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Research Report

Neonatal 3,4-methylenedioxymethamphetamine (MDMA) exposure alters neuronal protein kinase A activity, serotonin and dopamine content, and [^{35}S]GTPγS binding in adult rats

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ABSTRACT

Recreational use of methylenedioxymethamphetamine (MDMA) has dramatically increased among juveniles and young adults of child-bearing age, and the potential for fetal exposure has increased. For this reason, it is surprising that comparatively few studies have assessed the long-term impact of early MDMA exposure on serotonin (5-HT) and dopamine (DA) neurotransmitter systems. The purpose of this study was to determine whether repeated exposure to MDMA during the preweanling period would cause long-term changes in 5-HT and DA functioning. Rats were treated with saline or 20 mg/kg MDMA (two injections per day) from postnatal day (PD) 11–20. At PD 90, rats were killed, and their dorsal striatum, prefrontal cortex, and hippocampus were removed. 5-HT and DA content, as well as their metabolites, were measured using HPLC. In addition, cAMP-dependent protein kinase A (PKA) activity and agonist-stimulated [^{35}S]GTPγS binding was assayed using tissue homogenates from each brain region. Results indicated that early MDMA exposure caused a decrease in PKA activity and 5-HT content in the prefrontal cortex and hippocampus while increasing the efficacy of 5-HT$_{1A}$ receptors as measured by agonist-stimulated [^{35}S]GTPγS binding. Additionally, DA content was reduced in the dorsal striatum and prefrontal cortex. These data indicate that early MDMA exposure has long-term effects on the 5-HT and DA neurotransmitter systems that may be mediated, at least partially, by changes in 5-HT$_{1A}$ receptor sensitivity.

1. Introduction

Determining the consequences of 3,4-methylenedioxymethamphetamine (MDMA) exposure on brain development has become more important with the increased use of MDMA by juveniles and young adults of child-bearing age (Banken, 2004; Green et al., 2003; Landry, 2002). Although illicit drug use in the United States declined from 1991 to 2000, MDMA use among high school seniors nearly quadrupled during the same period (Landry, 2002). Smaller but significant increases in MDMA use occurred in 8th and 10th grade students as well as college students (Banken, 2004; Landry, 2002; NIDA, 2002).

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Because of these demographics, the rate of prenatal MDMA exposure is on the increase in the United States. That younger age groups are using MDMA is of special concern, since significant brain development, especially in forebrain structures, continues through the second decade (Bayer et al., 1993; Fuster, 2002). At present, however, there is limited information on the long-term effects of early MDMA exposure on later CNS functioning in humans. Those studies that are available suggest that human MDMA users exhibit a variety of cognitive impairments, including deficits in divided and selective attention, abnormal hippocampal responses during working memory tasks, and declines in both executive functioning and syllogistic reasoning (Jacobsen et al., 2004; Montgomery et al., 2005; von Geusau et al., 2004).

In adult rodents and nonhuman primates, MDMA has potent and long-lasting effects on serotonin (5-HT) neurons. In adult rats, MDMA causes persistent reductions in 5-HT content, 5-hydroxyindoleacetic acid (5-HIAA) levels, 5-HT transporters, and tryptophan hydroxylase activity (for reviews, see Green et al., 2003; McCann and Ricaurte, 2004; Simantov, 2004). Nonhuman primates show similar changes in 5-HT markers because 5-HIAA and 5-HT transporters are reduced after repeated MDMA exposure (for a review, see Lyles and Cadet, 2003). Importantly, these MDMA-induced changes in 5-HT neurochemistry are correlated with long-term declines in cognitive functioning in both rats and nonhuman primates, including impaired spatial memory, executive functioning, and attention (Cohen et al., 2005; McCann et al., 1999; Sprague et al., 2003; Vorhees et al., 2004; Williams et al., 2003). In addition to the well-documented effects of MDMA on 5-HT systems, it has also been reported that MDMA alters dopamine (DA) system functioning (Cohen et al., 2005; Koprich et al., 2003a,b; Miller and O'Callaghan, 1995); however, declines in DA content and DA metabolite levels are typically found in mice and not in rats or nonhuman primates (Colado et al., 2004; Green et al., 2003; Logan et al., 1988; but see Cohen et al., 2005; McGregor et al., 2003).

A very different pattern of effects occurs when MDMA exposure occurs during early ontogeny. Specifically, early MDMA exposure causes long-term impairments on a variety of cognitive tasks (Broening et al., 2001; Cohen et al., 2005; Vorhees et al., 2004; Williams et al., 2003) while not causing adult-like declines in 5-HT levels or 5-HT transporters (Broening et al., 1994, 2001; Cohen et al., 2005). Because of this apparent dissociation between the behavioral impact of MDMA and changes in the 5-HT system (e.g., reductions in 5-HT levels), it is possible that the behavioral changes occurring after early MDMA exposure are not due to the loss of monoamine containing terminals or neurons. Instead, early exposure to MDMA may affect learning and memory processes by causing long-term alterations in cyclic adenosine monophosphate (cAMP) signal transduction mechanisms. This hypothesis is supported by two sets of findings: (i) early exposure to other amphetamine analogs (D-amphetamine and D-methamphetamine) causes long-term declines in dorsal striatal and accumbal cAMP-dependent protein kinase A (PKA) activity (Crawford et al., 2000a,b, 2003); and (ii) decreased PKA activity is associated with hippocampal memory impairment (Abel et al., 1997; Nagakura et al., 2002; Wu et al., 2002). MDMA-induced changes in PKA activity are most likely due to altered receptor/G protein coupling because many monoamine receptors are coupled to the cAMP transduction system. Consistent with this suggestion, both adult and developmental studies have shown that repeated exposure to psychostimulants causes persistent alterations in receptor-mediated cAMP system functioning (Barnett et al., 1987; Roseboom et al., 1990; Unterwald et al., 2003).

The purpose of the present study, therefore, was to determine whether exposing rats to MDMA during the preweanling period would have long-term effects on 5-HT and DA functioning in adulthood. Specifically, rats were treated with saline or 20 mg/kg MDMA (two injections per day) from postnatal days (PD) 11–20. This injection period was chosen for two reasons: (i) exposing rats to MDMA from PD 11–20 disrupts performance on hippocampus-dependent memory tasks when assessed in adulthood (Broening et al., 2001; Cohen et al., 2005; Vorhees et al., 2004; Williams et al., 2003); and (ii) dramatic changes in CNS maturation, especially in forebrain structures, occur between PD 11 and PD 20: a period analogous to late third trimester human brain development (Altman et al., 1973; Bayer et al., 1993; Dobbing and Sands, 1979). At PD 90, rats were killed, and their dorsal striatum, prefrontal cortex, and hippocampus were removed. cAMP-dependent PKA activity, monoamine (5-HT and DA) and metabolite content, and agonist-stimulated [^{35}S]GTPγS binding assays were performed on tissue homogenates from each brain region. It was hypothesized that early MDMA exposure would cause persistent declines in PKA activity and increases in agonist-stimulated [^{35}S]GTPγS binding (a measure of receptor/G protein coupling) that would be detectable in adulthood.

2. Results

2.1. 5-HT and 5-HIAA content

Adult rats that had been exposed to MDMA on PD 11–20 showed long-term reductions of 5-HT in the prefrontal cortex [drug main effect: $F_{1,18} = 12.22$, $P < 0.01$] and hippocampus [drug main effect: $F_{1,18} = 87.06$, $P < 0.001$], but not in the dorsal striatum (Table 1). Although there was a trend towards a drug-induced decline in 5-HT metabolite levels, MDMA exposure did not significantly reduce 5-HIAA content in any of the brain areas tested. MDMA treatment did increase 5-HT turnover (5-HIAA/5-HT) in the hippocampus [drug main effect: $F_{1,18} = 8.49$, $P < 0.01$] but not in the prefrontal cortex or dorsal striatum. There was little evidence that 5-HT functioning differed according to sex, with the one exception being that hippocampal 5-HIAA levels were greater in females rats (107.57 ± 10.17 pg/mg wet weight tissue) than male rats (82.89 ± 7.44 pg/mg wet weight tissue) [sex main effect: $F_{1,18} = 7.53$, $P < 0.05$].

2.2. DA and DOPAC content

MDMA exposure during the neonatal period caused a long-term decrease in DA levels in the prefrontal cortex (Table 1) [drug main effect: $F_{1,18} = 5.83$, $P < 0.05$]. Likewise, the dorsal striatal DA levels of MDMA-treated adult male rats (20 586 ± 3097 pg/mg wet weight tissue) were reduced relative to male saline controls (26 496 ± 2644 pg/mg wet weight tissue)