assays. Membranes were resuspended in phosphate-buffered 0.32 M sucrose, pH 7.9 at 4 °C (Coffey and Reith, 1994) to a concentration of 1 μg/μl. Assays were performed in glass tubes containing 200 μl of [^{3}H]WIN 35428 diluted in phosphate-buffered 0.32 M sucrose (final radioligand concentration, 5 nM) and 50 μl of membranes. Incubation was done for 2 h at 4 °C. Non-specific binding was determined in the presence of 30 μM bupropion.

The density of SERT in the hippocampal and cortical membranes from male Sprague–Dawley rats was quantified by measuring the specific binding of 0.05 nM [^{3}H]paroxetine after incubation with 150 μg of protein at 25 °C for 2 h in a Tris–HCl buffer (50 mM, pH 7.4), containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 ml. Clomipramine (100 μM) was used to determine non-specific binding.

Glial activation was assessed by measuring [^{3}H]PK 11195 binding. Briefly, mice striatal or rat hippocampal membranes were resuspended in 50 mM Tris–HCl buffer, pH 7.4. Equilibrium binding assays were performed at 4 °C for 2 h in glass tubes containing 2 nM [^{3}H]PK 11195 and 50 μg of protein in a final volume of 0.25 ml. Unlabelled PK 11195 (10 μM) was used to determine non-specific binding.

All incubations were finished by rapid filtration under vacuum through GF-51 glass fiber filters (Schleicher and Schuell, Dassel, Germany). Tubes and filters were washed rapidly three times with 4 ml of ice-cold buffer, and the radioactivity in the filters was measured by liquid scintillation spectrometry.

2.4. In vitro neurotoxicity assays. ROS production

Striatal synaptosomes were obtained as described elsewhere (Pubill et al., 2005) with minor modifications. Briefly, on the morning of each day of the experiment, seven mice were decapitated and their striata were homogenized and centrifuged at 1000 × *g* at 4 °C for 10 min. The supernatant was recovered, and sucrose buffer was added to a final sucrose concentration of 0.8 M. Samples were then centrifuged at 13,000 × *g* for 30 min at 4 °C. The supernatant was discarded and the synaptosome layer was separated from mitochondria by carefully adding 1 ml of ice-cold 320 mM sucrose buffer and gently shaking. Finally, the synaptosome fraction was diluted in Hank's balanced saline solution (HBSS) buffer to a final protein concentration of about 0.1 mg/ml. Protein concentration was determined using Bio-Rad protein reagent. The final synaptosomes suspension was distributed in 1 ml aliquots in centrifuge tubes to perform the experiments.

The formation of intrasynaptosomal ROS was measured using DCFH-DA (50 μM). This fluorochrome was added to each tube, together with the drugs at the appropriate concentrations. The synaptosomes were incubated for 15 min in a shaking bath at 37 °C in the dark and MDMA or METH was then added at the desired concentrations. Incubation was continued in the dark for 1 h and finally stopped by centrifugation at 13,000 × *g* for 30 min at 4 °C. The pellets were resuspended in 1 ml ice-cold Tris–sucrose buffer (320 mM) and re-centrifuged. The final pellets were resuspended in 0.2 ml of cold HBSS and the tubes were kept on ice in the dark until fluorescence measurements were performed, within an hour. Fluorescence was measured on a Coulter Epics XL-MCL flow cytometer equipped with an argon laser. The excitation wavelength was 488 nm and the emission was detected at 525 nm. Samples were diluted in HBSS to obtain a flow rate of 500–900 synaptosomes per second and each sample was measured for 1 min. Fluorescence data were analyzed using Elite software (Coulter, USA).

Values were taken from triplicates of each experimental condition, and individual experiments were performed at least three times. Mean fluorescence values of each experimental condition are expressed as percentages of control (100%) of at least three separate experiments run on triplicates. In previous work, we ruled out the possibility that the presence of test compounds alters the ability of synaptosomes to accumulate the dye (Pubill et al., 2005).

Concentrations of MDMA, METH, MEM, MK-801 and PNU-2882987 were chosen on the basis of our previous results (Chipana et al., 2008a,b, 2006; Escubedo et al., 2005). GLU and PCP, an agonist and an antagonist, respectively, of the NMDA receptors, were used to evaluate the role of these receptors in the oxidative effect of MDMA or METH, and to test the role of these receptors on MEM effect.

2.5. [^{3}H]5-HT and [^{3}H]DA uptake assays

For measuring [^{3}H]5-HT uptake, synaptosomes from the hippocampus of Sprague–Dawley rats were obtained as described above and preincubated in a shaking water bath at 37 °C with MDMA (15 μM) for 1 h. Other compounds such as MLA, MEM, MK-801, PNU 282987, GF-209203X or EGTA were added, when appropriate, 10 min before MDMA. After preincubation, synaptosomes were centrifuged at 13,000 × *g* for 20 min, resuspended in 5 mM Tris–HCl/320 mM sucrose buffer, and re-centrifuged. Final pellets were resuspended in a volume of HBSS buffer containing 20 μM pargyline and 1 mM glutathione so that final protein content was approximately equivalent to 10 mg of tissue (wet weight) per ml. Reaction tubes consisted of 0.85 ml of HBSS buffer, 0.1 ml of synaptosome suspension, and 0.05 ml of [^{3}H]5-HT (final concentration 15 nM) added at the start of incubation.

Tubes were warmed 10 min at 37 °C before the addition of [^{3}H]5-HT, after which incubation was carried out for a further 5 min. Uptake reaction was stopped by rapid filtration as described for binding experiments. The radioactivity trapped on the filters was measured by liquid scintillation spectrometry. Non-specific uptake was determined at 4 °C in parallel samples containing fluoxetine (10 μM).

The remaining synaptosomal preparation (i.e. not used for the uptake assay) was kept and protein was determined as described above. Specific 5-HT uptake for each condition was normalized dividing by the protein concentration and expressed as percentage of the uptake in control tubes.

For measuring [^{3}H]DA uptake, synaptosomes from the striatum of Sprague–Dawley rats were preincubated in a shaking water bath at 37 °C with METH (1 μM) for 1 h. Experiments were carried out as described above, using a final concentration of [^{3}H]DA of 5 nM. Non-specific uptake was determined at 4 °C in parallel samples containing 100 μM cocaine and results were normalized by protein concentration. In both cases, specific uptake was calculated subtracting non-specific uptake values from those of total uptake (37 °C).

2.6. Nicotinic receptor interaction

Binding of [^{3}H]MLA to label alpha-7 nAChR was performed as described by Davies et al. (1999). Competition assays were carried out with 0.25 ml of whole rat brain membranes (300 μg). Membrane preparations (see Section 2.3.1) were incubated in glass tubes with 2 nM [^{3}H]MLA in the absence or presence of increasing concentrations of MDMA, METH or MEM, in a final volume of 0.5 ml for 2 h at 4 °C. Incubation buffer consisted of 50 mM Tris–HCl, 120 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$ and 0.1% bovine serum albumin. Non-specific binding was determined from tubes containing 1 μM unlabelled MLA and was subtracted from total binding values. Incubation was stopped as described above. Competition binding curves were plotted and calculated by non-linear regression using GraphPAD Prism (GraphPAD software, San Diego, USA). Data were best fitted to a 1- or a 2-site competition model. The K_i values (the concentration that occupies 50% of the receptor population) for competing drugs were calculated using the equation by Cheng and Prusoff: $K_i = IC_{50}/(1 + (L/K_d))$, where L is the total radioligand concentration and K_d is the dissociation constant of the radioligand. The K_d value for [^{3}H]MLA in rat brain membranes was that reported by Davies et al. (1999).

2.7. Statistical analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). Except in the lethality studies, differences between groups were compared using one-way analysis of variance (ANOVA, two-tailed). Significant ($P < 0.05$) differences were then analyzed by Tukey's post hoc test for multiple means comparisons where appropriate. All statistic calculations were performed using Graph Pad Instat (GraphPad Software, San Diego, USA).

3. Results

3.1. In vivo neurotoxicity studies

3.1.1. Lethality

None of the Dark Agouti rats receiving MDMA, MEM or MEM + MDMA died during the treatment. In the case of Swiss mice, although initially MEM seemed to diminish the incidence of death

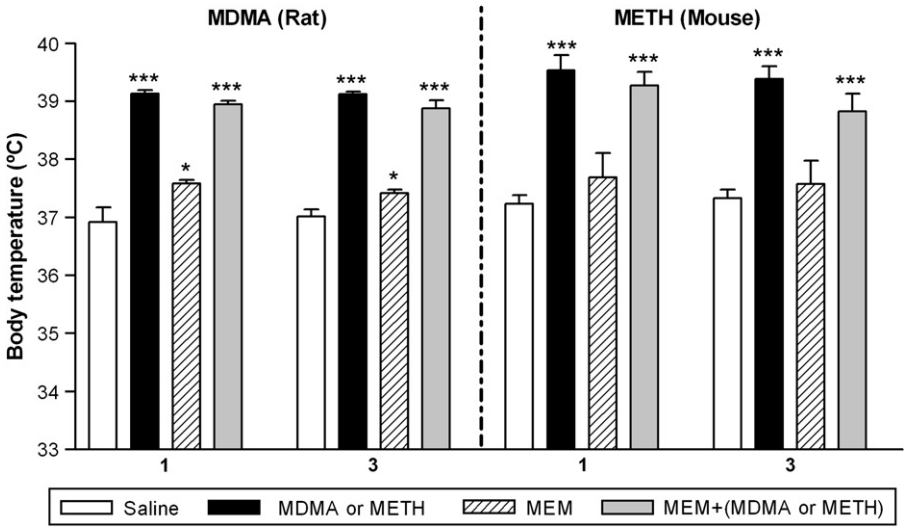

Fig. 1. Body temperature of Dark Agouti rats and Swiss CD1 mice treated with MDMA and METH, respectively. The ambient temperature remained constant at 26 ± 2 °C and body temperature was measured using a rectal probe 1 h and 3 h after the MDMA dose or 1 h after the first and the third dose of METH. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. rats or mice treated with saline (open bars). Data are mean ± S.E.M.; $n = 6–9$.

in METH-treated animals from 29.4% to 16.7% (MEM + METH group), this difference proved not to be significant ($P = 0.443$ Fisher's exact test).

3.1.2. Serotonergic injury in Dark Agouti rats

When the ambient temperature was maintained at 26 ± 2 °C, a single dose of 18 mg/kg of MDMA exerted a hyperthermic effect in Dark Agouti rats. This hyperthermic effect was detected 1 and 3 h post-treatment and was not affected by MEM pre-treatment. Moreover, treatment with MEM alone induced a slight, but significant, increase in body temperature (Fig. 1).

In animals killed 7 days post-treatment, we detected a significant decrease of about 30% and 43% in the density of [^{3}H]paroxetine binding sites in the hippocampus and the frontal cortex, respectively. MEM alone had no effect on the abundance of 5-HT uptake sites labeled by paroxetine, but prevented from any decrease being induced by MDMA treatment. Similarly, in these animals, an increase in the density of [^{3}H]PK 11195 binding sites was detected in these areas, indicating glial activation. This glial reaction was also prevented by MEM pre-treatment (Table 1).

3.1.3. Dopaminergic injury in Swiss CD1 mice

As in the case of Dark Agouti rats treated with MDMA, when the room temperature was kept at 26 ± 2 °C, treatment with METH induced a hyperthermic response in male Swiss CD1 mice, as measured 1 h after the first and the third dose. Pre-treatment with MEM did not affect this increase in body temperature (Fig. 1). Treatment with MEM alone did not induce any change in body temperature.

The dopaminergic neurotoxicity was determined 72 h post-treatment, revealing a significant decrease of about 25% in [^{3}H]WIN 3458 binding sites in striatum. MEM alone had not effect on the abundance of DA uptake sites labeled by [^{3}H]WIN 3458 but prevented any decrease from being induced by METH treatment (Fig. 2A). As for Dark Agouti rats, we detected glial activation which was evidenced by an increase of about 35% in [^{3}H]PK 11195 binding sites. This glial reaction was also prevented by MEM pre-treatment (Fig. 2B).

3.2. In vitro neurotoxicity studies. ROS production

Incubation of mouse striatal synaptosomes with MDMA-induced ROS production over a narrow concentration range (from 50 to 150 μM) (Fig. 3). This is consistent with the capacity of this amphetamine derivative to inhibit monoamine-oxidase A but not the B isoform at these concentrations (Chipana et al., 2008a). MDMA concentrations of 50 or 100 μM were chosen for further studies.

As can be seen in Fig. 3, MEM (0.3 μM) inhibited the ROS production induced by MDMA at all the concentrations tested. This inhibition was countered by the presence of PNU 282987 (0.5 μM), a specific agonist of the alpha-7 nAChR, in the incubation medium (148.74 ± 1.08% PNU + MEM + MDMA, n.s. vs. 143.17 ± 1.89% MDMA 50 μM). At a concentration of 0.3 μM, MEM had not antioxidant effect (data not shown). PNU 282987 at a concentration of 0.1 μM, enhanced the oxidative effect of MDMA (100 μM) (132.08 ± 1.09% MDMA vs. 143.40 ± 2.88% MDMA + PNU 282987; $P < 0.05$). PNU 282987 alone did not modify basal values of ROS production (95.87 ± 1.19%, n.s. vs. CTRL).

METH (from 1 μM to 10 mM) induced a concentration-dependent ROS production in mouse striatal synaptosomes yielding an EC_{50} value of 84.37 ± 2.08 μM. MEM (0.3 μM) inhibited the ROS

Table 1
Effect of memantine on MDMA-induced decrease in the abundance of 5-HT transporters (labeled by [^{3}H]paroxetine) in rat hippocampus and frontal cortex and also in the glial activation measured by the [^{3}H]PK11195 binding

Treatment	Hippocampus		Frontal cortex	
	[^{3}H]Paroxetine binding	[^{3}H]PK 11195 binding	[^{3}H]Paroxetine binding	[^{3}H]PK 11195 binding
Saline	100.00 ± 9.34	100.00 ± 6.35	100.00 ± 5.05	100.00 ± 2.94
MDMA	70.02 ± 7.85*	168.22 ± 20.65**	56.90 ± 2.57***	120.03 ± 6.70*
Memantine	116.40 ± 3.95	94.90 ± 7.05	88.93 ± 9.20	90.00 ± 9.12
MDMA + memantine	94.87 ± 4.96	104.41 ± 9.77#	74.00 ± 4.98**,#	97.70 ± 4.94*

$^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs Saline; $^{\#}P < 0.05$ vs. MDMA group. Results (in %) are expressed as mean ± S.E.M. from 6–9 different experiments.

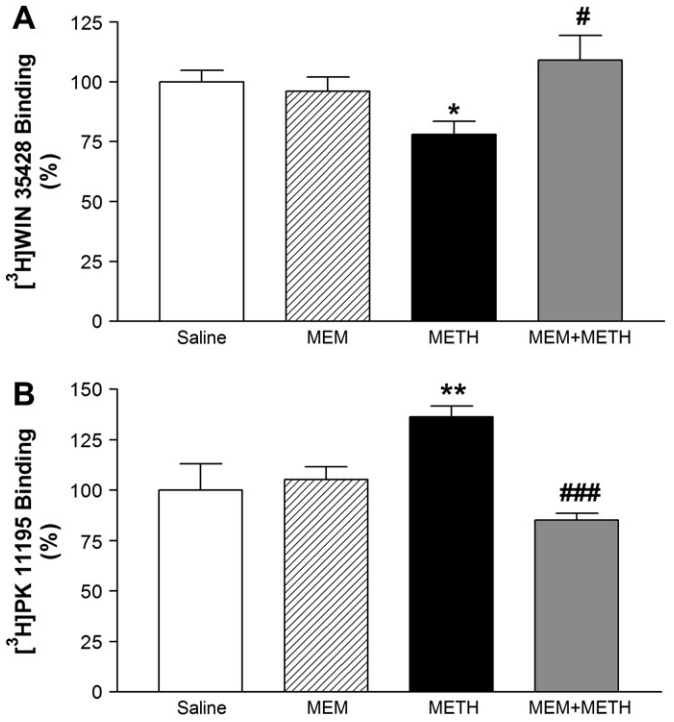

Fig. 2. Panel A. Effect of treatment with METH alone or in combination with MEM on the density of mouse striatal dopamine reuptake sites, measured as specific binding of [^{3}H]WIN 35428. $^*P < 0.05$ vs. saline-treated group; $^{\#}P < 0.05$ vs. METH group. Panel B. Effect of treatment with METH alone or in combination with MEM on glial activation, measured as the density of mouse striatal peripheral-type benzodiazepine receptors (specific binding of [^{3}H]PK 11195). $^{**}P < 0.01$ vs. saline-treated group; $^{\#\#\#}P < 0.001$ vs. METH group. In both panels data are mean ± S.E.M.; $n = 6$–9.

production induced by METH and the concentration–response curve was significantly shifted to the right ($P < 0.001$) giving an EC_{50} value of 208.2 ± 1.51 μM. As can be seen in Fig. 4, the inhibitory effect of MEM was countered by the presence in the incubation medium of PNU 282987 and the oxidative effect of METH was reinforced by this alpha-7 specific agonist.

Blocking of NMDA receptors with PCP (1 μM) did not modify the ROS production induced by MDMA (130.03 ± 0.32% MDMA vs. 130.35 ± 2.39% PCP + MDMA, n.s), nor by METH (174.2 ± 9.10% METH vs. 168.36 ± 7.50% PCP + METH, n.s.; 110 ± 4.5% PCP, n.s. vs. CTRL). Moreover, GLU (0.5 μM) alone induced ROS production (127.68 ± 6.36% GLU, $P < 0.05$ vs. CTRL) but this effect was not prevented by MEM (141.00 ± 7.37% MEM + GLU; n.s. vs. GLU). Additionally, MEM inhibition of MDMA- or METH-induced oxidative effect was not counteracted by the presence of GLU in the incubation medium (119.08 ± 1.74% GLU + MEM + MDMA, $P < 0.05$ vs. MDMA, n.s. vs. GLU), (127.23 ± 8.61% GLU + MEM + METH, $P < 0.001$ vs. METH; n.s. vs. GLU).

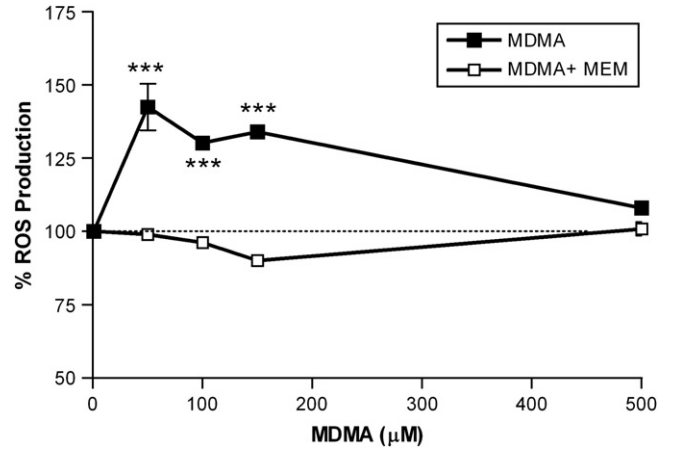

Fig. 3. Effect of MEM (0.3 μM) on the ROS production induced by different concentrations of MDMA in mouse striatal synaptosomes, expressed as percentage of control value (dotted line). $^{***}P < 0.001$ vs. control. Results are expressed as mean ± S.E.M. from at least three separate experiments run in triplicates, one-way ANOVA and Tukey's post hoc test.

3.3. Serotonin and dopamine uptake

In synaptosomes isolated from rat hippocampus, MDMA (15 μM) inhibited the 5-HT transporter function, as indicated by a significant decrease in 5-HT uptake of about 40% (Fig. 5). This inhibition was prevented by MEM (0.15 μM) as well as by MLA (0.1 μM). MK-801 (1 μM), an NMDA receptor antagonist, did not significantly modify 5-HT uptake or the effect of MDMA or MEM. In turn, the presence of PNU 282987 countered the preventive effect of MEM on the decrease in 5-HT uptake induced by MDMA. Moreover, at a concentration of 0.1 μM, PNU 282987 enhanced the decrease in 5-HT uptake induced by MDMA (5-HT uptake: 67.1 ± 1.6% MDMA vs. 59.7 ± 0.6% MDMA + PNU 282987; $P < 0.05$). When isolated synaptosomes were incubated with PNU 282987 alone, at a concentration of 0.1 μM, there was no significant change in 5-HT uptake. In contrast, at a concentration of 0.5 μM, this drug inhibited 5-HT uptake (100 ± 5.7% CTRL vs. 45.6 ± 15.3% PNU 282987, $P < 0.05$).

Incubation of synaptosomes with 7-NI (10 μM) (an inhibitor of neuronal NOS), GF-109203X (0.1 μM) (an inhibitor of PKC), or EGTA (2 mM) alone had no effect on basal levels of 5-HT uptake. In contrast, all of them prevented the decrease in this uptake induced by MDMA (100 ± 5.7% CTRL, 62.3 ± 2.2% MDMA, $P < 0.001$ vs. CTRL; 90.1 ± 2.8% 7-NI + MDMA, n.s. vs. CTRL; 102.9 ± 0.5% GF-109203X + MDMA, n.s. vs. CTRL; 92.6 ± 2.6% EGTA + MDMA, n.s. vs. CTRL).

Similarly, in synaptosomes isolated from rat striatum, METH (1 μM) inhibited the DA uptake by about 40% (Fig. 6). This inhibition was also prevented by MEM (0.15 μM) and MLA (0.10 μM). As above, MK-801 (1 μM) did not modify DA uptake, neither did it modify the effect of METH or MEM on this preparation. Furthermore, incubation of synaptosomes with 7-NI, GF-109203X or EGTA alone had no effect on basal levels of [^{3}H]DA uptake, but, all of them inhibited the decrease in DA uptake induced by METH (100 ± 1.7% CTRL, 66,1 ± 0.8% METH, $P < 0.001$ vs. CTRL; 95.9 ± 8.6% 7-NI + METH, n.s. vs. CTRL; 101.7 ± 1.7% EGTA + MDMA, n.s. vs. CTRL; 91.6 ± 1.9% GF-109203X + METH, $P < 0.05$ vs. CTRL).

3.4. Nicotinic receptor interaction

MDMA, METH and MEM, (from 10^{-7} to 3×10^{-3} M), each displaced [^{3}H]MLA binding in rat brain membranes in a concentration-dependent manner (Fig. 7). We performed competition assays by adding the membrane preparation to a mixture of the radioligand and the different competitors (MDMA, METH or MEM). None of the competitors fully displaced the bound [^{3}H]MLA at the highest concentration assayed (maximum radioligand displaced: MDMA 89 ± 5.5%, METH 70 ± 9.3% and MEM 68.7 ± 4.3%). This suggests that bound [^{3}H]MLA hamper ulterior binding of amphetamine derivatives. However, when membranes were preincubated with the competitor before adding the radioligand, displacement was complete. We therefore performed further competition experiments by incubating MDMA, METH or MEM with brain membranes for 15 min prior to the addition of [^{3}H]MLA.

Under these experimental conditions, MDMA fully and competitively (Hill coefficient not significantly different from unity)

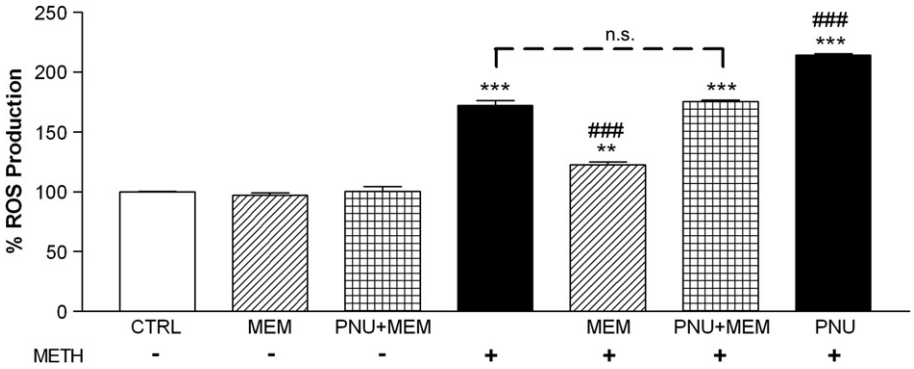

Fig. 4. Effect of PNU 282987 (0.5 μM) and MEM (0.3 μM), alone or in combination, on METH (3 mM)-induced ROS production in mouse striatal synaptosomes. Results are expressed as mean ± S.E.M. from at least three separate experiments run in triplicates. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. CTRL and $^{###}P < 0.001$ vs. METH n.s.: non significant, one-way ANOVA and Tukey's post hoc test.

displaced bound [^{3}H]MLA with a K_i value in the micromolar range. METH displaced a maximum of 88 ± 5.5% of bound [^{3}H]MLA with a Hill coefficient significantly different from unity. The displacement profile for METH better suited a two-site model and yielded the corresponding IC_{50} and K_i values. MEM also fully displaced bound [^{3}H]MLA in a biphasic manner and the displacement profile was also better fitted to a two-site model (Table 2).

4. Discussion

Alpha-7 nAChR is involved in the reinforcing actions of addictive drugs, such as nicotine and cocaine, and alpha-7 nAChR antagonists may be clinically useful in attenuating the rewarding effects of these addictive drugs (Panagis et al., 2000). Also, blocking this homomeric receptor has recently been suggested as useful in the development of drugs to treat cannabis dependence (Solinas et al., 2007).

In this paper we demonstrate that MEM inhibits the oxidative effects and the loss of [^{3}H]paroxetine binding sites induced by MDMA in Dark Agouti rats, and that it also prevents the effects of MDMA on SERT functionality. Apart from a brief publication advancing some of these results (Chipana et al., 2008b) this is the first time that this clinical drug (whose active mechanism involves blockade of alpha-7 nAChR and NMDA receptors) has been reported to prevent serotonergic impairment produced by MDMA. Similarly, we demonstrate that MEM also inhibits METH-induced dopaminergic deleterious effects, such as ROS production and decrease in [^{3}H]WIN 35428 binding, and it further prevents METH-induced DAT inhibition.

Although present in vivo experiments do not prove that the neuroprotective effects of MEM result only from blocking alpha-7 nAChR, our in vitro experiments support this hypothesis.

MEM is a specific, moderate affinity, uncompetitive NMDA receptor antagonist, with strong voltage-dependency and rapid blocking/unblocking kinetics. In rats, i.p. injection of MEM 5 mg/kg (the dose used in the present study) probably leads to CNS concentration of less than 1 μM (Parsons et al., 1999). However, a higher CNS concentration is probably reached after the dosage regimen applied to mice (5 mg/kg × 4, every 2 h).

4.1. In vivo neurotoxicity assays

MDMA and METH impair the thermoregulatory response, and hyperthermia is apparent at high ambient temperatures used in present studies (26 ± 2 °C). MEM administered alone produced a slight (though significant) increase in body temperature in rats but not in mice. When it was administered prior to MDMA or METH, the hyperthermic response to each amphetamine derivative was not modified. Thus, a neuroprotective effect based on an MEM antihyperthermic mechanism can be ruled out.

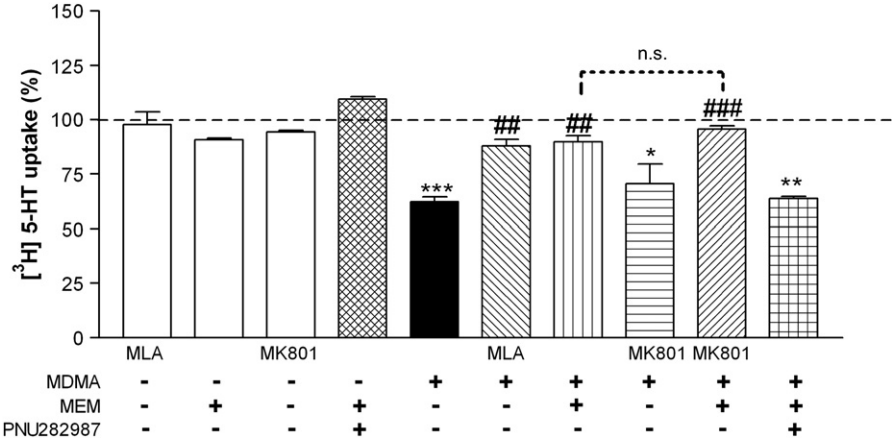

Fig. 5. Effect of MDMA (15 μM), MLA (0.1 μM), MEM (0.15 μM), MK-801 (1 μM) and PNU 282987 (0.5 μM), alone or in combination, on [^{3}H]5-HT uptake in synaptosomes from rat hippocampus. Data are presented as mean ± S.E.M. as percentage of control (dashed line) [^{3}H]5-HT uptake, from at least three separate experiments run in duplicate. Non-specific [^{3}H]5-HT uptake was determined at 4 °C in parallel samples containing 10 μM fluoxetine and was about 10% of total [^{3}H]5-HT uptake. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control, $^{##}P < 0.01$ and $^{###}P < 0.001$ vs. MDMA. n.s.: non significant, one-way ANOVA and Tukey's post hoc test.

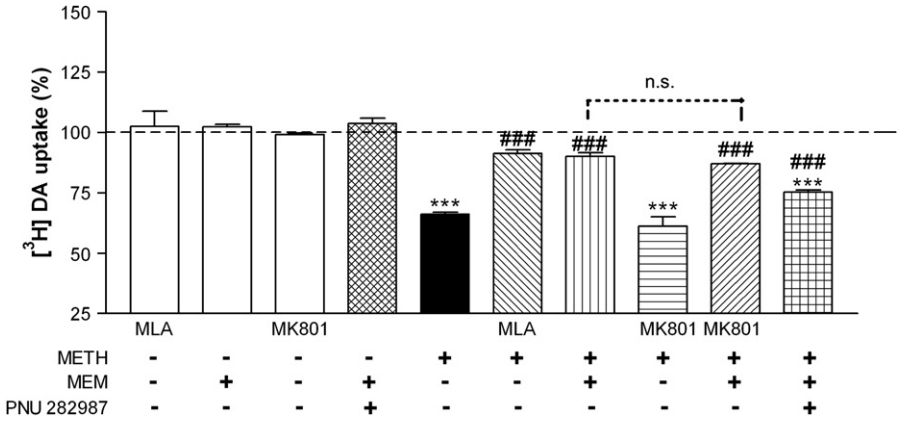

Fig. 6. Effect of METH (1 μM), MLA (0.1 μM), MEM (0.15 μM), MK-801 (1 μM) and PNU 282987 (0.5 μM), alone or in combination, on [^{3}H]DA uptake in rat striatal synaptosomes. Data are presented as mean ± S.E.M. as percentage of control (dashed line) [^{3}H]DA uptake, from at least three separate experiments run in duplicate. Non-specific [^{3}H]DA uptake was determined at 4 °C in parallel samples containing 100 μM cocaine and was about 7% of total [^{3}H]DA uptake. $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. METH. n.s.: non significant, one-way ANOVA and Tukey's post hoc test.

4.1.1. Serotonergic injury in Dark Agouti rats

A significant decrease in the density of SERT was observed in both, the hippocampus and frontal cortex of MDMA-treated animals killed 7 days post-treatment, but serotonergic injury was already apparent 24 h post-treatment (Chipana et al., 2008b). In both cases, MEM significantly prevented the loss of [^{3}H]paroxetine binding sites, suggesting a neuroprotective effect on serotonin terminals.

A characteristic that differentiates this vulnerable rat strain from Sprague–Dawley rats is that we detected not only the rapid appearance of the serotonergic injury, but also glial activation 7 days after treatment (characterized by an increase in [^{3}H]PK 11195 binding). In a previous study a neurotoxic regimen of MDMA was administered to Sprague–Dawley rats and we found no glial activation 3 or 7 days after treatment (Pubill et al., 2003). In the present study, a significant increase in [^{3}H]PK 11195 binding was detected in hippocampus and frontal cortex 7 days post-treatment but not in animals killed 24 h post-treatment. Therefore, this effect appears after the neuronal injury and suggests a phagocytic role rather than a pro-inflammatory response for this glial activation. Furthermore, MEM-pre-treated animals did not show serotonergic injury neither did they present glial activation.

4.1.2. Dopaminergic injury in Swiss CD1 mice

In parallel experiments, the extent of METH-induced injury was determined 3 days after concluding a dosage regimen that models chronic drug use. Using this schedule, METH decreased [^{3}H]WIN 35428 binding in mice striatum, which reflects a loss of DAT, due to the degeneration of dopaminergic terminals. Simultaneously, this drug significantly increased striatal [^{3}H]PK 11195 binding, suggesting glial activation. Both the dopaminergic injury and the consequent glial activation were prevented in animals pretreated with MEM, while the hyperthermia was not affected. MEM diminished the incidence of death in METH-treated animals, but this difference proved not to be significant.

Although MEM could directly prevent the MDMA- and the METH-induced neurotoxicity through antagonism of NMDA receptors, this is not a feasible hypothesis since antagonists of these receptors fail to prevent the oxidative stress and cell death induced by METH and MDMA (Jiménez et al., 2004). Protective effects of these compounds are secondary to the blocking of METH- or MDMA-induced hyperthermia (Albers and Sonsalla, 1995; Ali et al., 1994; Bowyer et al., 1994), which is not the case for MEM. However, MEM has a better protective effect in front of MDMA- and METH-induced neurotoxicity than MLA (Chipana et al., 2006; Escubedo et al., 2005), a specific alpha-7 nAChR antagonist. The double antagonism that MEM exerts on NMDA receptor and on alpha-7

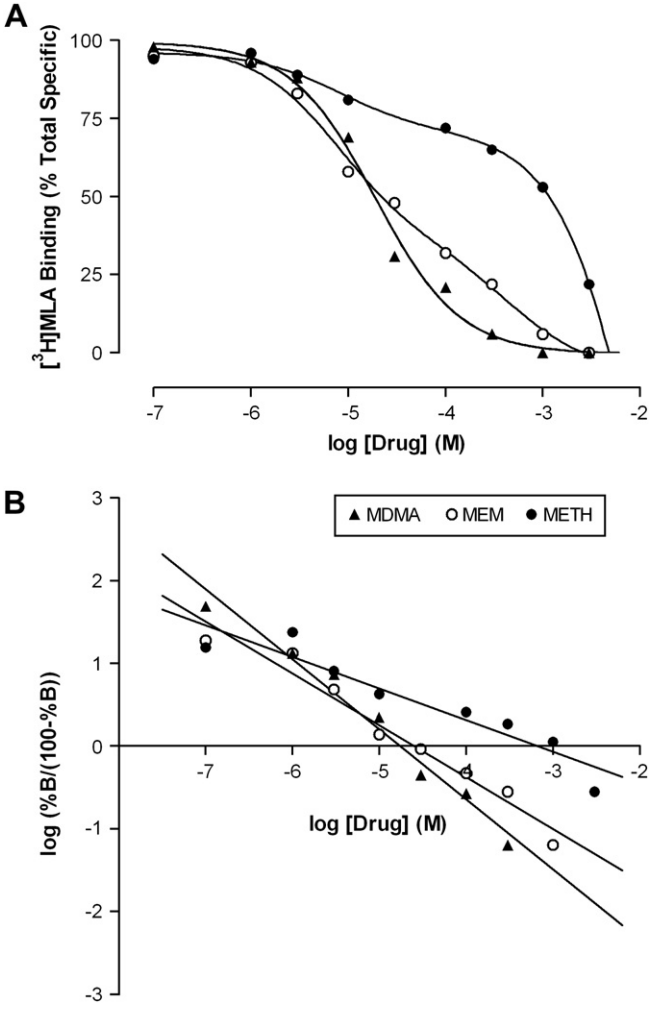

Fig. 7. Representative competition curves (panel A) and Hill plots (panel B) showing the inhibition of specific [^{3}H]MLA binding by MDMA, METH or MEM in membranes from rat brain. Membranes were incubated for 2 h at 4 °C with 2 nM [^{3}H]MLA, in the absence or presence of increasing concentrations of inhibitors, which were added 15 min prior to the radioligand. Inhibition curves were calculated using the non-linear least squares method and adjusted to the one- or two-site model. Data represent the mean of three different experiments performed in triplicate.

Table 2
IC_{50}, K_i and Hill coefficient (n_H) values of MDMA, METH and MEM against [^{3}H]MLA specific binding in rat brain membranes

Compound	IC_{50} (μM)		K_i (μM)		n_H
MDMA	19.03 ± 1.18		9.5		0.85 ± 0.06
	Site 1	Site 2	Site 1	Site 2	
METH	8.01 ± 1.56	15,000 ± 5	4.00	7500	0.38 ± 0.05**
MEM	7.78 ± 0.79	472 ± 0.5	3.89	236	0.63 ± 0.05*

*$P < 0.05$ and **$P < 0.01$ (one sample Student's t-test) vs unity. The K_i values from competition binding curves were adjusted to the one- or two-site model and were calculated using the Cheng-Prusoff equation. Data are presented as mean ± S.E.M. from three independent experiments carried out in duplicate.

nAChR, probably turns it into a better pharmacological tool to prevent amphetamines-induced damage.

DA neuron firing is modulated by glutamatergic (excitatory) afferents and DA release is evoked by NMDA. If MEM blocks NMDA receptors, a decrease in extracellular DA levels would take place. Following the Sprague et al. (1998) integrated hypothesis, the probability that released DA may be taken up into the depleted 5-HT terminals will be reduced by MEM. Consequently, the antagonism of NMDA receptors could contribute to the protective effects of MEM.

Even so, indirect mechanisms for MEM action based on the interplay between the various neurotransmission systems leading to a modification in basal ACh release should also be taken into account. Nair and Gudelsky (2006) described how MDMA stimulates hippocampal ACh release, involving non-dopaminergic and non-serotonergic mechanisms.

4.2. In vitro neurotoxicity studies. ROS production

Results obtained from mouse striatal synaptosomes demonstrate that MDMA and METH increase DCF fluorescence, suggesting that they induce ROS production inside the synaptosomes. Previous experiments (Chipana et al., 2006; Escubedo et al., 2005) characterize this oxidative process and show a dependence on DA, PKC, NOS, alpha-7 nAChRs and calcium entry. We concluded that ROS production induced by MDMA or METH is a consequence of a double mechanism: an increase in cytosolic free DA and an increase in cytosolic calcium. However, one mechanism seems to be a consequence of the other. Ca^{2+} entry through alpha-7 nAChRs specifically enhances synaptic vesicle mobilization at dopamine terminals (Turner, 2004). This phenomenon probably enhances the vesicular dopamine displaced by amphetamines that can be released or released and subsequently oxidized, depending on MDMA or METH concentration. Consequently, in the presence of an alpha-7 nAChR agonist, PNU 282987, this oxidative effect was enhanced, and it was inhibited by a specific alpha-7 nAChR antagonist (Escubedo et al., 2005; Chipana et al., 2006).

In the present study MDMA- and METH-induced ROS production was completely countered by MEM, and this was fully reversed by preincubation with PNU 282987. It has been suggested that alpha-7 nAChR located at striatal glutamatergic terminals and inducing glutamate release, could modulate DA release in the striatum (Kaiser and Wonnacott, 2000). Although in synaptosomal preparation the probability of neurochemical cross-talk is very low, we studied the possible involvement of NMDA receptors using GLU acid and PCP (a NMDA channel blocker). GLU acid alone induced ROS production that was not prevented by MEM, ruling out a role of NMDA receptors in the oxidative effects of GLU in this preparation, and for extension, NMDA-mediated effect of MEM. Additionally, MEM inhibition of MDMA- or METH-induced oxidative effect was not countered by the presence of GLU in the incubation medium, and PCP did not modify MDMA and METH effects. These results demonstrate a preventive effect of MEM on MDMA- and METH-induced oxidation in mouse striatum as a result of it blocking alpha-7 nAChRs.

4.3. Serotonin and dopamine uptake

In the present study, [^{3}H]5-HT uptake in rat hippocampal synaptosomes and [^{3}H]DA uptake in rat striatal synaptosomes were measured as indicative of the acute serotonergic effect of MDMA and the acute dopaminergic effect of METH, respectively. Preincubation of synaptosomes with a low concentration of each drug induced a significant reduction in [^{3}H]5-HT and [^{3}H]DA uptake, which persisted even after drug washout and therefore cannot be attributed to residual drug presence. This indicates that MDMA or METH induce persistent alterations in SERT and DAT, respectively, that are more complex than a simple blocking by direct interaction with each carrier.

The effect of the amphetamine derivatives on their respective transporters was prevented by EGTA, 7-NI and GF-109203X.

From present results we can establish that the inhibition of both 5-HT and DA uptake by MDMA and METH depends on calcium and also on the activity of NOS and PKC, both of which are calcium-dependent enzymes. If MDMA and METH act as alpha-7 nAChR agonists or positive modulators, calcium entry through this receptor would contribute to their effect on 5-HT or DA uptake. In accordance with this hypothesis, we studied the effects of MLA and MEM on the uptake inhibitory effects of MDMA and METH. MLA and MEM countered this inhibitory effect. Also, PNU 282987 prevented the effect of MEM but MK-801 did not modify it, confirming that the effect of MEM on MDMA/METH-induced uptake inhibition is mediated by alpha-7 receptor and not by NMDA receptor. Aznar et al. (2005) described the presence of alpha-7 nAChR at serotonin neurons, in terminals projecting into the hippocampus. Furthermore, PNU 282987 alone (0.5 μM, but not 0.1 μM) inhibited 5-HT uptake, which suggests that SERT functionality in the hippocampal serotonergic terminals can be regulated by alpha-7 nAChR. Accordingly, PNU 282987 (0.1 μM) potentiated the inhibitory effect of MDMA on SERT function.

In accordance with present results, David et al. (2006) described the effect of MEM on amphetamine-induced DA release using brain slices. Amphetamine derivatives induce DA release through reverse DA transport, a critical neurochemical event involved in its psychostimulant action. In this study, MEM, but not the prototypical NMDA antagonist, MK-801, blocked the increase in carrier-mediated DA release.

4.4. Nicotinic receptor interaction

Displacement of [^{3}H]MLA binding is more effective if the substances compete with [^{3}H]MLA on the external surface and may depend on the number of MLA molecules bound to the receptor. If test substances bind to a site inside the channel, the displacement will vary depending on the presence of [^{3}H]MLA and on voltage. A considerable discrepancy can therefore exist for this receptor between the K_d value obtained by binding and the IC_{50} value from a functional assay. This is the case of the substances assayed in this paper.

As we did not previously know where the binding site for amphetamine derivatives was localized, our objective was to use [^{3}H]MLA binding displacement as a tool to compare the affinities of MEM, MDMA and METH for alpha-7 nAChR. The order of affinities obtained was MDMA > MEM > METH. Hill coefficients (n_H) which differ from unity (in the METH and MEM curves) indicate either a form of negative cooperation in binding or binding of the competitor to two or more sites. The displacement profile for MEM and METH better suited a two-site model. However, for MDMA a low

micromolar value of K_i was obtained, which is compatible with the concentrations at which we observed that it inhibits [^{3}H]5-HT uptake or induces ROS production (both prevented by alpha-7 antagonists). Also, an n_H not significantly different from unity suggests competition with the MLA binding site.

Aracava et al. (2005) reported non-competitive inhibition of rat hippocampal alpha-7 receptor-mediated currents by MEM with an IC_{50} value of 0.34 μM. In the present study, MEM 0.15 or 0.3 μM was just enough to block the MDMA and METH effects on ROS production and on [^{3}H]5-HT and [^{3}H]DA uptake. However, it displaced [^{3}H]MLA binding with K_i values of 3.9 and 236 μM. Aracava et al. (2005) explained that MEM interacts with more than one class of sites on alpha-7 nAChR. One is voltage-sensitive and therefore probably within the receptor channel. The other is not voltage-sensitive and therefore is probably in the extracellular domain of the receptor. Thus, the interactions of MEM with alpha-7 nAChR depend on agonist binding and/or channel opening; this is reflected by a double binding site and an n_H different from unity.

When the effects of MEM on human alpha-7 nAChR were examined using two-electrode voltage-clamp recordings and ^{125}I-alpha-bungarotoxin binding (Maskell et al., 2003), inhibition of alpha-7 receptor function by MEM occurred at concentrations far lower than those needed to inhibit the binding of ^{125}I-alpha-bungarotoxin, suggesting that the effects of MEM on alpha-7 nAChR are non-competitive. A similar phenomenon would probably occur with METH. The effects of METH on alpha-7 nAChR receptor cannot be explained from the data for [^{3}H]MLA binding displacement. This experiment only allows us to conclude that METH seems to recognize two binding sites in alpha-7 nAChR; those with μM and mM affinities.

To sum up, in this paper we demonstrate that MEM prevents the oxidative effect and neurotoxicity induced by MDMA and METH in rodents, as well as their effect on their respective neurotransmitter transporters. We also show that these substances interact with alpha-7 nAChR. This agrees with recent results by Klingler et al. (2005) suggesting an agonistic action of MDMA on alpha-7 nAChR and results obtained by us in PC12 cells with MDMA and METH (García-Ratés et al., 2007).

Although previously we advanced some preliminary results that account for MEM protecting against MDMA-induced neurotoxicity (Chipana et al., 2008b), this is the first report of a protection against both METH and MDMA-induced neurotoxicity provided by a drug used in clinical practice, which acts by blocking alpha-7 nAChR.

Interest in MEM preventing MDMA- and METH-induced neurotoxicity is motivated by the hope that it could be useful to prevent the cognitive impairment associated with chronic consumption of amphetamine derivatives, as well as to treat addiction to these substances.

Acknowledgments

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