

Differential effects of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) on BDNF mRNA expression in rat frontal cortex and hippocampus

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Received 8 February 2006; received in revised form 20 March 2006; accepted 24 March 2006

Abstract

The serotonergic neurotoxin 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) produces rapid serotonin (5-HT) depletion in different areas of the forebrain after acute administration to rats and other animal species. We previously found that 5-HT depletion induced by acute MDMA treatment was transient in the frontal cortex, but not in the hippocampus, and recovery of cortical 5-HT levels correlated with an induction of CRE-binding activity and increased expression of tryptophan-hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis. As the brain-derived neurotrophic factor (BDNF) stimulates the growth and sprouting of serotonergic neurons, we sought the possible involvement of this neurotrophin in the region-specific increase in TPH mRNA expression induced by MDMA. We here report that, 24–48 h after acute MDMA treatment, the expression of BDNF in the frontal cortex is increased by ~33–70%, and the levels of the transcription factor phospho-CREB are also increased. In the hippocampus, however, a time-dependent decrease in BDNF mRNA expression (maximal decrease of ~73%) is found in all subfields examined 2–7 days after treatment in spite of increased phospho-CREB levels, perhaps as a consequence of corticosterone release by the serotonergic neurotoxin. The differential regulation of BDNF mRNA expression in the two brain regions examined appears to account for the enhanced TPH expression and the recovery of 5-HT levels in the frontal cortex, but not in the hippocampus, after neurotoxin treatment.

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Keywords: BDNF; MDMA; Serotonin; CREB

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”), is a widely used recreational drug that, in rats and other animal species, induces a rapid reduction in the content of brain serotonin (5-HT) [2,22], in the activity of tryptophan-hydroxylase (TPH), the rate-limiting enzyme in 5-HT synthesis [23], and in 5-HT transporter density [2]. On chronic treatment, it has been repeatedly shown that MDMA produces a degeneration of serotonergic nerve terminals [2,19,22].

The cellular and molecular mechanisms involved in MDMA actions have been extensively studied although many aspects remain to be elucidated (see [7] for review). In a previous study [6], we found that acute MDMA treatment produced a rapid reduction of 5-HT content in rat frontal cortex and hippocampus. In the cortex, but not in the hippocampus, 5-HT levels

returned to normal values 48 h after injection, and this recovery was correlated with an increase in binding to the CRE consensus sequence and in mRNA levels of TPH. It is known that the neurotrophin brain-derived neurotrophic factor (BDNF), which is upregulated in response to various types of neuronal injury as a compensatory effect after brain damage [10], augments 5-HT synthesis by enhancing TPH mRNA levels [24]. Many other studies point to the mutual interactions between brain 5-HT and BDNF (reviewed in [15]). This neurotrophin influences the survival and function of 5-HT neurons in the rat brain [5,13] whereas blockade of the 5-HT transporter and activation of 5-HT receptor subtypes induces transcription of the BDNF gene [15]. Serotonergic neurotoxins such as *p*-chloroamphetamine (PCA) and 2'-NH₂-MPTP may alter the expression of BDNF mRNA and protein in the rat hippocampus and cortical areas [12,32]. A single study showed that neonatal administration of MDMA moderately increased BDNF protein on postnatal day 21 [11]. On the basis of the previously observed differential changes in 5-HT and TPH mRNA levels [6], the current study

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sought to determine the possible effects of acute MDMA administration on BDNF mRNA expression in the rat frontal cortex and hippocampus. Given that MDMA also increased the activity of protein binding to CRE [6] and the known regulation by cAMP response element-binding protein (CREB) of the BDNF-induced gene expression in the cortex [28], we also looked for eventual changes in the levels of phospho-CREB in response to the neuronal injury caused by MDMA.

Male Wistar rats (Harlan, Barcelona, Spain) weighing 200–220 g were used. Animals were housed three to a cage in a temperature-controlled room (22–23 °C) with a 12 h light/dark cycle and had free access to food and water. All procedures were in accordance with the guidelines established by the normative of the European Community of November 24, 1986 (86/609/EEC). Rats were injected with saline (5 ml/kg, i.p.) or with a single dose of MDMA.HCl (10 mg/kg, i.p.) and killed by decapitation at different times. The brains were removed and placed on ice. Western-blot analyses were carried out with fresh tissue from the frontal cortex and hippocampus. In rats assigned to in situ hybridization studies, the brains were immediately frozen in isopentane on dry ice. All tissues were stored at –80 °C until use.

The levels of 5-HT were measured in rat brain regions by high-performance liquid chromatography with electrochemical detection, as described [20].

For in situ hybridization, 14 µm coronal sections were cut serially with a cryostat at the level of frontal cortex (3.7 mm relative to bregma) and dorsal hippocampus (–3.3 mm relative to bregma), according to the atlas of Paxinos and Watson. The sections were mounted onto Superfrost/Plus slides and then processed for the in situ hybridization of BDNF mRNA as previously described [14]. For detection of BDNF mRNA, an oligonucleotide complementary to bases of the coding region of rat BDNF exon V (Invitrogen Custom primers) was used: 5'-AGT TCC AGT GCC TTT TGT CTA TGC CCC TGC AGC CTT TGG TGT AAC-3'. The probe was 3'-tail labeled with αS[³⁵S]dATP (specific activity >1000 Ci/mmol, Amersham Pharmacia) using terminal deoxynucleotide transferase (Roche). A negative control included hybridization of sections using an oligonucleotide in the sense orientation, which showed minimal background signals. Hybridization densities were measured from films autoradiograms, with labeling densities calibrated relative to film images of ¹⁴C-labeled standards (Amersham Pharmacia), using the Microcomputer Imaging Device (Imaging Research, St Catharines, Ontario, Canada). Densitometric values measured from four sections of each animal were averaged and expressed as nCi/g tissue, and mRNA abundance in drug treated groups was expressed as a percentage of their respective controls.

Nuclear protein extracts from rat frontal cortex and hippocampus were prepared as previously described [6,26]. For Western blotting, 25 µg of nuclear protein extract were separated onto SDS-polyacrilamide gel (12%). Proteins were transferred to a nitrocellulose membrane using a Trans-Blot® SD semidry (BioRad) system for 30 min at 16 V. The membranes were blocked and incubated at 4 °C overnight with the primary antibody, diluted in 5% non-fat dried milk in TBST:

Table 1

Effect of MDMA on 5-HT levels in rat frontal cortex and hippocampus

Time after treatment	Frontal cortex	Hippocampus
Control	527.0 ± 36.2	470.2 ± 21.5
24 h	326.8 ± 29.5*	135.8 ± 23.6**
48 h	502.2 ± 40.5	142.5 ± 30.1**
7 days	510.3 ± 29.2	237.0 ± 20.3**

Animals received saline (controls) or a single injection of MDMA (10 mg/kg). Data, expressed as pg/mg wet tissue, are mean ± S.E.M. (n = 6–8).

* $P < 0.05$ vs. controls (one-way ANOVA followed by post hoc Scheffé test).

** $P < 0.01$ vs. controls (one-way ANOVA followed by post hoc Scheffé test).

mouse anti-phospho-CREB (Ser133) (1B6) monoclonal (Cell Signaling Technology®). After washing, an HRP-conjugated anti-mouse antibody (Dako; dilution 1:1000) was added and incubated for 60 min at room temperature. Immunolabeled protein bands were detected using an enhanced chemiluminescence system (ECL Amersham Pharmacia). The quantification of signals was determined by densitometry.

Twenty-four hours later, acute MDMA treatment reduced 5-HT levels by ~38% in the frontal cortex and by ~72% in the hippocampus. Two days after treatment, cortical levels did not significantly differ from controls, but hippocampal 5-HT levels were reduced to a similar extent (~70%) and were still significantly reduced by ~50% one week after MDMA treatment (Table 1).

In the frontal cortex, one-way ANOVA revealed a significant effect of MDMA treatment on BDNF mRNA expression [$F_{3,160} = 19.76$; $P < 0.001$]. The levels of BDNF mRNA were significantly increased (~33%) 24 h after injection and were further increased (~70%) at the 48 h time point. Seven days after MDMA administration, BDNF mRNA expression returned to control levels (Fig. 1). In the hippocampus, ANOVA also revealed a significant treatment effect on BDNF mRNA expression in CA1 [$F_{3,53} = 218.40$; $P < 0.001$], CA3 [$F_{3,52} = 82.21$; $P < 0.001$], and dentate gyrus [$F_{3,52} = 51.73$; $P < 0.001$]. However, in sharp contrast with the effects observed in the frontal cortex, the levels of BDNF mRNA in the hippocampus were moderately decreased 48 h after MDMA treatment, and were further decreased (~73%) 7 days after injection (Fig. 2).

In the frontal cortex, acute MDMA treatment significantly increased (~35%) pCREB levels 24 and 48 h later. In the hippocampus, the effect was more pronounced and significantly increased pCREB levels were also detected 24 and 48 h after MDMA injection (Table 2).

Cortical and hippocampal cells respond to MDMA by modulating transcription of several genes that may lead to long-term changes in the brain [3,6,29]. The present results show that BDNF gene transcription is increased or decreased by MDMA treatment in the frontal cortex and hippocampus respectively. A modest increase in BDNF protein levels in different forebrain structures (~19–21%) after chronic treatment of neonatal rats with high MDMA doses (20 mg/kg bid for 10 consecutive days) had been reported [11]. We here show that a single administration of a lower MDMA dose (10 mg/kg) produced much more marked changes in BDNF mRNA expression in adult animals.

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