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METHAMPHETAMINE INDUCES ALTERATIONS ON HIPPOCAMPAL NMDA AND AMPA RECEPTOR SUBUNIT LEVELS AND IMPAIRS SPATIAL WORKING MEMORY

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Abstract—Methamphetamine (METH) is a powerful psychostimulant that increases glutamate (Glu) levels in the mammalian brain and it is currently known that hippocampi are particularly susceptible to METH. Moreover, it is well established that the overactivation of *N*-methyl-D-aspartate (NMDA) and AMPA ionotropic Glu receptors causes excitotoxicity. In the present study, we investigated the effect of acute (30 mg/kg) versus escalating dose (ED) administration of METH on NMDA receptor 1, NMDA receptor 2 and glutamate receptor 2 (GluR2) subunit expression in the hippocampus and on memory. Adult Sprague–Dawley rats were injected s.c. during six consecutive days with saline (control and acute groups) or with a growing dose of METH (10, 15, 15, 20, 20, 25 mg/kg/day; ED group). On the 7th day, both METH groups were injected with a 'bolus' of 30 mg/kg METH whereas controls received saline. Western blot analysis showed an increase of GluR2 and NR2A expression levels and no alterations on NR1 subunit in the acute group. On the other hand, in the ED group, GluR2 and NR2A expression levels were unaltered and there was a decrease on NR1 levels. Moreover, we did not observe neurodegeneration with both administration paradigms, as assessed by Fluoro-Jade C staining, but we did observe a strong astrogliosis in the acute administration group by using both immunohistochemistry and Western blot analysis. The impact of METH on working memory was evaluated using the Y maze test and revealed significant mnemonic deficit in the rats acutely treated with the drug. Overall, our results suggest a protection mechanism under conditions of METH administration by decreasing permeability and/or functionality of NMDA and AMPA receptors, which has implications on memory. So, the participation of the glutamatergic system should be considered as an important pharmacological target to design new strategies to prevent or diminish the harmful effect of drug consumption.

Key words: psychostimulants, GluR2, NR1, NR2A, astrogliosis.

Methamphetamine (METH) is a worldwide abused psychostimulant with a strong action on the CNS (Hanson et al., 2004). A concerning neuropsychological consequence of METH abuse is the cognitive impairment, with working memory deficits remaining long after withdrawal (Meredith et al., 2005). Indeed, evidence of neurotoxicity has been found in the hippocampus of METH chronic abusers, as they presented hippocampus atrophy and impairment in hippocampal-dependent memory tasks (Ernst et al., 2000). However, METH neurochemical, behavioral and psychological consequences depend largely on the pattern of abuse (Davidson et al., 2005). Most of the studies underlying METH-induced neurotoxicity have used the single-day–single-dose (20–100 mg/kg) or single-day–multiple-dose administrations (5–10 mg/kg, given four times at 2 h intervals) (Cappon et al., 2000; Chapman et al., 2001; Pereira et al., 2006). The acute paradigm mimics the common human overdose scenario, when a new abuser will take the same high dose that a tolerant abuser is capable of, but which will be lethal to the first one. Yet, another common scenario is the human 'binger' condition, which is characterized by compulsive high dose administrations of the drug over several days. This pattern of abuse results in steady-state plasma levels and is thought to induce a great tolerance to the lethal effects of the drug (Davidson et al., 2001; Maxwell, 2005).

METH noxious effects on the brain have long been associated to dopamine (DA) and 5-HT (Yamamoto and Bankson, 2005), but over the last decades, the focus has been shifting toward glutamate (Glu). Indeed, it is known that METH evokes an initial increase of DA release in the striatum (Pereira et al., 2002, 2006) and a delayed increase in extracellular Glu both in the striatum and in the hippocampus (Nash and Yamamoto, 1992; Rocher and Gardier, 2001). Increases in extracellular Glu can activate ionotropic receptors, such as *N*-methyl-D-aspartate (NMDA) and AMPA, and result in increased intracellular Ca^{2+} levels, which in turn, leads to the overactivation of Ca^{2+} dependent proteases, lipases, phosphatases and endonucleases that break down cytoskeletal proteins and damage DNA. Also, overactivation of NMDA receptors increases the production of NO via activation of nNOS, thus contributing to reactive

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Abbreviations: AMPH, amphetamine; CA1, regio superior cornu ammonis; CA3, regio inferior cornu ammonis; DA, dopamine; ED, escalating dose/s; F-J C, Fluoro-Jade C; GFAP, glial fibrillary acidic protein; Glu, glutamate; LTP, long term potentiation; METH, methamphetamine; MK-801, dizocilpine; NMDA, *N*-methyl-D-aspartate; TBS, 0.05 M Trizma base buffer containing 150 mM of NaCl, pH 7.2.

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nitrogen species formation, which has been implicated in METH neurotoxicity (Quinton and Yamamoto, 2006; Cadet et al., 2007). Glu-mediated excitotoxicity is believed to be one of the mechanisms leading to METH-induced apoptotic and/or necrotic cell death (Davidson et al., 2001; Cadet et al., 2003). Indeed, the toxicity to monoaminergic systems induced by amphetamines can be attenuated/prevented by NMDA receptor competitive and noncompetitive antagonists: Fuller et al. (1992) prevented the amphetamine (AMPH)-induced DA-prolonged depletion in the mice striatum using a competitive antagonist of NMDA receptor, LY274614; Ohmori et al. (1996) also observed a prevention of METH-repeated dose-consequent behavioral sensitization and the loss of monoamines and their metabolites in the rat striatum, using both competitive MK-801 and noncompetitive D-CPP-ene antagonists. The neuroprotection afforded with these chemicals was thought to be related to their hypothermic effects but, more recently, Bowyer et al. (2001) found that MK-801 still exerted its neuroprotection, preventing DA and TH (tyrosine hydroxylase) depletion in the mice striatum, 7 days after a regimen of 4×15 mg/kg METH, independent of thermoregulation. Moreover, Eisch et al. (1996) observed a decrease in Glu binding to NMDA receptor in the striatum and an increase in the parietal cortex of rats, 1 week after administrating 4×4 mg/kg s.c. METH. Furthermore, the NMDA receptor binding increase in parietal cortex layers II/III prevailed 1 month after drug administration, confirming long-term changes in glutamatergic transmission after a neurotoxic regimen of METH. In addition, Yamamoto et al. (1999) found that KCl-evoked Glu release from rat hippocampus slices, 7 days after a sensitizing regimen of METH, was diminished by 31% whereas it was increased by 34% in the striatum. Moreover, these authors found that NMDA receptor functionality was decreased at the striatum, since NMDA-evoked DA release and NMDA-stimulation dependent cleavage of fodrin alpha-subunit were significantly lowered. Moreover, NMDA and AMPA receptors are known for their essential role in mediating memory and learning consolidation in the hippocampus (Malinow and Malenka, 2002).

Another measure of METH-induced toxicity is astroglia activation which has been observed in the cortex, striatum and hippocampus of rodents (Pubill et al., 2003). Astrocytes become reactive in the first few days after injury by becoming hypertrophic and expressing elevated levels of glial fibrillary acidic protein (GFAP) (O'Callaghan and Miller, 1993; Pennypacker et al., 2000). The reactive astrocytes are not only important for rescuing neurons in the injured area but also for clearing up excessive Glu at the synaptic level. However, astrogliosis may also contribute to aggravate injury (Benarroch, 2005).

The aim of the present study was to investigate the effect of two different administrations of METH—acute versus subchronic with ED—on the levels of NMDA receptor obligatory NR1 and modulatory NR2A subunits, and of AMPA receptors GluR2 subunit (which controls the channel permeability to Ca^{2+}) in the rat hippocampus. It was also assessed whether these METH treatments would evoke hippocampal astrogliosis and/or neurodegeneration. Finally, the impact of METH on spontaneous alternation behavior in a Y-maze, an index of hippocampal-dependent spatial working memory, was also evaluated.

EXPERIMENTAL PROCEDURES

Animals and METH treatments

Male, 8-week-old, Sprague–Dawley rats (Charles River Laboratories Inc., Barcelona, Spain) weighting between 250 and 300 g were housed one per cage under controlled environmental conditions (12-h light/dark schedule, at room temperature of 21±1 °C) with food and water supplied *ad libitum*. A group of animals (the ED group) received a subchronic administration, being injected for seven consecutive days with increasing doses of METH (10, 15, 15, 20, 20, 25, 30 mg/kg, s.c.). The total daily doses were given in three injections per day at 6 h intervals (08:00, 14:00 and 20:00) except for the last dose of 30 mg/kg METH, given on the 7th day, which was administrated in a single injection, 'bolus' (at 14:00). A second group of animals (the acute group) was injected with saline, 0.9% NaCl s.c., for six consecutive days, three times per day, receiving the 'bolus' of 30 mg/kg METH s.c. on the 7th day. Finally, a third group of animals (the control group) received saline throughout the 7 days of treatment. All animals survived these dosing regimens. All procedures involving experimental animals were performed in accordance with European Community guidelines (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used. METH HCl was synthesized in the Organic Chemistry Department, University of Porto, Portugal.

Perfusion and histological processing

A different set of animals was anesthetized with 45 mg/kg pentobarbital 24 h after the last METH/saline administration and intracardially perfused with 250 ml of a saline solution (0.9% NaCl, 4% sucrose, pH 7.4) followed by 250 ml of 4% paraformaldehyde solution (4% paraformaldehyde, 0.9% NaCl, 4% sucrose, pH 7.4). Brains were post-fixed for 24 h and 20 μm thick coronal sections from the beginning to the end of the hippocampus were cut in a cryostat and collected in a sodium phosphate buffer (1× PBS, 25% sucrose, pH 7.3). Tissue sections were mounted onto gelatin-coated slides, dried at room temperature and stored at −20 °C until processed.

Fluoro-Jade C (F-J C) staining and GFAP immunohistochemistry

Neurodegeneration was assessed by F-J C staining as it evidences degenerating neurons, dendrites, axons, and terminals (Schmued et al., 2005). Briefly, the slides with the hippocampal slices were immersed 1×5 min in a basic alcohol solution (0.1% NaOH in 80% of absolute ethanol), 1×2 min in 70% ethanol and 1×2 min in distilled water. Slides were transferred to 0.06% potassium permanganate for 10 min, under constant shaking, rinsed in distilled water 1×2 min and incubated for 10 min in 0.0001% F-J C (Histo-Chem Inc., AR, USA), freshly prepared. After 3×1 min rinse in distilled water, the slices were air-dried on a 50 °C slide warmer during 5 min, dehydrated in xylene and coverslipped with DPX (Sigma-Aldrich, Sintra, Portugal). Finally, the staining was examined under a Zeiss (PG-HITEC, Mem Martins, Portugal) Axioskop 2 plus microscope equipped with a EBQ100 isolated fluorescent lamp and analysis were performed using the Axiovision Release 4.2 software.

In order to evaluate METH-evoked astrocytic response, immunohistochemistry for GFAP was performed, as this protein is a sensitive marker of astrogliosis and consequently, of a neurotoxic condition (O'Callaghan and Sriram, 2005). Slides were rinsed 1×5