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Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence

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Abstract

The cannabis gateway hypothesis purports that early exposure to cannabis is a risk factor for subsequent use of other addictive drugs, e.g., psychostimulants. Neurobiological sensitization, consistent with a gateway hypothesis, was currently studied in regard to amphetamine response. Rats were exposed to the cannabinoid receptor agonist WIN 55,212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone] 1.25 mg/kg, intraperitoneally; i.p.) for 5 days during early adolescence. Amphetamine (0.5 mg/kg, i.p.) or WIN 55,212-2 (1.25 mg/kg, i.p.) was administered in late adolescence and in vivo dopamine levels were simultaneously measured in the nucleus accumbens. Locomotor and stereotyped behaviors were also monitored in rats pretreated with WIN 55,212-2 (0.625, 1.25 or 2.5 mg/kg) or Δ-9-tetrahydrocannabinol (0.75, 1.5 or 3.0 mg/kg, i.p.) for 5 days during early adolescence and challenged with amphetamine (0.5 or 2.0 mg/kg) in late adolescence or as adults. Pretreatment with WIN 55,212-2 or Δ-9-tetrahydrocannabinol during early adolescence did not alter the dopaminergic or behavioral responses to amphetamine in adolescence or adulthood. In conclusion, these findings do not support the cannabis gateway hypothesis in regard to subsequent amphetamine exposure.

Keywords: THC; Psychostimulants; Cross-sensitization; Locomotion; Microdialysis, in vivo; (Rat)

1. Introduction

Cannabis is one of the most widely used illicit drugs in the world. It is self-administered by various animal species (Braida et al., 2001; Fattore et al., 2001; Tanda et al., 2000) and repetitive cannabis use by humans is associated with addiction and impairment of cognitive functions. Early use of cannabis has been hypothesized to be a risk factor for the onset of use of even more highly addictive drugs of abuse, e.g., psychostimulants and opiates. This is termed the cannabis gateway hypothesis and is substantiated by a number of epidemiological studies, which suggest that prior exposure to cannabis encourages use of other illicit drugs (Fergusson and Horwood, 2000; Lynskey et al., 2003; Yamaguchi and Kandel, 1984). The gateway effect could be due to neurobiological factors such that cannabis exposure sensitizes reward pathways in the brain thereby leading to stronger effects of subsequent drug use. It could also be due to social factors, e.g., cannabis users may be exposed to an environment with easier access to other illicit drugs. In this study, we designed an experimental rat study to focus on the neurobiological factors underlying a possible cannabinoid gateway effect. Because cannabis use most commonly is initiated during adolescence, we studied young rats at an age corresponding to human adolescence and examined the interaction with the highly addictive drug amphetamine.

The mechanisms underlying the euphoric and addictive properties of cannabinoids are still quite unclear. However, previous studies have documented increased firing rate of mesolimbic dopaminergic neurons after admin-

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istration of Δ-9-tetrahydrocannabinol, the psychoactive constituent of cannabis or the synthetic cannabinoid CB1 receptor agonists WIN 55,212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone] and CP 55,940 [(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol] (Gessa et al., 1998), as well as increased levels of extracellular dopamine in the nucleus accumbens after acute administration of Δ-9-tetrahydrocannabinol or WIN 55,212-2 (Chen et al., 1990; Tanda et al., 1997). In addition, chronic Δ-9-tetrahydrocannabinol treatment followed by an acute challenge with a cannabinoid CB1 receptor antagonist, SR 141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride], has been shown to reduce extracellular dopamine in the nucleus accumbens and to precipitate a physical withdrawal syndrome in rats (Tanda et al., 1999). Altogether, such findings suggest that exposure to cannabinoids modulates the dopaminergic activity in the nucleus accumbens and subsequently mesolimbic reward circuits and behavior. Increased locomotor activity is a common feature of many drugs of abuse, including cannabinoids that induce a time- and dose-dependent biphasic locomotor activity response. Fairly low doses (1.5–2 mg/kg) of Δ-9-tetrahydrocannabinol have been shown to produce an initial increase in locomotor activity that over time develops into an inhibition of locomotor activity, whereas higher doses induce catalepsy (Sanudo-Pena et al., 2000).

Drug reinforcement and motivational components of drug addiction have been linked with alterations in various neurotransmitter systems, in particular, the mesolimbic dopamine system including the nucleus accumbens and the ventral tegmental area. Sensitization of mesolimbic dopamine efflux during repeated exposure to drugs of abuse has been hypothesized to be a key neural adaptation underlying the development of pathological drug dependence (Robinson and Berridge, 1993). Exposure to one type of drug may induce cross-sensitization to another type, so if cannabis exposure has the capacity to induce cross-sensitization to amphetamine, that could contribute to the observed gateway effect.

Amphetamine is well documented to induce a marked increase of dopamine in reward-related areas, such as the nucleus accumbens, to increase locomotor activity and to induce stereotypes (Di Chiara and Imperato, 1988; Hurd and Ungerstedt, 1989; Kuczenski and Segal, 1989; Zetterstrom et al., 1983). Repeated amphetamine administration has been shown to induce sensitization of the dopaminergic and behavioral responses (Robinson et al., 1988).

A limited number of rat studies have indicated that chronic pretreatment with Δ-9-tetrahydrocannabinol or WIN 55,212-2 induces behavioral cross-sensitization to amphetamine (Gorriti et al., 1999; Muschamp and Siviy, 2002). These studies were conducted using adult rats, whereas in humans, as emphasized earlier, most individuals start smoking cannabis in adolescence. No information is known to date about the consequences of early adolescent cannabinoid pretreatment on subsequent stimulant exposure. As such, we wanted to assess whether there is cross-sensitization of locomotor behavior between cannabis and amphetamine in adolescent rats, and to take the question a step further, we also wanted to look at interactions in the mesolimbic dopamine system. Adolescence in rats is considered to take place approximately around day 28–42, a time period where the rats show behavioral differences from younger and older rats, a growth spurt, and peak in gonadotropin levels (Maeda et al., 2000; Spear, 2000).

Specifically, three questions were addressed in this study:

(1) Does pretreatment with the synthetic cannabinoid receptor agonist WIN 55,212-2, modulate the dopaminergic responsiveness to amphetamine in adolescent rats?
(2) Are the behavioral effects of amphetamine altered by pretreatment with WIN 55,212-2 or Δ-9-tetrahydrocannabinol in adolescent rats?
(3) Does the effects of Δ-9-tetrahydrocannabinol pretreatment on the behavioral responsiveness to amphetamine in rats depend on the age at which they are amphetamine-challenged?

2. Materials and Methods

2.1. Subjects

Male adolescent Sprague–Dawley rats (21-days old) were obtained from B&K Universal, Sweden. They were housed in a temperature-controlled environment on a 12-h light/dark cycle (lights on 7:00 AM) with ad libitum access to food and water. They were allowed to acclimate for a week in their new environment before the start of the experiment. All animal experiments were performed in accordance with the guidelines of the European Community and The Swedish National Board for Laboratory Animals (CFN), under a protocol approved by the Ethical Committee of Northern Stockholm.

2.2. Drugs

WIN 55,212-2 mesylate (Sigma-Aldrich, Sweden) was dissolved in Tween 80 and then diluted with 0.9% NaCl to the concentrations of 1 mg/ml WIN 55,212-2 and 1% Tween 80. Δ-9-tetrahydrocannabinol (10 mg/ml in ethanol solution; Sigma-Aldrich, Sweden) was evaporated under nitrogen gas, dissolved in Tween 80 and diluted with 0.9% NaCl to the concentrations of 1 mg/ml Δ-9-tetrahydrocannabinol and 1% Tween 80. Amphetamine sulphate (Apoteket, Sweden) was dissolved in 0.9% NaCl. All drugs were administered intraperitoneally (i.p.).

2.3. Stereotaxic surgery

Two days prior to the microdialysis testing, animals were anesthetized with isoflurane (Forene, Apoteket, Sweden) and a guide cannula (CMA Microdialysis, Sweden) was implanted above the nucleus accumbens using stereotaxic surgery. The coordinates for the position of the guide cannula were AP +1.5 mm, ML +1.0 mm and DV −2.5 mm from bregma or the dura, according to the atlas of Paxinos and Watson (1986) and to empirical tests in 150-g rats. The guide cannula was secured to the scull with stainless steel screws and dental cement (AgnTho's, Sweden). After the surgery, the rats were kept in single cages.

2.4. In vivo microdialysis

On the evening prior to the test day, a microdialysis probe (CMA 12, CMA Microdialysis, Sweden) was lowered into the nucleus accumbens (DV −7.5) via the guide cannula. In the morning, the probe was connected to an infusion pump (Univentor syringe pump 801, AgnTho's, Sweden) and artificial cerebrospinal fluid (148 mM NaCl, 2.7 mM KCl, 0.85 mM $MgCl_2$, 1.2 mM $CaCl_2$, pH 7.1; Apoteket, Sweden) was pumped through the probe at a constant rate of 1 μl/min. After approximately 30 min of equilibration, dialysate samples were collected every 20 min (Univentor microsampler 810, AgnTho's, Sweden) in vials containing 2.2 μl of 1 M perchloric acid (to give a final concentration of 0.1 M perchloric acid) to minimize catecholamine degradation. The microdialysis experiment was performed in the rat's home-cage, and immediately after the end of the experiment, the rats were anaesthetized with CO_2, followed by decapitation. The brain was removed, frozen in ice-cold isopentane and sliced in a cryostat. The sections were validated for correct probe localization with the guidance of the brain atlas of Paxinos and Watson (1986).

2.5. HPLC analysis

The dialysate samples were injected into a high-performance liquid chromatograph (HPLC) equipped with a reverse phase column (Reprosil, 150×4 mm, 3 μm particle size) for separation and a coulometric detector (Coulochem II, ESA) to quantitate the dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels. The oxidation electrode was set to 400 mV, and the reduction electrode to −200 mV. The mobile phase consisted of 55 mM sodium acetate, 0.5 mM octanesulfonic acid, 0.01 mM Na_2EDTA and 10% methanol (pH was adjusted to 4.1 with acetic acid) and was perfused at a constant flow rate of 0.7 ml/min.

2.6. Locomotor activity

Locomotor activity was measured in two activity chambers (45×45 cm; ActiMot, TSE Systems, Germany) with transparent walls. Behavioral activity was monitored by three sets of 32 infrared emitters and receivers with 14 mm distance between beams, two sets in the *x–y* axis to register horizontal activity and one set 8–15 cm above the floor to monitor rearing behavior. Novelty was measured during the first 30 min of the session, and thereafter, a challenge amphetamine injection was given. Locomotor activity was measured for an additional 90 or 120 min. All tests were carried out between 8:00 AM and 12:30 PM.

2.7. Stereotyped behavior

The rats were videotaped during the locomotor activity tests and stereotyped behavior was scored manually according to a scale developed by Kalivas et al. (1988). The scale ranged from 1–10 as follows:

1. asleep or still;
2. inactive, grooming or mild licking;
3. locomotion, rearing or sniffing;
4. any combination of locomotion, rearing or sniffing;
5. continuous sniffing without locomotion or rearing;
6. continuous sniffing with locomotion or rearing;
7. patterned sniffing for 5 s;
8. patterned sniffing for 10 s;
9. continuous gnawing;
10. bizarre diskinetic movements or seizures.

The behavior was analyzed for 10 s every 10 min for 90 min, giving a maximum score of 90.

2.8. Experimental designs

2.8.1. Experiment 1: effects of subchronic WIN 55,212-2 pretreatment on the dopaminergic response to an amphetamine challenge in adolescent rats

28-day-old rats (n=46) were treated with WIN 55,212-2 (1.25 mg/kg, i.p.) or saline once a day for 5 days. Following a 7-day drug-free period, a challenge injection of amphetamine (0.5 mg/kg, i.p.), WIN 55,212-2 (1.25 mg/kg, i.p.) or saline was given, while extracellular dopamine, DOPAC and HVA in the nucleus accumbens were monitored using in vivo microdialysis. Because adolescence in rats is considered to take place approximately from day 28 to day 42 (Spear, 2000), the age of the rats at the time of cannabis pretreatment (day 28–32) corresponds to early adolescence in humans and when they receive the drug challenge (day 40) to late adolescence.

2.8.2. Experiment 2: effects of subchronic WIN 55,212-2 pretreatment on amphetamine-induced locomotor activity and stereotypy in adolescent rats

28-day-old rats (n=23) were treated with WIN 55,212-2 (0, 0.625, 1.25 and 2.5 mg/kg, i.p.) once a day for 5 days. After a 7-day drug-free period, the animals were given an amphetamine injection (0.5 mg/kg, i.p.), and locomotor activity and stereotypic behavior were monitored.

2.8.3. Experiment 3: effects of subchronic Δ-9-tetrahydrocannabinol pretreatment on amphetamine-induced locomotor activity and stereotypy in adolescent rats

28-day-old rats (n=24) were treated with Δ-9-tetrahydrocannabinol (0, 0.75, 1.5 and 3.0 mg/kg, i.p.) once a day for 5 days. After a 7-day drug-free period, the animals were given an amphetamine injection (0.5 mg/kg, i.p.), and locomotor activity and stereotypic behavior were monitored.

2.8.4. Experiment 4: effects of subchronic Δ-9-tetrahydrocannabinol pretreatment on amphetamine-induced locomotor activity in rats challenged with amphetamine as adolescents or adults

28-day-old rats (n=62) were treated with Δ-9-tetrahydrocannabinol (1.5 mg/kg, i.p.) or saline once a day for 5 days. After 7 or 35 drug-free days, the animals were given an amphetamine injection (0.5 or 2.0 mg/kg, i.p.), and locomotor activity was monitored.

2.9. Statistical analysis

The dopamine, DOPAC and HVA levels were expressed as percent changes from baseline levels, where the baseline was calculated as the mean of the three samples preceding the challenge injection. The mean±S.E.M. percent changes were calculated for each 20-min sample for all rats in each group. Data were analyzed with three-way analysis of variance (ANOVA; pretreatment×challenge×time), followed by Tukey's HSD test when appropriate. Locomotor activity was measured as forward locomotion, recorded in 10-min intervals and analyzed with two- (pretreatment×time) or three-way (pretreatment×challenge×time) ANOVA. The total locomotor activity was analyzed with one- or two-way (pretreatment×challenge) ANOVA. When appropriate, the analysis was followed by Tukey's HSD test. Median stereotypic rating was calculated for each group and was analyzed using nonparametric Kruskal–Wallis ANOVA. All statistical calculations were performed using the StatSoft Statistica software. A P-value<0.05 was considered significant.

3. Results

3.1. Experiment 1: effects of subchronic WIN 55,212-2 pretreatment on the dopaminergic response to an amphetamine or WIN 55,212-2 challenge in adolescent rats

The mean baseline levels±S.E.M. of dopamine were 1.38±0.07 nM; DOPAC, 1.30±0.08 μM; and HVA, 0.55±0.03 μM. The baseline levels did not differ significantly between groups.

The overall statistical analysis revealed significant treatment [F(5,37)=54.0, P<0.0001], time [F(4,149)=84.4, P<0.0001] and interaction [F(20,149)=27.4, P<0.0001] effects on the dopamine levels. There were also significant effects for DOPAC (treatment [F(5,41)=23.6, P<0.0001], time [F(4,146)=46.2, P<0.0001] and interaction [F(18,146)=15.0, P<0.0001]) and HVA (treatment [F(5,40)=11.0, P<0.0001], time [F(3,117)=4.2, P<0.0001] and interaction [F(15,117)=7.2, P<0.0001]). An acute injection with WIN 55,212-2 showed a trend towards causing a small increase of extracellular dopamine (130% of baseline 40 min after injection, P<0.1) but there was no significant difference from the saline group (Fig. 1A). Fig. 1B shows that an amphetamine challenge lead to markedly increased dopamine levels in both saline (982% of baseline 40 min after injection, P<0.0001) and WIN 55,212-2 (882% of baseline 40 min after injection, P<0.0001) pretreated animals, but there was no difference between the increases induced by the two pretreatments. A

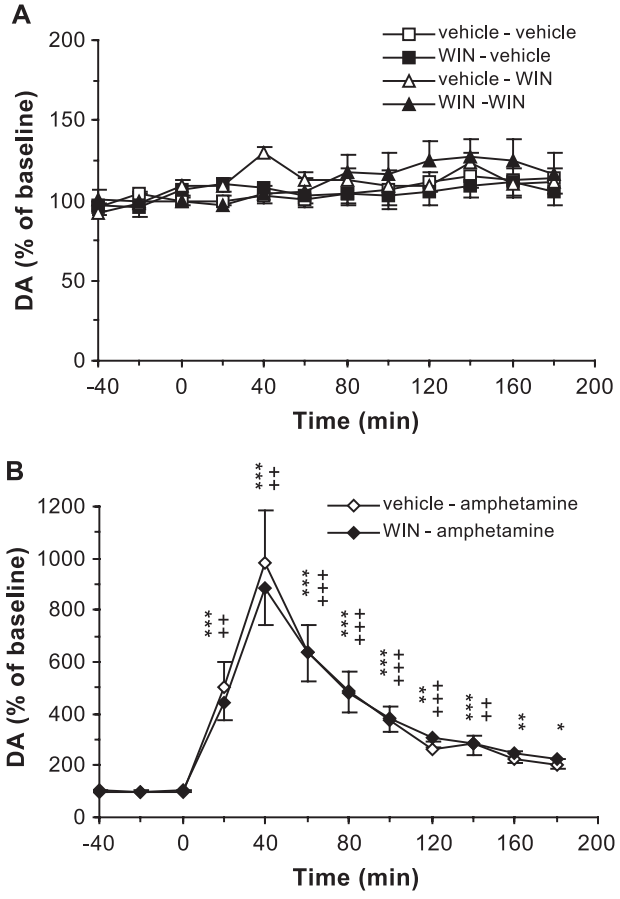

Fig. 1. Effects of (A) amphetamine (0.5 mg/kg) and (B) WIN 55,212-2 (1.25 mg/kg) or vehicle administration on extracellular dopamine levels in the nucleus accumbens in adolescent rats, 7 days after subchronic (5 days) WIN 55,212-2 (1.25 mg/kg) or vehicle pretreatment. The challenge injection was given at time 0. Data are expressed as mean (±S.E.M.) percent change from baseline levels. Symbols indicate significant challenge effect; *P<0.05, **P<0.01, ***P<0.001 WIN 55,212-2–amphetamine vs. WIN 55,212-2–saline; ^{+}P<0.05, ^{++}P<0.01 vehicle–amphetamine vs. vehicle–vehicle. n=6–9.

challenge injection with WIN 55,212-2 did not alter the levels of the dopamine metabolites DOPAC or HVA. Amphetamine administration led to a significantly decreased levels of DOPAC (49% of baseline 40 min after injection in the WIN 55,212-2-pretreated group, $P<0.0001$ and 55% of baseline 40 min after injection in the saline-pretreated group, $P<0.0001$) and HVA levels (73% of baseline 60 min after injection in the WIN 55,212-2-pretreated group, $P<0.01$ and 75% of baseline 60 min after injection in the saline-pretreated group, $P<0.0001$; Fig. 2A and B). There was no difference in the metabolite response between animals pretreated with WIN 55,212-2 or saline. The microdialysis probes were validated to be located predominantly in the medial nucleus accumbens shell.

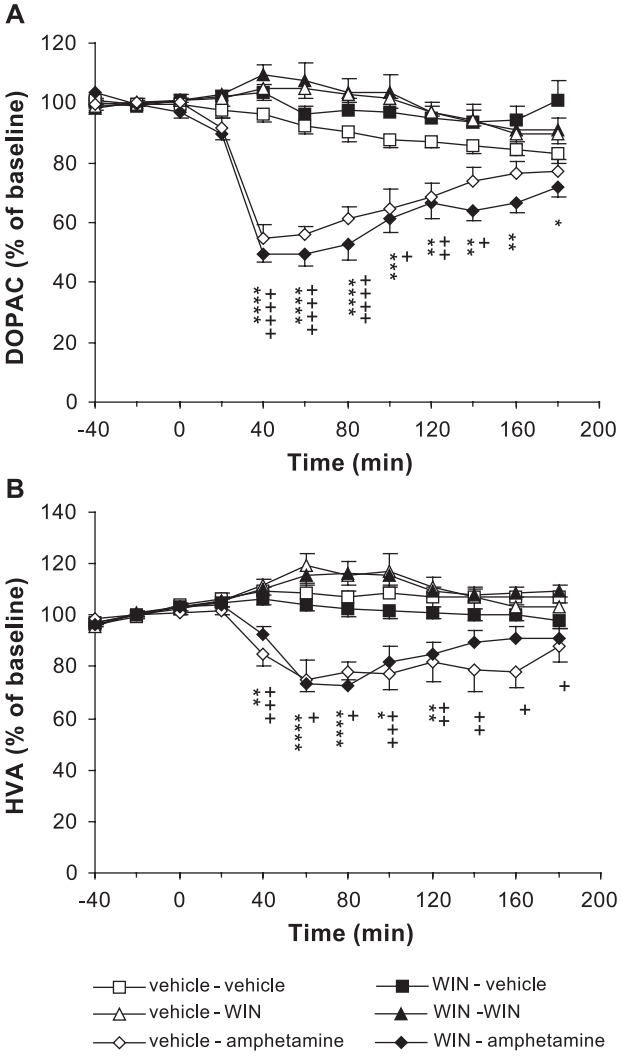

Fig. 2. Effects of WIN 55,212-2 (1.25 mg/kg), amphetamine (0.5 mg/kg) or vehicle on extracellular (A) DOPAC and (B) HVA levels in the nucleus accumbens in adolescent rats, 7 days after subchronic (5 days) WIN 55,212-2 (1.25 mg/kg) or vehicle pretreatment. The challenge injection was given at time 0. Data are expressed as mean (±S.E.M.) percent change from baseline levels. Symbols indicate significant challenge effect; *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ WIN 55,212-2–amphetamine vs. WIN 55,212-2–saline; $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$, $^{++++}P<0.0001$ vehicle–amphetamine vs. vehicle–vehicle. n=6–9.

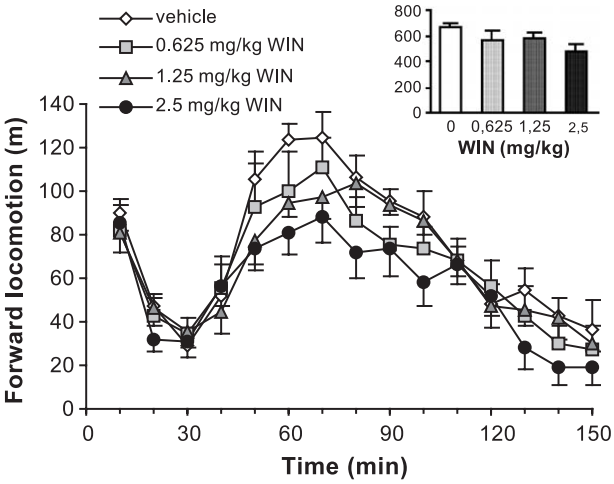

Fig. 3. Amphetamine (0.5 mg/kg)-induced locomotor activity in adolescent rats, 7 days after subchronic (5 days) pretreatment with 0, 0.625, 1.25 or 2.5 mg/kg WIN 55,212-2. The amphetamine injection was given after 30 min. Data are presented as mean forward locomotion (±S.E.M.). The insert shows total forward locomotion (±S.E.M.) during the period 20–80 min after amphetamine administration. n=5–6.

3.2. Experiment 2: effects of subchronic WIN 55,212-2 pretreatment on amphetamine-induced locomotor activity and stereotypy in adolescent rats

As shown in Fig. 3, rats pretreated with WIN 55,212-2 showed a small tendency towards reduced locomotor activity response to amphetamine in a dose dependent matter, but there was no significant pretreatment effect [$F(3,19)=0.33$]. Total forward locomotion after the amphetamine challenge was 27% lower in rats pretreated with the highest dose of WIN 55,212-2 (2.5 mg/kg) than in vehicle pretreated rats, but the attenuation was not significant. WIN 55,212-2 pretreatment did not affect the stereotypy response to amphetamine (Table 1). Because the amphetamine dose used was quite low (0.5 mg/kg), only mild stereotypic behavior was elicited, and hence, no rat reached a stereotypy score of 7 or higher.

Table 1
Effect of early subchronic WIN 55,212-2 pretreatment on amphetamine-induced stereotypy behavior[a]

WIN preexposure dose (mg/kg)	Total stereotypy score
0	39
0.625	35.5
1.25	34.5
2.5	34

[a] Rated during 20–80 min following amphetamine (0.5 mg/kg) administration and presented as the group median total stereotypy score. n=5–6.

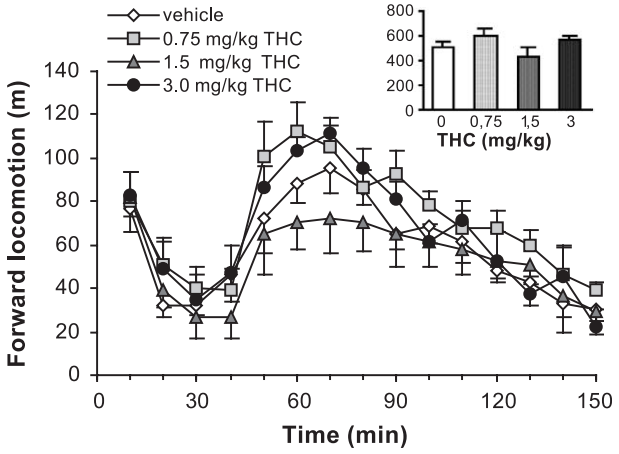

Fig. 4. Amphetamine (0.5 mg/kg)-induced locomotor activity in adolescent rats, 7 days after subchronic (5 days) pretreatment with 0, 0.75, 1.5 or 3.0 mg/kg Δ-9-tetrahydrocannabinol. The amphetamine injection was given after 30 min. Data are presented as mean forward locomotion (±S.E.M.). The insert shows total forward locomotion (±S.E.M.) for the period 20–80 min after amphetamine administration. n=6.

3.3. Experiment 3: effects of subchronic Δ-9-tetrahydrocannabinol pretreatment on amphetamine-induced locomotor activity and stereotypy in adolescent rats

The results from the locomotor activity tests are shown in Fig. 4. There was no significant pretreatment effect on the amphetamine-induced locomotor activity response [F(3,20)=0.38]. Δ-9-tetrahydrocannabinol pretreatment did not affect the stereotypy response to the amphetamine challenge (Table 2). Due to the rather low dose of amphetamine, no rat reached a stereotypy score of 7 or higher.

3.4. Experiment 4: effects of subchronic Δ-9-tetrahydrocannabinol pretreatment on amphetamine-induced locomotor activity in rats challenged with amphetamine as adolescents or adults

Overall, 2.0 mg/kg amphetamine induced a greater locomotor activity and rearing response than the 0.5-mg/kg dose (Figs. 5 and 6). An overall statistical analysis revealed significant challenge [F(1,28)=59.4, P<0.0001], time [F(3,97)=70.6, P<0.0001] and challenge×time [F(3,97)=31.2, P<0.0001] effects of locomotor activity in adolescent rats. In adult rats, there were significant effects for challenge [F(1,26)=43.1, P<0.0001], time [F(4,95)=47.1, P<0.0001], challenge×time [F(4,95)=23.2, P<0.0001]. Post hoc analysis (Tukey's HSD) showed a significant challenge effect on locomotor activity 40–70 min after the injection in the adolescent rats (P<0.05). There was a significant challenge effect in both adolescent [F(1,30)=70.0, P<0.0001] and adult rats [F(1,28)=44.2, P<0.0001] on the total forward locomotion following the amphetamine challenge. Pretreatment with Δ-9-tetrahydrocannabinol had no modulatory effect on the amphetamine-induced locomotor response. The amphetamine challenge also induced rearing behavior. Statistical analysis revealed significant time [F(4,44)=16.6, P<0.0001], time×challenge [F(4,44)=5.2, P<0.0001], pretreatment×challenge [F(1,12)=6.7, P<0.05] and time×pretreatment×challenge [F(4,44)=1.9, P<0.05] effects in adolescent rats. Post hoc analysis (Tukey's HSD) showed a significant challenge effect in the Δ-9-tetrahydrocannabinol-pretreated adolescent rats (P<0.05). In adult rats, significant effects were found for time [F(5,124)=15.9, P<0.0001], challenge [F(1,26)=18.5, P<0.001] and time×challenge

Table 2
Effect of early subchronic Δ-9-tetrahydrocannabinol pretreatment on amphetamine-induced stereotypy behavior[a]

WIN preexposure dose (mg/kg)	Total stereotypy score
0	39
0.625	35.5
1.25	34.5
2.5	34

[a] Rated during 20–80 min following amphetamine (0.5 mg/kg) administration and presented as the group median total stereotypy score. n=6.

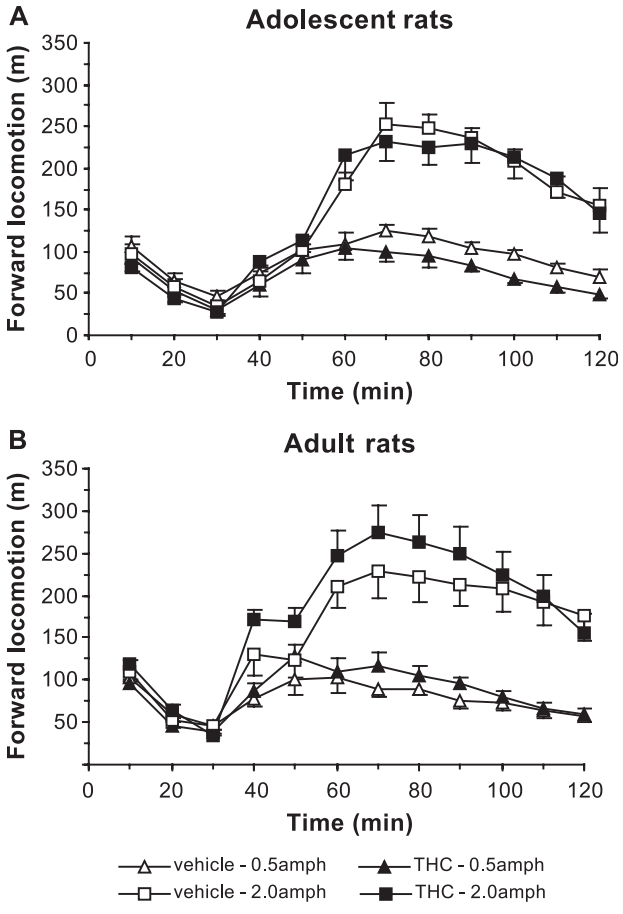

Fig. 5. Locomotor activity response to amphetamine (0.5 or 2.0 mg/kg) in (A) adolescent (40-days old) rats and (B) adult (68-days old) rats pretreated with 1.5 mg/kg Δ-9-tetrahydrocannabinol or vehicle for 5 days during early adolescence. The amphetamine injection was given after 30 min. Data are presented as mean forward locomotion (±S.E.M.). n=7–8.

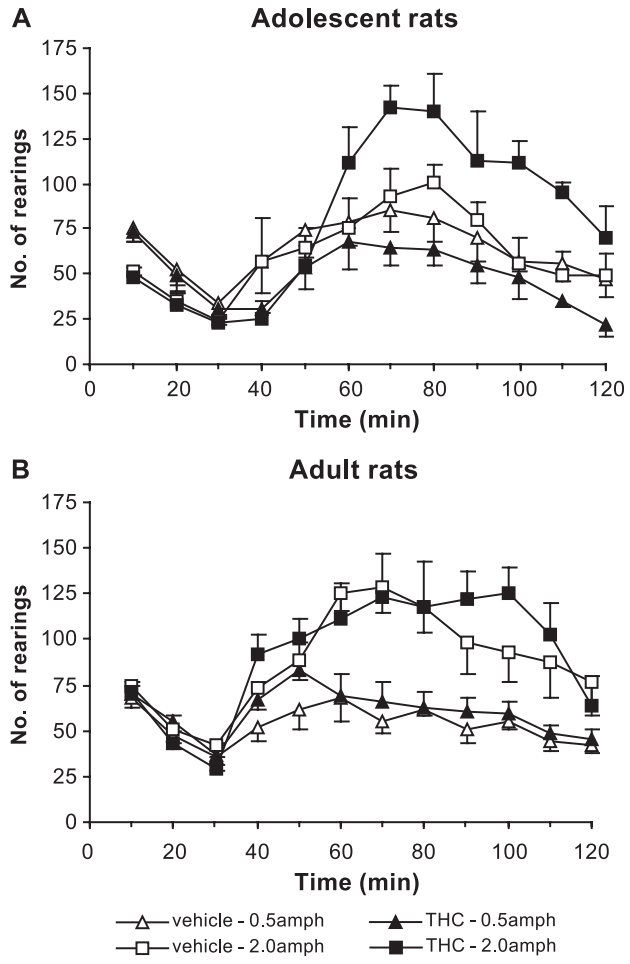

Fig. 6. Rearing behavior response to amphetamine (0.5 or 2.0 mg/kg) in (A) adolescent (40-days old) rats and (B) adult (68-days old) rats pretreated with 1.5 mg/kg Δ-9-tetrahydrocannabinol or vehicle for 5 days during early adolescence. The amphetamine injection was given after 30 min. Data are presented as mean number of rearings (±S.E.M.). n=4–8.

[$F(5,124)=7.1$, $P<0.0001$]. Significant challenge effects on total number of rearings were found in both adolescent [$F(1,14)=9.1$, $P<0.05$] and adult [$F(1,28)=21.7$, $P<0.0001$] rats. Post hoc analysis (Tukey's HSD) revealed a significant difference between the response to 0.5 mg/kg amphetamine and 2.0 mg/kg amphetamine in the Δ-9-tetrahydrocannabinol-pretreated adolescent rats ($P<0.01$). Δ-9-tetrahydrocannabinol pretreatment did not modulate amphetamine-induced rearing behavior.

4. Discussion

This is the first study to experimentally evaluate the cannabis gateway hypothesis of enhanced sensitivity to future drug use, as a consequence of cannabis exposure during adolescence. The present findings do not support the gateway hypothesis because there was no sensitization to the effects of amphetamine on dopamine levels or behavior in rats pretreated with cannabinoids during adolescence. Amphetamine administration lead to a marked elevation of dopamine levels in the nucleus accumbens, with a peak response at 40 min, concomitant with reduced levels of the metabolites DOPAC and HVA. This pattern resembled the amphetamine-induced response normally observed in adult rats (Di Chiara and Imperato, 1988; Hurd and Ungerstedt, 1989; Kuczenski and Segal, 1989; Zetterstrom et al., 1983). Our results also show that subchronic WIN 55,212-2 pretreatment during early adolescence did not alter the dopaminergic response to the subsequent amphetamine challenge.

Adolescent rats have been documented to express sensitization (Fujiwara et al., 1987; Tsuchida et al., 1994; Ujike et al., 1995), but cross-sensitization between cannabis and other drugs during adolescence has not been previously studied. Because most humans start smoking marijuana in their teens, it is critical to study animals at an age corresponding to human adolescence when evaluating the cannabis gateway hypothesis. The age of the rats used in the current investigation corresponded to early adolescence in humans at the time of the cannabis pretreatment (day 28–32) and late adolescence at the time of the drug challenge (day 40; Spear, 2000). Our results show that neither subchronic WIN 55,212-2 nor Δ-9-tetrahydrocannabinol pretreatment during early adolescence induced cross-sensitization to the behavioral effects of amphetamine. Multiple doses of WIN 55,212-2 (0.625–2.5 mg/kg) as well as Δ-9-tetrahydrocannabinol (0.75–3.0 mg/kg) and amphetamine (0.5–2.0 mg/kg) in the behavioral experiments were used, but there was no sensitization with any of the doses tested. Earlier investigations of behavioral cross-sensitization between chronic cannabinoid pretreatment and psychostimulants in adult rats have shown diverse results. Amphetamine-induced locomotor activity was found to be increased following chronic pretreatment, with a rather high dose of Δ-9-tetrahydrocannabinol (6.4 mg/kg; Gorriti et al., 1999) but not WIN 55,212-2 (1 mg/kg; Muschamp and Siviy, 2002), although stereotypic behavior was enhanced in both studies. The locomotor activity response to amphetamine was also found to be increased after chronic Δ-9-tetrahydrocannabinol pretreatment in rats, defined as "high responders" to a novel environment (Lamarque et al., 2001). However, because they examined cross-sensitization to both amphetamine and heroin in the same experiment, the rats had also received a single heroin injection before the amphetamine administration. Furthermore, preexposure to either of the synthetic cannabinoid CB1 receptor agonists CP 55,940 or HU-210 [$R(-)$-7-hydroxy-delta-6-tetra-hydrocannabinol-dimethylheptyl] did not induce cross-sensitization to the locomotor activity effects of cocaine (Arnold et al., 1998; Ferrari et al., 1999). We also assessed whether rats challenged with amphetamine, as young adults (day 68), would respond differently than adolescent rats. The results were similar, showing that the amphetamine

response in adults was not sensitized after Δ-9-tetrahydrocannabinol pretreatment during early adolescence. Thus, it appears that cannabis exposure during adolescence does not induce behavioral cross-sensitization to amphetamine.

In the present study, acute or subchronic administration of the cannabinoid agonist WIN 55,212-2 to adolescent rats had no or little effect by itself on dopamine levels in the nucleus accumbens, in contrast to the significant increase in dopamine induced by acute administration shown earlier in adult rats (Tanda et al., 1997). It is a possibility that the dopamine-elevating properties of WIN 55,212-2 depend on the route of administration. In the study by Tanda et al. (1997) where WIN 55,212-2 was shown to elevate extracellular dopamine in nucleus accumbens, the drug was given intravenously; while in this study, WIN 55,212-2 was administered by an intraperitoneal route. The intraperitoneal administration of WIN 55,212-2 would be expected to have a reduced capacity to increase dopamine release because of slower kinetic access to the brain.

Taken altogether, preadministration of cannabinoids to rats during early adolescence had no modulatory effect on the dopaminergic or behavioral responsiveness to amphetamine. These findings are not compatible with the cannabis gateway hypothesis, at least for subsequent amphetamine sensitization. Future studies are needed to further evaluate the gateway hypothesis on an experimental level such as in regard to the duration of THC pretreatment or route of administration (e.g., intravenous). Future experimental investigations of the cannabis gateway hypothesis could also evaluate the interactions with other drugs of abuse such as opioids which have a tighter neurobiological interaction with the cannabinoid system.

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