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Effects of a short-course MDMA binge on dopamine transporter binding and on levels of dopamine and its metabolites in adult male rats

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ABSTRACT

Although the recreational drug 3,4-methylenedioxymethamphetamine (MDMA) is often described as a selective serotonergic neurotoxin, some research has challenged this view. The objective of this study was to determine the influence of MDMA on subsequent levels of two different markers of dopaminergic function, the dopamine transporter (DAT) as well as dopamine and its major metabolites. In experiment I, adult male Sprague–Dawley rats were administered either a low or moderate dose MDMA binge (2.5 or 5.0 mg/kg × 4 with an inter-dose interval of 1 h) or saline, and were killed 1 week later. The moderate dose dramatically reduced [^{3}H]WIN 35,428 binding to striatal DAT by 73.7% ($P \leq 0.001$). In experiment II, animals were binged with a higher dose of MDMA (10 mg/kg × 4) to determine the drug's effects on concentrations of serotonin (5-HT), dopamine, and their respective major metabolites 5-hydroxyindoleacetic acid (5-HIAA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) in the striatum and frontal cortex 1 week later. As expected, MDMA significantly reduced 5-HT and 5-HIAA (≥ 50%) in these structures, while only a marginal decrease in dopamine was noted in the striatum. In contrast, levels of DOPAC (34.3%, $P < 0.01$) and HVA (33.5%, $P < 0.001$) were reduced by MDMA treatment, suggesting a decrease in dopamine turnover. Overall, these findings indicate that while serotonergic markers are particularly vulnerable to MDMA-induced depletion, significant dopaminergic deficits may also occur under some conditions. Importantly, DAT expression may be more vulnerable to perturbation by MDMA than dopamine itself.

1. Introduction

The illicit substance 3,4-methylenedioxymethamphetamine (colloquially known as Ecstasy) is a commonly used amphetamine derivative known to cause long term deficits to serotonergic function (Lyles and Cadet, 2003). These effects have been documented in several species, including rats (Xie et al., 2006), guinea pigs (Commins et al., 1987), non-human primates (Ricaurte and McCann, 1992), and humans (Kish et al., 2000; McCann et al., 1994, 2000, 2008), and include prolonged decreases in serotonin (5-hydroxytryptamine; 5-HT) levels, activity of tryptophan hydroxylase, and serotonin transporter expression. In contrast, rats typically show few long-term effects of MDMA on catecholamine neurons, as exemplified by an absence of changes in levels of dopamine or norepinephrine (Colado et al., 2004), the dopamine transporter (DAT) (Battaglia et al., 1987; Lew et al., 1996), or catecholaminergic fiber density when these projections were stained for tyrosine hydroxylase (O'Hearn et al., 1988). For this reason, MDMA is often described as a selective 5-HT neurotoxin (Cole and Sumnall, 2003; Ricaurte et al., 1988).

Despite this prevailing view that the adverse effects of MDMA are mainly limited to serotonergic neurons, several studies have found evidence for significant, drug-induced deficits in dopamine neurochemistry (reviewed in Colado et al., 2004; Piper, 2007). For example, Commins et al. (1987) reported decreased dopamine content in the striatum two weeks following high, repeated doses of MDMA in rats, while a similar decrease in levels of striatal dopamine was also noted at either 1 (Able et al., 2006) or 3 months (McGregor et al., 2003) following repeated drug administration. Another study showed that the extent of mazindol binding to DAT was decreased in the midbrain, but not the striatum,

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of rats given a similar dosing regimen (Battaglia et al., 1987). A postmortem case study determined that the dopamine content of an MDMA abuser was approximately half that in comparison subjects in the nucleus accumbens, while abnormal quantities of DOPAC were noted in the putamen (Kish et al., 2000). Additionally, McCann et al. (1994) identified a reduction in the dopamine metabolite homovanillic acid (HVA) in the cerebral spinal fluid of female, but not male, Ecstasy users. Importantly, two investigations have documented MDMA-induced decreases in the vesicular monoamine transporter 2 (VMAT-2), a protein that primarily marks dopaminergic terminals in the striatum (Nirenberg et al., 1997). Levels of VMAT-2 were reduced in the caudate/putamen and frontal cortex of baboons previously treated with multiple doses of MDMA (Ricaurte et al., 2000), with similar deficits also observed in the striatum 1 day following an MDMA binge in rats (Hansen et al., 2002). Taken together, these findings highlight the possibility that MDMA may have the potential to cause long-term neurochemical changes to dopaminergic neurons.

Given that all documented cases of dopaminergic deficits following MDMA exposure involved administration of high, repeated doses of the compound, this particular dosing pattern may prove to be important in unmasking the effects of MDMA on this population of neurons. To test this hypothesis, we administered four doses of MDMA (1 h apart) to rats and measured two different indices of dopaminergic neuron function, either DAT binding (experiment I) or dopamine and its major metabolites (experiment II) 1 week later. In experiment II, to show that this binge paradigm yielded the anticipated deficits in serotonergic markers, we also measured levels of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA).

2. Methods

2.1. Animals

Adult male Sprague–Dawley rats were obtained from Charles River Laboratories (Kingston, NY). Animals were pair-housed in standard plastic tubs under a reverse 12 h light/dark cycle with food and water freely available. All animals were habituated to experimenters by gentle handling for at least 3 days prior to drug administration, and all care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Experimental protocols were approved by the University of Massachusetts—Amherst Institutional Animal Care and Use Committee.

2.2. MDMA administration

The cohort of animals in experiment I was also part of a separate investigation examining the potential for various pretreatments to influence the extent of serotonergic marker depletion following a subsequent MDMA binge (Piper et al., 2010). For simplicity, only the animals that were pretreated with saline by twice-daily injections of the vehicle (4 h apart) are presented here. This regimen was repeated every fifth day from postnatal day (PD) 60–85, after which animals were assigned to one of three binge groups (N=7–10/group) on PD92.The binge consisted of four subcutaneous doses with an inter-dose interval of 1 h of either 0.0, 2.5, or 5.0 mg/kg ±MDMA HCl (Research Triangle Institute, Research Triangle Park, NC) in 0.9% NaCl vehicle. All animals were killed for DAT binding 1 week later.

Given the controversy surrounding the potential of MDMA to adversely affect the dopamine system (see Ricaurte et al., 2002 and subsequent retraction), it was important to investigate whether our MDMA binge regimen could cause similar changes in other markers of dopaminergic neuron function. As such, additional information regarding the effects of a similar binge regimen on levels of dopamine and its major metabolites was determined in a separate cohort of animals. In experiment II, adult rats were binged with MDMA or saline as before (PD92) without undergoing saline pretreatment. In this study, however, animals (N=10/group) were given a higher dose of the compound (10 mg/kg × 4, also with an inter-dose interval of 1 h) to accentuate any potential dopaminergic deficits arising from MDMA exposure. As in experiment I, all animals were killed a week later for endpoint examination.

2.3. Core temperature analysis

Temperature measurements were taken using a rectal probe (RET-2, Physitemp Instruments, Clifton, NJ) connected to a digital thermometer (Thermalert TH-5, Physitemp Instruments, Clifton, NJ) 30 min prior to, and then every 30 min during and following drug administration, terminating at 3 h after the final injection of the MDMA binge. All rats exceeding 40.5 °C during dosing were briefly cooled, either by temporary placement in a 4 °C cold-room (experiment I) or by application of ice packs to both sides of the animal (experiment I and II).

2.4. Dopamine transporter binding

One week following the MDMA binge (PD99), animals in experiment I were lightly anesthetized with CO_2 and decapitated. Striatal samples were rapidly dissected over ice according to Piper et al. (2005) and stored at −70 °C for later analysis of DAT binding. On the day of the assay, frozen tissues were homogenized in 100 vols of homogenization buffer (20 mM phosphate buffer, 0.32 M sucrose, pH 7.4) using a Polytron. Homogenates were centrifuged at 20,000 × g for 20 min at 4 °C, the supernatant was decanted, and the pellet was resuspended in 50 vols of homogenization buffer. Centrifugation and resuspension were repeated twice more to yield a crude washed membrane fraction. Transporter binding was measured using tritiated (−)-2-beta-carbomethoxy-3-beta-(4-fluorophenyl)tropane 1,5-napthalenedisulfonate([^{3}H]WIN 35,428; 87.0 Ci/mmol, Perkin Elmer, Boston, MA) at a final concentration of 5.0 nM. For nonspecific binding measurements, cocaine HCl was additionally present at a final concentration of 30 μM. Incubations were performed at a final volume of 500 μL, including 100 μL of membrane suspension, for 90 min at 4 °C on a shaking platform. The reaction was terminated by rapid filtration using Whatman GF/B filters presoaked in 0.05% polyethyleneimine, followed by two 5.0-mL rinses with phosphate buffer. Filters were then placed in scintillation vials with 4.7 mL of ScintiSafe cocktail (Perkin Elmer, Waltham, MA) and counted the following day using a Packard 1900CA liquid scintillation analyzer. The protein concentration in each sample was determined by means of the Bio-Rad© DC protein assay (Hercules, CA) using bovine gamma globulin as the standard. Mean data were expressed as a percentage of the saline group.

2.5. High-performance liquid chromatography

One week following the MDMA binge, animals in experiment II were decapitated and a 2-mm slice beginning 1 mm from the anterior pole was removed, encompassing the entire extent of the frontal cortex up to the most rostral boundary of the caudate-putamen (Paxinos and Watson, 1998). The striatum was dissected as above. All samples were immediately frozen at −70 °C until analysis. Concentration of 5-HT and dopamine and their metabolites 5-hydroxyindole acetic acid (5-HIAA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were quantified by a modified method of high-performance liquid chromatography (HPLC) combined with electrochemical detection (EC) as described