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3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) produces edema due to BBB disruption induced by MMP-9 activation in rat hippocampus

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ABSTRACT

The recreational drug of abuse, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) disrupts blood-brain barrier (BBB) integrity in rats through an early $P2X_7$ receptor-mediated event which induces MMP-9 activity. Increased BBB permeability often causes plasma proteins and water to access cerebral tissue leading to vasogenic edema formation. The current study was performed to examine the effect of a single neurotoxic dose of MDMA (12.5 mg/kg, i.p.) on *in vivo* edema development associated with changes in the expression of the perivascular astrocytic water channel, AQP4, as well as in the expression of the tight-junction (TJ) protein, claudin-5 and Evans Blue dye extravasation in the hippocampus of adult male Dark Agouti rats. We also evaluated the ability of the MMP-9 inhibitor, SB-3CT (25 mg/kg, i.p.), to prevent these changes in order to validate the involvement of MMP-9 activation in MDMA-induced BBB disruption. The results show that MDMA produces edema of short duration temporally associated with changes in AQP4 expression and a reduction in claudin-5 expression, changes which are prevented by SB-3CT. In addition, MDMA induces a short-term increase in both tPA activity and expression, a serine-protease which is involved in BBB disruption and upregulation of MMP-9 expression. In conclusion, this study provides evidence enough to conclude that MDMA induces edema of short duration due to BBB disruption mediated by MMP-9 activation.

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) is a recreational psychostimulant drug of abuse. According to the United Nations Office on Drugs and Crime data, published in the World Drug Report of 2016, the availability of high dose "ecstasy" formulations appears to have increased in recent years, particularly in Europe. Although MDMA is often thought of as a safe drug, it produces hepatic and brain toxicity. Fatalities caused by MDMA consumption are generally associated with hyperthermia (Hall and Henry, 2006) and cerebral edema (Ghatol and Kazory, 2012; O'Connor et al., 1999; Parr et al., 1997).

MDMA administration in rats activates a short-term neuro-inflammatory response, induces hyperthermia and enhances oxidative stress (Colado et al., 1997; O'Shea et al., 2005; O'Shea et al., 2014; Orio et al., 2004), all of which are factors that

Abbreviations: AQP4, aquaporin 4; GFAP, glial fibrillary acidic protein; LRP-1, lipoprotein receptor-related protein 1; MDMA, 3,4-methylenedioxymethamphetamine; MMP, metalloproteinase; TJ, tight junction; tPA, tissue plasminogen activator.

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mediate blood-brain barrier (BBB) disruption in diverse pathologies. Proinflammatory cytokines and oxygen reactive species can modify BBB permeability through the induction of protease expression and activity (Candelario-Jalil et al., 2009). Metalloproteinases (MMPs) are the main proteases associated with BBB disruption in pathological conditions through basal lamina and tight-junction (TJ) protein degradation (Rosenberg, 2009). MMP-9 is the main inducible MMP upregulated during BBB disruption in cerebral ischemia (Cui et al., 2012; Yang et al., 2007) and traumatic brain injury (TBI) rodent models (Vilalta et al., 2008). It degrades extracellular matrix and TJ protein leading to BBB disruption, an increase in permeability to plasmatic proteins and vasogenic edema in rodent models of pathological conditions such as status epilepticus (Kim et al., 2015), acute liver failure (Chen et al., 2009) or ischemia (Rosenberg and Yang, 2007).

We recently showed that a single neurotoxic dose of MDMA increases BBB permeability in rat hippocampus due to microglial activation through $P2X_7$ receptor-mediated signaling, which in turn increases MMP-9 activity (Rubio-Araiz et al., 2014). MDMA increases plasmatic immunoglobulin G extravasation and decreases expression of the basal lamina proteins laminin and collagen type IV, indicative of altered BBB permeability and integrity (Rubio-Araiz et al., 2014). Considering that vasogenic edema formation could be a consequence of BBB disruption, we determine, for the first time, the effect of MDMA on the time-course of edema formation by *in vivo* magnetic resonance imaging (MRI). In addition, we examine the expression of AQP4, a perivascular astrocytic water channel which is associated with both formation and resolution of vasogenic edema in other models (Fukuda et al., 2012; Steiner et al., 2012; Tourdias et al., 2011). Furthermore, to assess TJ integrity we evaluate the expression of claudin-5, the most abundant protein of neuroendothelial TJs (Nitta et al., 2003), as well as Evans Blue dye extravasation following MDMA. To implicate MMP-9 in BBB disruption and edema formation following MDMA, rats are treated with SB-3CT, a BBB-permeable selective gelatinase inhibitor (Gooyit et al., 2012).

We also examine the MDMA effect on the tissue plasminogen activator (tPA)/plasmin system since both tPA (Moser et al., 1993) and plasmin (Skrzypiec et al., 2009) are capable of degrading laminin. tPA activity and expression, as well as the expression of the plasmin zymogen, plasminogen, are determined. We also study the effect of MDMA on low density lipoprotein receptor-related protein 1 (LRP-1) expression. The interaction between tPA and LRP-1 is a widely described mechanism upregulating MMP-9 during BBB disruption (Yepes et al., 2003).

2. Material and methods

2.1. Animals and drug administration

Male Dark Agouti rats (175–200 g, Envigo, Barcelona) were used. In this strain, MDMA induces a reproducible acute hyperthermic response and a long-term neurotoxic loss of 5-HT after a single dose (O'Shea et al., 1998). Rats were housed in groups of 6 in conditions of constant temperature (21 ± 2 °C) and a 12 h light/dark cycle (lights on: 08 h 00min) and given free access to food and water. Animals were sacrificed 1 h, 3 h, 6 h or 24 h after treatment. Room temperature during the experiment was 21–22 °C. All experimental procedures were performed in accordance with the guidelines of the Animal Welfare Committee of the Universidad Complutense de Madrid and of the Comunidad de Madrid (following EU Directive 2010/63/UE for animal experiments).

(±) MDMA.HCl (12.5 mg/kg, Ministerio de Sanidad, Servicios Sociales e Igualdad, Spain) was dissolved in saline (0.9% NaCl). Dose is reported in terms of the base. SB-3CT (25 mg/kg, S7430, Selleck Chemicals, Spain), a potent and selective inhibitor of MMP-2 and MMP-9 activities which crosses the BBB (Gooyit et al., 2012), was suspended in a vehicle solution (5% DMSO, 10% Tween-80 in saline). Both treatments were administered intraperitoneally in a volume of 1 ml/kg.

2.2. Evans Blue extravasation

Evans Blue dye (E2129, Sigma-Aldrich, Spain) was dissolved in saline and administered intravenously (80 mg/kg; 4 ml/kg), 1 h after MDMA, in the lateral tail vein of anesthetized rats (isoflurane 2.5%, Isoba®vet, Global Vet, Spain; in a mixture of oxygen and nitrogen protoxide 0.5:1 L/min, Alphagaz™, Spain). Two hours after MDMA, rats were sacrificed with sodium pentobarbital (120 mg/kg, Dolethal®, Ventoquinol, Spain) and perfused transcardially through the left ventricle with phosphate-buffered saline (0.1 M PBS, pH7.4). Brains were removed and hippocampi dissected over ice and stored at −80 °C. Tissue was homogenized by sonication 1:3 w/v in 50% trichloroacetic acid. Homogenates were centrifuged at 30 000 × g for 20 min at 4 °C. Absorbance at 620 nm was determined in duplicate for each sample and interpolated from a standard curve of Evans Blue.

2.3. Magnetic resonance imaging

All MRI experiments were performed on a BIOSPECT BMT 47/40 (Bruker, Ettlingen, Germany) spectrometer operating at 4.7 T, equipped with a 6 cm gradient system capable of reaching a 450 mT/m gradient strength. A 3.5 cm birdcage radiofrequency probehead was used for transmission and reception.

During the MRI procedure, the rats were anesthetized with 1–2.5% isoflurane in oxygen mixture and kept at the temperature registered just after induction of anesthesia. In order to minimize movement artifacts, the head of the rat was immobilized and the breathing progress was monitored and kept over 60 breaths per minute to synchronize the respiration and the image acquisition.

Before drug treatment (basal) and from 2.5 to 6 h after MDMA administration, a series of spin echo images were obtained to assess T2 relaxation time. In the MMP-9 inhibition experiment only basal T2 images and those obtained 2.5 h after MDMA administration were evaluated.

For assessment of the edema formation, T2 relaxation time (ms) values of 3 coronal slices of hippocampus (16 images per slice) were obtained using the following parameters: variable echo time = 20–320 ms, interecho interval = 20 ms, constant repetition time = 2.5s (changing with animal breathing due to image acquisition synchronization), field of view = 3 × 3cm^2, slice thickness = 1.0 mm and image matrix = 256 × 256.

Image Sequence Analyze tool of the ParaVision 3.1 package (Bruker) was used to analyze the T2 series. Three regions of interest were assigned to each hippocampal slice (bregma −5, −3.5 and −2 mm) to determine T2 values.

2.4. Immunohistochemistry

Rats were anesthetized with sodium pentobarbital (120 mg/kg, Dolethal® Ventoquinol) and perfused transcardially through the left ventricle with 0.1 M PBS (pH7.4) followed by 4% paraformaldehyde-PBS. Brains were removed, postfixed in the same paraformaldehyde solution for 4 h and cryoprotected in 30% sucrose-PBS at 4 °C. After freezing at −20 °C brains were sliced at 30 μm in the coronal plane. Slices were stored floating in cryoprotectant solution at −20 °C. Immunohistochemical studies were performed in sections containing the hippocampal regions dentate gyrus, CA1 and CA3 (Paxinos and Watson, 2005) but only those in