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3,4-Methylenedioxymethamphetamine enhances kainic acid convulsive susceptibility

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ABSTRACT

Kainic acid (KA) causes seizures and neuronal loss in the hippocampus. The present study investigated whether a recreational schedule of 3,4-methylenedioxymethamphetamine (MDMA) favours the development of a seizure state in a model of KA-induced epilepsy and potentiates the toxicity profile of KA (20 or 30 mg/kg). Adolescent male C57BL/6 mice received saline or MDMA t.i.d. (s.c. every 3 h), on 1 day a week, for 4 consecutive weeks. Twenty-four hours after the last MDMA exposure, the animals were injected with saline or KA (20 or 30 mg/kg). After this injection, we evaluated seizures, hippocampal neuronal cell death, microgliosis, astrogliosis, and calcium binding proteins. MDMA pretreatment, by itself, did not induce neuronal damage but increased seizure susceptibility in all KA treatments and potentiated the presence of Fluoro-Jade-positive cells in CA1. Furthermore, MDMA, like KA, significantly decreased parvalbumin levels in CA1 and dentate gyrus, where it potentiated the effects of KA. The amphetamine derivative also promoted a transient decrease in calbindin and calretinin levels, indicative of an abnormal neuronal discharge. In addition, treatment of cortical neurons with MDMA (10–50 μM) for 6 or 48 h significantly increased basal Ca^{2+}, reduced basal Na^{+} levels and potentiated kainate response. These results indicate that MDMA potentiates KA-induced neurodegeneration and also increases KA seizure susceptibility. The mechanism proposed includes changes in Calcium Binding Proteins expression, probably due to the disruption of intracellular ionic homeostasis, or/and an indirect effect through glutamate release.

1. Introduction

A substantial number of studies have been performed on the neuropharmacological mechanisms involved in the adverse effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") in laboratory animals, in which it has been shown to be neurotoxic to dopamine (DA) and serotonin (5-HT) terminals (Chipana et al., 2006; Cuyas et al., 2014; Esteban et al., 2001; O'Shea et al., 2006; Sanchez et al., 2004). It is well known that these neurotoxic effects are species-dependent (Logan et al., 1988). In mice, it is generally agreed that MDMA, at high doses (three doses of 25–30 mg/kg at 3 h intervals), induces dopaminergic terminal injury in the striatum (Chipana et al., 2006) and disrupts 5-HT neurochemistry in the hippocampus, depending on schedule dose used. In contrast, administration of a neurotoxic regimen of this amphetamine derivative to rats results in a selective reduction in cerebral tissue concentrations of 5-HT and also in 5-HT uptake sites in cortex and hippocampus, pointing to a selective injury of serotonergic terminals (Green et al., 2003; Pubill et al., 2003).

Likewise recent reports documented the finding that repeated exposure of rats to MDMA increases glutamate release in the hippocampus (Anneken and Gudelsky, 2012). Sustained increases in extracellular glutamate have the potential to promote excitotoxicity and could be involved in the neurotoxic effects of MDMA in the brain (Capela et al., 2006).

Collectively all these data provide support to the suggestion that the consequences of chronic exposure to MDMA are not limited to specific neuron terminals, but that other elements are also susceptible to damage. In this sense, changes in the EEG records have been described in long-term MDMA users and epileptic seizures associated with these altered records are one of the most frequent disturbances in ecstasy abusers (Zagnoni and Albano, 2002). Giorgi et al. (2005) reported, for the first time, that MDMA lowered the threshold for kainate-induced seizures. These data are important because MDMA might predispose

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; KA, Kainic acid; CaBP, calcium binding proteins; PV, parvalbumin; CB, calbindin; CR, calretinin.

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to seizures due to alterations in brain excitability (Brown et al., 2011; Zagnoni and Albano, 2002) or increasing basal calcium levels (Garcia-Ratés et al., 2010). However there have been few studies evaluating the proconvulsant effect of MDMA in experimental models of epilepsy (Giorgi et al., 2005) and they use a different schedule of MDMA administration than that of the present study.

Kainic acid (KA) has been widely used for its ability to replicate many of the phenomenological features of human temporal lobe epilepsy (Ben-Ari and Cossart, 2000; Leite et al., 2002). Seizures cause extensive brain damage concomitant with an increase in reactivity of the glia, as well as failure of the cellular homeostasis (Cavazos et al., 2004; Junyent et al., 2011a, 2011b; Kondratyev and Gale, 2004; Niquet et al., 1994; Represa et al., 1995). KA has direct excitatory effects on neurons but its potent neurotoxic action involves also the activation of presynaptic receptors on glutamatergic terminals, thereby releasing Asp and Glu. Endogenous glutamate, by activating NMDA, AMPA or mGluR1 receptors, may contribute to the brain damage occurring acutely after *status epilepticus* (Meldrum, 2000).

The aim of the present study was to evaluate whether MDMA favours the development of a seizure state in adolescent mice treated with the neurotoxin KA. We used a regimen of MDMA that differed from the classic neurotoxic exposure, trying to simulate classical adolescent weekend binge use of this substance. We observed a decrease in time to first seizure and an increase in seizure activity induced by KA. We also investigated whether hippocampal neurotoxicity after two different doses of KA is potentiated by MDMA. We also investigated by immunohistochemistry the effects of these treatments on the expression of calcium binding proteins (CaBP), which are crucial for calcium homeostasis in neurons, as well as the impact on Ca^{2+} levels in cortical neuron cultures.

Our data indicate that MDMA potentiates KA-induced neurodegeneration and also increases KA seizure susceptibility.

2. Experimental procedures

2.1. Animals

Adolescent male C57/BL6 mice (4–5 weeks old) (Charles River Laboratories, France) were kept under controlled temperature, humidity and light conditions with food and water provided ad libitum. They were treated according to European Community Council Directive 86/609EEC and the procedure registered at the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drug treatments and sample preparation

To model recreational MDMA use, we used adolescent mice. It could also be considered appropriate to simulate the widespread practice of "boosting" (taking supplemental doses over time in order to maintain the drug's effect) (Hammersley et al., 1999; Meyer et al., 2008). To select the appropriate MDMA dose we took into account that the dose regimen used in neurotoxic experiments with mice is 25 mg/kg three times per day (tid) every 3 h (Colado et al., 2001) or 20 mg/kg every 2 h for a total of four injections (O'Callaghan and Miller, 1994). Consequently, a maximal dose of 10 mg/kg tid was choosen. Treatment schedule started with a standard psychostimulant dose of MDMA (5 mg/kg) (Spanos and Yamamoto, 1989) that increased over the treatment, imitating the classic consumption of a reinforcing compound. In order to simulate its recreational use, MDMA was administered once a week during all the periadolescent period (from week 4–8 of age) (Smith, 2003). Drug administration was carried out at high environmental temperature, thus simulating the hot environments in which this substance tends to be consumed (clubs, raves).

Initially, the animals were randomly assigned to the following treatment groups: saline + saline (saline), MDMA + saline (M), saline + Kainate 20 mg/kg (KA20), MDMA + Kainate 20 mg/kg (M + KA20), saline + Kainate 30 mg/kg (KA30), MDMA + Kainate 30 mg/kg (M + KA30).

The animals (n = 7–10) for every treatment group in each experiment received a chronic dosage regimen of saline (5 ml/kg) or MDMA t.i.d. (s.c, every 3 h) on 1 day a week (the same day every week), for 4 consecutive weeks. MDMA and KA doses and the treatment schedule were as illustrated in Fig. 1. On the day of treatment, the environmental temperature was maintained at 26 ± 1 °C until 2 h after the last dose. Thereafter, the animals were returned to normal housing conditions (22 ± 1 °C). One hour after the second daily dose of saline or MDMA, rectal temperature was measured using a lubricated, flexible rectal probe inserted into the rectum and attached to a digital thermometer (0331 Panlab SL, Barcelona, Spain). The dose of MDMA increased every week. Accordingly, the doses were: 5 mg/kg, 7.5 mg/kg (for 2 consecutive weeks) and 10 mg/kg. Twenty-four hours after the last dose of MDMA or saline, the animals were exposed to low/moderate (20 mg/kg) or high (30 mg/kg) epileptogenic KA i.p. doses (Santos and Schauwecker, 2003; Sonn et al., 2010).

After the KA injection, the animals were put in individual plexiglas cages and observed for a period of 4 h to evaluate the occurrence and intensity of seizures. Seizures were assessed according to an adaptation of the Racine's scale (Racine, 1972) consisting of seven stages (0–6), which correspond to the successive developmental stages of motor seizures: (0) normal non-epileptic activity; (1) Still and crouched in a corner, staring; (2) Stretches body out, tail becomes straight and rigid, ears laid back, bulging eyes; (3) Repetitive head bobbing, rears into a sitting position with forepaws resting on belly (4) Rearing and falling tonic clonic seizures broken by periods of total stillness, jumping clonus, running clonus; (5) Continuous Level 4 seizures and (6) Body in clonus, no longer using limbs to maintain posture, usually a precursor to death.

The animals were killed 24 h or 72 h later. All mice were anaesthetized by i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and were perfused with paraformaldehyde 4% in phosphate buffer 0.1 M, after which the brains were removed. These were subsequently rinsed in paraformaldehyde 4% with 30% sucrose for 24 h and then frozen. Coronal sections of 30 μm were obtained. Brains from mice killed after 24 h of KA treatment were used for microgliosis immunohistochemistry, and brains from mice killed after 3 days of KA treatment were used for GFAP immunohistochemistry. Fluoro-Jade B staining, calbindin-D28k, calretinin and parvalbumin immunohistochemistry were performed either at 24 or 72 h.

2.3. Fluoro-Jade B staining

Slides were defatted by dehydration in ethanol before being rehydrated, rinsed in phosphate-buffered saline (PBS), and incubated with 5 nmol/l of Hoechst 33342 for 10 min in the dark. After two washes in distilled water, the slides were immersed in 0.06 g/l of potassium permanganate ($KMnO_4$) for 15 min in the dark. After two washes, the slides were transferred to the staining solution containing 0.1 ml/l of acetic acid and 4 μl/l Fluoro-Jade B for 30 min, in the dark. The slides were rinsed in distilled water, dried, and then submerged directly in xylene and mounted in DPX medium. They were analysed with an epifluorescence microscope (Olympus BX61).

2.4. Immunohistochemistry

Free-floating coronal sections were rinsed in 0.1 mol/l PB, pH 7.2, and then treated with 5 ml/l H_2O_2 and 100 ml/l methanol in PBS for 15 minutes. After that, they were preincubated in a blocking solution (10% fetal bovine serum (FBS), 0.2 mol/l of glycine, Triton X-100 0.2% in 0.2% PBS-gelatin). Then, the sections were incubated overnight at 4 °C with different primary antibodies: rabbit anticalbindin-D28k, rabbit anticalretinin and rabbit antiparvalbumin (1:1,000; Swant, Belinzona, Switzerland), rabbit antilba-1 (Wako Chemicals, Japan