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ORIGINAL INVESTIGATION

Cannabinoid-induced conditioned place preference in the spontaneously hypertensive rat–an animal model of attention deficit hyperactivity disorder

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Abstract
Rationale Cannabis preparations are the most widely consumed illicit drugs, and their use typically begins in adolescence. The prevalence of cannabis abuse is higher in patients with attention deficit/hyperactivity disorder (ADHD) than in the general population, yet, knowledge about the motivational properties of cannabinoids in animal models of ADHD are lacking.
Objective To compare the motivational effects of the synthetic cannabinoid agonist WIN55,212-2 (WIN) in adolescent and adult spontaneously hypertensive rats (SHR), a validated animal model of ADHD, and Wistar rats, representing a "normal" genetically heterogeneous population. We also asked whether the effects of WIN depended (1) on the activation of the cerebral subtype of cannabinoid receptors, namely, the CB_1 cannabinoid receptor and (2) on putative changes by WIN in blood pressure.
Methods WIN was tested under an unbiased conditioned place preference (CPP) paradigm. Blood pressure after WIN administration was also monitored in additional groups of rats.
Results In the Wistar rats, WIN produced place aversion only in the adult but not adolescent rats. In contrast, WIN produced CPP in both adolescent and adult SHR rats. The behavioral effects of WIN were CB_1-mediated and not related to blood pressure.
Conclusion The contrasting effects of WIN in Wistar and SHR, and the higher resistance of adolescent rats to the aversive and rewarding effects of WIN in these two strains suggests that both adolescence and the ADHD-like profile exhibited by the SHR strain constitute factors that influence the motivational properties of cannabinoids.

Keywords Attention deficit hyperactivity disorder (ADHD) · Drug addiction · Adolescence · Cannabinoid system · Dopamine · Reward · Aversion · Conditioned place preference · Inbred strain · Spontaneously hypertensive rat (SHR)

Introduction

Attention deficit hyperactivity disorder (ADHD) is the most commonly diagnosed psychiatric disorder in children, yet its primary symptoms of hyperactivity, inattention and impulsivity (Taylor 1998; Himelstein et al. 2000) can persist into adolescence and adulthood (Biederman et al. 1994). Moreover, comorbidity in ADHD is very common with other psychiatric disorders, such as drug addiction. Notably, marijuana (*Cannabis sativa*) is the most popular illicit drug used by ADHD patients (Crowley et al. 1998; Biederman et al. 2006). It has been hypothesized that hypofunctionality of the mesolimbic dopamine system in ADHD results in an altered reinforcement of behavior and in an inadequate extinction of a previously reinforced behavior (Sagvolden et al. 2005). The presence of these characteristics may contribute to the earlier onset and the

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higher prevalence of drug abuse observed in ADHD patients compared to the normal population (Crowley et al. 1998; Biederman et al. 2006). Nonetheless, the biological mechanisms underlying the comorbidity between ADHD and drug abuse is largely unknown due in part to the lack of appropriate animal models.

One of the most validated animal models of ADHD is the spontaneously hypertensive rat (SHR). This strain is derived from the Wistar Kyoto (WKY) rat strain (Okamoto and Aoki 1963), and as reviewed by Sagvolden (2000), the SHR presents good face, construct, and predictive validity. Accordingly, SHR rats display hyperactivity, impulsivity, novelty seeking, and sustained attention deficits in comparison with their normotensive controls, the WKY rats (Davids et al. 2003; Russell et al. 2005; Sagvolden et al. 2005). Moreover, the SHR strain has been proposed to be potentially useful for the study of the relationship between ADHD and drug addiction (Vendruscolo et al. 2009) as these rats show increased sensitivity to psychostimulants (Pamplona et al. 2007) and opioids (Hoffmann et al. 1998) and exhibit greater ethanol consumption (Da Silva et al. 2005) than other rat strains. Furthermore, we have reported that an acute dose of the cannabinoid agonist WIN55,212-2 (WIN) promoted locomotor stimulation in adolescent SHR, but not in adult SHR and Wistar rats of any age (Pandolfo et al. 2007). This latter finding suggests that sensitivity to cannabinoids may depend on the age and strain of rats tested.

Despite the well-known abuse liability of cannabinoids in humans, controversial data concerning the discrimination of addictive properties in experimental animals have been reported. For example, cannabinoid agonists induce either aversive (Parker and Gillies 1995; McGregor et al. 1996; Sanudo-Pena et al. 1997) or rewarding (Lepore et al. 1995; Braida et al. 2004) effects in the conditioned place preference (CPP) paradigm. Similarly, discrepant findings have been reported regarding the hedonic effects of cannabinoids in intracranial self-stimulation (Gardner et al. 1988; Vlachou et al. 2007) and self-administration (Takahashi and Singer 1979; Martellotta et al. 1998; Fattore et al. 2001; Tanda et al. 2000; Deiana et al. 2007; Zangen et al. 2006) procedures.

In view of the limited number of studies concerning ADHD and cannabinoid addiction in humans and the lack of studies directly examining the motivational properties of cannabinoids in animal models of ADHD, the objective of this study was to compare the motivational properties of the cannabinoid receptor agonist WIN in adolescent and adult SHR rats using the CPP paradigm (Tzschentke 2007). It is particularly important to study drug abuse in adolescent subjects because of the tight interactions between the dopaminergic and the endocannabinoid systems in the maturating brain (Maharajan et al. 2001; Crews et al. 2007; Köfalvi and Fritzsche 2008), and because human drug consumption often starts before adulthood (Crowley et al. 1998; Spear 2000). The involvement of CB_1 receptors and the influence of blood pressure on the effects of WIN were also investigated. Furthermore, given the controversial data regarding the effects of cannabinoid in animal models, rats of the widely used Wistar strain, representing a "normal" genetically heterogeneous population, were chosen as controls in all tests, as previously reported (Pandolfo et al. 2007).

Materials and methods

Subjects

Adolescent and adult male outbred Wistar and inbred SHR rats (n=7–10 for each age and strain) bred in our own facilities were used. Rats were considered adolescent between post-natal days 28 and 45 (Spear and Brake 1983) and adult between post-natal days 90–110. The average (±S.E.M.) weight of the animals was 53±2 and 310±5 g for adolescent and adult SHR rats, and 124±2 and 369±8 g for adolescent and adult Wistar rats, respectively. The animals were housed in groups of four or five per cage and were maintained in a room under controlled temperature (22±2°C) on a 12-h light/dark cycle (lights on at 7:00 am), with free access to food and water. 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 National Institute of Health (NIH).

Drugs

WIN 55,212-2 [*R*-(+)-(2,3-dihydro-5-methyl-3-[is methyl] pyrrol [1,2,3-de-]-1,4-benzoxazin-6-yl)(1-naphthalenyl) methanone mesylate] and the cannabinoid antagonist AM 251 [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] were purchased from Tocris. The drugs were dissolved in saline (NaCl, 0.9%) with 10% dimethylsulfoxide plus 0.1% Tween 80, and administered intraperitoneally (i.p.) in a volume of 0.2 ml/100 g of body weight. The control solution consisted of the drug vehicle.

Conditioned place preference

The motivational properties of WIN were evaluated using an unbiased conditioned place preference (CPP) paradigm (Tzschentke 2007). CPP was tested in four identical rectangular wooden boxes covered with Formica. Each of the CPP boxes consisted of three different compartments

separated by guillotine doors. The two conditioning compartments (30×25×40 cm) had different tactile and visual cues: one compartment was black with a smooth wooden floor and the other was black with vertical white stripes and aluminum floor. The central "neutral" compartment (15×25×40 cm) was gray with a smooth wooden floor and had openings (10×10 cm) that gave access to any of the two other compartments. The test was conducted under low-light conditions (10 lx). The behavior of each animal was recorded via a video camera positioned above the boxes and monitored in an adjacent room via a closed-circuit TV camera. The experimenter was unaware of the drug treatment of the animals during behavioral evaluation. The apparatus was cleaned with a 70% ethanol solution and then dried with a paper towel after each trial.

The CPP protocol consisted of a schedule of 11 days divided into three different phases: pre-conditioning, conditioning, and post-conditioning. In the pre-conditioning phase (the first 2 days), the rats were allowed to freely explore the three compartments for 15 min each day. The time spent by the animal (with all four paws) in each of the three compartments on the second day was recorded.

The conditioning phase consisted of eight 25-min sessions, one per day. Immediately after i.p. administration of WIN (0.125, 0.25, 1.25 or 2.5 mg/kg), the rats were confined in one compartment and, on alternate days, received vehicle and were then confined in the opposite compartment. The control group received vehicle before conditioning in each compartment. For the experiment with the CB_1 antagonist, another group of rats was given AM 251 (0.25 or 1.25 mg/kg, i.p.) or vehicle 20 min before conditioning with WIN or vehicle. Drug doses were selected based on our previous studies (Pamplona et al. 2006; Pandolfo et al. 2007).

In the post-conditioning phase, each animal was placed in the neutral compartment and had free access to all three compartments. The time spent in each compartment was recorded for 15 min.

Blood pressure

In an additional group of Wistar and SHR rats, the arterial blood pressure (BP) (mmHg) was measured after i.p. injection of WIN (0.125–2.5 mg/kg) or vehicle, as previously described (Ramos et al. 2002). Under anesthesia with ketamine and xylazine (90 and 15 mg/kg, respectively), a heparinized PE20 polyethylene catheter was inserted into the right carotid artery for recording of systolic and diastolic arterial pressure. To prevent clotting, an i.p. dose of heparin (300 IU) was injected 10 min before the ketamine/xylazine injection. Animals were allowed to breathe spontaneously via a tracheal cannula and body temperature (maintained at 37±1°C) was monitored by a rectal thermometer. After the surgical procedure, a period of 5 min was allowed for stabilization and immediately after the i.p. administration of saline or WIN, the systolic and diastolic arterial BP were recorded for 30 min. BP data were recorded with a catheter pressure transducer (Mikro-Tip®, Millar Instruments, Inc., Huston, Texas, USA) coupled to a Powerlab 8/30 (AD Instruments Pty Ltd., Castle Hill, Australia). At the end of the experiment, animals were sacrificed with a pentobarbital overdose.

Statistical analysis

All results are expressed as means and S.E.M. For the CPP data, the statistical analysis of results for the cannabinoid agonist WIN was carried out using a three-way ANOVA (age, treatment, and strain) on the percentage of time spent in the drug compartment during the post-conditioning test according to the following formula: time in the drug-paired compartment/(time in the drug-paired compartment+time in the saline-paired compartment)×100. The data for the antagonism with AM 251 were analyzed by a one-way ANOVA with treatment as the independent variable. For blood pressure data, analyses were carried out using a three-way ANOVA (age, treatment, and strain). Following significant ANOVAs, multiple post-hoc comparisons were performed using the Duncan's test. The accepted level of significance for all tests was $P \leq 0.05$. The analyses were performed using the Statistica® software package (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Conditioned place preference

The results regarding the effects of the cannabinoid agonist WIN (0.125, 0.25, 1.25, or 2.5 mg/kg) in adolescent and adult SHR and Wistar rats tested in the CPP test are shown in Fig. 1. The three-way ANOVA revealed a significant overall effect of strain [$F(1,153)=14.47$, $P<0.01$], but not of treatment [$F(4,153)=2.26$, $P=0.06$] and age [$F(1,153)=3.60$, $P=0.06$] for the percentage of time spent in the drug-paired compartment. A significant interaction between age vs. treatment vs. strain [$F(4,153)=3.87$, $P<0.01$] was detected. The post-hoc comparisons indicated that vehicle-treated SHR and Wistar rats did not differ in terms of place preference (regardless of age). However, the highest tested dose of WIN (2.5 mg/kg) induced a significant CPP in SHR adolescents, whereas the dose of 0.25 mg/kg produced a significant CPP in SHR adults, compared to their respective control groups ($P<0.05$). Moreover, a significant place aversion at doses of 0.25, 1.25, and 2.5 mg/kg was

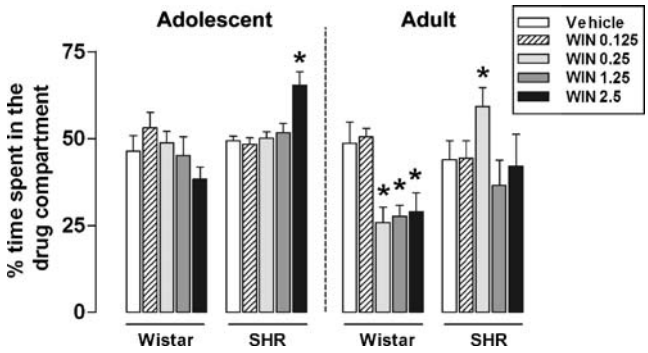

Fig. 1 Effects of treatment with the cannabinoid receptor agonist WIN (0.125, 0.25, 1.25 or 2.5 mg/kg, i.p.) on place conditioning of adolescent and adult SHR, and Wistar rats. Histograms represent the means and S.E.M. of the percentage of time spent in the drug-paired compartment during the post-conditioning test of animals, grouped by age, strain, and treatment (n=8–10). *P≤0.05 compared to the respective vehicle-treated control groups

observed in Wistar adults, as indicated by a reduction in the percentage of time spent in the drug-paired compartment compared to vehicle-treated controls (P<0.05). No effects were observed for Wistar adolescents.

Figure 2 illustrates the effects of the CB_1-selective antagonist AM-251 on the rewarding responses of WIN in adolescent and adult SHR rats, and on the aversive responses of WIN in Wistar adults. For SHR rats, the one-way ANOVA revealed a significant effect of treatment [adolescents: $F(3,27)=10.4$, $P<0.01$; adults: $F(3,26)=8.64$, $P<0.01$] for the percentage of time spent in the drug-paired compartment. As expected, the post-hoc comparisons indicated that WIN induced CPP in SHR adolescents and in SHR adults (P<0.05). More importantly, AM 251, which was ineffective when administered alone, prevented the WIN-induced CPP (P>0.05). For adult Wistar rats, the ANOVA also revealed a significant effect of treatment [$F(3,29)=9.34$, $P<0.01$] for the percentage of time spent in the drug-paired compartment. The post-hoc comparisons indicated that WIN induced place aversion in adult Wistar rats (P<0.05), and that AM 251 prevented this effect (P>0.05).

Blood pressure

Table 1 shows the results of WIN administration (0.125-2.5 mg/kg i.p.) on the arterial blood pressure (BP) of adolescent and adult SHR and Wistar rats. The three-way ANOVA revealed a significant effect of age [$F(1,54)=79.95$, $P<0.01$], but not of treatment [$F(4,54)=0.96$, $P=0.43$] and strain [$F(1,54)=0.39$, $P=0.53$] for BP. Moreover, a significant interaction between age vs. treatment vs. strain [$F(4,54)=4.40$, $P<0.01$] was detected. The post-hoc comparisons indicated that SHR adults were hypertensive in relation to adolescents (P<0.05). Moreover, only the dose of 1.25 mg/kg of WIN induced a significant decrease in the BP of adult SHR rats. No other significant effects of WIN were found on BP measurements.

Discussion

In the present study, we compared the motivational effects of the cannabinoid receptor agonist WIN in adolescent and adult male SHR and Wistar rats using the CPP test. The main finding was the opposing responses exhibited by SHR

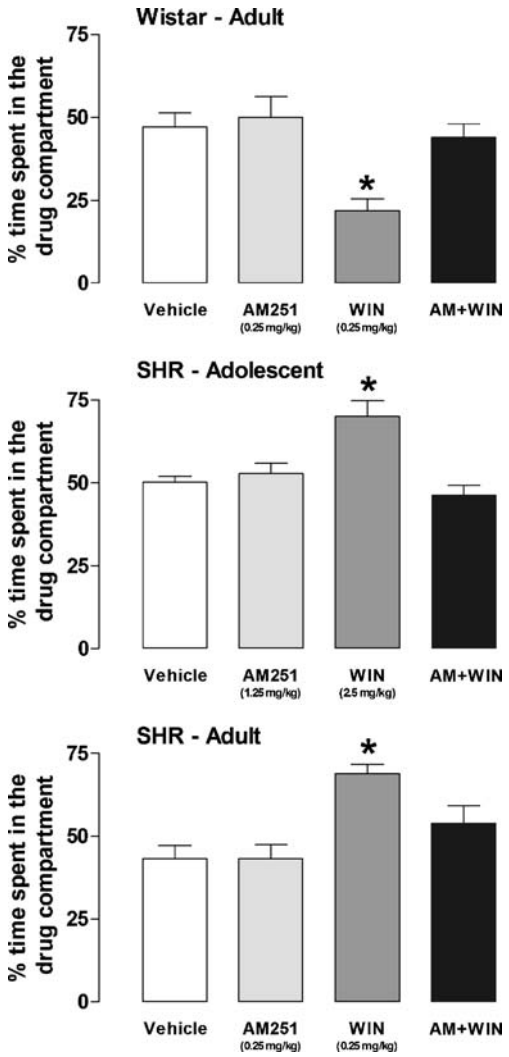

Fig. 2 Effects of pre-treatment with the cannabinoid receptor antagonist AM251 (0.25 or 1.25 mg/kg, i.p.) on place conditioning of adult Wistar rats, and adolescent and adult SHR rats treated with WIN (0.25 or 2.5 mg/kg, i.p.). Effects of treatment with the cannabinoid receptor agonist WIN (0.25 or 2.5 mg/kg, i.p.) and/or AM 251 (0.25 or 1.25) on place conditioning of adult Wistar rats, and adolescent and adult SHR rats. Histograms represent the means and S.E.M. of the percentage of time spent in drug-paired compartment during the post-conditioning test of animals grouped by age, strain and treatment (n=8–10). *P≤0.05 compared to the respective vehicle-treated control groups

Table 1 Effects of the i.p. administration of WIN on mean arterial pressure (mean±S.E.M.) of adolescent and adult SHR and Wistar rats

Age	Strain	Treatment (mg/kg)	Mean arterial pressure (mmHg)
Adolescent	Wistar	Vehicle	85±2.1
		WIN 0.125	80.7±1.5
		WIN 0.25	85.0±3.3
		WIN 1.25	86.3±3.7
		WIN 2.5	84±1
	SHR	Vehicle	78.3±0.8
		WIN 0.125	92±1
		WIN 0.25	82.5±0.5
		WIN 1.25	86.5±6.5
		WIN 2.5	78.3±2.4
Adult	Wistar	Vehicle	100±2.3
		WIN 0.125	91.7±1.7
		WIN 0.25	95.3±4.4
		WIN 1.25	104.3±4.7
		WIN 2.5	107.3±4.7
	SHR	Vehicle	110.1±3.2**
		WIN 0.125	97±3.6
		WIN 0.25	120.8±6.4
		WIN 1.25	87.3±5.9*
		WIN 2.5	99±5.9

**$P \leq 0.05$ compared to vehicle-treated SHR adolescents; *$P \leq 0.05$ compared to vehicle-treated SHR adults

and Wistar rats under identical experimental conditions. In the Wistar strain, included as a "normal" genetically heterogeneous population, WIN clearly produced place aversion in adults, while no effects were observed in adolescents. In contrast, in the SHR strain, WIN produced rewarding effects in both adult and adolescent rats. The selective CB_1 receptor antagonist, AM 251, prevented the rewarding and aversive effects of WIN regardless of strain and age, indicating that these behavioral effects were mediated by CB_1 receptors. Furthermore, administration of WIN at doses which altered behavior did not change blood pressure in any experimental group.

It has been reported that cannabinoids can induce both rewarding and aversive responses in a variety of animal models, such as drug self-administration, intracranial self-stimulation, CPP, and reinstatement procedures. However, aversion is the predominant effect of cannabinoids (Vlachou et al. 2005). For example, in contrast to the typical effects of most drugs of abuse (e.g., cocaine and heroin), cannabinoids failed to produce self-administration behavior (Leite and Carlini 1974; Carney et al. 1977; van Ree et al. 1978; Mansbach et al. 1994), induce place aversion (Parker and Gillies 1995; McGregor et al. 1996; Sanudo-Pena et al. 1997), and increase intracranial self-stimulation thresholds (Vlachou et al. 2005), a sign of negative affective state, in laboratory animals. Conversely, Justinova et al. (2003) have demonstrated unambiguously positive reinforcing effects of Δ^9-THC in squirrel monkeys using the intravenous self-administration paradigm. Furthermore, in some particular experimental conditions (e.g., pre-exposure to the drug or the homeostatic state of the animal) rewarding effects of cannabinoids can be achieved (e.g., Takahashi and Singer 1979; Lepore et al. 1995; Tanda et al. 2000; Valjent and Maldonado 2000).

In our experimental conditions, adult Wistar rats clearly showed an aversion for the place previously paired with WIN, thus indicating a dysphoric effect in "normal" rats. In sharp contrast, SHR rats showed preference for the place paired with WIN, a result that can be interpreted as an increased motivation for cannabinoids in these animals. Interestingly, both the rewarding and aversive effects of WIN in SHR and Wistar rats, respectively, were mediated by CB_1 receptors. It has been reported that CB_1 receptors are involved in the primary reinforcing effects of cannabinoids, alcohol, nicotine, and opioids (Maldonado et al. 2006). In addition, these receptors are densely expressed in brain regions related to motivation and reward (Chambers et al. 2003), and play a modulatory role in the dopamine system (Robbe et al. 2002; Köfalvi and Fritzsche 2008). Thus, an altered function of the cannabinoid system in SHR rats may result in an increased sensitivity to the rewarding effects of cannabinoids. The characteristics of the brain cannabinoid system in adult SHR rats, however, remain to be investigated.

It has been reported that SHR rats present abnormalities in the dopamine system, including increased density of dopamine D2-receptors (Chiu et al. 1982), increased striatal dopamine turnover (McKeon and Hendley 1988), increased expression of dopamine transporter (Watanabe et al. 1997), and altered release of dopamine in some brain regions compared to other rat strains (Russell et al. 1995; Russell et al. 2000; Viggiano et al. 2003). Considering that dopamine dysfunction is a major factor in the etiology of both ADHD and drug addiction, alterations in this system may explain, at least in part, the ADHD-like behavior displayed by SHR rats, and eventually the rewarding effect of cannabinoids observed specifically in this strain (Vendruscolo et al. 2009). Future studies are needed to test directly the role of the dopamine system on cannabinoid-induced CPP in SHR rats.

When comparing adult and adolescent rats, it was observed herein that a moderate dose of WIN (0.25 mg/kg) induced CPP in SHR adults, whereas a tenfold-higher dose (2.5 mg/kg) elicited CPP in SHR adolescents. As with adults, the CPP effect of WIN in SHR adolescents was prevented by pre-treatment with the CB_1-receptor antagonist AM 251. It is noteworthy that the behavioral effects of WIN were not dose-related. However, a non-linear dose–response relation-

ship is common in behavioral tests with cannabinoid agonists (Sanudo-Pena et al. 1997; Valjent and Maldonado 2000; Braida et al. 2004; Quinn et al. 2008). As discussed above, alterations in the cannabinoid (Adriani et al. 2003) and dopamine (Papa et al. 2002) systems that have been reported in adolescent SHR rats may contribute to the rewarding effects of cannabinoids specifically observed in these animals. In Wistar rats, WIN induced place aversion (0.25–2.5 mg/kg) in adults, whereas no effects were observed in adolescents. Taken together, these results suggest that adolescent rats were more resistant to both the rewarding and aversive properties of the cannabinoid receptor agonist WIN than adult rats. In agreement with these results, Quinn et al. (2008) have recently demonstrated that adult Wistar rats displayed a long-lasting avoidance to a Δ^9-THC-paired environment, yet this effect was not observed in adolescent rats. On the other hand, it has been reported that adolescent rats are more sensitive than adults to enduring changes induced by drugs of abuse. For example, adolescent rats previously exposed to Δ^9-THC showed a significant memory impairment and greater hippocampal alterations than adult rats (Quinn et al. 2008). Andersen et al. (2002) have reported that adolescent Sprague–Dawley rats, compared to adults, are more susceptible to persistent behavioral and neurobiological changes after repeated exposure to methylphenidate, a psychostimulant drug often prescribed for individuals with ADHD. With respect to SHR, repeated methylphenidate treatment during adolescence was able to decrease the place preference induced by cocaine (Augustyniak et al. 2006), but produced anxious-like behavior and enhanced ethanol intake in adult rats (Vendruscolo et al. 2008). To test whether methylphenidate influences cannabinoid-induced conditioned place preference in SHR rats is an interesting approach for future studies. Therefore, although adolescent rats appear to be more resistant to the rewarding and aversive drug effect than adult rats, they are more sensitive to the enduring drug-induced behavioral and brain changes. These effects might favor a higher drug intake during adolescence and contribute to the development of drug addiction.

The hypertension displayed by SHR rats is frequently questioned as a possible confounding factor in behavioral studies (Adriani and Laviola 2004). Thus, it was of interest in the present study to test the effects of WIN on blood pressure. The results provide evidence supporting a dissociation between the behavioral effects of WIN and blood pressure. First, WIN produced CPP in adolescent SHR rats that had not yet developed hypertension, and administration of WIN did not alter blood pressure in these animals. Second, as expected, adult SHR rats were hypertensive, yet WIN injection in the dose that produced CPP (0.25 mg/kg) did not significantly alter blood pressure. Only a fivefold-higher dose of WIN (1.25 mg/kg) produced a hypotensive effect in SHR adults. Furthermore, the blood pressure was not significantly altered in WIN-treated adolescent and adult Wistar rats. Because some studies have reported cardiovascular effects of cannabinoids in normotensive and SHR rats (Lake et al. 1997; Batkai et al. 2004; Wheal et al. 2007) and because blood pressure measurements in the present study were carried out in anesthetized rats, the influence of blood pressure on the behavioral effects of WIN cannot be completely ruled out.

Previous research has shown that WIN at doses of 0.25 and 1.25 mg/kg selectively promoted locomotor stimulation in adolescent SHR in the open field test (Pandolfo et al. 2007). Indeed, WIN at doses that increased locomotion in our previous studies did not induce place preference in the present study. Moreover, it is important to note here that our rats were tested in a drug-free state. More specifically, these observations indicate that locomotor activity was not a confounding factor in the interpretation of the present results.

In conclusion, the present findings indicate that the cannabinoid receptor agonist WIN produces opposite behavioral effects in the SHR and Wistar strains. We have demonstrated for the first time that cannabinoids induced rewarding effects in adolescent and adult SHR rats. This result also confirms and extends previous research that demonstrates the aversive effects of cannabinoids in adult Wistar rats and a resistance to these effects in adolescent rats. In addition, the behavioral effects of WIN were mediated by CB_1 cannabinoid receptors and not related to hypertension. Taken together, these results suggest that both adolescence and the ADHD-like profile exhibited by the SHR strain may constitute factors that alter the motivational properties of cannabinoids. Although additional research is necessary, the SHR strain may constitute a useful tool for the study of the behavioral aspects underlying the relationship between ADHD and cannabis vulnerability.

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