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Chronic administration of THC prevents the behavioral effects of intermittent adolescent MDMA administration and attenuates MDMA-induced hyperthermia and neurotoxicity in rats

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ABSTRACT

Most recreational users of 3, 4-methylenedioxymethamphetamine (MDMA or "ecstasy") also take cannabis, in part because cannabis can reduce the dysphoric symptoms of the ecstasy come-down such as agitation and insomnia. Although previous animal studies have examined the acute effects of co-administering MDMA and Δ^9-tetrahydrocannabinol (THC), which is the major psychoactive ingredient in cannabis, research on chronic exposure to this drug combination is lacking. Therefore, the present study was conducted to investigate the effects of chronic adolescent administration of both THC and MDMA on behavior and on regional serotonin transporter (SERT) binding and serotonin (5-HT) concentrations as indices of serotonergic system integrity. Male Sprague-Dawley rats were divided into four drug administration groups: (1) MDMA alone, (2) THC alone, (3) MDMA plus THC, and (4) vehicle controls. MDMA (2×10 mg/kg $\times$ 4 h) was administered every fifth day from postnatal day (PD) 35 to 60 to simulate intermittent recreational ecstasy use, whereas THC (5 mg/kg) was given once daily over the same time period to simulate heavy cannabis use. THC unexpectedly produced a modest hyperthermic effect when administered alone, but in animals co-treated with both THC and MDMA, there was an attenuation of MDMA-induced hyperthermia on dosing days. Subsequent testing conducted after a drug washout period revealed that THC reduced MDMA-related behavioral changes in the emergence and social interaction tests of anxiety-like behavior and also blunted the MDMA-induced decrease in exploratory behavior in the hole-board test. THC additionally attenuated MDMA -induced decreases in 5-HT levels and in SERT binding in the frontal cortex, parietal cortex, and striatum, but not in the hippocampus. These results suggest that chronic co-administration of THC during adolescence can provide some protection against various adverse physiological, behavioral, and neurochemical effects produced by MDMA.

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1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA or "ecstasy") is used by many teenagers, especially at "rave" parties (Lyles and Cadet, 2003). Despite MDMA's popularity, there is ample evidence that high doses of this compound produce long-lasting serotonergic deficits in rats and monkeys (Green et al., 2003), and neuroimaging studies suggest that serotonergic toxicity may also occur in heavy ecstasy users (Cowan, 2007). Moreover, a variety of neuropsychological and behavioral abnormalities have been associated with heavy ecstasy use, including cognitive impairments, heightened anxiety, and increased impulsivity (Morgan, 2000; Morgan et al., 2006; Quednow et al., 2007; Parrott, 2001). It is possible, though not yet proven, that these abnormalities stem from MDMA-induced dysfunction in serotonergic pathways in the forebrain.

Most ecstasy users also consume other drugs, with cannabis being the most popular followed by alcohol and stimulants (Lenton et al., 1997; Boys et al., 1997). Wish et al. (2006) surveyed a sample of East Coast college students and found that 98% of the ecstasy users also had taken cannabis. Moreover, toxicologic screening of urine samples collected from 198 ecstasy users between 2005 and

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2007 found the Δ^9-tetrahydrocannabinol (THC) metabolite 11-nor-9-carboxy-THC in approximately 62% of the samples (Black et al., 2009). The percentage of concomitant ecstasy and cannabis use is also high in other countries (Sala and Braida, 2005). Drug users report that cannabis helps reduce the dysphoric symptoms of the ecstasy come-down, such as agitation and insomnia (Strote et al., 2002; Winstock et al., 2001).

The prevalence of cannabis consumption among ecstasy users is an important potential confound in interpreting reported ecstasy-associated cognitive and neuropsychiatric abnormalities, making the cause of such abnormalities difficult to determine (Croft et al., 2001; Daumann et al., 2004; Parrott et al., 2007). Cannabis could affect MDMA action in at least three ways. First, THC may interact directly with serotonergic neurons, thereby altering their sensitivity to MDMA. This possibility is supported by the demonstration of CB_1 receptor expression in at least some serotonergic neurons (Häring et al., 2007; Lau and Schloss, 2008), along with evidence that presynaptic CB_1 receptor activation inhibits serotonin (5-HT) synthesis and release and reduces 5-HT turnover (Balázsa et al., 2008; Moranta et al., 2004; Nakazi et al., 2000). Second, cannabinoids also inhibit the release of other neurotransmitters, including dopamine (DA) (Nava et al., 2000; Schlicker and Kathmann, 2001). As DA is hypothesized to play a role in the serotonergic neurotoxic effects of MDMA (Breier et al., 2006; Sprague et al., 1998), THC-induced reductions in DA release might ameliorate such effects. Finally, MDMA neurotoxicity is due, in part, to drug-induced oxidative stress (Quinton and Yamamoto, 2006), whereas THC has been shown to possess antioxidant activity (Chaperon and Thiébot, 1999; van der Stelt and Di Marzo, 2005). This is a third mechanism, therefore, by which THC could alter the effects of MDMA exposure.

Despite the above mentioned evidence for possibilities of ecstasy and cannabis co-use and interaction, little is known about the modulation of MDMA effects by cannabinoids. Two previous studies reported on the effects of acute co-administration of THC and MDMA in adult rats (Morley et al., 2004; Young et al., 2005), and more recently Robledo and coworkers (2007) examined both the acute neurochemical effects of THC and MDMA co-administration and the combined effects of these compounds in a conditioned place paradigm in mice. However, to our knowledge there is no published information regarding the consequences of recurrent adolescent co-administration of these compounds in a rodent model. The present study begins to address this gap in the literature by examining the physiological, behavioral, and neurochemical effects of repeated co-administration of MDMA and THC in adolescent rats. We used an intermittent MDMA dosing regimen previously developed and characterized by our laboratory (Meyer et al., 2008; Piper and Meyer, 2004) along with daily THC dosing to simulate heavy cannabis use (Block and Ghoneim, 1993). Outcome measures included drug-induced changes in body weight, core body temperature, tests of anxiety-like and exploratory behaviors, and neurochemical measures of 5-HT neurotoxicity. All of these measures have previously been used to investigate the effects of acute and/or chronic MDMA administration in rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were obtained from the Charles River Laboratory (Wilmington, MA) at postnatal day (PD) 25–28. Animals were pair-housed in $44.5 \times 23.5 \times 20.0$ cm plastic tubs and habituated to the animal colony room for at least 1 week prior to the beginning of each experiment. Animals were maintained under a reversed 12-h light/dark cycle (lights off at 0800 h), with drug administration and behavioral testing performed during the dark phase of the cycle. They were provided with water and food *ad libitum* and maintained at a temperature of 26 ± 1 °C in Experiment I and 23 ± 1 °C in Experiment II. The protocol for these experiments was approved by the University of Massachusetts-Amherst Institutional Animal Care and Use Committee. Animal care conformed to the standards set forth in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

2.2. Drug treatments

(±) MDMA-HCL and THC (20 mg/ml in ethanol) were provided by the National Institute on Drug Abuse (NIDA). MDMA was dissolved in sterile physiological saline for administration to the animals. THC was prepared according to the procedure of Singh et al. (2005) described as follows. Briefly, a small amount of Tween-80 was added to the ethanolic THC solution, after which the mixture was placed under nitrogen gas and continuously stirred until the ethanol evaporated. The resulting residue was mixed thoroughly with saline, resulting in a fine suspension of THC in a vehicle of 0.75% Tween-80 in saline. Both MDMA and THC were freshly prepared on the day of dosing and administered at a volume of 1 ml/kg.

Animals received MDMA, THC, or their respective vehicles from PD 35 to 60, thus forming four treatment groups: (1) MDMA alone, (2) THC alone, (3) MDMA plus THC, and (4) vehicle controls. The MDMA alone group received two subcutaneous (s.c.) injections of 10 mg/kg (based on the weight of the salt) of MDMA every fifth day along with THC vehicle every day. On MDMA treatment days, the first MDMA dose was given in the morning, and the second MDMA dose was given 4 h later as previously described by Piper and Meyer (2004). The THC alone group received a single daily intraperitoneal (i.p.) injection of 5 mg/kg of THC as well as a s.c. saline injection every fifth day. The THC dose was chosen based on previous reports of cognitive impairment in rats given the same daily dose during adolescence (Cha et al., 2006; Nakamura et al., 1991). The MDMA plus THC group received THC daily and MDMA every fifth day. On MDMA/THC co-administration days, the THC injection was administered 2 h after the second MDMA dose. This procedure was adopted to simulate the use of cannabis to ameliorate the dysphoric symptoms of the ecstasy "come-down" by drug users (Strote et al., 2002; Topp et al., 1999; Winstock et al., 2001). The control group received a dosing pattern similar to that of the MDMA plus THC group but with vehicle only (i.e., daily afternoon injections of the Tween-80/saline vehicle along with two saline injections every fifth day).

2.3. Experiment I

Sixty Sprague–Dawley rats were included in Experiment I. Behavioral testing began 10 days following the end of drug treatment in order to allow ample time for THC washout and for any potential acute withdrawal symptoms to subside. In this experiment, the tests consisted of an open-field test (locomotor activity), emergence and social interaction tests (anxiety-like behaviors), and the hole-board test (exploratory behavior). Testing was conducted according to the following schedule: the open-field activity and emergence tests were conducted on PD 70, the social interaction test on PD 72, and the hole-board test on PD 74. On PD 75, rats were euthanized by means of rapid decapitation under light CO_2 anesthesia. Brains were removed, dissected into frontal cortex, parietal cortex, hippocampus, and striatum, and the tissues were then frozen over dry ice and stored at −70 °C for subsequent analysis. Each brain region was collected from both sides of the brain, and in the case of the frontal and parietal cortices and the hippocampus, samples from the two sides were stored and analyzed separately either for SERT binding or for concentrations of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) as indices of MDMA-induced neurotoxicity. As striatal samples were too small to permit both analyses, striatal tissues were used only for SERT binding.

2.3.1. Body weight

Body weights were recorded approximately 1 h prior to drug administration on a daily basis from PD 35 to 60, after which weights continued to be obtained at 5-day intervals for the remainder of the experiment. For simplicity, only the weight data from PD 35, 40, 45, 50, 55, 60, 65, 70, and 75 were used for presentation and statistical analysis.

2.3.2. Core body temperature

Core body temperature responses to drug administration were measured in all animals on PD 35, 45, and 60 using a rectal probe (RET-2; Physiotemp Instruments, Clifton, NJ) and a digital thermometer (TH-5; Physiotemp) according to the procedures of Piper et al. (2005). Temperature measurements were obtained at 30-min intervals beginning 30 min prior to the first MDMA or vehicle injection and ending 2 h after the THC or vehicle injection.

2.3.3. Social interaction test

Rats were placed in individual housing on PD 65, 7 days prior to the social interaction test. Testing was conducted under 40W red light in a wooden black arena measuring $60 \times 60 \times 30$ cm. Animals were tested in pairs in the arena for 10 min. The pairs were of similar weight and were selected from the same treatment groups, but from different home cages, so that the animals were unfamiliar with each other. Each session was videotaped for later coding of the following social interactions: sniffing, adjacent lying, following, crawling, mutual grooming, and aggression.