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Increased sensitivity of adolescent spontaneously hypertensive rats, an animal model of attention deficit hyperactivity disorder, to the locomotor stimulation induced by the cannabinoid receptor agonist WIN 55,212-2

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Abstract

Converging evidence points to adolescence as a critical period for the onset of a wide range of neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD) and drug abuse. Spontaneously hypertensive rats (SHR) are generally considered to be a suitable genetic model for the study of ADHD, since they display hyperactivity, impulsivity, poorly sustained attention, cognitive deficits and increased novelty seeking. Despite the high prevalence of ADHD among adolescents, studies using SHR have mainly been performed on adult animals. The aim of the present study was to evaluate the effect of acute intraperitoneal (i.p.) administration of the cannabinoid receptor agonist WIN 55,212-2 (0.25–2.5 mg/kg) on locomotor activity and anxiety-like behavior in male adolescent and adult SHR and Wistar rats using the open field and elevated plus-maze tests. WIN 55,212-2 at doses of 0.25 and 1.25 mg/kg (i.p.) selectively promoted locomotor stimulation in adolescent SHR in the open field, but not in adult SHR or Wistar rats (regardless of age). The effect of WIN 55,212-2 (0.25 mg/kg, i.p.) on locomotion of adolescent SHR was reversed by pretreatment with the selective cannabinoid CB_1 receptor antagonist AM 251 (0.25 mg/kg, i.p.). Moreover, although the present doses of WIN 55,212-2 had no effect on anxiety-related behaviors in any of the animal groups evaluated in the open field (central locomotion) or elevated plus-maze (time and entries in open arms), the highest dose of WIN 55,212-2 tested (2.5 mg/kg, i.p.) significantly decreased the number of closed-arm entries (an index of locomotor activity) of adolescent rats of both the Wistar and SHR strains in the elevated plus-maze. The present results indicate strain- and age-related effects of cannabinoids on locomotor activity in rats, extending the notion that adolescence and ADHD represent risk factors for the increased sensitivity to the effects of drugs.

Keywords: Cannabinoid; Adolescence; Attention deficit hyperactivity disorder (ADHD); Locomotor activity; Anxiety; Spontaneously hypertensive rat (SHR)

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood psychiatric disorders affecting between 1.3 and 5% of grade-school children (Taylor, 1998). The disorder usually manifests before the child is 7 years old and often persists into adulthood. ADHD is characterized by the presence of three primary symptoms: hyperactivity, inattention and impulsivity (Taylor, 1998; Himelstein et al., 2000). The behavioral profile of ADHD children has been suggested to be secondary to a dysfunction of reinforcement mechanisms (Sagvolden et al., 1992) which, associated with poor judgment and/or impulsive behavior in social settings, seems to predispose the ADHD adolescent to the earlier onset of drug consumption and abuse (for review see Sullivan and Rudnik-Levin, 2001; Schubiner, 2005).

Spontaneously hypertensive rats (SHR) have often been used as an animal model of ADHD, since they display hyperactivity, impulsivity, impaired ability to withhold responses, poorly sustained attention and reduced performance in different learning and memory paradigms (Sagvolden and Sergeant,

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1998; Sagvolden, 2000; Prediger et al., 2005a,b). Additionally, SHR also show increased behavioral responses to psychostimulant drugs (Amini et al., 2004), as well as increased consumption of alcohol in a free-choice self-administration paradigm (Da Silva et al., 2005). However, despite the high prevalence of ADHD among adolescents, studies using SHR have mainly been performed on adult animals. Adriani and Laviola (2004) have recently reviewed the advantages of testing the SHR strain during adolescence rather than in adulthood, such as a temporary rearrangement in functional dopaminergic parameters (Teicher et al., 1995) and reduced influence of elevated blood pressure on behavioral responsiveness.

The unique characteristics of adolescent rodents make them a good model for the study of early-onset neuropsychiatric disorders including ADHD and drug abuse. Periadolescence is classically defined in rodents as the ontogenetic period including the week preceding the onset of puberty and few days thereafter (Spear and Brake, 1983). During this period, brain areas show proliferation and maturation of axon terminals and synapses (Stamford, 1989; Teicher et al., 1995). Adolescent rodents present elevated levels of novelty seeking (Adriani et al., 1998), impulsivity (Adriani and Laviola, 2003), and risk-taking behavior (Macrı et al., 2002), as well as increased oral intake of alcohol (Ferris et al., 1998) and nicotine (Adriani et al., 2002).

Cannabis is one of the most widely used illicit drugs in the world and its use frequently starts during adolescence (Crowley et al., 1998). The target of psychoactive cannabinoids is the endogenous brain cannabinoid system which seems to be present since the early stages of life, playing a relevant role in brain organization (Fernandez-Ruiz et al., 2000). It is noteworthy that cannabinoid exposure in adult rodents is associated with biphasic effects on locomotion (Navarro et al., 1993; Rodriguez de Fonseca et al., 1998; Sañudo-Peña et al., 2000; Drews et al., 2005) and anxiety-related behaviors (Onaivi et al., 1990; Berrendero and Maldonado, 2002; Haller et al., 2004; Rodgers et al., 2005; Viveros et al., 2005). However, although used predominantly by adolescents and young adults, there have been very few studies that directly compare the effects of cannabinoids between youngsters or adolescents and adults in either humans or in animal models. Therefore, the present study focused on the acute effects of intraperitoneal (i.p.) administration of the cannabinoid receptor agonist WIN 55,212-2 (0.25–2.5 mg/kg) on the performance of male adolescent and adult SHR and Wistar rats in the open field and elevated plus-maze, two tests that have been validated for the evaluation of locomotor activity and anxiety-like responses in rats (Hall, 1936; Pellow et al., 1985).

2. Materials and methods

2.1. Animals

Adolescent and adult male Wistar and SHR rats ($n=8$–9 for each strain) from our own colony were used in the experimental procedures. Adolescence was defined as the period between postnatal days 28 and 35 (Spear and Brake, 1983) and adulthood (period between postnatal days 90 and 100). The animals were housed in groups of five animals per cage and were maintained in a room under controlled temperature (23 ± 1 °C) on a 12-h light/dark cycle (lights on at 7:00 am), with free access to food and water. All experiments were carried out between 9:00 and 12:00 am. All procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals, which follows the principles of laboratory animal care of the NIH.

2.2. Treatments

The cannabinoid receptor agonist WIN 55,212-2 [*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinyl}methyl] pyrrol [1,2,3-de-]-1,4-benzoxazin-6-yl)(1-naphthalenyl) methanone mesylate] (Tocris, USA) and the selective cannabinoid CB_1 receptor antagonist AM 251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] (Tocris, USA) were dissolved in 0.9% NaCl (saline) with 10% dimethylsulfoxide plus 0.1% Tween 80. The control solution consisted of drug vehicle. All drug doses, selected according to the literature (Rodriguez de Fonseca et al., 1998; Haller et al., 2004; Pamplona and Takahashi, 2006), were administered i.p. in a volume of 0.2 ml/100 g body weight. WIN 55,212-2 was administered 30 min before the behavioral tests, and AM 251 was administered 20 min prior to WIN 55,212-2 injection.

2.3. Open field

The open field test consists of a novel large arena containing an aversive central area and represents a widely used model for the evaluation of both locomotor activity and anxiety-like behavior. The apparatus was made of wood covered with impermeable Formica, had a white floor measuring 100 × 100 cm (divided by black lines into 25 squares of 20 × 20 cm) and was surrounded by 40-cm high walls. The experiments were conducted in a sound-attenuated room under low-intensity light (12 lx). Each rat was placed in the center of the open field and the following variables were recorded for 5 min: number of peripheral squares (adjacent to the walls) crossed (peripheral locomotion), number of central squares (away from the walls) crossed (central locomotion), time in the central area (central time), and percentage of central locomotion in relation to the total locomotion (peripheral locomotion plus central locomotion). The experiments were recorded with a video camera positioned above the arena and monitored in an adjacent room by an experimenter who was unaware of the drug treatment of the animals during behavioral evaluation.

2.4. Elevated plus-maze

The elevated plus-maze test, which is based on the conflict displayed by rodents between the drive to explore a new environment and the fear of open elevated areas, was used on the basis of its documented ability to detect both anxiolytic- and anxiogenic-like drug effects in rats (Pellow et al., 1985). Briefly, the apparatus was made of wood covered with impermeable Formica and was placed 52 cm above the floor. The four arms

were 50 cm long and 10 cm wide. Two opposite arms were surrounded by walls (10 cm high, closed arms); while the other two were devoid of enclosing walls (open arms). The four arms were connected by a central platform (10 × 13.5 cm). Each subject was placed in the central area of the maze facing an open arm. The animals were observed for a 5-min test period and anxiolytic-like effects were defined as an increase in the proportion of open-arm entries divided by the total number of arm entries, and the time spent in the open arms relative to the total time spent in both arms. Whenever a rat placed all four paws into an arm, one entry was recorded. The total number of closed-arm entries was used as a measure of locomotor activity (Cruz et al., 1994). The experiments conducted in a sound-attenuated room under low-intensity light (12 lx) were recorded with a video camera positioned above the maze and monitored in an adjacent room by an experimenter who was unaware of the drug treatment of the animals during behavioral evaluation.

2.5. Statistical analysis

All values are expressed as means ± S.E.M. Statistical comparison of the results was carried out using three-way ANOVA; with strain, age and treatment as independent variables. The results of the open field parameters for the antagonism with AM 251 were analyzed by one-way ANOVA, with treatment as independent variable. Following significant ANOVAs, multiple post hoc comparisons were performed using Duncan's test. The accepted level of significance for the tests was $P \leq 0.05$. All tests were performed using the Statistica® software package (StatSoft Inc., Tulsa, Oklahoma, USA).

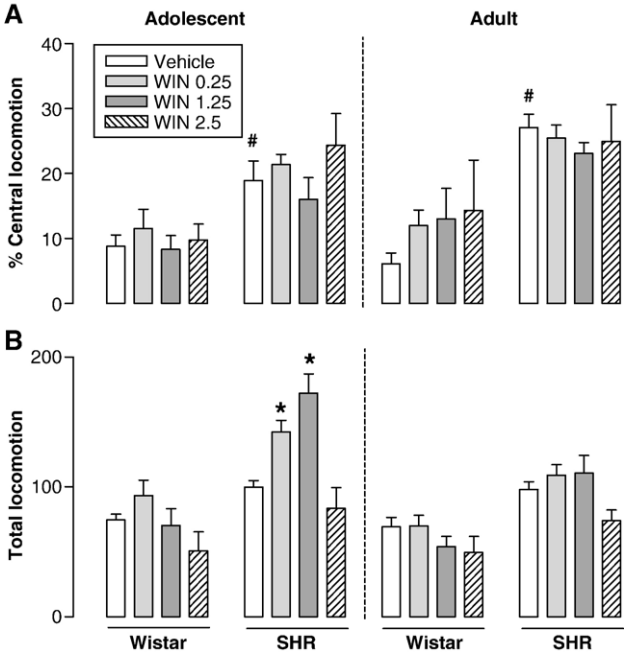

Fig. 1. Effects of acute administration of the cannabinoid receptor agonist WIN 55,212-2 (WIN, 0.25, 1.25 or 2.5 mg/kg, i.p.) on the percentage of central locomotion (A) and total locomotion (B) of adolescent and adult spontaneously hypertensive rats (SHR) and Wistar rats in the open field (for 5 min). Bars represent the means and S.E.M. of animals grouped according to strain, age and treatment ($n = 8–9$). $^{\#}P \leq 0.05$ compared to the vehicle-treated Wistar rats of the same age. $^{*}P \leq 0.05$ compared to the respective vehicle-treated group of the same strain and age (Duncan's test).

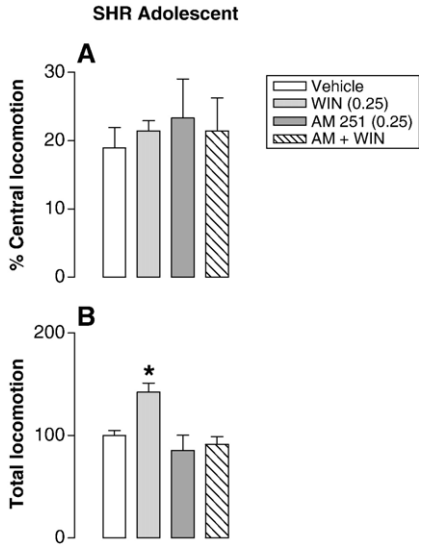

Fig. 2. Effects of acute administration of the cannabinoid receptor agonist WIN 55,212-2 (WIN, 0.25, 1.25 or 2.5 mg/kg, i.p.) and/or the selective cannabinoid CB_1 receptor antagonist AM 251 (0.25 mg/kg, i.p.) on the percentage of central locomotion (A) and total locomotion (B) of adolescent spontaneously hypertensive rats (SHR) in the open field (for 5 min). Bars represent the means and S.E.M. of animals grouped according to treatment ($n = 8–9$). $^{*}P \leq 0.05$ compared to the vehicle-treated group (Duncan's test).

3. Results

3.1. Open field test

The results regarding the effects of acute i.p. injection of WIN 55,212-2 (0.25, 1.25 or 2.5 mg/kg) into adolescent and adult SHR and Wistar rats on the percentage of central locomotion (an index of anxiety) in the open field test are shown in Fig. 1A. Three-way ANOVA (strain vs. age vs. treatment) revealed a significant effect for the strain factor [$F(1,114) = 47.92$, $P \leq 0.0001$]. However, it indicated a nonsignificant effect for the age factor [$F(1,114) = 3.59$, $P = 0.06$], for the treatment factor [$F(3,114) = 0.87$, $P = 0.46$], and for the interaction factor between strain vs. age [$F(1,114) = 0.85$, $P = 0.36$], strain vs. treatment [$F(3,114) = 0.62$, $P = 0.61$], age vs. treatment [$F(3,114) = 0.23$, $P = 0.87$] and strain vs. age vs. treatment [$F(3,114) = 0.75$, $P = 0.52$] on the percentage of central locomotion in the open field. Subsequent Duncan's test indicated that vehicle-treated SHR crossed significantly more squares in the aversive central area of the open field than vehicle-treated Wistar rats (regardless of age) (Fig. 1A).

Fig. 1B summarizes the effects of acute administration of WIN 55,212-2 (0.25, 1.25 or 2.5 mg/kg, i.p.) into adolescent and adult SHR and Wistar rats on the total locomotion (an index of locomotor activity) in the open field test. Three-way ANOVA (strain vs. age vs. treatment) revealed a significant effect for the three main factors: strain [$F(1,114) = 70.08$, $P \leq 0.0001$], age [$F(1,114) = 12.68$, $P \leq 0.001$], and treatment [$F(3,114) = 11,54$, $P \leq 0.0001$], and for the interaction factor

between strain vs. treatment [$F(3,114)=5.22$, $P\leq 0.01$)], and age vs. treatment [$F(3,114)=2.66$, $P\leq 0.05$]. However, it indicated a nonsignificant effect for the interaction factor between strain vs. age [$F(1,114)=1.97$, $P=0.16$], nor strain vs. age vs. treatment [$F(3,114)=1.00$, $P=0.59$] on the total locomotion in the open field. Post hoc comparison indicated that, although vehicle-treated SHR and Wistar rats did not differ in terms of total locomotion (regardless of age), this parameter was selectively increased in adolescent SHR after acute injection of WIN 55,212-2 at doses of 0.25 and 1.25 mg/kg (Fig. 1B).

The effects of acute administration of WIN 55,212-2 (0.25, 1.25 or 2.5 mg/kg, i.p.) and/or AM 251 (0.25 mg/kg, i.p.) into adolescent SHR on the percentage of central locomotion and total locomotion in the open field test are summarized in Fig. 2A and B, respectively. One-way ANOVA revealed a nonsignificant effect for the treatment factor [$F(3,27)=2.13$, $P=0.12$] on the percentage of central locomotion in the open field. However, it indicated a significant effect for the treatment factor [$F(3,27)=8.89$, $P\leq 0.001$] on the total locomotion. Post hoc comparisons indicated that a *per se* ineffective dose of the selective cannabinoid CB_1 receptor antagonist AM 251 (0.25 mg/kg, i.p.) antagonized the effect of WIN 55,212-2 (0.25 mg/kg, i.p.) on the total locomotion of adolescent SHR in the open field, suggesting that this effect was related to the activation of cannabinoid CB_1 receptor.

3.2. Elevated plus-maze test

Table 1 summarizes the effects of acute i.p. administration of WIN 55,212-2 (0.25, 1.25 or 2.5 mg/kg) on the behavioral parameters of adolescent and adult SHR and Wistar rats in the elevated plus-maze test. Three-way ANOVA (strain vs. age vs. treatment) revealed a significant effect for the strain factor [$F(1,114)=71.23$, $P\leq 0.0001$] and for the age factor [$F(1,114)=4.27$, $P\leq 0.05$] on the percentage of time spent in the open arms. However, it indicated a nonsignificant effect for the treatment factor [$F(3,114)=0.50$, $P=0.60$], and for the interaction factor between strain vs. age [$F(1,114)=0.09$, $P=0.76$], strain vs. treatment [$F(3,114)=0.57$, $P=0.63$], age vs. treatment [$F(3,114)=0.74$, $P=0.53$] and strain vs. age vs. treatment [$F(3,114)=1.66$, $P=0.18$] on the percentage of time spent in the open arms. Post hoc comparisons indicated that SHR displayed a higher percentage of time spent in the open arms than control Wistar rats of the same age (Table 1).

Three-way ANOVA (strain vs. age vs. treatment) revealed a significant effect for the strain factor [$F(1,114)=17.57$, $P\leq 0.0001$] on the percentage of entries in the open arms. However, it indicated a nonsignificant effect for the age factor [$F(1,114)=3.91$, $P=0.05$], for the treatment factor [$F(3,114)=1.14$, $P=0.34$], and for the interaction factor between strain vs. age [$F(1,114)=0.19$, $P=0.66$], strain vs. treatment [$F(3,114)=0.28$, $P=0.84$], age vs. treatment [$F(3,114)=0.50$, $P=0.69$] and strain vs. age vs. treatment [$F(3,114)=0.51$, $P=0.68$] on the percentage of entries in the open arms.

Moreover, three-way ANOVA (strain vs. age vs. treatment) revealed a significant effect for the age factor [$F(1,114)=13.29$, $P\leq 0.001$], for the treatment factor [$F(3,114)=6.26$, $P\leq 0.001$], and for the interaction factor between age vs. treatment [$F(3,114)=6.92$, $P\leq 0.001$] on the number of closed-arm entries (an index of locomotor activity). However, it indicated a nonsignificant effect for the strain factor [$F(1,114)=0.02$, $P=0.88$], and for the interaction factor between strain vs. age [$F(1,114)=0.33$, $P=0.57$], strain vs. treatment [$F(3,114)=0.47$, $P=0.70$], and strain vs. age vs. treatment [$F(3,114)=0.25$, $P=0.86$] on the number of closed-arm entries. Subsequent application of Duncan's test indicated that the highest dose of WIN 55,212-2 tested (2.5 mg/kg, i.p.) significantly decreased the number of closed-arm entries of adolescent rats of both Wistar and SHR strains in the elevated plus-maze, suggesting a

Table 1
Effects of acute administration of the cannabinoid receptor agonist WIN 55,212-2 (WIN, 0.25, 1.25 or 2.5 mg/kg, i.p.) on behavioral parameters of adolescent and adult spontaneously hypertensive rats (SHR) and Wistar rats evaluated in the elevated plus-maze (for 5 min)

Age	Strain	Treatment (mg/kg)	% Open-arm time	% Open-arm entries	Closed-arm entries	*N*
Adolescent	Wistar	Vehicle	31.94±5.80	41.78±3.85	8.13±0.72	8
		WIN 0.25	37.39±4.78	41.52±4.28	9.75±1.13	8
		WIN 1.25	33.94±6.84	41.91±2.17	7.00±0.46	8
		WIN 2.5	28.12±9.48	47.30±8.43	3.75±1.10[b]	8
	SHR	Vehicle	55.65±2.32[a]	52.77±1.78	8.25±0.59	8
		WIN 0.25	57.75±2.11	50.93±3.00	11.25±2.09	8
		WIN 1.25	71.76±5.45	60.12±4.46	7.37±1.59	8
		WIN 2.5	79.16±10.62	68.11±9.57	3.38±1.52[b]	8
Adult	Wistar	Vehicle	28.65±6.96	33.84±5.84	5.11±1.16	9
		WIN 0.25	22.60±8.17	38.19±7.99	5.13±1.09	8
		WIN 1.25	26.85±8.19	37.86±5.69	5.38±0.65	8
		WIN 2.5	26.49±6.90	42.90±4.99	6.13±0.81	8
	SHR	Vehicle	57.06±5.34[a]	47.78±5.81	5.88±0.61	8
		WIN 0.25	59.01±9.72	49.66±6.26	4.88±0.77	8
		WIN 1.25	64.86±5.43	56.27±4.74	5.67±0.65	9
		WIN 2.5	47.36±14.28	47.22±13.43	4.38±1.57	8

[a] $P\leq 0.05$ compared to the vehicle-treated Wistar rats of the same age.
[b] $P\leq 0.05$ compared to the respective vehicle-treated group of the same strain and age (Duncan's test).

reduced locomotor activity following WIN 55,212-2 at this dose in adolescent animals (Table 1).

4. Discussion

In the present study, we compared the effects of the cannabinoid receptor agonist WIN 55,212-2 on the behavior of male adolescent and adult SHR and Wistar rats evaluated in the open field and elevated plus-maze tests. Our results provide evidence that systemic injection of WIN 55,212-2 elicited distinct behavioral responses depending on the age and strain of the animals. Adolescent SHR, a valid animal model of ADHD, displayed higher sensitivity to the hyperlocomotion induced by low doses of WIN 55,212-2 (0.25 and 1.25 mg/kg) in the open field test, suggesting that the occurrence of these two factors (adolescence and ADHD-like behavior) might be associated with an increased sensitivity to behavioral effects of cannabinoids. A single injection of AM 251 (0.25 mg/kg, i.p.) antagonized the motor stimulation induced by WIN 55,212-2 (0.25 mg/kg, i.p.) in adolescent SHR, suggesting that this effect was mediated by cannabinoid CB_1 receptor.

Adolescence is often associated with elevated levels of sensation-seeking, reckless and risk-taking behaviors, as well as with changes in harm-avoidance and anxiety (Arnett, 1992). The development of brain circuits underlying motivation and decision-making renders this age period critically vulnerable to the development of neuropsychiatric disorders such as ADHD and drug abuse (Chambers et al., 2003). Moreover, children diagnosed with ADHD have been found to be more at risk for using illicit drugs later in life than children without ADHD (for review see Sullivan and Rudnik-Levin, 2001; Schubiner, 2005).

Animal models can provide an important perspective on the issue. The unique characteristics of adolescent rodents make them a good model for the study of early-onset neuropsychiatric disorders including ADHD and drug abuse. Whereas adolescence in humans and non-human primates extends over several years, rodent models of this developmental stage, termed 'periadolescence', are limited to the 7–10 days preceding the onset of puberty (at about 40 days of age) and the first few days thereafter (Spear and Brake, 1983). Periadolescence in rodents has been largely recognized as an ontogenic period of increased self-administration and responsiveness to behavioral effects of different drugs such as nicotine, ethanol, cocaine and delta^9-tetrahydrocannabinol (THC) (Adriani and Laviola, 2004; Barron et al., 2005; Caster et al., 2005; Cha et al., 2006). Indeed, despite the high prevalence of ADHD among adolescents, studies using the SHR model of ADHD have mainly been performed on adult animals, with increased sensitivity to psychostimulant effects (Amini et al., 2004) and alcohol intake (Da Silva et al., 2005) being documented for this rat strain.

As mentioned earlier in the introduction, cannabis is one of the most widely used illicit drugs in the world and its use frequently starts during adolescence (Crowley et al., 1998). However, there are controversial data in the literature concerning the ability of cannabinoid receptor agonists to reinforce behavioral responses in experimental animals, i.e., to reduce self-stimulation thresholds and to induce self-administration, or conditioned place preference (for review see Gardner, 2002, 2005). A variety of animal models, such as place preference, drug self-administration and reinstatement procedures, are used to investigate the reinforcement properties of commonly abused drugs (see Gardner, 2002, 2005). Indeed, the activity of rodents in novel environments, presumably related to the novelty-seeking trait in humans, is one of the most studied factors that may predict a predisposition to drug self-administration (Klebaur et al., 2001; Nadal et al., 2002).

In the present study, we provided evidence that the acute administration of the cannabinoid receptor agonist WIN 55,212-2 (0.25, 1.25 or 2.5 mg/kg, i.p.) promoted strain- and age-related effects on locomotor activity of rats exposed to novel environments. Low doses of WIN 55,212-2 (0.25–1.25 mg/kg) selectively stimulated the locomotor activity of adolescent SHR as indicated by a significant increase in the number of squares crossed (total locomotion) in the open field test. Moreover, the highest dose of WIN 55,212-2 tested (2.5 mg/kg, i.p.) significantly decreased the number of closed-arm entries, an index of locomotor activity (Pellow et al., 1985), of adolescent rats of both Wistar and SHR strains in the elevated plus-maze. These findings are in line with several studies showing that acute administration of cannabinoid receptor agonists in rodents stimulates locomotion at low doses and inhibits motor activity at higher doses (reviewed in Rodriguez de Fonseca et al., 1998). The cannabinoid CB_1 receptor is densely expressed in brain structures such as the cerebellum and the basal ganglia, which are known to mediate initiation and coordination of movement (Breivogel and Childers, 1998). Especially the high cannabinoid CB_1 receptor density on the axon terminals of striatal GABAergic neurons of the basal ganglia and of the glutamatergic granule cells of the cerebellum is probably involved in motor control (Howlett, 1995). Thus, cannabinoid receptors can modulate both inhibitory and excitatory neuronal transmission in the basal ganglia and may thus provide dual regulation of movement (Sañudo-Peña et al., 1996; van der Stelt and Di Marzo, 2003).

The fact that the highest dose of WIN 55,212-2 (2.5 mg/kg, i.p.) did not reduce motor activity of adult rats seems to be in contrast to previous studies (Sañudo-Peña et al., 2000; Lichtman et al., 2001) showing cataleptic effects of THC at high doses. Since we only tested locomotion and did not investigate catalepsy with tests specifically designed to detect this condition, we cannot rule out cataleptogenic effects of WIN 55,212-2. Moreover, time- and dose-dependent biphasic effects of cannabinoid receptor agonists have been frequently observed. Increased motor activity has been associated with low doses or immediately after administration of high doses of cannabinoid receptor agonists. Later after administration, high doses of cannabinoid receptor agonists inhibit movement and induce catalepsy (Rodriguez de Fonseca et al., 1998; Sañudo-Peña et al., 2000). Therefore, since we only evaluated the early minutes of open-field locomotion, our data do not allow us to discard completely a decline in locomotor activity in later time points. The present findings are in accordance with recent studies (Drews et al., 2005; Pamplona and Takahashi, 2006) that also failed to demonstrate inhibitory effects of WIN

55,212-2 in a similar dose range on locomotor activity of adult Wistar rats in the open field test. Moreover, the incongruent responses of the cannabinoid receptor agonists WIN 55,212-2 and THC on motor activity may be attributed to their differential pharmacological profile, since WIN 55,212-2 is a full agonist while THC is probably a partial agonist at cannabinoid CB_1 receptor (Shen and Thayer, 1999).

The present findings confirm a developmental sensitivity to the effects of WIN 55,212-2 on locomotion, extending previous literature data indicating an increased response to cannabinoid receptor agonists during adolescence in rodents. For example, Cha et al. (2006) have recently demonstrated that systemic administration of THC induces more pronounced deficits in both spatial and non-spatial learning in the water maze test in adolescent rats compared to adults. O'Shea et al. (2004) observed an increased anxiety-like behavior and a lasting impairment of working memory in adolescent, but not adult Wistar rats treated with the cannabinoid receptor agonist (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol (CP 55,940) for 21 days. Moreover, the repeated treatment (25 days) with WIN 55,212-2 during pubertal development causes persistent deficits in memory, sensory-motor gating and performance in a progressive ratio task that continue into adulthood, while the same treatment in adulthood does not have persistent effects (Schneider and Koch, 2003). In accordance with these behavioral results, Rodriguez de Fonseca et al. (1993) demonstrated that the peak of cannabinoid receptor binding in the rat brain is reached during adolescence, between postnatal days 30 and 40, and then the number of cannabinoid receptors begins to slightly decline until reaching adult levels. Conversely, although our data showed increased locomotor responsiveness to cannabinoids in adolescent SHR, the only report of the expression of cannabinoid CB_1 receptor in this rat strain failed to show any difference in the striatum compared to adult rats (Adriani et al., 2003). However, the expression of cannabinoid CB_1 receptor in the brain of adolescent SHR remains to be determined.

Although at present it is not possible to determine the exact mechanisms and site of action for the stimulatory effect of WIN 55,212-2 on locomotor activity of adolescent SHR, cannabinoid stimulation of dopamine release represents one possibility. Both *in vivo* (Meschler and Howlett, 2001) and *in vitro* (Szabo et al., 1999) studies point to the regulation of dopaminergic signaling in the striatum by cannabinoid CB_1 receptor. Moreover, cannabinoid receptor antagonists enhance locomotor activation induced by dopamine agonists, whereas cannabinoid receptor agonists appear to counteract the psychomotor and hyperarousing effects induced by dopamine agonists (Ferrari et al., 1999; Giuffrida et al., 1999; Masserano et al., 1999; Hayase et al., 2001). Previous studies suggest that the activity of the nigrostriatal dopaminergic system is altered in the brain of SHR, including reduced release of dopamine in the striatum (Russell et al., 1995) and increased striatal dopamine turnover (McKeon and Hendley, 1988). Dopamine D_2-receptor binding was also found to be elevated in the striatum of SHR (Chiu et al., 1982), suggesting that the inhibition of dopamine release in the SHR striatum is due to an increased number of dopamine D_2-autoreceptors. Therefore, these alterations in the dopaminergic system may be responsible for the higher sensitivity displayed by SHR rats to the hyperlocomotion induced by drugs like amphetamine (Tsai and Lin, 1988), and also for the present increased sensitivity to the effects of WIN 55,212-2.

Additionally, we also investigated the acute effects of WIN 55,212-2 (0.25–2.5 mg/kg, i.p.) on anxiety-like behavior in adolescent and adult SHR and Wistar rats. SHR (regardless of age) presented an increased exploration of the open arms in the elevated plus-maze and crossed significantly more squares in the aversive central area of the open field, a response pattern that is consistent with a reduced anxiety profile (Hall, 1936; Pellow et al., 1985). Thus, the main finding at this point was that the reduced anxiety profile previously reported for adult SHR can be extended to adolescent rats of this strain (Ramos et al., 1997, 2002). However, despite the notion that cannabinoid exposure in rodents is associated with biphasic effects on anxiety-related behaviors (Onaivi et al., 1990; Berrendero and Maldonado, 2002; Haller et al., 2004; O'Shea et al., 2004; Rodgers et al., 2005; Viveros et al., 2005), the present doses of WIN 55,212-2 had no effect on anxiety-related behaviors in any of the animal groups evaluated in the open field (central locomotion) or elevated plus-maze (time in open arms).

In conclusion, the results of the present study indicate that acute systemic administration of the cannabinoid receptor agonist WIN 55,212-2 induces age- and strain-dependent locomotor effects in rats. At low doses, it selectively increases the locomotion of adolescent SHR, with no effects in adult SHR or Wistar rats (regardless of age). At higher dose, it induces hypoactivity in adolescent rats of both strains. These results suggest that both adolescence and the ADHD-like profile exhibited by the SHR strain may constitute factors of increased responsiveness to cannabinoid drugs. Further research is needed to explore these findings using predictive models of rewarding effects of drugs such as place preference and drug self-administration in order to establish the interplay between age and genetic factors that may contribute to the use and abuse of cannabinoid derivatives.

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