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Methamphetamine decreases dentate gyrus stem cell self-renewal and shifts the differentiation towards neuronal fate

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Abstract Methamphetamine (METH) is a highly addictive psychostimulant drug of abuse that negatively interferes with neurogenesis. In fact, we have previously shown that METH triggers stem/progenitor cell death and decreases neuronal differentiation in the dentate gyrus (DG). Still, little is known regarding its effect on DG stem cell properties. Herein, we investigate the impact of METH on mice DG stem/progenitor cell self-renewal functions. METH (10 nM) decreased DG stem cell self-renewal, while 1 nM delayed cell cycle in the G0/G1-to-S phase transition and increased the number of quiescent cells (G0 phase), which correlated with a decrease in cyclin E, pEGFR and pERK1/2 protein levels. Importantly, both drug concentrations (1 or 10 nM) did not induce cell death. In accordance with the impairment of self-renewal capacity, METH (10 nM) decreased $Sox2^+/Sox2^+$ while increased $Sox2^-/Sox2^-$ pairs of daughter cells. This effect relied on N-methyl-D-aspartate (NMDA) signaling, which was prevented by the NMDA receptor antagonist, MK-801 (10 μM). Moreover, METH (10 nM) increased doublecortin (DCX) protein levels consistent with neuronal differentiation. In conclusion, METH alters DG stem cell properties by delaying cell cycle and decreasing self-renewal

Abbreviations: bFGF2, basic fibroblast growth factor 2; BrdU, 5-bromo-2′-deoxyuridine; Cdk, cyclin-dependent kinase; DCX, doublecortin; DG, dentate gyrus; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinases 1/2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; MAPK, mitogen-activated protein kinase; MDMA, 3,4-methylenedioxymethamphetamine; MEK1, MAPK/ERK kinase 1; METH, methamphetamine; MK-801, (5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cylcohepten-5,10-imine maleate; NMDA, N-methyl-D-aspartate; pEGFR, phospho-epidermal growth factor receptor; pERK1/2, phospho-extracellular-signal-regulated kinases 1/2; SGZ, subgranular zone; Sox2, SRY (sex determining region Y)-box 2; SVZ, subventricular zone; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

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capacities, mechanisms that may contribute to DG neurogenesis impairment followed by cognitive deficits verified in METH consumers.

Introduction

Methamphetamine (METH) is a highly addictive drug whose consumption has been increasing worldwide and turned to be a public health problem (Silva et al., 2010). Several studies have extensively described the negative effects of METH in the Central Nervous System (Gonçalves et al., accepted for publication; Krasnova and Cadet, 2009), concluding that METH abusers exhibit smaller hippocampal volume, which was positively correlated with poorer memory performance (Thompson et al., 2004). Accordingly, animal studies have clearly demonstrated hippocampal neuronal dysfunction (Gonçalves et al., 2010), as well as cognitive deficits induced by this psychostimulant (Simões et al., 2007). Nevertheless, the mechanisms of METH-induced memory deficits are still poorly understood, but pieces of evidence show that neurogenesis is tightly related to memory. In fact, reduction in the number of immature neurons induces deficits in long-term retention of spatial cognitive functions (Deng et al., 2009), and ablation of hippocampal neurogenesis impairs memory performance related to pattern separation functions (Clelland et al., 2009).

The information available regarding the effect of METH on neurogenesis describes that, in the dentate gyrus (DG), cell proliferation is decreased in gerbils (postnatal day 30) administered once with the drug at postnatal day 14–20 (50 mg/kg) (Hildebrandt et al., 1999). On the other hand, a lower dose of METH (25 mg/kg) transiently decreased cell proliferation in the same region (Teuchert-Noodt et al., 2000). Furthermore, a chronic METH administration (1 mg/kg/day for 14 days) had no effect on the number of proliferating cells in mice DG (Maeda et al., 2007). Interestingly, Wistar rats self-administered with METH (0.05 mg/kg/infusion, 1 h intermittent access, 2 × a week during 28 days) displayed an increase in DG cell proliferation as well as in neuronal differentiation, whereas both short (1 h/day) and long (6 h/day) access decreased proliferation and differentiation followed by a reduced number of DG granule cell neurons (Mandyam et al., 2008). Furthermore, self-administration of METH (1 h/day access METH for 13 days) increased the number of radial glia-like cells (type 1 cells), but decreased the proportion of preneuronal neuroblasts (type 2a cells) (Yuan et al., 2011) showing that at different maturation stages cells respond differently to an external stimuli (Tashiro et al., 2007). Also, daily access to METH (6 h/day for 4 or 13 days) decreased the number of proliferating cells in the DG without changing, however, the length of S-phase of the cell cycle (Yuan et al., 2011). In vitro studies also point that METH reduced proliferation of rat hippocampal neural progenitor cells (Tian et al., 2009; Venkatesan et al., 2011). Additionally, our group recently verified that a nontoxic concentration of METH (1 nM for 7 days) decreased the number of mature neurons in DG-derived neurosphere cultures (Baptista et al., 2012). Concerning the subventricular zone (SVZ), we have also shown that METH decreases cell proliferation, neuronal differentiation and maturation of stem/progenitor cells (Bento et al., 2011).

Overall, it seems clear that METH interferes with hippocampal neurogenesis, but many questions remain unanswered. In fact, the direct effect of this drug on stem cell self-renewal capacities has never been addressed before. Herein, we show that METH delays cell cycle progression from G0/G1-to-S phase. This effect could be due to the down-regulation of cyclin E, a cyclin involved in the progression through the G1 phase and initiation of DNA replication in the S phase, and to the decrease of epidermal growth factor receptor (EGFR) and extracellular-signal-regulated kinases 1/2 (ERK1/2) phosphorylation, mediators in the MAPK signaling pathway involved in cell proliferation progression. Also, METH decreases DG stem cell self-renewal capacities, which seems to involve NMDA receptors. In conclusion, the present work reveals a negative impact of METH on DG stem cell capacities that can contribute to memory deficits upon METH consumption.

Material and methods

Dentate gyrus neurosphere cultures

Post-natal 1–3-day-old C57BL/6J mice were sacrificed by decapitation and the brains were placed in sterile saline solution. Afterwards, the meninges were removed and DG fragments were dissected from 450 μm-thick brain coronal sections, digested in 0.025% trypsin and 0.265 mM EDTA (both from Life Technologies, Carlsbad, CA, USA), and single cells were obtained by gentle trituration. Then, cells were diluted in serum-free culture medium (SFM) composed of Dulbecco's modified Eagle's medium/Ham's (DMEM) F-12 medium GlutaMAX-I supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 1% B27 supplement, 5 ng/ml epidermal growth factor (EGF) and 2.5 ng/ml basic fibroblast growth factor (bFGF-2) (all from Life Technologies). Afterwards, cells were plated in uncoated Petri dishes and neurospheres were allowed to develop for 6 days in a 95% air–5% CO_2 humidified atmosphere at 37 °C. At 6 days, the neurosphere mean diameter was 90.22 ± 2.24 μm (measurements performed on 2 independent cultures).

Experimental procedures were performed according to the guidelines of the European Communities Council Directives (2010/63/EU) and the Portuguese law for the care and use of experimental animals (DL no. 129/92). All efforts were made to minimize animal suffering and to reduce the number of animals.

Cell death assay

DG neurospheres were exposed to 10 or 100 nM METH (Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal) for 24 h (Fig. 1A)