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Research report

CB_1 cannabinoid receptor agonist WIN 55, 212-2 decreases intravenous cocaine self-administration in rats

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Abstract

The effect of the CB_1 cannabinoid receptor agonist WIN 55, 212-2 on intravenous cocaine self-administration (IVSA) in rats was evaluated. Male Long Evans rats were implanted with silastic catheters through the external jugular vein. The IVSA was conducted in 3-h daily sessions with a fixed ratio (FR1) schedule: the experimental apparatus had a nose-poking response-like operandum. Intravenous pre-treatment with WIN 55, 212-2 (0.25, 0.5 and 1 mg/kg) to rats self-administering cocaine (0.25 or 0.5 mg/kg/inj) at stable baseline, reduces cocaine intake in a dose-dependent manner. The CB_1 receptor antagonist SR 141716A (3 mg/kg i.p.) completely reversed the WIN 55, 212-2-induced decrease of cocaine intake. However, pre-treatment of SR 141716A alone (up to dose of 9 mg/kg i.p.) was unable to modify cocaine IVSA. These results indicate that stimulation of CB_1 cannabinoid receptors activates rewarding mechanisms which produce reinforcing effects additional to those induced by cocaine.

Keywords: Cannabinoid receptors; Cocaine; Intravenous self-administration; WIN 55, 212-2; SR 141716A; Reward; Rat

1. Introduction

Over the last decade great efforts have been made to understand the molecular basis of the behavioral effects of cannabis, beginning with the identification, cloning and expression of selective central cannabinoid receptors (CB_1) both in rats [28,38] and humans [23,25,34], followed by isolation of the endogenous cannabinoid ligand anandamide [13] and synthesis of a selective CB_1 antagonist SR 141716A [44].

It is now well-documented that, at low doses, CB_1 receptor stimulation produces a mixture of depressant and excitatory effects, while higher doses predominantly elicit depression of the central nervous system [1]. Nevertheless, animal studies have showed that cannabinoid agonists induce both rewarding and aversive effects, such as conditioned place preference [32], place aversion [9,35,39,42,48], taste aversion [39,42] and anxiogenic effects in the elevated plus maze test in rodents [41].

Controversial data also exist concerning the ability of cannabinoids to lower the threshold for electrical self-stimulation [22,51] and to support self-administration in animals [19,26,36,37,52,53].

A recent epidemiological study revealed that despite the general decline in drug use seen in the general population, marijuana abuse had increased primarily in youths and young adults [45]. Moreover, it has been assessed that in humans cannabinoids exert discriminative properties and possess subjective and reinforcing effects [7,8,27]. Since the practice of using two or more drugs concurrently (polydrug abuse) has become widespread [4,5,15], the combination of marijuana and cocaine has been identified as one of the most popular among recreational abusers. Clinical studies of this interaction have pointed out that marijuana is able to

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not only induce considerable cardiovascular effects [16,17] but also to potentiate cocaine's subjective effects. In fact, it appears not only to enhance the positive effects of cocaine, but also to minimize the dysphoria frequently associated with the resultant 'crash' [33]. Therefore, healthy subjects who first smoked marijuana and then received cocaine described themselves as being 'stimulated' and 'high' to a higher degree than when they received these drugs separately [18].

The purpose of the present study was to investigate the effect of CB_1 receptor stimulation induced by the synthetic agonist WIN 55, 212-2 on intravenous cocaine self-administration (IVSA) in rats. The IVSA is the most reliable and commonly used model of addiction, while cocaine is known to act as a positive reinforcer to maintain the IVSA in a variety of species, including humans.

2. Methods and procedures

Male Long Evans rats (Charles-River) weighing 300–350 g at the start of experiments were individually housed with ad libitum access to food and water, and maintained on a 12-h reversed light–dark cycle (dark from 09:00 to 21:00 h). Environment temperature (22 ± 1°C) and humidity (60%) were constant.

Experiments were carried out in strict accordance with both the local Animal Care Committee and the E.C. regulations for animal use in research (CEE NE 86/609).

Cocaine-hydrochloride (Sigma, Italy) was dissolved in heparinized (1%) saline solution in a volume of 0.5 ml/kg/inj. Doses were adjusted by injection volume prior to each self-administration session, according to the animal's body weight. WIN 55, 212-2 (RBI, MA, USA) and SR 141716A (Sanofi, Montpellier, France) were freshly dissolved in two drops of Tween 80 and diluted in heparinized (1%) saline solution. The first was administered i.v. in a volume of 1 ml/kg immediately prior to start of the IVSA session, while the second was administered i.p. in a volume of 5 ml/kg 30 min before the start of the IVSA session. As a control study, a group of six rats were injected with the same vehicles.

Animals were anesthetized with chloral hydrate (400 mg/kg i.p.) and under sterile conditions implanted with silastic catheters inserted into the right external jugular vein. The catheter was passed under the skin and exited in the mid-scapular region.

Before the start of each self-administration session, free passage of heparinized (1%) saline solution through the catheters was checked for the subsequent intravenous drug infusions.

The self-administration apparatus (Ecos, Italy) consisted of eight Plexiglas cages (30 × 30 × 30 cm). Two holes, 15 cm apart, provided with fotobeam detectors, were made 2 cm above the floor. The catheter was connected to an infusion pump via a silastic tube. Nose-poking in one of the holes (defined as active) switched on the infusion pump, injecting the cocaine solution into the animal's venous system. Nose-poking in the other hole (defined as passive) did not activate the pump. Assessment of self-administration schedules and the collection of data were programmed by means of PC software.

Starting 5 days after surgery, each rat was allowed access to cocaine at a dose of 0.5 mg/kg/inj under a continuous reinforcement (FR1) schedule during 3-h daily sessions. Time-outs lasting 5 s, corresponding to the infusion time, were imposed immediately after drug delivery: during this time the nose-poking response was inactivated. Only rats developing a stable pattern of cocaine intake with a variation of less than 10% over three consecutive baseline sessions were selected for this study. Each dose was tested, once for each animal, in a random order and a minimum of 3 no-pre-treatment days separated each test day. Subsequently, rats were allowed access to the drug at the lower dose of 0.25 mg/kg/inj, under the same experimental conditions.

Due to the fact that not all animals completed the entire set of experiments (due to catheter blockades), data were computed as for independent rather than correlated samples. Each treatment, however, included a minimum of six subjects. The number of reinforcers earned during the 180-min session was recorded and normalised with respect to baseline performances. These data were submitted to a one-way analysis of variance (ANOVA) followed by the Newman–Keuls test to compare the different doses and vehicle. Apart from the first days of the training period, nose-poking activity in the passive hole was virtually absent and was therefore excluded from data analysis.

3. Results

Fig. 1 shows the effect of WIN 55, 212-2 (0.25, 0.5 and 1 mg/kg, i.v.) on cocaine i.v. self-administration. WIN 55, 212-2 pre-treatment significantly reduced cocaine intake compared with both vehicle-treated rats ($F(2,10) = 7.56$, $^*P < 0.01$) and to their previous baseline levels of cocaine intake (broken line). The maximum decrease of cocaine intake observed was $-38 \pm 6.32\%$ with the WIN 55, 212-2 dose of 0.5 mg/kg/inj. A higher dosage (1 mg/kg/inj) did not produce any further decrease.

As shown in Fig. 2, a similar decrease of cocaine intake subsequent to WIN 55, 212-2 pre-treatment was observed when cocaine concentration was reduced (0.25

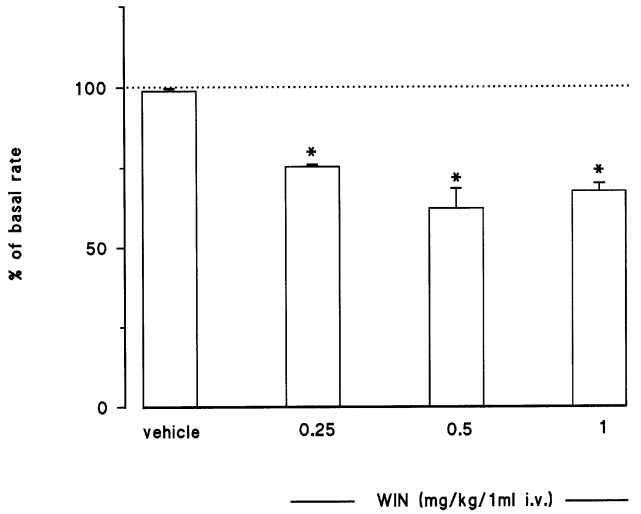

Fig. 1. Effect of intravenous WIN 55, 212-2 acute pre-treatment on cocaine (0.5 mg/kg/inj) self-administration in rats. Data are expressed as percentage of the basal rate of reinforcement (= mean of cocaine injections over three consecutive IVSA sessions). Each bar represents mean ± S.E.M. of six animals. *Significant difference from vehicle-treated group ($P < 0.01$ ANOVA followed by Newman–Keuls test).

mg/kg/inj) suggesting that this effect is independent of cocaine concentration.

Pre-treatment with CB_1 receptor antagonist SR 141716A dose-dependently antagonizes the WIN 55, 212-2-induced decrease of cocaine self-administration (Fig. 3). However, as shown in Fig. 4, pre-treatment with SR 141716A, up to a dose of 9 mg/kg, was

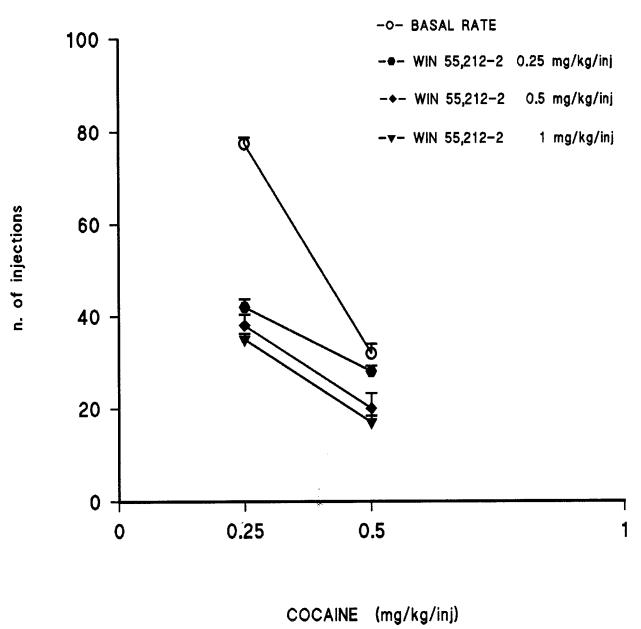

Fig. 2. Effect of intravenous WIN 55, 212-2 acute pre-treatment on different doses of cocaine self-administration. Each point represents mean ± S.E.M. of six experiments. All doses of WIN 55, 212-2 are expressed as mg/kg and are significantly different from basal values (*$P < 0.05$ Newman–Keuls test).

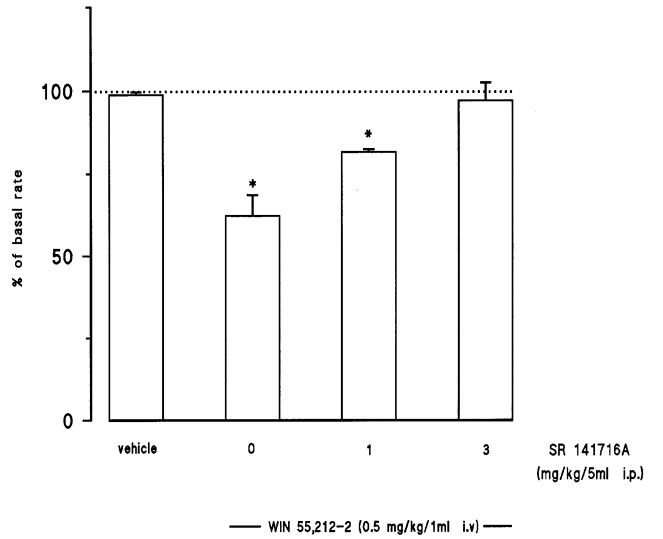

Fig. 3. Effect of intraperitoneal SR 141617A acute pre-treatment in WIN 55, 212-2 0.5 mg/kg pre-treated rats self-administering cocaine 0.5 mg/kg/inj. Each bar represents mean ± S.E.M. of the percentage of the basal rate of reinforcements of six animals. *Significant difference from vehicle-treated group ($P < 0.05$ ANOVA followed by Newman–Keuls test).

unable to modify cocaine intake in rats self-administering cocaine (0.5 mg/kg/inj).

Fig. 5 illustrates an example of the pattern of cocaine self-administration and the effects of WIN 55, 212-2, SR 141716A + WIN 55, 212-2 and SR 141716A throughout the 3-h session in a representative rat. In this figure, each mark represents an infusion of the drug. It may be observed how WIN 55, 212-2 pre-treated rats maintained a regular response pattern, albeit characterized by longer intra-reinforcement

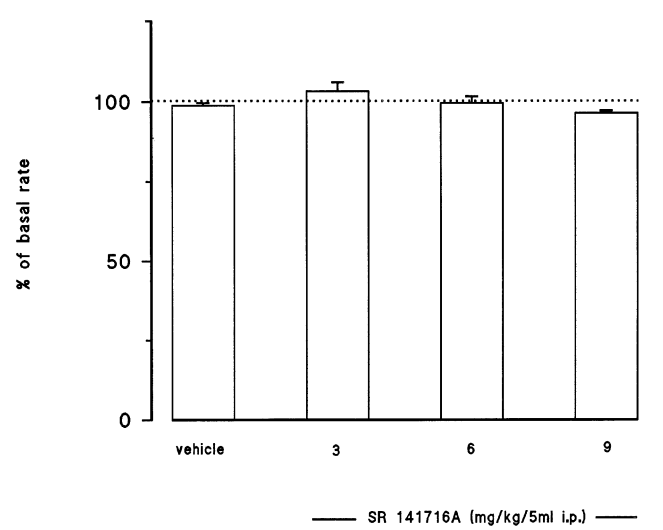

Fig. 4. Effect of intraperitoneal SR 141716A pre-treatment on cocaine 0.5 mg/kg/inj self-administration in rats. Each bar represents mean ± S.E.M. of the percentage of the basal rate of reinforcements of six animals.

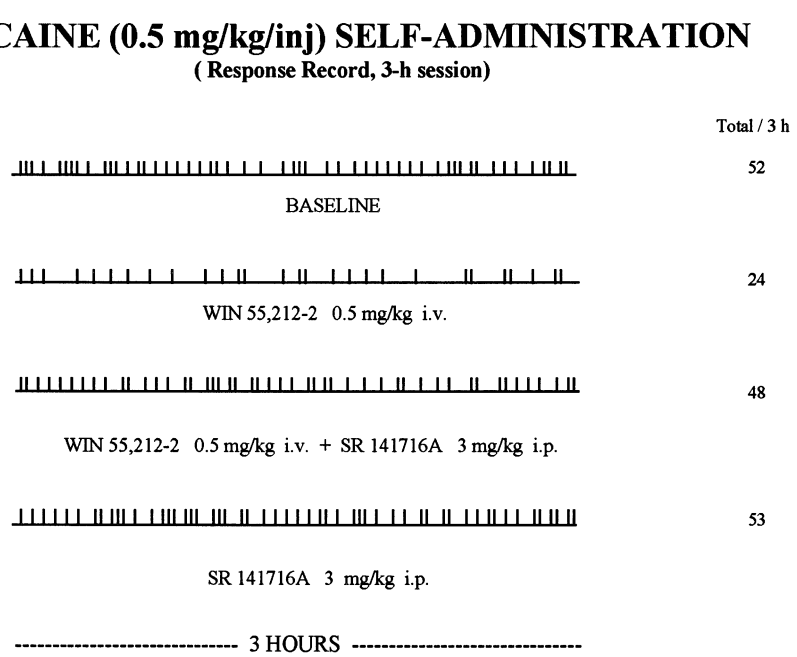

Fig. 5. Pattern of cocaine infusions delivered during self-administration sessions for representative rats pre-treated with WIN 55, 212-2 and/or SR 141716A. Each record represents a separate 3-h session and each small vertical mark represents an intravenous infusion of cocaine 0.5 mg/kg/inj. Pre-treatment of each animal is indicated at the foot of each record, while number of injections per sessions is shown on the right side of each record.

intervals. Patterns of response after WIN 55, 212-2 + SR 141716A and SR 141716A pre-treatment were pratically indistinguishable from those observed at baseline values.

4. Discussion

Results obtained in the present study reveal that pre-treatment with the CB_1 receptor agonist WIN 55, 212-2 alters the i.v. cocaine self-administration in rats, significantly reducing the cocaine intake respect to basal and control vehicle values. This effect was present in rats self-administering the cocaine unit dose of both 0.25 and 0.5 mg/kg/inj, thus indicating that it is independent of cocaine concentration.

It is well-known that rats self-administering drugs at stable levels tend to adjust the dose during the session by modifying the response frequency [31]: a decrease of the responding rate usually occurs when the unit dose of the reinforcer is increased and viceversa. By causing a shift of the cocaine intake, WIN 55, 212-2 pre-treatment seems to mimic the effect of changes in the unit dose of the reinforcer, suggesting a synergistic action of WIN 55, 212-2 on reinforcing properties of cocaine. Furthermore, as can be observed in Fig. 5, pre-treatment with WIN 55, 212-2 specifically increases inter-reinforcement intervals without altering the regularity of the pattern of response. We also excluded the possibility that aspecific sedative effects could be responsible for the decreased cocaine intake since no signs of sedation or motor impairment were observed in these rats. On the other hand, significant sedative effects induced by WIN 55, 212-2 were reported at much higher doses (>2.5 mg/kg) than those used in the present experiments [2,43,50].

The effect of WIN 55, 212-2 on cocaine self-administration was completely reversed by the CB_1 receptor antagonist SR 141716A, indicating that this effect is specifically mediated at CB_1 receptor level.

Pre-treatment with SR 141716A alone, even at high doses, did not modify cocaine intake. This lack of effect by the CB_1 receptor antagonist might indicate that the mechanism regulating cocaine reinforcing effects is not under an endogenous tonic control by cannabinoid systems. Nevertheless, the possibility that stimulation of CB_1 receptors could produce additional effects to cocaine reinforcing properties by acting on the dopaminergic system cannot be ruled out. Accordingly, it has been shown that cannabinoid receptors are localized mainly in the dopaminergic structures of the brain [47,49] and participate in the regulation of dopamine synthesis [3], release [6,10–12,14,20,21,24,30,40,46] and turnover [29].

In conclusion, results obtained in the present study show that despite its specific mechanism, stimulation of CB_1 receptors seems to potentiate cocaine reinforcing effects in cocaine self-administering rats. This positive interaction between cannabinoids and cocaine possibly accounts for the combined taking of marijuana and

cocaine by polydrug users in order to increase the duration of the positive effects of cocaine and to overcome the unpleasant effects which often occur as the initial euphoria dissipates [18,33].

Finally, these data further suggest that cannabinoids may interfere with the brain systems responsible for the expression of both acute reinforcing properties of drugs of abuse and the neuroadaptative changes of the dependence process.

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