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Effects of the cannabinoid receptor agonist win 55,212-2 on operant behavior and locomotor activity in rats

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Abstract

Cannabinoids influence the motivational state of a subject and affect motor behavior. In the present study, we examined the acute effects of the cannabinoid (CB) receptor agonist WIN 55,212-2 (WIN) in three different doses (0.6, 1.2 and 1.8 mg/kg) on the performance of rats in a progressive ratio operant behavior task and on locomotor activity.

WIN dose-dependently reduced the break point and the total number of lever-presses under a progressive ratio schedule. A food preference test revealed a preference for freely available casein pellets over lab chow in all treatment groups, indicating no WIN-effects on primary motivation. There was a significant reduction in the amount of casein pellets consumed by animals treated with 1.8 mg/kg WIN. Locomotor activity in the open field was increased by 0.6 mg/kg, but not by higher doses of WIN.

These data show that administration of the synthetic cannabinoid receptor agonist WIN leads to dose-dependent alterations of the performance in an operant behavior task and of motor behavior. We confirm previous findings of dose-dependent motor stimulating and inhibiting effects of cannabinoids, and show an impairment of a complex operant behavior at higher doses of WIN.

Keywords: Progressive ratio schedule; Reward; Open field; Break point; Cannabinoid

1. Introduction

Cannabinoids are implicated in the regulation not only of basic physiological processes such as food intake, pain perception and motor coordination, but also of cognitive functions such as learning (Ameri, 1999; Iversen, 2003; Elphick and Egertova, 2001). They exert their effects through interaction with specific G protein-coupled cannabinoid (CB) receptors (Devane et al., 1988). Two subtypes of CB receptors have been identified so far: the CB1 and the CB2 receptor (Childers and Breivogel, 1998; Ameri, 1999; but see Breivogel et al., 2001). It is known that cannabinoids influence motivational and reward-related behavior and thus probably affect the performance in operant behavior tasks like the progressive ratio (PR) test. In PR schedules, the instrumental demand for a constant food reward increases progressively, until eventually responding ceases and reinforcers are no longer obtained. This "break point" is an operational measure for a shift in motivation where the rewarding value is lower than the effort the animal is willing to expend to obtain this reward (Hodos, 1961; Ellenbroek and Cools, 2000). The PR test of operant responding is a complex task, where animals are forced to adjust their instrumental behavior (lever-pressing) from continuous reinforcement to an incremental schedule of responding. This task implies switching from a fixed ratio to an increasing schedule of reinforcement and the dynamic adjustment of behavior according to a cost–benefit analysis. Therefore, this is a useful test for the brain mechanisms underlying the translation of motivation and changing values of reinforcement into an appropriate behavior.

PR schedules were developed first with non-drug reinforcers like food (Hodos, 1961), and later modified to study other reinforcing events such as brain self-stimulation or self-administration of drugs of abuse. Hodos and

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Kalman (1963) noted that the break point measure might be particularly suitable for studying the effects of drugs, since drugs could impair the rate of responding.

Many recent studies investigated the effects of cannabinoid receptor ligands on self-administration of various drugs and other measures of drug effects on operant responding, while data about the effects of cannabinoids per se in these tasks are rare. Schulze et al. (1988) observed impaired break points in a PR task after acute administration of Δ^9-tetrahydrocannabinol (THC) in monkeys. Moreover, Schneider and Koch (2003) showed a reduced break point after chronic pubertal exposure to the CB1/2-receptor synthetic full agonist WIN 55,212-2 (WIN).

CB receptor agonists show a different profile compared to other drugs of abuse regarding their reinforcing properties. There are controversial data in the literature concerning the ability of CB receptor agonists to reinforce behavioral responses in experimental animals, i.e. to reduce self-stimulation thresholds and to support self-administration, or conditioned place preference (reviewed in Vlachou et al., 2003). Cannabinoid agonists and antagonist have modulatory effects on the PR performance in self-administration studies with heroin (De Vries et al., 2003; Solinas et al., 2003), cocaine (Vlachou et al., 2003) and alcohol (Gallate et al., 1999). More detailed knowledge about cannabinoid-induced disturbances of PR performance are essential for an objective interpretation of cannabinoid actions on drug self-administration behavior. Therefore, the aim of the present study was to investigate the acute effects of the synthetic cannabinoid agonist WIN in different doses (0.6, 1.2 and 1.8 mg/kg) on the performance in an operant behavior task. We have used this synthetic full agonist already in our previous studies, mainly because of its high affinity to the CB1-receptor (Schneider and Koch, 2002, 2003).

Since a well-investigated pharmacological effect of cannabinoids is the change of motor behavior (Rodriguez de Fonseca et al., 1998), we also tested the effects of WIN on locomotor behavior in the open field. The CB1 receptor is densely expressed in structures like the cerebellum and the basal ganglia, which are known to mediate initiation and coordination of movement (Breivogel and Childers, 1998). Especially the high CB1 receptor density on the axons-terminals of the striatal GABAergic neurons of the basal ganglia and of the glutamatergic granule cells of the cerebellum are probably involved in motor control (Howlett, 1995). Several studies showed that acute administration of cannabinoids stimulate locomotion at low doses, and inhibit motor activity at higher doses (reviewed in Rodriguez de Fonseca et al., 1998). Therefore, in order to examine the dose-dependent effects of WIN on motor behavior and to exclude possible motor impairment in the PR task, locomotor activity in an open field was recorded.

2. Methods

2.1. Subjects and housing

Forty naive adult male Wistar rats (Hannover strain, Harlan-Winkelmann, Borchen, Germany) were used in this study. The rats were housed in groups of 3–6 in Macrolon cages (type IV) under standard conditions under a 12-h light–dark schedule (lights on 7:00–19:00). They received free access to tap water and were maintained on a bodyweight of 250–300 g by controlled feeding of 12 g rodent chow/rat/day.

The experiments were done in accordance with the NIH ethical guidelines for the care and use of laboratory animals for experiments and with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

2.2. Priming

In order to exclude possible adverse effects of the first exposure to the cannabinoid (Sanudo-Pena et al., 1997; Chaperon and Thiebot, 1999), each rat of the WIN group was submitted to a priming procedure once 24 h before the beginning of the whole test session, in accordance with the observations of Valjent and Maldonado (2000). The rats received one injection of the same dose of WIN as in the subsequent experiments (0.6, 1.2 or 1.8 mg/kg) and were immediately returned to their home cages.

2.3. Behavioral testing

After priming, the rats were tested in the following order, with at least 48 h of rest in between: locomotor activity (open field), food preference test, continuous reinforcement training, and progressive ratio test.

2.4. Locomotor activity

Locomotor activity was measured in a infrared-beam operated open field (44.7×44.7×44 cm, ActiMot-System, TSE, Bad Homburg, Germany) for 35 min. At the beginning of the test sessions, each rat was placed in the center of the testing chambers. Number of rearings, time spent in the center of the open field [s], and distance travelled [m] were recorded. Testing arenas were cleaned with 70% ethanol solution between subjects.

2.5. Food preference test

Each rat was placed in a standard Macrolon cage (type II) and the amount of freely available pellets and lab chow consumed was measured for 10 min. The caloric content of chow and pellets was similar.

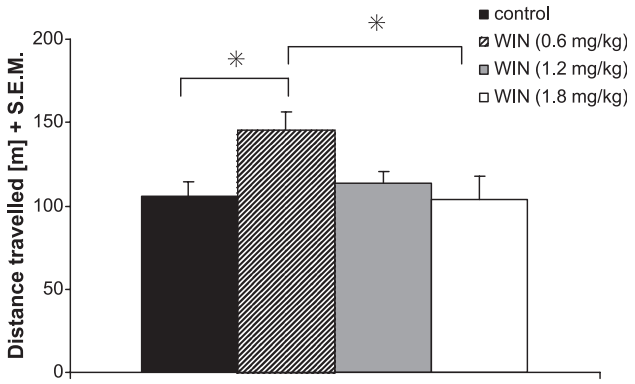

Fig. 1. Effects of acute WIN administration on mean distance travelled (±standard error of mean) in the open field. WIN (0.6 mg/kg) caused a significant increase of locomotor activity compared to controls (*p=0.017) and compared to animals treated with 1.8 mg/kg WIN (*p=0.026) (0.6 mg/kg, n=11; 1.2 mg/kg, n=8; 1.8 mg/kg, n=8; control, n=13).

2.6. Progressive ratio

The PR test was conducted in an operant chamber (24×28×28 cm, Operant Behavior System, TSE, Bad Homburg, Germany). First, subjects were habituated for 1 day to the test chamber, the palatable casein pellets and the noise of the magazine response (shaping). After shaping, rats were trained over 3 days for lever pressing in sessions of 30 min on a continuous reinforcement schedule (CRF) until they reached a stable baseline. After lever pressing training was completed, one PR test session (for 30 min) was conducted on the next day. The PR schedule (i.e. lever presses required for one pellet) was changed every second minute according to the following exponential progression: 1, 2, 4, 6, 9, 12, 15,. . ., derived from the formula $5 \cdot e^{0.2n} - 5$, where n is the position in the sequence of ratios (Mobini et al., 2000). The so-called "break point", the conventional index of performance on a PR schedule of reinforcement (Reilly, 1999), was defined as the first PR sequence where lever-presses decreased ≤50% relative to the previous phase without increasing ≥100% in the following phase (Schneider and Koch, 2003).

2.7. Drugs

WIN 55,212-2 (Tocris Biotrend, Köln, Germany) was dissolved in 0.1% Tween 80 and diluted in saline (0.9%). The drug was administered intraperitoneally (i.p.) at a dose of 0.6, 1.2 or 1.8 mg/kg. Injection volumes were 1 ml/kg. During the test, the experimenter was not aware of the drug treatment of the animals.

2.8. Data analysis

For the statistical analysis of locomotor activity [s], rearings, time spent in the center of the open field [s], distance travelled [m], break points and the total number of lever-presses, a one-way repeated measure analysis of variance (ANOVA) was used, followed by post-hoc Tukey t-test for pairwise comparison.

The amount of food consumed [g] in the preference test was analysed using a two-way repeated measure ANOVA, also followed by post-hoc Tukey t-test. A value of P<0.05 was considered to represent a significant effect.

3. Results

3.1. Locomotor activity

Distance travelled by 0.6 mg/kg WIN-treated rats was significantly increased compared to controls and compared to animals treated with 1.8 mg/kg WIN (Fig. 1) (F(3.36)=6.1, p<0.05). Moreover, treatment with 0.6 mg/kg WIN resulted in a significant increase in the number of rearings compared to 1.8 mg/kg WIN (Fig. 2) (F(3.36)=3.9, p<0.05). No drug-effects on the time spent in the center of the open field were found [data not shown; (F(3.36)=0.68, p>0.05)].

3.2. Progressive ratio

Acute treatment with 1.2 and 1.8 mg/kg WIN significantly reduced the break point in the PR task compared to vehicle treatment, as well as compared to rats treated with 0.6 mg/kg WIN (F(3.33)=7.5, p<0.05) (Fig. 3). Moreover, we detected a significant decrease in the total number of lever presses in animals treated with 1.2 and 1.8 mg/kg WIN compared to those treated with 0.6 mg/kg WIN (F(3.33)=4.5, p<0.05) (Fig. 4).

3.3. Food preference

A two-way repeated measure ANOVA revealed a significant preference for casein pellets in all treatment groups compared to lab chow (Table 1) (F(1.24)=534.9, p<0.05). Additionally, there was a significant reduction in

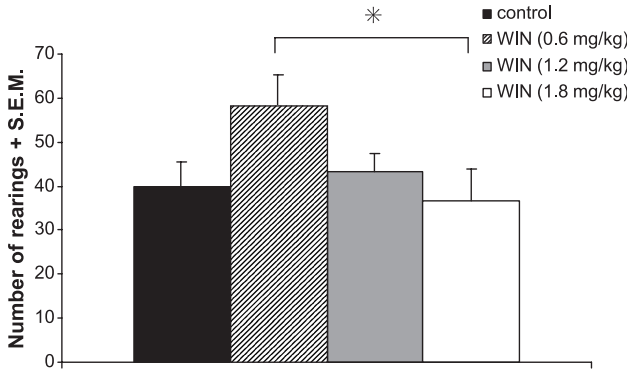

Fig. 2. Acute administration of 0.6 mg/kg WIN resulted in a significant increase in the mean number of rearings (±standard error of mean) compared to animals treated with 1.8 mg/kg WIN (*p=0.032) (0.6 mg/kg, n=11; 1.2 mg/kg, n=8; 1.8 mg/kg, n=8; control, n=13).

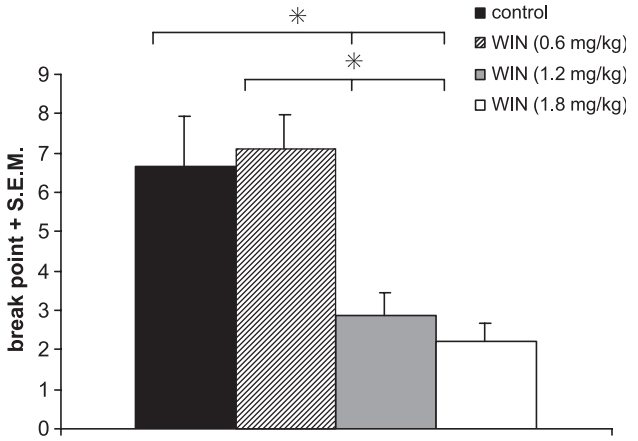

Fig. 3. Effects of acute WIN-treatment on the performance in a PR test. The bars indicate the last sequence in which the animals responded according criterion. A significant reduction in the break point was detected in rats treated with 1.2 mg/kg (*p=0.044) and 1.8 mg/kg WIN (*p=0.011) compared to controls. Likewise, treatment with 1.2 mg/kg WIN resulted in a significant reduction in the break point compared to 0.6 mg/kg WIN (*p=0.015), as well as 1.8 mg/kg WIN compared to 0.6 mg/kg WIN (*p=0.003) (0.6 mg/kg, n=11; 1.2 mg/kg, n=8; 1.8 mg/kg, n=9; control, n=10).

the amount of casein pellets consumed by animals treated with 1.8 mg/kg WIN compared to controls and to animals treated with 0.6 mg/kg WIN ($F(3.24)=3.9$, $p<0.05$).

4. Discussion

We shall first discuss the results of the open field test in order to include possible motor effects of WIN in the interpretation of the PR performance. The present data show that the CB receptor agonist WIN at 0.6 mg/kg increases motor activity, whereas high doses of WIN had no effect on the open field performance.

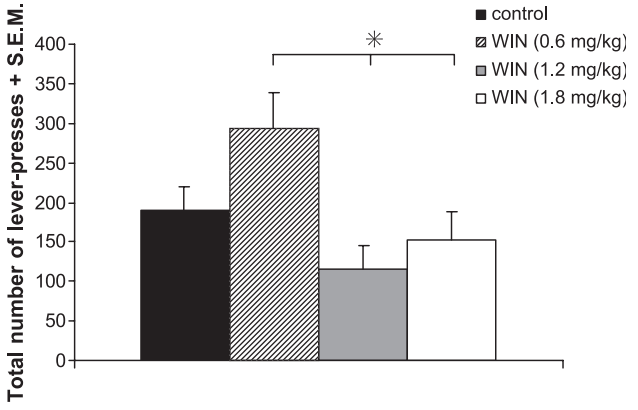

Fig. 4. Acute administration of 0.6 mg/kg WIN caused a significant increase in the mean total number of lever presses (±standard error of mean) compared to animals treated with 1.2 mg/kg WIN (*p=0.010). Likewise, the total number of lever presses was significantly increased in 0.6 mg/kg WIN-treated animals compared to rats treated with 1.8 mg/kg WIN (*p=0.043) (0.6 mg/kg, n=11; 1.2 mg/kg, n=8; 1.8 mg/kg, n=9; control, n=10).

Table 1
The table shows the total amount of food consumed [g]

	Casein pellets	(±) S.E.M.	Lab chow	(±) S.E.M.
Control	10.06*·◆	(+0.56)	0.03	(+0.03)
WIN (0.6 mg/kg)	8.64*·◆	(+1.06)	0.00	(+0.00)
WIN (1.2 mg/kg)	8.31*	(+0.36)	0.03	(+0.03)
WIN (1.8 mg/kg)	6.59*	(+0.72)	0.03	(+0.03)

Acute administration of WIN did not affect the preference for casein pellets, i.e. animals showed a preference for casein pellets compared to lab chow in all treatment groups (control: *p=0.001, 0.6 mg/kg WIN: *p=0.001, 1.2 mg/kg WIN: *p=0.001, 1.8 mg/kg WIN: *p=0.001). Yet, 1.8 mg/kg WIN led to a significant reduction in the amount of consumed casein pellets compared to controls (♦p=0.001) and to animals treated with 0.6 mg/kg WIN (♦p=0.032) (0.6 mg/kg, n=7; 1.2 mg/kg, n=7; 1.8 mg/kg, n=7; control, n=7).

These findings are in line with earlier reports of stimulatory as well as inhibitory effects of cannabinoids on locomotion (reviewed in Rodriguez de Fonseca et al., 1998). A study by Sanudo-Pena et al. (2000) described a triphasic effect of THC which includes decreased locomotor activity in low doses (up to 0.2 mg/kg), increased locomotion in doses of 1–2 mg/kg THC, and catalepsy after administration of high doses THC (5 mg/kg). In the present study, a stimulating effect of WIN was observed in rats treated with 0.6 mg/kg WIN. Since WIN shows a more than four times higher potency than THC (Hampson and Deadwyler, 2000), the stimulating effect seen in the present study is in line with the observations of Sanudo-Pena et al. Additionally, Sulcova et al. (1998) reported a biphasic effect of the endogenous CB receptor ligand anandamide on motor behavior in mice. Administration of 10–100 mg/kg anandamide resulted in inhibition of movement, whereas 0.01 mg/kg anandamide stimulated behavioral activities in the open field.

The fact that higher doses of WIN did not reduce motor activity in the present study seems to be in contrast to the study by Sanudo-Pena et al. (2000) showing cataleptic effects of THC in high doses. Likewise, Lichtman et al. (2001), as well as Prescott et al. (1992) described catalepsy after administration of 4 mg/kg THC. Since we only tested locomotion and did not investigate catalepsy with tests specially designed to detect catalepsy, we cannot exclude cataleptogenic effects of WIN. However, even high doses WIN induced no inhibitory effects on locomotor behavior. Likewise, Darmani (2001) also found no inhibitory effects of WIN on locomotor activity and rearing behavior in shrews at doses below 2–3 mg/kg. Since the affinity of WIN for the CB1 receptor in shrews is similar to that in rodents (Darmani et al., 2003), these findings are in line with the results of our study. The observation that THC has cataleptogenic effects whereas WIN does not, suggests a different profile of action of WIN and THC on motor behavior. These differences might be due to the activation of a novel CB receptor that is stimulated by anandamide and WIN, but not by THC (Breivogel et al., 2001; Hajos et al., 2001).

Cannabinoids affect motor behavior via CB receptors that are abundant in different parts of the basal ganglia, by regulating glutamatergic and GABAergic systems within the same neuronal network. Thus, cannabinoid receptors can modulate both the inhibitory and the excitatory neuronal transmission in the basal ganglia and may thus provide dual regulation of movement (Sanudo-Pena et al., 1996; Sanudo-Pena and Walker, 1998; van der Stelt and Di Marzo, 2003).

Notably, we found no effect of WIN-treatment on the time spent in the center of the open field (which can be considered as an index of reduced anxiety), indicating no overt anxiogenic or anxiolytic effect of the cannabinoid receptor stimulation.

In order to assess the effects of WIN on operant behavior, the PR task was chosen. The administration of 0.6 mg/kg increased the total number of lever-presses compared to administration of 1.2 and 1.8 mg/kg WIN. However, this effect of WIN may be due to the general motor stimulating effect of 0.6 mg/kg WIN, as seen in the open field behavior.

Administration of 1.2 and 1.8 mg/kg WIN impaired the performance in the PR task, as indicated by a significant reduction in break points compared to controls and to 0.6 mg/kg WIN. These results are consistent with earlier findings in monkeys of impaired break points in a PR task after acute administration of THC (Schulze et al., 1988). Since measurement of locomotor activity did not reveal a motor impairment in rats receiving 1.2 and 1.8 mg/kg WIN, the reduction in break point cannot be due to motor deficits.

The food preference test showed a reduced amount of consumed palatable pellets after administration of 1.8 mg/kg WIN compared to the lowest WIN dose and to controls. These findings may be explained by a general reduction in food motivation and thus in food intake in rats receiving 1.8 mg/kg WIN. Similar results were reported in a study by Biscaia et al. Here, chronic treatment with the cannabinoid receptor agonist CP 55,940 led to reduced food intake and body weight during the treatment period (Biscaia et al., 2003). Notably, a recent paper showed *hyperphagia* in rats in 4-h test sessions after systemic (0.4–10 mg/kg) but not after intracerebral WIN (0.1–10 μg) administration (Gomez et al., 2002). However, this effect was only found in food deprived, partially satiated rats. Our findings of reduced food intake during 10 min of the food preference test in rats treated with 1.8 mg/kg WIN does not necessarily contradict this paper, since the rats in our study were on a restricted feeding regimen, but were neither food deprived nor partially satiated.

A deficit in food motivation in rats treated with 1.8 mg/kg WIN could explain the reduction in break point in the PR task. Due to the reduced food motivation, animals cease responding at low levels of instrumental demand. However, the reduced break point after administration of 1.2 mg/kg WIN cannot be explained by a deficit in food motivation, since here the food preference test did not reveal a reduction in the amount of consumed casein pellets.

The adequate performance of animals in a PR task is influenced by numerous variables that are not directly associated with reward value or the motivational state of the animal. The kinetic effort of lever-pressing, extinction or frustration (an aversive emotional reaction to the reduction of reward) are factors which may affect the animals' response rate and break point (Stewart and Blampied, 1975; Salinas et al., 1996; Mobini et al., 2000). It is presently unclear which of these factors are responsible for the reduction of the break point by WIN. Since there is evidence for a functional cross-talk between cannabinoid and opioid systems (Navarro et al., 1998; Manzanares et al., 1999) and some studies already reported an impaired break point after treatment with opioids (Poling et al., 1996; Jarema et al., 1999), the PR impairment in the present study could also be caused by alterations in the endogenous opioid system.

Taken together, our results show a dose-dependent influence of the synthetic cannabinoid receptor agonist WIN on motor behavior and on the performance in a PR operant behavior task. We demonstrate an increase in motor behavior after application of a low dose of WIN, whereas higher doses impaired instrumental behavior under a PR schedule and point towards a complex role of cannabinoids in the control of reward-related behavior. It is necessary in self-administration studies investigating the interactions of cannabinoids with other drugs of abuse to disambiguate the cannabinoid effects on operant behavior per se and drug interaction effects.

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