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Pretreatment with Δ9-tetrahydrocannabinol (THC) increases cocaine-stimulated activity in adolescent but not adult male rats

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ABSTRACT

Marijuana (*Cannabis sativa*) remains one of the most widely used illegal drugs, with adolescents being particularly vulnerable to its use and abuse. In spite of this, most studies are conducted in adult animals even though the effects might be quite different in adolescents. Additionally, the use of marijuana often precedes the use of other psychoactive drugs including cocaine, especially when marijuana exposure begins during early adolescence. The purpose of this study was to examine the effects of repeated Δ9-tetrahydrocannabinol (THC), the major active ingredient in marijuana, in adolescents compared to adults and to determine its subsequent effects on cocaine-stimulated activity. To this end, adolescent (postnatal day PND 34) and adult (PND 66) rats were administered 3 mg/kg/day THC for 8 days and locomotor activity was measured on days 1, 2, 7 and 8 after dosing. On day 12 (4 days after the last dose of THC), rats were injected with escalating doses of cocaine and behavior was recorded. Results show that THC depressed locomotor activity in adult rats but not in adolescents. However, following a cocaine challenge, adolescents exposed to THC showed increased locomotor responses to cocaine compared to chronic vehicle-injected controls. This was not seen in adults. These results show that the effects of cocaine are enhanced after THC in adolescents, but not adults, and that this might account for the greater transition to cocaine after early, as opposed to later, marijuana use.

1. Introduction

Marijuana (*Cannabis sativa*) remains one of the most widely used illegal drugs, with adolescents being particularly vulnerable to its use and abuse. The percentage of adolescents reporting lifetime use of marijuana rises steadily from 14.2% in eighth grade to 41.8% by twelfth grade, with a majority of eighth through twelfth graders reporting that they are at a 'great risk' to try marijuana regularly (Johnston and O'Malley, 1997). Both acute and chronic doses of THC have been shown to cause impairment in attention and motor coordination in a maze task in humans (Weinstein et al., 2008), inhibition of movement and basal ganglia neuronal activity in adult rats (Shi et al., 2005), and decreased locomotor activity in rats (Romero et al., 1996; Whitlow et al., 2002). The decrease in locomotor activity after THC administration has been reported in both adult and adolescent rats, with the effect being much smaller in adolescents (Schramm-Sapyta et al., 2007). In addition, it also has been reported that low doses of THC increased activity in adolescence and had no effect in adults (Wiley et al., 2008).

The National Survey on Drug Use and Health showed that adults who initiated marijuana use prior to age 15 were 6 times more likely to be dependent on an illicit drug than adults who first used marijuana at age 21 or older (NSDUH, 2002). In addition, of adults initiating marijuana use prior to age 15, 62% reported lifetime cocaine use, compared to 16% in marijuana users who reported first smoking the drug after age 20 (a four-fold difference) or 0.6% among those who had never used marijuana (a 100-fold difference). These data show that the earlier the first marijuana use, the more likely one is to use other illicit drugs. While these studies do not unequivocally show causality, the data suggest that there may be fundamental differences in the effects of marijuana in preadolescents and young adolescents compared to adults. Laboratory studies show that the adolescent period may in fact be a period of development of increased vulnerability. Earlier studies from our lab and others have shown that drugs such as nicotine (Collins and Izenwasser, 2004; Collins et al., 2004a; Collins et al., 2004b; McQuown et al., 2007) and MDMA (Aberg et al., 2007; Achat-Mendes et al., 2003; Daza-Losada et al., 2008) increase the subsequent response to cocaine in adolescent rats. These studies suggest that it is possible to model and study the effects of drugs during adolescence and to determine whether there are differential effects than during adulthood.

Within the central nervous system, the greatest density of CB1 receptors is found in the cerebellum, basal ganglia and CA1, CA3 and dentate gyrus areas of the hippocampal formation (Herkenham, 1991; Herkenham et al., 1990). Normally stimulated by endogenous cannabinoids (endocannabinoids), the binding of cannabinoids to presynaptic G-protein coupled receptors can alter the release of neurotransmitters at the synapse and is responsible for many of

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the behavioral effects seen with marijuana consumption. Ontogenetic studies show that cannabinoid receptor density in whole brain increases progressively from birth to PND 60 (Belue et al., 1995; McLaughlin et al., 1994). Additionally, although cannabinoid receptors are present in forebrain from PND 10 on, there are regional variations in the ontogeny of these receptors. For example, receptors in the striatum, limbic forebrain and mesencephalon peak between PND 30 and 40 and then progressively decrease to adult levels (Rodriguez de Fonseca et al., 1993). In adults, in the ventral mesencephalon, CB1 receptors are located on GABA terminals and serve to dampen the GABA inhibition of DA firing rates (Szabo et al., 2002; Wu and French, 2000). That is, cannabinoids inhibit GABA's effects on DA neurons in the ventral mesencephalon producing a net increase in firing of the ascending DA projections (Szabo et al., 2002). In striatum in adults, CB1 receptors are co-localized with DA receptors (Herkenham, 1991). Cannabinoids have been shown to inhibit release of DA from striatal synaptosomes (Poddar and Dewey, 1980). Therefore, in striatum cannabinoids appear to directly reduce DA function while in the ventral mesencephalon, cannabinoids appear to enhance DA function. The ventral mesencephalon develops before the striatum and may be selectively activated in adolescents producing enhanced DA function as opposed to dampened DA function in the adult striatum. In addition, these relationships may undergo extensive modification from adolescence into adulthood.

Several groups have examined the effects of chronic THC on behavioral responses to psychostimulants and reported variable results. For example, administration of THC for 14 days led to increased amphetamine-stimulated activity 30 min and 24 h after the last dose (Gorriti et al., 1999). Acutely, CP 55, 940 or THC did not alter the locomotor responses to cocaine (Panlilio et al., 2007) although they did block the development of sensitization to cocaine (Arnold et al., 1998) in adult rats. The present study was done to examine the effects of daily THC in adolescent and adult rats on locomotor activity and on the subsequent response to cocaine. To this end, adolescent (PND 34) and adult (PND 66) rats were injected with 3 mg/kg/day THC for 8 days and locomotor activity was measured on the first two and last two days of the daily dosing. On day 12 (4 days after the last dose of THC) rats were injected with exponentially increasing doses of cocaine and behavior recorded.

2. Materials and methods

2.1. Chemicals

Drugs were obtained from the following sources: Δ9-tetrahydrocannabinol (THC) and cocaine hydrochloride from Research Triangle Institute, Research Triangle Park, NC courtesy of the National Institute on Drug Abuse (Bethesda, MD).

2.2. Treatments

Sprague–Dawley rats (Charles River, NC) were used in these studies. Periadolescent male rats at postnatal day 34 (PND 34) and adult male rats (PND 66) were injected once daily for eight days with i.p. injections of either 3.0 mg/kg THC or vehicle (60% saline:20% ethanol:20% cremophor EL). Periadolescence is a period of early adolescence, which begins in rats at approximately postnatal day 28 and ends at postnatal day 40 (Spear and Brake, 1983). This period of early adolescence was chosen because it is during this stage of development that marijuana use is often initiated (The National Center on Addiction and Substance Abuse at Columbia University, 2003). All rats were housed two per cage in a temperature and humidity-controlled environment under a 12 h light/dark cycle with lights on at 7 a.m. and off at 7 p.m. All behavioral testing was done during the light schedule between 9 a.m. and 4 p.m. with each group tested at the same hour each day and the groups randomized over the course of the day. Food and water were available ad libitum. Administration of THC did not alter body weight compared to vehicle in either adolescent or adult rats.

On day 12 of the experiment, all rats were injected with saline, followed by 1.0, 3.0, 10.0, 20, and 30 mg/kg cocaine (i.p.) in a cumulative dosing regimen (actual injections of 1.0, 2.0, 7.0, 10, and 10 mg/kg cocaine), as described previously (Collins and Izenwasser, 2004). Following each injection, locomotor activity was measured for a total of 10 min for vehicle and for each cumulative dose of cocaine. Thus, there were 10 min between each dose of cocaine and the entire session lasted 50 min. This procedure allows a full dose–response curve to be determined in a single day and greatly reduces the number of animals used, since full curves are determined in each animal.

2.3. Locomotor activity testing

On the first two and last two days (days 1–2 and 7–8) of administration of THC or vehicle, locomotor activity was measured for 30 min. Rats were placed in clear acrylic chambers (16×16 in.) inside Digiscan activity monitors (Omnitech Electronics, Columbus, OH) that were equipped with infrared light sensitive detectors mounted 2.5 cm apart along two perpendicular walls. Mounted along the opposing walls were infrared light beams that were directed at the detectors. The pattern of beam breaks provides information on the distance that the animal has traveled. Locomotor activity was analyzed by a three-factor (pretreatment × age × day) analysis of variance (ANOVA) with repeated measures for day. Stereotypy also was measured, both by machine, which measures repeated beam breaks and by experimenter observation. For the cocaine cumulative dosing curve on day 12, a three-way analysis of variance (pretreatment × age × dose of cocaine) with repeated measures for dose was done. Significant interactions were followed by tests for simple treatment (drug) effects. Comparisons of data on individual days were made by one-way ANOVA, and followed by post hoc analysis using Fisher's Protected Least Significant Difference (PLSD) when warranted. P values less than 0.05 were considered significant for all tests.

3. Results

3.1. THC pretreatment (days 1–8)

Locomotor activity: THC (3 mg/kg) decreased activity in adult rats, but not in adolescent rats (Fig. 1). An overall analysis of pretreatment × age × day, with day as a repeated measure, was not significant. However, there was a significant effect of pretreatment ($F[1,52] = 11.29$, $p \leq 0.002$) and a significant pretreatment × age interaction ($F[1,52] = 5.75$, $p \leq 0.02$). Post-hoc tests showed that there were no significant differences in the effect of vehicle on locomotor activity in the adult and adolescent rats. There was, however, a significant difference in the effect of THC, with an overall decrease in activity compared to vehicle in adult ($p \leq 0.0005$) but not adolescent rats. On day 1 of administration, THC did not have a significant effect on activity in either group and on none of the other test days was there a significant effect of THC in adolescents (Fig. 1A). However on days 2, 7, and 8 THC treatment significantly decreased activity in adult rats (Fig. 1B) in response to the THC ($p \leq 0.05$).

3.2. Cocaine-stimulated activity (day 12)

Cocaine increased activity in both adult and adolescent rats and the dose–effect curve for cocaine-stimulated activity was shifted upward in adolescent rats that had been treated previously with THC (Fig. 2A). In contrast, there was no difference in cocaine-stimulated activity in the adult rats that had received THC compared to vehicle. There was a significant pretreatment × age × dose interaction ($F[4,232] = 3.385$, $p \leq 0.01$) on cocaine-stimulated activity on day 12 of the experiment. There was no significant main effect of pretreatment, but there was a significant main effect of age ($F[1,58] = 4.76$, $p \leq 0.03$). There were

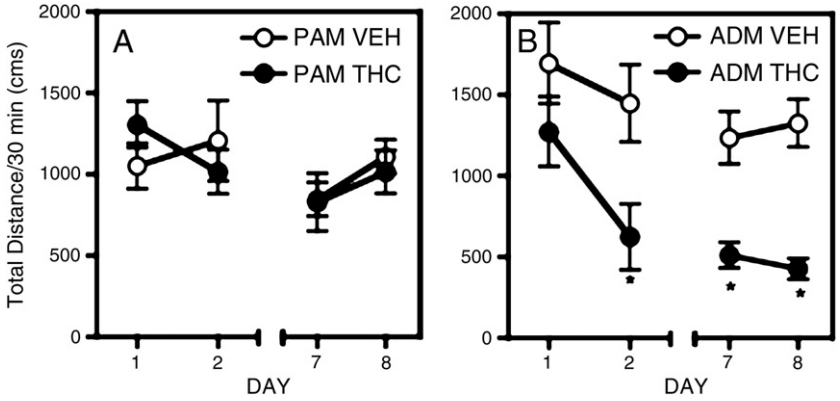

Fig. 1. (A) THC had no effect on locomotor activity in periadolescent male rats (n = 14) compared to periadolescent male rats treated with vehicle (n = 16) on days 1, 2 and 7, 8 of an eight-day period of daily injections. In addition, activity levels in the periadolescent rats did not change significantly across days regardless of pretreatment. (B) Adult male rats injected daily with THC (n = 16) for 8 days showed daily decreases in locomotor activity on all days tested after the first day compared to adult male rats treated with vehicle (n = 16). *Represents a significant difference from vehicle ($p \leq 0.05$).

also significant dose × age ($F[4,232] = 3.37$, $p \leq 0.01$) and pretreatment × age ($F[1,58] = 5.643$, $p \leq 0.02$) effects. Post hoc tests showed that there was a significant effect of pretreatment with THC vs vehicle in adolescent ($p \leq 0.02$) but not in adult rats. In addition, adolescent and adult rats exhibited different levels of activity in response to cocaine after pretreatment with vehicle ($p \leq 0.002$). There were no differences in stereotypy across groups.

4. Discussion

Chronic THC administration reduced locomotor activity in adult but not adolescent rats, however, THC increased the responses to a cocaine challenge only in adolescents but not adults. Differences in the interactions between the cannabinoid system and the systems responsive to cocaine (including the dopamine system) undoubtedly underlie these findings.

There were differences in the effects of cocaine after pretreatment with vehicle in that adolescent rats had a diminished response to the highest doses of cocaine tested compared to adults. Previous studies have shown that there are no differences in response to a single acute injection of cocaine in adult vs periadolescent male rats, however, adult rats are more likely than adolescents to develop sensitization to cocaine-stimulated activity (or are sensitized to a greater degree) upon repeated administration of cocaine (e.g. Collins and Izenwasser, 2002; Laviola et al., 1995). Thus, it is possible that this sensitization is reflected in the effects of cocaine in the vehicle-treated rats in this study. To our knowledge, a cumulative dosing procedure has not been used in adolescent rats previously, although it has been shown in adults that it produces a curve similar to that of separately tested doses (Terry, 1992). Since the adolescent rats exhibited an enhanced response to cocaine after THC compared to vehicle, it appears that THC sensitized the adolescent rats such that they now resemble adult rats, in that there were no significant differences between the adolescent and adult rats that received THC.

The finding that repeated administration of THC did not alter cocaine-stimulated activity in adult rats compared to vehicle is consistent with earlier studies showing that repeated administration of THC to Sprague–Dawley rats (Panlilio et al., 2007), as in the present experiment, or the cannabinoid agonist CP 55,940 to Wistar rats (Arnold et al., 1998), produced no change in the subsequent locomotor response to cocaine in adult rats. Thus, this lack of interaction between chronic cannabinoid agonist administration and cocaine-stimulated activity appears to cross drugs and strains of rat. The current study goes on to extend the literature by showing that in contrast to the adults, there are increases in the effects of cocaine after prior THC administration in adolescents. It is interesting to note that an earlier study showed that in rats treated for 14 days with THC, there were increases in amphetamine-stimulated locomotor activity either 30 min or 24 h after the last injection of THC (Gorriti et al., 1999). The difference in this finding compared to the above-mentioned studies with cocaine could be due to several reasons, including that the actions of amphetamine to reverse the dopamine transporter differ from that of cocaine, or that there was residual THC present, since it would not have cleared by 24 h and certainly not by 30 min

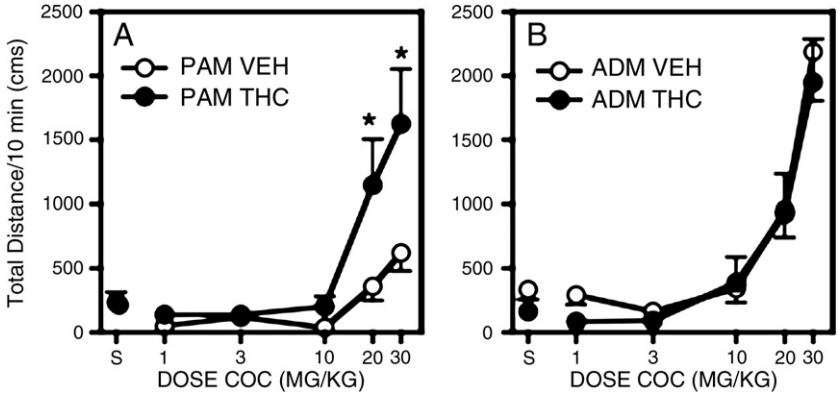

Fig. 2. (A) Periadolescent male rats pretreated with THC were sensitized to the locomotor-activating effects of cocaine and were significantly different from the periadolescent male rats pretreated with saline ($p < 0.02$). (B) In contrast, there were no significant differences in adult male rats pretreated with THC compared saline. S = saline. *Represents a significant difference from vehicle ($p \leq 0.05$).

post injection. In the Panlilio study (Panlilio et al., 2007), rats were tested 1 week after the last administration of THC and in the Arnold study (Arnold et al., 1998), they were challenged with cocaine 4 days after CP 55,940 administration, as was the case in the present study.

Our findings also are consistent with other studies showing that administration of a cannabinoid agonist during adolescence leads to subsequent increases in the effects of cocaine on other behavioral measures, predominantly in male rats (Higuera et al., 2005; Higuera-Matas et al., 2008; Izenwasser, 2005). Numerous studies have shown that behavioral and physiological alterations in response to drug administration differ in adolescents compared to adults, suggesting that this period is one of increased vulnerability to drug effects (for review see Izenwasser, 2005).

THC increases firing of dopamine neurons in VTA and substantia nigra in adult rats (Wu and French, 2000) by inhibiting GABA inhibition of mesencephalic neurons (Szabo et al., 2002). In striatum, CB1 receptors are co-localized with DA receptors (Herkenham, 1991) and cannabinoids have been shown to inhibit release of DA from striatal synaptosomes (Poddar and Dewey, 1980). However, following chronic exposure, CB1 receptors have been found to decrease in number in striatum (Rodriguez de Fonseca et al., 1994) thus reducing the dampening capacity of the endocannabinoids. Cocaine increases anandamide levels in striatum, which presumably serves to dampen the cocaine response (Giuffrida et al., 1999). Following chronic THC, the reduced CB1 receptor number hypothetically permits less dampening of the cocaine response. Thus, equal doses of cocaine might be expected to produce greater locomotor responses in animals chronically exposed to THC. We found this in adolescent males but not adult males. One possible explanation for this difference is that the dose of THC used in the current study did not produce a down regulation in striatum of adults. In adolescence, there was no decrease in activity following the THC administration perhaps due to the immaturity of the striatal DA/CB1 interactions where cannabinoids inhibit dopamine release from DA terminals thus decreasing activity (Cadogan et al., 1997). If these neurons did not possess their mature complement of CB1/DA interactions, the reduced locomotor activity may not have occurred during the period of THC administration. In fact, it has been reported that low doses of THC increased activity in adolescents (Wiley et al., 2008), an effect that we have seen with younger male rats as well (Harte and Dow-Edwards, 2010). Actions of cannabinoids to inhibit GABA inhibition of ventral mesencephalic neurons and increase DA neuronal firing should increase activity. In the adult, the effects of cannabinoids in striatum may override the effects in ventral mesencephalon due to direct inhibition of DA release in striatum. However, in the adult, the dose/duration of THC administered may not have been sufficient to reduce CB1 receptors following chronic exposure thus producing no increase in response to cocaine challenge.

In summary, we found that repeated administration of THC decreased locomotor activity in adult male Sprague–Dawley rats, an effect not seen in adolescence. However, subsequent to the repeated regimen of THC administration, adolescents show increased cocaine stimulated locomotor activity, an effect not seen in adults. The results suggest that in adolescence, the mesolimbic/nigrostriatal DA systems are not mature and although there is no change in locomotor activity during cannabinoid administration, homeostasis in the system is disrupted since the response to cocaine is increased. Thus, it appears that there are differences in modulation of the dopaminergic system in adolescent vs adult rats by THC and this might account, at least in part, for the increased effect of cocaine after THC administration in adolescents but not adults.

Acknowledgments

The animals used in this study were maintained and the studies were conducted in accordance with the guidelines of the Guide for Care and Use of Laboratory Animals, National Research Council, Department of Health, Education and Welfare, NIH Publication 85-23, revised 1985 and the Institutional Animal Care and Use Committee at the University of Miami. This work was supported by the National Institute on Drug Abuse and the NIH Office of Research on Women's Health (grants DA 024584-0001 and DA 024584-0002).

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