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Extended-access methamphetamine self-administration elicits neuroinflammatory response along with blood-brain barrier breakdown

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ABSTRACT

Methamphetamine (METH) is a highly addictive psychostimulant drug that can lead to neurological and psychiatric abnormalities. Several studies have explored the central impact of METH use, but the mechanism(s) underlying blood-brain barrier (BBB) dysfunction and associated neuroinflammatory processes after chronic METH consumption are still unclear. Important findings in the field are mainly based on *in vitro* approaches and animal studies using an acute METH paradigm, and not much is known about the neurovascular alterations under a chronic drug use. Thus, the present study aimed to fill this crucial gap by exploring the effect of METH-self administration on BBB function and neuroinflammatory responses. Herein, we observed an increase of BBB permeability characterized by Evans blue and albumin extravasation in the rat hippocampus and striatum triggered by extended-access METH self-administration followed by forced abstinence. Also, there was a clear structural alteration of blood vessels showed by the down-regulation of collagen IV staining, which is an important protein of the endothelial basement membrane, together with a decrease of intercellular junction protein levels, namely claudin-5, occludin and vascular endothelial-cadherin. Additionally, we observed an up-regulation of vascular cell and intercellular adhesion molecule, concomitant with the presence of T cell antigen CD4 and tissue macrophage marker CD169 in the brain parenchyma. Rats trained to self-administer METH also presented a neuroinflammatory profile characterized by microglial activation, astrogliosis and increased pro-inflammatory mediators, namely tumor necrosis factor-alpha, interleukine-1 beta, and matrix metalloproteinase-9. Overall, our data provide new insights into METH abuse consequences, with a special focus on neurovascular dysfunction and neuroinflammatory response, which may help to find novel approaches to prevent or diminish brain dysfunction triggered by this overwhelming illicit drug.

1. Introduction

Methamphetamine (METH) is a psychostimulant drug of abuse that causes severe alterations in the central nervous system (CNS). Human METH abusers usually show structural brain abnormalities, namely grey matter loss and white matter hypertrophy that are associated with learning and working memory deficits (Cadet et al., 2014; Thompson et al., 2004). Although oxidative stress and both dopaminergic and glutamatergic systems deregulation are usually identified as the main factors contributing for METH neurotoxicity, it is now unquestionable that the impact of METH on the CNS is much broader than previously thought (Cadet and Krasnova, 2009; Gonçalves et al., 2014; Silva et al., 2010; Thomas et al., 2004). In fact, recent studies have shown significant alterations in glial cells and blood-brain barrier (BBB) function (Coelho-Santos et al., 2015; Gonçalves et al., 2008, 2010; Kiyatkin et al., 2007; Krasnova et al., 2016; Martins et al., 2011, 2013; Sharma and Ali, 2006). Kousik et al. (2014), demonstrating that METH self-administration followed by forced abstinence, as well as acute METH treatment, reduces microvessels diameter and volume in the rat striatum. Also, it was stated that dopamine receptor

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D2 (D2R) activation was largely responsible for the vasoconstrictor effects of METH (Kousik et al., 2014). Accordingly, abstinent METH abusers present a reduction in both striatal and cortical cerebral blood flow (Chung et al., 2010; Hwang et al., 2006). Despite evidence that METH causes neurovascular dysfunction both in humans and animals, the mechanisms underlying such effects remain unclear, particularly in which concerns with chronic drug use.

We have previously reported that acute METH treatment (30 mg/kg) triggers a neuroinflammatory response and BBB disruption in the mouse hippocampus (Gonçalves et al., 2008, 2010; Martins et al., 2011). Furthermore, we and others observed that METH-induced BBB impairment involves a downregulation of tight (TJ) and adherens junctions (AJ) (Eugenin et al., 2013; Mahajan et al., 2008; Ramirez et al., 2009; Rosas-Hernandez et al., 2013; Urrutia et al., 2013), as well as an increase in the activity and expression of matrix metalloproteinase-9 (MMP-9) (Martins et al., 2011; Urrutia et al., 2013). Up-regulation of MMP-2 and MMP-9 was also detected in postmortem human brains after METH intoxication (Wang et al., 2014). More recently, our group demonstrated that METH concentrations relevant to human abuse increase BBB permeability by interfering not only with paracellular pathway but also with vesicular transport across endothelial cells (ECs) (Coelho-Santos et al., 2015; Martins et al., 2013). In addition, nitric oxide (Martins et al., 2013), reactive oxygen species (Ramirez et al., 2009), and tumor necrosis factor-alpha (TNF-α) (Coelho-Santos et al., 2015) were identified as key players in METH-induced BBB permeability. Despite these important data, the available studies were based on *in vitro* approaches (Coelho-Santos et al., 2015; Martins et al., 2013) or acute regiment of METH (Coelho-Santos et al., 2015; Emoto et al., 2015; Eugenin et al., 2013; Kiyatkin et al., 2007; Martins et al., 2011, 2013; Northrop et al., 2016; Northrop and Yamamoto, 2012, 2015). Noteworthy, to date only two works have simultaneously explored both neuroinflammatory processes and BBB alterations triggered by METH. Nevertheless in both studies *in vitro* models were used (Jumnongprakhon et al., 2016; Parikh et al., 2015), which may not mirror what is occurring in a more complex system as an animal model.

Understanding the cellular mechanisms involved in METH-induced neurodegeneration is of great interest to scientist and clinicians, since it may contribute to the identification of new therapies to diminish the consequences of METH abuse. Chronic consumption of METH causes not only neuropsychiatric disorders, but also increases the vulnerability to other disease states, such as human immunodeficiency virus (HIV) and hepatitis C (Loftis and Janowsky, 2014) infections. In fact, METH users tend to have a higher brain rates of bacterial and viral invasion as compared with non-users (Gavrilin et al., 2002; Letendre et al., 2005), which can result in cognitive decline and dementia. Accordingly, animal studies have demonstrated that METH potentiates the vascular oxidative stress induced by the HIV-1 proteins, gp120 and Tat (Flora et al., 2003; Mahajan et al., 2008). Thus, unraveling the impact of METH abuse on BBB function may provide new insight into the neuropathology and behavioral alterations that occur under METH abuse.

Herein, we reveal that METH increases of BBB permeability, with alterations on the expression of endothelial intercellular proteins and vascular adhesion molecules, also promoting gliosis and up-regulation of inflammatory mediators. Such effects were observed 24 h after the last drug self-administration (SA) session, and some remained until 7 days of abstinence. Overall, we concluded that METH abuse leads to hippocampal and striatal neuroinflammation and barrier permeability with up-regulation of tissue macrophage marker (CD169) and T cell antigen CD4 in the brain parenchyma.

2. Materials and methods

2.1. Animals

Twenty-two male wild-type Wistar rats (Charles River Laboratories, Barcelona, Spain) aged 3 months and weighing 280–320 g at the beginning of the experiments were used. All animals were experimentally naïve and were housed individually in a temperature-controlled room (23 °C) with a 12-h light–dark cycle (8:00–20:00 lights on) and with free access to food and tap water prior to initiation of the experiments. Importantly, at the time-points that rats were sacrificed (24 h and 7 d post-last SA session) there were no significant alterations in body temperature and locomotor activity. Moreover, the survival rate was 100%. The present studies were carried out in accordance with the European Union Council Directives (86/609/EEC and 2010/63/EU). All the experimental procedures were approved by the local Animal Welfare Committee of School of Psychology (UNED, Madrid, Spain).

2.2. Extended-access to methamphetamine self-administration

Twelve operant chambers (Coulburn Instruments, Allentown, PA, USA) were used for extended-access to METH self-administration (SA) studies. Wistar rats were food-deprived to 95% of their free-feeding weight before surgery (Miguens et al., 2013), and they were submitted to a fixed ratio (FR) 1 schedule of food reinforcement in several 30 min sessions. When rats showed a stable rate of lever pressing, they were given *ad libitum* access to food and surgery was performed. Rats were then prepared with intravenous catheters in the right jugular vein as previously described (Higuera-Matas et al., 2008) with few modifications. Briefly, polyvinylchloride tubing (0.064 i.d.) was surgically implanted in the jugular vein approximately at the level of the atrium under isoflurane (Forane®, Abbott; 0.6 L/min) anesthesia. All subjects were housed individually following surgery and catheters were flushed daily with 0.5 mL of antibiotic (gentamicin, 0.10 mg/mL) dissolved in heparinized saline to prevent infections and to maintain catheter patency. After at least 7 days postoperative recovery period, rats were once again food-deprived to 95% of their free feeding body weight. Subsequently, and based on a study by Kousik et al. (2011), animals were trained to self-administer METH (0.1 mg/kg per infusion; methamphetamine hydrochloride; Sigma-Aldrich, St. Louis, MO) or saline (0.9% NaCl) in 100 μL volume, according to a FR1 schedule of reinforcement during daily sessions, with a timeout period of 10 s to prevent overdose. From day 1 to day 3, the sessions lasted 2 h or until 25 METH infusions (short-term access to the drug), followed by daily 6 h sessions or 50 METH infusions during 7 d (from day 4 to day 10; long-term access to the drug; Jang et al., 2013). Drug and food delivery, operant data acquisition and storage were performed using IBM compatible computers (MED Associates, Georgia, VE, USA). The animals were sacrificed 24 h or 7 d after the last operant session.

2.3. Assessment of Evans blue extravasation and immunohistochemistry

Evans blue (EB; Sigma-Aldrich) dye was used to evaluate BBB permeability. After 24 h or 7 d of abstinence, rats were anaesthetized with chloral hydrate 16% (0.2 kg/mL, intraperitoneally) and injected in the tail vein with 2% EB (4 mL/kg). After 30 min, they were perfused intracardially with 0.01 M phosphate-buffered saline solution (PBS, pH 7.4) followed by 4% paraformaldehyde (PFA, pH 7.4). The brains were removed, immersed in 30% sucrose during 24 h at 4 °C, post-fixed overnight