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Dopamine D_1 receptor deletion strongly reduces neurotoxic effects of methamphetamine

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ABSTRACT

Methamphetamine (METH) is a potent, highly addictive psychostimulant consumed worldwide. In humans and experimental animals, repeated exposure to this drug induces persistent neurodegenerative changes. Damage occurs primarily to dopaminergic neurons, accompanied by gliosis. The toxic effects of METH involve excessive dopamine (DA) release, thus DA receptors are highly likely to play a role in this process. To define the role of D_1 receptors in the neurotoxic effects of METH we used D_1 receptor knock-out mice ($D_1R^{-/-}$) and their WT littermates. Inactivation of D_1R prevented METH-induced dopamine fibre loss and hyperthermia, and increases in gliosis and pro-inflammatory molecules such as iNOS in the striatum. In addition, D_1R inactivation prevented METH-induced loss of dopaminergic neurons in the substantia nigra. To explore the relationship between hyperthermia and neurotoxicity, METH was given at high ambient temperature (29 °C). In this condition, $D_1R^{-/-}$ mice developed hyperthermia following drug delivery and the neuroprotection provided by D_1R inactivation at 23 °C was no longer observed. However, reserpine, which empties vesicular dopamine stores, blocked hyperthermia and strongly potentiated dopamine toxicity in $D_1R^{-/-}$ mice, suggesting that the protection afforded by D_1R inactivation is due to both hypothermia and higher stored vesicular dopamine. Moreover, electrical stimulation evoked higher DA overflow in $D_1R^{-/-}$ mice as demonstrated by fast scan cyclic voltammetry despite their lower basal DA content, suggesting higher vesicular DA content in $D_1R^{-/-}$ than in WT mice. Altogether, these results indicate that the D_1R plays a significant role in METH-induced neurotoxicity by mediating drug-induced hyperthermia and increasing the releasable cytosolic DA pool.

Introduction

Methamphetamine (METH), a synthetic derivative of amphetamine, is a psychostimulant with high addictive potential used by between 13.7 and 52.9 million people world-wide (UNODC, World Drug Report, 2010). METH is a known neurotoxin, causing damage primarily to the dopaminergic system in all species studied: monkeys (Seiden et al., 1976), rodents (Granado et al., 2010, 2011a,b; Krasnova et al., 2011) and humans (McCann et al., 1998; Volkow et al., 2001). In mice, repeated exposure to METH causes persistent neurotoxicity to dopaminergic terminals and neurons, evidenced by reduced tyrosine hydroxylase (TH) (Bowyer et al., 2008; Deng et al., 1999; O'Callaghan and Miller, 1994; Xu et al., 2005; Zhu et al., 2005) and dopamine transporter (DAT) levels (Achat-Mendes et al., 2005; Deng et al., 1999; Fumagalli et al., 1999) in the striatum. In addition, this drug induces neuronal death in the striatum, which occurs by a process resembling neuronal apoptosis (Cadet and Krasnova, 2009; Cadet et al., 2005, 2007).

METH also causes cell body loss in the substantia nigra (Granado et al., 2011a,b; Sonsalla et al., 1996), affecting the same nigrostriatal dopaminergic neurons that undergo selective degeneration in Parkinson's disease (Granado et al., 2010). We have recently shown that METH selectively damages the nigrostriatal pathway, sparing the mesolimbic dopaminergic pathway, and that the striosomes are more sensitive than the striatal matrix, as indicated by greater TH/DAT-immunoreactivity loss (Granado et al., 2010). METH also causes reactive astrocytosis and microgliosis (Cadet and Krasnova, 2009; Fantegrossi et al., 2008; O'Callaghan and Miller, 1994; Thomas et al., 2004, 2008a), providing additional evidence for neuronal injury.

Although the exact molecular mechanisms of METH-induced dopaminergic neurotoxicity are not established, dopamine itself appears to play a significant role (Albers and Sonsalla, 1995; Thomas et al., 2008b). Specifically, it has been suggested that newly synthesized DA in the cytoplasmic pool (Thomas et al., 2008b) can be metabolized via auto-oxidation to produce DA quinones, superoxide anions and hydrogen oxygen species, with subsequent generation of oxidative stress, mitochondrial dysfunction, and damage within dopaminergic

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terminals (Albers and Sonsalla, 1995; Cadet and Krasnova, 2009; LaVoie and Hastings, 1999). Alternatively, neurotoxicity may result from the increase in extracellular DA induced by METH. Excessive release of DA following METH administration has been shown to activate stress pathways in the ER (endoplasmic reticulum) in a D_1R-dependent manner (Jayanthi et al., 2009).

Pharmacological studies with dopamine receptor antagonists also implicate dopamine receptors in the neurotoxicity induced by METH. We recently demonstrated that genetic inactivation of dopamine D_2R reduced the damage to nigrostriatal terminals and cell bodies induced by METH (Granado et al., 2011a). However, the protective effect provided by inactivation of D_2R is incomplete, suggesting that D_1R may be also involved since SCH23390, a D_1/D_5R antagonist, can also prevent neurotoxicity induced by METH (Angulo et al., 2004; Broening et al., 2005; Bronstein and Hong, 1995; Metzger et al., 2000; Sonsalla et al., 1986; Xu et al., 2005).

To determine the role of D_1R in neurotoxicity induced by METH, we examined the effect of this drug on loss of dopaminergic fibres and nigrostriatal dopamine neurons in D_1R knockout mice ($D_1R^{-/-}$), revealing a protective effect of D_1R inactivation. D_1R inactivation also blocked METH-induced hyperthermia, a hallmark of the response to amphetamine derivatives. To further explore the role of the D_1R and the relationship between hyperthermia and neuronal damage, we compared the effects of this drug given at normal (23 °C) or elevated (29 °C) ambient temperature by examining dopaminergic parameters and the glial response in the striatum and substantia nigra in D_1R knock-out animals. In addition, we did fast scan cyclic voltammetry (FSCV) and pretreatment with αMPT or reserpine to study how inactivation of D_1R might protect against neurotoxicity induced by this drug.

Methods

Animals and treatment

Experiments were carried out in male and female dopamine D_1 receptor knockout ($D_1R^{-/-}$) mice generated by homologous recombination as described previously (Granado et al., 2008c; Moratalla et al., 1996; Xu et al., 1994) and in their wild type (WT) littermates. Mice used in this study were derived from mating heterozygous mice. We used adult males and females initially weighing 20–25 g whose genotype was determined by PCR analysis. Mice were housed in groups of 4–6 per cage, in conditions of constant room temperature (21–22 °C) and a 12 h light/dark cycle (lights on at 7:00 h) and given free access to food and water. Animals were treated in accordance with European Community guidelines (2003/65/CE), and all experimental procedures were approved by the Bioethics Committee of the Instituto Cajal.

Mice received three injections of either (+)-METH (5 mg/kg i.p.) or saline (control) at 3 h intervals, a neurotoxic regimen previously shown to produce marked depletion of mouse striatal DA. METH was dissolved in 0.9% w/v NaCl (saline) and injected in a volume of 10 ml/kg. METH hydrochloride was obtained from Sigma-Aldrich (Madrid, Spain). Doses are quoted in terms of the base. Reserpine (3 mg/kg, i.p. Sigma-Aldrich, Madrid, Spain) was dissolved in 0.1% acetic acid and administered 18 h before METH (3 injections of 3 mg/kg i.p). This lower dose of METH was used in order to better see the potentiation of METH toxicity induced by reserpine. Animals in this experiment were sacrificed 5 days after treatment with METH or saline.

For the elevated ambient temperature experiment, $D_1R^{-/-}$ mice receiving METH were maintained at a room temperature of 29 ± 2 °C for 2 h prior to treatment, during the injection paradigm, and for 1 h following the last injection. The α-methyl-p-tyrosine (αMPT) was obtained from Sigma-Aldrich (Madrid, Spain). αMPT was dissolved in 0.9% w/v NaCl and administered i.p. (100 mg/kg × 4) at 24, 16, 2 and 1 h before treatment with METH (5 mg/kg, i.p. 3 injections at 3 hour intervals). Animals were sacrificed 1 or 7 days after drugs administration.

Measurement of rectal temperature

Rectal temperature was measured using a digital readout thermocouple (BAT-12 thermometer, Physitemp Instruments, Clifton, NJ, USA) with a resolution of 0.1 °C and accuracy of +/−0.1 °C attached to a RET-3 Rodent Sensor. The sensor was inserted 2 cm into the mouse rectum, while the mouse was lightly restrained by holding in the hand. A steady readout was obtained within 10 s of probe insertion. Temperature readings were taken every 30 min immediately before and after each METH injection and then 1, 2 and 3 h after drug administration.

Measurement of monoamines and their metabolites in the striatum

One or seven days after the treatment, mice were killed by cervical dislocation and decapitation (n = 4–6 per group). The brains were rapidly removed and the striatum dissected out on ice. Dopamine, serotonin, and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA) were measured by high-performance liquid chromatography and electrochemical detection as described previously (Granado et al., 2010; Gutierrez-Lopez et al., 2010). The mobile phase consisted of KH_2PO_4 (0.05 M), octanesulfonic acid (0.4 mM), EDTA (0.1 mM), and methanol (16%) and was adjusted to pH 3.7 with phosphoric acid, filtered, and degassed. The flow rate was 1 ml/min. The high-performance liquid chromatography system consisted of a pump (Waters 510) linked to an automatic sample injector (Loop 200 µl, Waters 717 plus Autosampler) and a stainless steel reversed-phase column (Spherisorb ODS2, 5 µm, 150×4.6 mm^2; Waters, Milford, MA, USA) with a precolumn and a coulometric detector (Coulochem II; Esa, Chelmsford, M, USA). The working electrode potential was set at 400 mV with a gain of 2 µA. The current produced was monitored by means of integration software (Clarity Software, DataApex, Prague, Czech Republic).

Immunohistochemistry

One or seven days after the treatment, animals (n = 3–6 per group) were deeply anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then transcardially perfused with 4% paraformaldehyde dissolved in PB (Buffer phosphate, pH 7.4). After perfusion, brains were removed and immersed overnight in the same fixative solution. Coronal brain sections (30 µm) were obtained on a slicing vibratome (Leica, Madrid, Spain) and kept in PB solution at 4 °C until use. Immunostaining was carried out on free-floating sections with standard avidin–biotin immunocytochemical protocols (Darmopil et al., 2008, 2009; Ortiz et al., 2010; Pavon et al., 2006). Endogenous peroxidase activity was removed by incubation in 3% H_2O_2 for 10 min. Sections were preblocked for 1 h with normal goat serum (NGS) (Vector Laboratories, Burlingame, CA, USA). Sections were incubated overnight with specific primary antibodies (Ab-I): rabbit tyrosine hydroxylase antiserum (TH, used at 1:1000 Chemicon International, Temecula, CA, USA); rat monoclonal antibody against dopamine transporter (DAT, used at 1:1000, Chemicon International); rabbit anti-glial fibrillary acidic protein antibody (GFAP, used at 1:1000, DakoCytomation, Denmark); rat monoclonal anti-Mac1 or CD11b (1:500, Serotec, Kinlington, Oxford, UK); and a polyclonal antiserum against iNOS (1:4000, gift from Dr. R Martínez, Instituto Cajal, CSIC, Madrid, Spain). All primary antibodies were prepared in Buffer phosphate with triton (PBST) solutions containing NGS. After careful washing, sections were incubated with the appropriate secondary biotinylated antiserum (Vector) at room temperature and developed using diaminobenzidine (DAB). The reaction was monitored every 5 min using an optical microscope (Leica). After washing, sections were mounted on gelatin-coated slides, air-dried and dehydrated in ascending concentrations of ethanol, cleared with xylene and coverslipped under Permount.

Quantification of TH and DAT expression was carried out using an image analysis system (Analytical Imaging Station (AIS), Imaging