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3,4-Methylenedioxy-methamphetamine induces *in vivo* regional up-regulation of central nicotinic receptors in rats and potentiates the regulatory effects of nicotine on these receptors

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ABSTRACT

Nicotine (NIC), the main psychostimulant compound of smoked tobacco, exerts its effects through activation of central nicotinic acetylcholine receptors (nAChR), which become up-regulated after chronic administration. Recent work has demonstrated that the recreational drug 3,4-methylenedioxy-methamphetamine (MDMA) has affinity for nAChR and also induces up-regulation of nAChR in PC 12 cells. Tobacco and MDMA are often consumed together. In the present work we studied the *in vivo* effect of a classic chronic dosing schedule of MDMA in rats, alone or combined with a chronic schedule of NIC, on the density of nAChR and on serotonin reuptake transporters. MDMA induced significant decreases in [^{3}H]paroxetine binding in the cortex and hippocampus measured 24 h after the last dose and these decreases were not modified by the association with NIC. In the prefrontal cortex, NIC and MDMA each induced significant increases in [^{3}H]epibatidine binding (29.5 and 34.6%, respectively) with respect to saline-treated rats, and these increases were significantly potentiated (up to 72.1%) when the two drugs were associated. Also in this area, [^{3}H]methyllycaconitine binding was increased a 42.1% with NIC + MDMA but not when they were given alone. In the hippocampus, MDMA potentiated the α7 regulatory effects of NIC (raising a 25.5% increase to 52.5%) but alone was devoid of effect. MDMA had no effect on heteromeric nAChR in striatum and a coronal section of the midbrain containing superior colliculi, geniculate nuclei, substantia nigra and ventral tegmental area. Specific immunoprecipitation of solubilised receptors suggests that the up-regulated heteromeric nAChRs contain α4 and β2 subunits. Western blots with specific α4 and α7 antibodies showed no significant differences between the groups, indicating that, as reported for nicotine, up-regulation caused by MDMA is due to post-translational events rather than increased receptor synthesis.

1. Introduction

3,4-Methylenedioxy-methamphetamine (MDMA, ecstasy) is an amphetamine derivative used illicitly in developed countries for recreational purposes, usually by young people in night clubs and at extended dance parties (known as raves).

A number of fatalities have been reported after acute consumption of this drug but there also exists experimental evidence that chronic MDMA can induce serotonergic and, to a lesser extent, dopaminergic neurotoxicity in rats and primates (see Capela et al., 2009 for a review). Also, serotonergic (Erritzoe et al., 2011; Reneman et al., 2002) and cognitive (Adamaszek et al., 2010; Nulsen et al., 2010; Parrott et al., 1998; Quednow et al., 2006) deficits have been reported in human chronic MDMA users, which could be due to neurotoxicity or to drug-induced long-lasting regulatory changes (Biezonski and Meyer, 2011).

The neurotoxicity of amphetamine derivatives can be a consequence of coordinated oxidative stress, metabolic compromise and inflammation (see Capela et al., 2009 and Yamamoto and Raudensky, 2008 as reviews), and we have recently reported that neuronal acetylcholine nicotinic receptors (nAChR), mainly the homomeric α7 subtype, also play a key role in MDMA-induced neurotoxicity as the blockade of these receptors by the antagonists methyllycaconitine (MLA) or memantine prevents *in vitro* and *in vivo* MDMA-induced neurotoxicity (Chipana et al., 2006, 2008a,b,c) as well as cognitive impairment in rats (Camarasa et al., 2008). Also, using radioligand binding experiments, we have demonstrated that

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MDMA has affinity for both homomeric and heteromeric nAChRs and behaves as a partial agonist at α7 nAChR (Chipana et al., 2008b,c; Garcia-Rates et al., 2007, 2010).

NAChR are a family of ligand-gated cation channels widely distributed in the brain and the peripheral nervous system, whose subunit composition and signalling effects depend on subtype and localisation (Albuquerque et al., 2009; Gotti et al., 2007). They exert a number of effects on brain functions, involving fast synaptic transmission, cognitive enhancement, memory or reinforcement, and they are the main targets of smoked nicotine. In the brain, nAChRs are pentameric structures formed by the association of α and β subunits and can be either homomeric or heteromeric. The homomeric family is made up of the α7–α10 subunits and is sensitive to α-bungarotoxin (αBgTx), while the heteromeric receptors consist of combinations of α2–α6 and β2–β4 subunits, and are insensitive to αBgTx. Of these combinations, the most abundant are homomeric α7 and heteromeric $(\alpha4)_2(\beta2)_3$ receptors. A particular feature of some nAChR subtypes is that, after chronic nicotine exposure, they undergo radioligand binding up-regulation, changes in stoichiometry and increase in their functional state (functional up-regulation) (reviewed by Gaimarri et al., 2007). Such up-regulation occurs at a post-translational level and several mechanisms have been proposed to explain it, including a chaperone-like maturation enhancing effect of nicotine (Lester et al., 2009; Kuryatov et al., 2005; Sallette et al., 2005; Srinivasan et al., 2011;) and stabilisation of the high-affinity state of the receptors (Vallejo et al., 2005). Moreover, nAChR play a key role in addiction to nicotine (Govind et al., 2009), so up-regulation could enhance addiction to nicotine by increasing the pleasant effects of the drug.

In a previous study on PC12 cells, we demonstrated that MDMA pretreatment induces *in vitro* up-regulation of both homomeric and heteromeric receptors (Garcia-Rates et al., 2007) through a mechanism that seemed to mimic that of nicotine. Then it was of interest to assess whether MDMA induces nAChR up-regulation *in vivo* as well, as changes in these receptors could have a role in drug addiction and explain some psychiatric effects of this drug, such as memory impairment and psychoses, among others in which nAChRs have been found to play a role (Levin and Rezvani, 2002; Martin et al., 2004; Ripoll et al., 2004).

Consequently, the aim of this study was to determine whether treatment with MDMA induces *in vivo* nAChR up-regulation and, moreover, to investigate whether it affects or potentiates the up-regulatory effects of nicotine, as MDMA and tobacco are very often associated (Scholey et al., 2004) and this could have implications on the addiction induced by both drugs.

2. Materials and methods

2.1. Drugs and radioligands

MDMA hydrochloride, obtained from the National Health Laboratory (Barcelona, Spain), was dissolved in saline (0.9% NaCl). Nicotine bitartrate dihydrate, purchased from Sigma–Aldrich (St. Louis, MO, USA), was also dissolved in saline. [^{3}H]MLA came from American Radiolabeled Chemicals (St. Louis, MO, USA), while [^{3}H]paroxetine, and [^{3}H]epibatidine came from Perkin-Elmer (Boston, MA, USA). All buffer reagents were of analytical grade and purchased from several commercial sources.

2.2. Animals and treatment

The experimental protocols for the use of animals in this study follow the guidelines set out by the European Communities Council (86/609/EEC) and were supervised by the ethics committee of the University of Barcelona. Male Sprague–Dawley rats weighing 200–230 g (Harlan Ibérica, Barcelona, Spain) were used. They were housed at 21 ± 1 °C under a 12 h light/dark cycle with free access to food and drinking water.

At the beginning of the treatment they were housed one per cage and a combined nicotine and MDMA dosing schedule was carried out for 10 days as follows. Six animals were used in each treatment group. The control (Ctrl) group received saline (1 ml/kg s.c.) twice daily (7-h interval) for the 10 days; the nicotine (NIC) group received 2 mg/kg nicotine bitartrate dihydrate (s.c.) twice daily (7-h interval) for 10 days (Flores et al., 1992); the MDMA group was given saline (s.c.) twice a day from days 1 to 6, and 20 mg/kg MDMA (s.c., b.i.d., 7-h interval) from days 7 to 10 (Battaglia et al., 1987). The MDMA + NIC group received nicotine bitartrate for the 10 days as stated for the NIC group, and MDMA (same dosing as above) was also injected during the last 4 days, 15 min after nicotine and at a different puncture site. The rats were weighed at days 1, 4, 6 and 11 and the percentage increase calculated throughout the treatment.

The rats were killed by decapitation under isoflurane anaesthesia on day 11. The brains were rapidly removed from the skull and dissected on a refrigerated surface. Prefrontal and parietal cortex, striatum, hippocampus, and a coronal block delimited by the thickness of superior colliculi, after removal of cortex and hippocampus (contains the colliculi, the geniculate nuclei, the substantia nigra and the ventral tegmental area, VTA), were excised, frozen on dry ice and stored at −80 °C until use.

These areas were selected on the basis of their abundance in the different types of nAChR and the amount of protein to perform binding assays in homogenates. Thus heteromeric nAChR were measured in cortex, striatum and the section containing the colliculi, as they express high levels of these receptors. As for α7 nAChR, they were assessed in the hippocampus (where they are more abundant and there are low levels of α4β2) and in the cortex as well (Tribollet et al., 2004).

2.3. Tissue processing

When required, tissue samples were thawed and homogenised at 4 °C in 10 volumes of buffer consisting of 5 mM Tris–HCl, 320 mM sucrose, and protease inhibitors (aprotinin 4.5 μg/μl, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate), pH 7.4, with a Polytron homogeniser. The homogenates were centrifuged at 15,000 × *g* for 30 min at 4 °C. The resulting pellets were resuspended in fresh buffer, incubated 5 min at 37 °C to degrade remaining endogenous ligands and recentrifuged twice. The final pellets of membrane homogenates were resuspended in 50 mM Tris–HCl buffer (plus protease inhibitors) and stored at −80 °C until use in radioligand binding assays or receptor solubilisation for Western blotting or immunoprecipitation. Protein content was determined using the Bio-Rad Protein Reagent (Bio-Rad Labs, Inc., Hercules, CA, USA), according to the manufacturer's instructions.

2.4. [^{3}H]Paroxetine binding

The density of serotonin transporters (SERT) in each rat's cortex and hippocampus was determined to assess the serotonergic changes/neurotoxicity induced by MDMA (Pubill et al., 2003). This was accomplished by measuring the specific binding of 0.05 nM [^{3}H]paroxetine after incubation with 150 μg protein at 25 °C for 2 h in a Tris–HCl buffer (50 mM, pH 7.4), containing 120 mM NaCl and 5 mM KCl to a final volume of 1.6 ml. Clomipramine (100 μM) was used to determine non-specific binding.