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Original article

Opioid gene expression changes and post-translational histone modifications at promoter regions in the rat nucleus accumbens after acute and repeated 3,4-methylenedioxy-methamphetamine (MDMA) exposure

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ABSTRACT

The recreational drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) has been shown to produce neurotoxic damage and long-lasting changes in several brain areas. In addition to the involvement of serotoninergic and dopaminergic systems, little information exists about the contribution of nociceptin/orphaninFQ (N/OFQ)-NOP and dynorphin (DYN)-KOP systems in neuronal adaptations evoked by MDMA. Here we investigated the behavioral and molecular effects induced by acute (8 mg/kg) or repeated (8 mg/kg twice daily for seven days) MDMA exposure.

MDMA exposure affected body weight gain and induced hyperlocomotion; this latter effect progressively decreased after repeated administration. Gene expression analysis indicated a down-regulation of the N/OFQ system and an up-regulation of the DYN system in the nucleus accumbens (NAc), highlighting an opposite systems regulation in response to MDMA exposure.

Since histone modifications have been strongly associated to the addiction-related maladaptive changes, we examined two permissive (acH3K9 and me3H3K4) and two repressive transcription marks (me3H3K27 and me2H3K9) at the pertinent opioid gene promoter regions. Chromatin immunoprecipitation assays revealed that acute MDMA increased me3H3K4 at the pN/OFQ, pDYN and NOP promoters. Following acute and repeated treatment a significant decrease of acH3K9 at the pN/OFQ promoter was observed, which correlated with gene expression results. Acute treatment caused an acH3K9 increase and a me2H3K9 decrease at the pDYN promoter which matched its mRNA up-regulation.

Our data indicate that the activation of the DYNergic stress system together with the inactivation of the N/OFQergic anti-stress system contribute to the neuroadaptive actions of MDMA and offer novel epigenetic information associated with MDMA abuse.

1. Introduction

MDMA (3,4-methylenedioxy-methamphetamine), popularly known as “ecstasy”, is a synthetic psychoactive drug which exhibits similarities to amphetamine and to the hallucinogen mescaline [1]. It produces feelings of euphoria, emotional warmth and distortions in sensory and time perceptions [2,3]. MDMA was initially popular among adolescents and young adults in the nightclub scene or at “raves” (long dance parties), but currently it affects a broader range of users. The powerful effects of MDMA on the serotonergic system seem to be responsible for most actions of this drug of abuse. The massive release of serotonin (5-HT) is mainly due to MDMA action as a substrate-type releaser on the serotonin transporter (SERT) [4]. Furthermore, MDMA rapidly increases dopamine (DA) release [5,6]

Abbreviations: 5HT, serotonin; BSA, bovine serum albumin; ChIP, chromatin immunoprecipitation; CPP, conditioned place preference; CREB, cyclic-AMP response-element-binding protein; DA, dopamine; DDCt, delta–delta threshold cycle; DYN, dynorphin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H, histone; K, lysine; KOP, κ-opioid receptor; MDMA, 3,4-methylenedioxy-methamphetamine; N/OFQ, nociceptin/orphaninFQ; NOP, nociceptin receptor; PBS, phosphate-buffered saline; PIC, protease inhibitor cocktail; pDYN, prodynorphin; pN/OFQ, pronociceptin/orphaninFQ; SDS, sodium dodecyl sulfate; SERT, serotonin transporter.

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and several studies demonstrated the involvement of other neurotransmitter circuits such as the glutamatergic, cholinergic and opioid systems in MDMA-mediated behavioral and neurochemical effects [7–9]. Notably, the opioid antagonist naloxone is able to block the locomotor stimulation caused by MDMA in mice [10] and it can attenuate MDMA ability to produce conditioned place preference (CPP) in rats [11]. In addition, the δ-opioid antagonist naltrindole blocks the increase of intracranial brain stimulation induced by MDMA [12].

In vivo studies demonstrated that MDMA treatment alters the dynorphinergic system in specific rat brain regions. It has been observed that a single MDMA administration increases dynorphin (DYN) A and prodynorphin (pDYN) mRNA levels in the striatum [13–15] and markedly up-regulates pDYN levels in the prefrontal cortex and in the brainstem [8,15]. Conversely, a single MDMA treatment promoted pDYN down-regulation in the ventral tegmental area [15] suggesting a specific response depending on the brain area analyzed.

The nociceptin/orphaninFQ (N/OFQ) peptide shows high structural homology to DYN A and interacts with a G protein-coupled receptor known as the nociceptin (NOP) receptor, which is closely related to the κ-opioid (KOP) receptor [16]. It has been previously suggested that the N/OFQ system is involved in neuronal adaptations evoked by amphetamine since N/OFQ treatment can inhibit amphetamine-induced CPP acquisition in rats [17].

Gene expression alterations contribute to the long-lasting changes induced by MDMA in the brain reward circuitry but the detailed molecular steps through which such alterations are induced are unknown. Several studies identify chromatin remodeling as one of the main gene expression regulators for many drugs of abuse including alcohol, cocaine and amphetamine [18–21], contributing substantially to the addictive phenotype. Chromatin modifications can activate or repress the transcriptional activity through post-translational histone modifications, which play a crucial role in the maintenance of the steady-state of chromatin [22–24]. It has been demonstrated that the increase of histone-3 (H3) and histone-4 (H4) acetylation on lysine (K) residues is linked to increased gene expression, whereas histone de-acetylation has been connected with a decrease of several mRNA levels in addiction paradigms [25,26]. Conversely, H3 or H4 K methylation may act as permissive or repressive marker depending on the methylation site [27].

Although histone modifications induced by cocaine or ethanol in specific brain areas have been characterized using the chromatin immunoprecipitation (ChIP) technique [28–30], histone changes induced by MDMA exposure have not been sufficiently investigated.

Thus, this study examined the effects of acute and repeated MDMA exposure on the opioid genes expression, as well as the underlying chromatin remodeling mechanisms. The evidence of MDMA-induced mRNA changes prompted us to investigate whether post-translational modifications of histone proteins at the corresponding opioid promoter regions were involved in the regulation of gene expression. Two distinct histone modifications acting as permissive transcription marks were chosen: the acetylation of histone 3 at lysine 9 (acH3K9) and the trimethylation of histone 3 at lysine 4 (me3H3K4). In addition, two histone modification acting as repressive marks were examined: the trimethylation of histone 3 at lysine 27 (me3H3K27) and the dimethylation of histone 3 at lysine 9 (me2H3K9). The brain area designated for the analyses was the nucleus accumbens (NAc) because of its pivotal role in drug addiction. Due to its connectivity with other brain regions, NAc integrates information from cortical and limbic structures to mediate goal-directed behaviors. Accordingly, repeated exposure to several classes of drugs of abuse, including MDMA, may disrupt the plasticity in this region allowing a pathologic motivation for drug seeking.

2. Materials and methods

2.1. Drug

MDMA was supplied from the National Institute on Drug Abuse/National Institutes of Health (Research Triangle Institute, Research Triangle Park, NC, USA) and dissolved in saline (0.9% NaCl).

2.2. Animals and treatments

Male Sprague-Dawley rats (Harlan, Italy) weighing 200–250 g at the beginning of the experiment were used. Animals were housed two per cage in standard cages (Tecniplast Gazzada, Buguggiate, Italy) and kept in a temperature- and humidity-controlled room with a 12-h light/dark cycle (lights on at 7 a.m.); standard lab chow and tap water were available *ad libitum*. Rats were allowed to acclimatize for 1 week before the start of experiments. Care was taken to minimize the number of rats and to avoid animal stress and discomfort during handling and procedures.

All experiments were carried out in accordance with the European Communities Council Directives on the protection of animals used for scientific purposes of (86/609/EEC – 2010/63/EU) and Italian National (Ministry of Health, Italy) laws and policies. This study has been approved by the "Ethic Scientific Committee for the Animal Experiments" of the University of Bologna.

A total number of twenty-four rats was used and divided into four groups. For the acute treatment, six rats per group received a single i.p. injection of vehicle (0.9% NaCl) or MDMA (8.0 mg/kg). In the repeated treatment experiment, six rats per group received twice daily i.p. injections of vehicle or MDMA (8.0 mg/kg) for seven days. During the entire period of MDMA exposure rats were weighed every day before treatments. Two hours after the last injection rats were sacrificed; the brains were rapidly removed and NAc was dissected and quickly frozen on dry ice.

2.3. Locomotor activity

The effects of MDMA on locomotor activity were measured daily at 9 am for 50 min after MDMA injection. Rats were placed into locomotor activity chambers (Actometric cages: $38 \times 30 \times 25$ cm) for 15 min habituation, after which they were injected with 8 mg/kg MDMA or vehicle. Horizontal movements were recorded every 5 min. A direct current (65 V, 25 μA) was continuously delivered to the stainless steel grid floor of the cage and every closure of the circuit, performed by the rat feet, was recorded as one motility count by an electronic counter.

2.4. RNA isolation and qRT-PCR

Total RNA was extracted from NAc according to the method of Chomczynski and Sacchi [31]. The integrity of RNA was checked by 1% agarose gel electrophoresis and the concentrations were measured by spectrophotometry. RNA samples with OD260/OD280 ratio >1.8 and <2.0 were subsequently subjected to DNase treatment and converted to cDNA using the GeneAmp RNA PCR kit (Life Technologies Italia, Monza, Italy). Relative abundance of each mRNA species was assessed by real-time qRT-PCR using SYBR Green MasterMix (Life Technologies) on a Step One PCR System (Life Technologies). Relative expression of different gene transcripts was calculated by the Delta–Delta Ct (DDCt) method and converted to relative expression ratio (2^{-DDCt}) for statistical analysis [32]. All samples were run in triplicate and data are expressed as means $\pm$ SEM. After PCR, a dissociation curve (melting curve) was