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Research report

WIN 55,212-2 decreases the reinforcing actions of cocaine through CB_1 cannabinoid receptor stimulation

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Abstract

CB_1 cannabinoid receptor agonists show a different profile compared to other drugs of abuse on the basis of experimental data that reveal their reinforcing properties. Thus, there are controversial data in the literature concerning the ability of CB_1 receptor agonists to reinforce behavioral responses in experimental animals, i.e. to lower self-stimulation thresholds, and to support self-administration or conditioned place preference. The aim of the present study was to examine the effects of WIN 55,212-2, a potent CB_1 receptor agonist (graded doses 0.1, 0.3, 1 mg/kg, i.p.), on the rewarding efficacy of lateral hypothalamic self-stimulation and on the systemic cocaine-induced potentiation of brain-stimulation reward. WIN 55,212-2 did not affect lateral hypothalamic self-stimulation thresholds both in drug naïve rats and in rats pretreated with the drug, whereas it produced a significant dose-dependent decrease in the maximal rate of responding, i.e. in the performance of the animals. Cocaine (5.0 mg/kg, i.p.) produced a significant reduction in self-stimulation threshold, without altering maximal rates of responding. Importantly, WIN 55,212-2 attenuated the effect of cocaine at the two higher doses tested. The effects of the CB_1 receptor agonist were reversed by pretreatment with the selective CB_1 receptor antagonist SR 141716A (0.02 mg/kg, i.p.) that did not by itself affect cocaine's action. These results indicate that acute stimulation of CB_1 receptors per se does not affect baseline self-stimulation, but reduces the reinforcing effects induced by cocaine. Taken together these findings suggest that cannabinoids may interfere with brain-reward systems responsible for the expression of acute reinforcing properties of drugs of abuse, such as cocaine, and provide evidence that the cannabinoid system could be an interesting drug discovery and development target for the treatment of drug addiction.

Keywords: Intracranial self-stimulation; Cannabinoid; Medial forebrain bundle; Reward; WIN 55,212-2; SR 141716A; Cocaine

1. Introduction

Nearly every drug that humans find pleasurable can be shown to have positive reinforcing properties in animal behavioral models. However, it has been rather difficult to demonstrate the rewarding properties of cannabis and its main psychoactive component Δ^9-tetrahydrocannabinol (THC) in the currently used rodent models of addictive behavior. A number of studies failed to show self-administration of cannabis or THC in rodents or primates [12,27,32,37,58]. However, some reports indicate a facilitation of brain-stimulation reward [23,34] sustained self-administration [55,56] and conditioned place preference [8,33,36,57], by THC in experimental animals.

Experimental research during the past decade has shown the existence of an endogenous cannabinoid system. Since the discovery of the brain cannabinoid$_1$ (CB_1) receptors [41,46], the discovery of synthetic cannabinoid ligands has stimulated research towards the identification of the role of the endogenous cannabinoid system in reward processes as well as in the pathophysiological processes leading to drug abuse. Thus, sustained self-administration of the selective CB_1 receptor agonist WIN 55,212-2 has been reported in drug naïve mice [40] and rats [18], whereas various CB_1 agonists have been shown to establish both place conditioning [4] and place aversion [10,43]. In this context, some recent studies have examined the effects of cannabinoid receptor agonists and antagonists on intracranial self-stimulation behavior [1,14]. Arnold et al. [1] have reported that the CB_1 receptor agonist CP 55,940 did not affect the reinforcing properties of medial forebrain bundle (MFB) self-stimulation, whereas the CB1 receptor antagonist SR

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141716, in higher doses than those used in the present study, appears to decrease the reinforcing efficacy of the stimulation [1,14].

Recent evidence suggests that the endogenous cannabinoid system may play a key role in determining the reinforcing effects of not only cannabis, but of other drugs of abuse as well. Cannabinoids may, thus, influenc the sensitivity to other addictive drugs, e.g. psychostimulants. Indeed, chronic treatment with THC or the CB_1 receptor agonist WIN 55,212-2 enhances the locomotor response to amphetamine and heroin [30,51], while pretreatment with heroin produces cross-sensitization to WIN 55,212-2 [52]. Acute administration of the cannabinoid agonist HU 210 potently counteracted acute and subchronic cocaine induced hyperlocomotion [20]. Similarly, THC has been shown to antagonize the hyperlocomotion produced by cocaine and amphetamine [53]. In addition, repeated administration of the CB_1 receptor agonist CP 55,940 did not sensitize locomotor activity in the same way as cocaine, while pre-exposure to CP 55,940 did not enhance sensitivity to the behavioral effects of cocaine and co-administration of CP 55,940 with cocaine reduced the locomotor hyperactivity produced by the psychostimulant [2]. Furthermore, the acquisition of conditioned place preference induced by cocaine, morphine or food was blocked by pre-pairing administration of the CB_1 receptor antagonist SR 141716A [8], whereas the CB_1 receptor agonist WIN 55,212-2 has been shown to decrease cocaine self-administration in rats [19]. Recently, an important role of the cannabinoid system in the neuronal processes underlying relapse to cocaine seeking was revealed using selective CB_1 receptor agonists and antagonists [15]. A recent study by Braida and Sala [6] has confirme that the combination of CP 55,940 with MDMA reduced the number of drug-associated lever pressings compared to the single drug injections.

In the present study, we investigated the role that the CB_1 receptors might play in reward. To this end, we examined (a) the effect of WIN 55,212-2, a potent CB_1 receptor agonist, on the rewarding efficacy of lateral hypothalamic self-stimulation (in drug naïve rats as well as in rats pretreated with the drug) and on the reward-facilitating action of cocaine; and (b) whether the selective CB_1 receptor antagonist SR 141716A can reverse any observed actions of WIN 55,212-2.

2. Methods

2.1. Animals and surgery

Male Sprague–Dawley rats weighing 300–350 g at the time of surgery were used. Before surgery they were housed in groups of three under a 12:12-h light–dark cycle with free access to food and water. The animals were anaesthetized with ketamine hydrochloride (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.). Atropine sulphate (0.6 mg/kg, i.m.) was injected to reduce bronchial secretion. Moveable monopolar stimulating electrodes (Model SME-01, Kinetrods, Ottawa, Ont., Canada) were lowered into the MFB at the level of lateral hypothalamus (coordinates: AP, −2.5 mm from bregma; L, −1.7 mm from the midline; VD, −8.0 mm from a fla skull), according to Paxinos and Watson [50].

The electrodes consisted of a plastic guiding base and a 0.25 mm diameter moveable stainless-steel wire, which were insulated with Epoxylite except from the conically shaped tip. The anode was an Amphenol pin connected to fi e miniature skull screws. Following implantation and for the entire duration of the experiments, the animals were housed individually.

Animal care and the procedures used were in accordance with NIH public document 85-23 (1985).

2.2. Apparatus and procedures for self-stimulation

One week after surgery, the animals were tested for self-stimulation in an operant chamber that was made of transparent Plexiglas (25 cm wide, 25 cm deep, 30 cm high). A stainless-steel rodent lever protruded 2 cm from the left wall at a height of 4 cm from the floor. Each bar-press triggered a constant current generator that delivered a 0.4-s train of rectangular cathodal pulses of constant duration (0.1 ms) and intensity (250 μA) and variable frequency (15–100 Hz, i.e. 6–40 number of pulses/0.4 s). The pulse frequency, i.e. the number of pulses within a train, was progressively increased up to 40 per stimulation train until the subject showed vigorous self-stimulation. If the implantation site failed to support self-stimulation, the electrode was lowered by steps of 0.16 mm (one step every 24 h), until a self-stimulation site was found. The electrode position was held unchanged in all subsequent testing. The animals were then trained to self-stimulate for at least 3 consecutive days (1 h daily), using stimulation parameters that maintained near maximal bar-pressing rates. The animals were subsequently trained to self-stimulate using four alternating series of ascending and descending pulse frequencies. The pulse frequency was varied by steps of approximately 0.1 log units. Each frequency was tested within trials of 60 s in duration, followed by an extinction period of 30 s. At the beginning of each trial, the animals received three trains of priming stimulation, at the frequency of the stimulation which was available for that trial. Thus, drug-induced changes in the rewarding efficacy of self-stimulation were inferred using the rate–frequency curve shift method. This procedure has enabled us to distinguish between reward and performance, while allowing quantification of the drug effect [38,39,44].

2.3. Drugs

Cocaine hydrochloride (National Monopoly for Narcotics, Ministry of Health, Greece) (5 mg/kg, i.p.) was dissolved in 0.9% saline and injected intraperitoneally (i.p.) at a volume

of 1 ml/kg of body weight. WIN 55,212-2 (Tocris) and SR 141716A (synthesized by Lilly Research Laboratories) were dissolved in a solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL in 0.9% NaCl and injected i.p. at a volume of 3 ml/kg of body weight.

2.4. Experimental procedures

Drug testing began for each animal when the function relating bar-pressing rate to pulse frequency (the rate–frequency function) was stable for at least 3 consecutive days. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log units. Each drug or vehicle self-stimulation test consisted of a baseline and a drug rate–frequency function determination (for 45 min each). Following the baseline period, each animal was injected with the drug or its vehicle.

2.4.1. Experiment 1: effects of systemically administered WIN 55,212-2 on brain-stimulation reward in drug-naïve rats

Six rats received multiple doses of WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) or its vehicle. The sequence of injections for the different drug doses was counterbalanced with respect to order and a 3-day period was allowed between injections.

2.4.2. Experiment 2: effects of systemically administered WIN 55,212-2 on brain-stimulation reward in rats pretreated with the drug

Four rats were pretreated in the home cage with WIN 55,212-2 (1 mg/kg) for 3 consecutive days before drug testing to examine whether repeated administration affected the reinforcing effects of the drug. Then, the subjects received multiple doses of WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) or its vehicle. The sequence of injections for the different drug doses was counterbalanced with respect to order and a 3-day period was allowed between injections.

2.4.3. Experiment 3: effects of WIN 55,212-2 on the cocaine-induced lowering of brain-reward thresholds

Eight rats received various doses of WIN 55,212-2 (0, 0.1, 0.3, 1 mg/kg, i.p.) followed 5 min later by cocaine (5.0 mg/kg, i.p.). The sequence of injections for the different drug doses was counterbalanced with respect to order and a 3-day period was allowed between injections.

2.4.4. Experiment 4: reversal of the action of WIN 55,212-2 on the cocaine-induced lowering of brain-reward thresholds by pretreatment with the CB_1 receptor antagonist SR 141716A

Nine rats received a combination treatment of SR 141716A (0, 0.02, 0.3 mg/kg, i.p.) and WIN 55,212-2 (0.1 mg/kg, i.p.) followed 5 min later by cocaine (5.0 mg/kg, i.p.), with a minimum of 3 days between consecutive combination drug treatments. The sequence of injections for the different drug doses was counterbalanced with respect to order.

2.5. Data analysis and statistical treatment

Data gathered from pre- and post-injection portions of each session were curve fitted and threshold and asymptote estimates were obtained using the Gompertz sigmoid model [13]:

$$f(x) = \alpha\, e^{-e^{b(x_i - x)}}$$

In this equation, α represents the maximum rate (asymptote), whereas x_i (x at inflection represents the threshold frequency. The latter is the pulse number producing 36.7% of the asymptotic rate, i.e. the rate lying on the fastest-accelerating region of the curve. Parameter b represents an index of the slope whereas e is the base of natural logarithms.

The post-treatment threshold and asymptote values were expressed as percentage of pre-drug values. The results were statistically evaluated by using one-way analysis of variance (ANOVA) followed, whenever appropriate, by LSD test.

2.6. Histology

At the end of the experiment, the animals were given a lethal dose of sodium pentothal. The location of the terminal stimulation site was then marked according to the following procedure: a direct anodal current of 0.1 mA and 15-s duration was passed through the electrode tip. The animals were perfused intracardially with saline 0.9%, which was followed by a 50 ml solution of potassium ferrocyanide (3%), potassium ferricyanide (3%), and trichloroacetic acid (0.5%) in 10% formalin. The brains were then removed and stored in 10% formalin for 3 days, and 30% sucrose solution for 2 days. Finally, the brains were sliced in a cryostat microtome and the sections containing the electrode tract were mounted on slides and stained with cresyl violet. Only the rats in which tracks from the electrode were verified to be located in the MFB were included in this study.

3. Results

Electrode tips were examined in all animals tested in this study. The location of the stimulating electrodes within the MFB at the level of the lateral hypothalamus is illustrated in Fig. 1.

3.1. Experiment 1: effects of systemically administered WIN 55,212-2 on brain-stimulation reward in drug-naïve rats

The changes of self-stimulation threshold and asymptotic rate of responding after acute administration of the CB_1 receptor agonist WIN 55,212-2 are presented in Fig. 2A

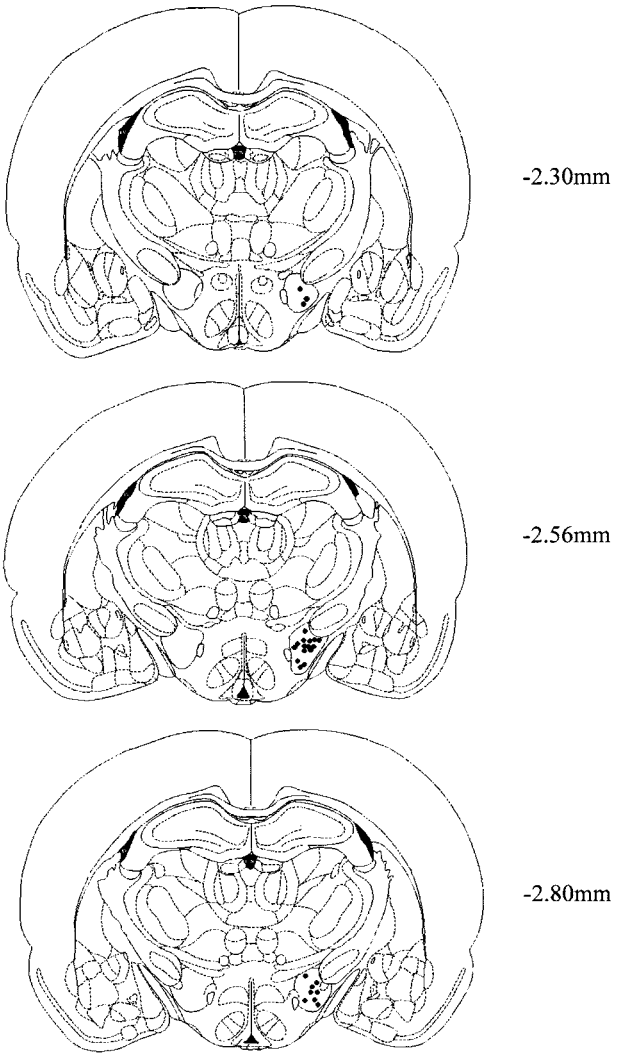

Fig. 1. Histological localization of the electrode tips aimed at the lateral hypothalamic level of the MFB. The reconstructions are based on the stereotaxic atlas of [50]. The numbers beside each section indicate the distance (mm) from bregma.

and B, respectively. WIN 55,212-2 did not significantl affect self-stimulation thresholds ($F(3, 23) = 1.626$, $P = 0.215$) at any of the doses tested. However, it altered significantly the asymptotic rate of responding between the groups ($F(3, 23) = 4.402$, $P = 0.016$). Post-hoc analysis with the LSD test showed that the group receiving 1 mg/kg of WIN 55,212-2 had significantl lower bar-pressing rate. These finding indicate that WIN 55,212-2 did not affect the rewarding efficacy of the stimulation but rather decreased the performance of the animals.

3.2. Experiment 2: effects of systemically administered WIN 55,212-2 on brain-stimulation reward in rats pretreated with the drug

The changes of self-stimulation threshold and asymptotic rate of responding after administration of the CB_1

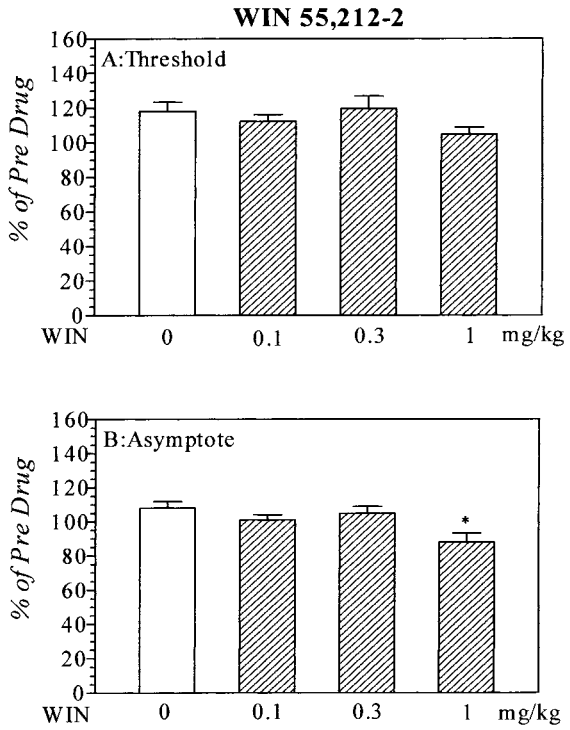

Fig. 2. Changes in self-stimulation threshold (A) and asymptotic rate (B) of responding (expressed as percentage of pre-drug values) following acute vehicle or WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) administration. Vertical bars represent the standard errors of the mean. The asterisk (*) signifie an ICSS asymptote value significantl different from the control condition.

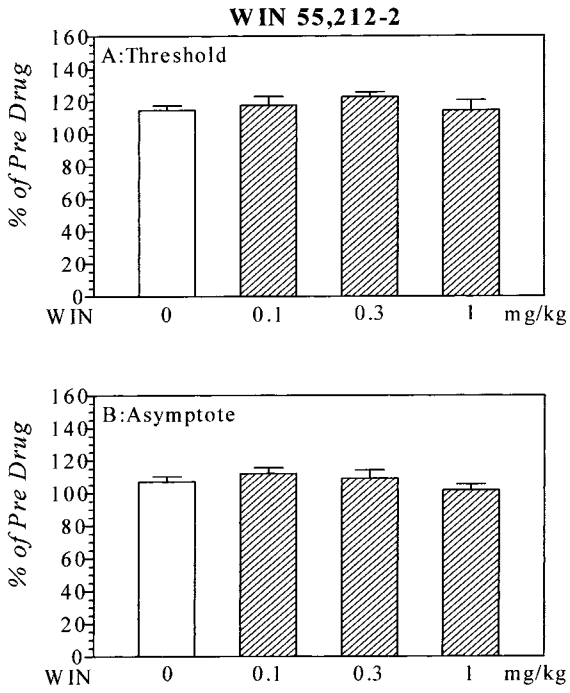

Fig. 3. Changes in self-stimulation threshold (A) and asymptotic rate (B) of responding (expressed as percentage of pre-drug values) following acute vehicle or WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) administration, in animals pretreated with the drug. Vertical bars represent the standard errors of the mean.

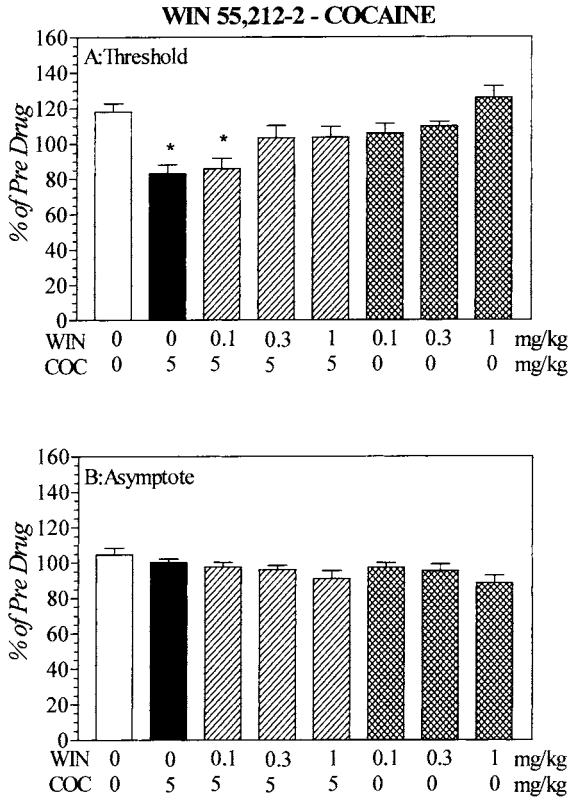

Fig. 4. Changes in self-stimulation threshold (A) and asymptotic rate (B) of responding (expressed as percentage of pre-drug values) following WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) or its vehicle and cocaine (5 mg/kg, i.p.) administration. Vertical bars represent the standard errors of the mean. The asterisk (*) signifie an ICSS threshold value significantl different from the control condition.

receptor agonist WIN 55,212-2 in rats pretreated with the drug are presented in Fig. 3A and B, respectively. WIN 55,212-2 did not significantl affect self-stimulation thresholds ($F(3, 15) = 0.733$, $P = 0.552$), or asymptotic rates of responding ($F(3, 15) = 1.195$, $P = 0.353$) at any of the doses tested.

3.3. Experiment 3: effects of WIN 55,212-2 on the cocaine-induced lowering of brain-reward thresholds

Fig. 4 shows the changes in self-stimulation threshold and asymptotic rate of responding after i.p. administration of WIN 55,212-2 or its vehicle and cocaine. Cocaine produced a significan decrease in self-stimulation threshold. Pretreatment with WIN 55,212-2 significantly blocked this facilitation ($F(7, 55) = 6.252$, $P < 0.05$). Post-hoc analysis with the LSD test showed that this effect was significan at the doses of 0.3 (WIN 55,212-2 0.3 mg + cocaine) and 1 mg/kg (WIN 55,212-2 1 mg + cocaine) as compared to the groups receiving either vehicle or cocaine alone. There were no significant differences in the asymptotic rate of responding between the different groups ($F(7, 55) = 2.103$, $P = 0.061$).

3.4. Experiment 4: reversal of the action of WIN 55,212-2 on the cocaine-induced lowering of brain-reward thresholds by pretreatment with the CB_1 receptor antagonist SR 141716A

It was found that the CB_1 receptor antagonist SR 141716A (0.02 mg/kg) by itself did not affect self-stimulation thresholds, while completely reversed the inhibitory action of WIN 55,212-2 (1 mg/kg) on the cocaine-induced lowering of brain-reward thresholds ($F(7, 71) = 10.893$, $P < 0.05$; Fig. 5A). Co-administration of SR 141716A (0.02 mg/kg) with WIN 55,212-2 (1 mg/kg) reduced maximal rates of responding compared to the vehicle group or the SR 141716A (0.02 mg/kg) + vehicle group ($F(7, 71) = 3.273$, $P = 0.005$; Fig. 5B).

Fig. 6 depicts rate–frequency functions from representative animals obtained before and after administration of various treatment combinations of cocaine, WIN 55,212-2 and SR 141716A. As indicated in the figure the vehicle+cocaine treatment produced a parallel curve shift to the left, indicating a clear increase in the rewarding efficacy of the stimulation. On the other hand, the WIN 55,212-2 + cocaine

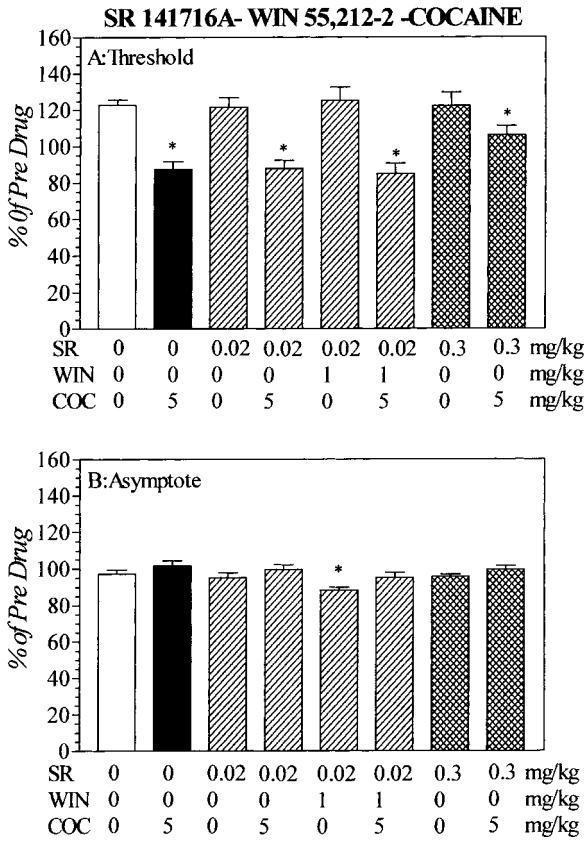

Fig. 5. Changes in self-stimulation threshold (A) and asymptotic rate (B) of responding (expressed as percentage of pre-drug values) following WIN 55,212-2 (0.1, 0.3, 1 mg/kg, i.p.) or its vehicle and SR 141716A (0.02, 0.3 mg/kg, i.p.) or its vehicle and cocaine (5 mg/kg, i.p.) administration. Vertical bars represent the standard errors of the mean. The asterisk (*) signifie an ICSS threshold or asymptote value significantl different from the control condition.

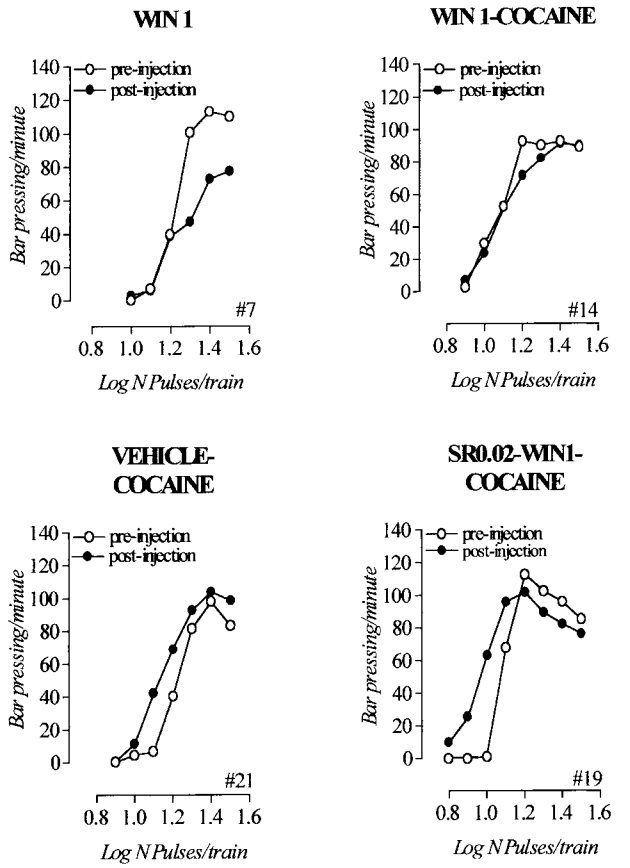

Fig. 6. Rate–frequency functions (rate of lever pressing as a function of stimulation frequency) taken from representative animals before and after administration of various treatment combinations of cocaine, WIN 55,212-2 and SR 141716A. Each plot represents data from a single animal under pre-drug and drug conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing.

treatment produced no effect on MFB stimulation reward, blocking the potentiating effect of cocaine. This effect was reversed by co-administration of SR 141716A. Acute administration of WIN 55,212-2 did not produce a parallel shift in the rate–frequency function, but rather a vertical shift, which indicates a decrease in the motor/performance capacity of the animal.

4. Discussion

The major findin of the present study is that acute stimulation of CB_1 receptors per se does not affect baseline self-stimulation, but reduces the reward-facilitating effect of systemic cocaine. CB_1 receptor stimulation was induced by WIN 55,212-2, which by itself was ineffective in altering brain-stimulation reward thresholds. The effects of the CB_1 receptor agonist were completely reversed by pretreatment with the selective CB_1 receptor antagonist SR 141716A in a dose that did not affect cocaine's action. This indicates that the action of WIN 55,212-2 is specifically mediated through CB_1 receptor stimulation.

Lateral hypothalamic brain stimulation is thought to be rewarding because it activates a brain circuit that normally mediates the reinforcing effects of natural rewards, such as food and sexual contact (for a review, see [3,16,21,59]). A basic property of drugs of abuse is that they increase the rewarding efficacy of LH self-stimulation, an effect reflecte in the shift of the rate–frequency function to the left (for review, see [60,61]). There is strong evidence, that the presently used intracranial self-stimulation paradigm provides reward-threshold estimates that are unaffected by performance effects of drug treatments or other experimental manipulations [17,38,39,45]. This is also evident in the present study. Thus, the reductions in self-stimulation thresholds produced by cocaine were not accompanied by significan changes in asymptotic rates of responding.

The present results are consistent with previous studies that showed that the synthetic cannabinoid receptor agonists levonantradol and CP 55,940 [1,29] did not affect brain-stimulation reward thresholds. However, the present results contradict previous studies by Gardner and colleagues [23,34], showing that THC increases the rewarding efficacy of self-stimulation. These seemingly contrasting results could be attributed to differences in the pharmacological properties and the dose range of the compounds tested, the strain of the animals used and the methods followed. For example Lepore et al. [34] found the most pronounced action of THC in Lewis rats, while in Sprague–Dawley rats, used also in the present study, the effect was marginal.

Controversial data also exist in the literature concerning the ability of WIN 55,212-2 to support self-administration or to produce conditioned place preference. WIN 55,212-2 has been shown to establish a robust place aversion in rats and this aversive effect was antagonized by SR 141716A [8]. However, two recent studies reported intravenous self-administration of WIN 55,212-2 in mice and rats [18,40]. Interestingly, divergent results on reward and reinforcement of behavior have also been reported with other synthetic cannabinoids [4,5,10,43].

As is the case with various other addictive drugs, systemic cocaine decreased lateral hypothalamic stimulation reward thresholds and caused parallel leftward shifts in the rate–frequency functions (see Figs. 4–6). In other words, cocaine reduced the amount of stimulation necessary to sustain responding at a given criterion level (see [44]), increasing the rewarding efficacy of brain stimulation. This effect of cocaine is consistent with other studies that have examined its actions on self-stimulation elicited from MFB or other nuclei [11,35,42,48,49,54]. The fact that WIN 55,212-2 attenuated the potentiating effect of cocaine in lateral hypothalamic self-stimulation is not entirely unexpected. Several studies have reported an interaction between cannabinoids and other drugs of abuse, including cocaine [4–7,15,19,22,25,26,30,47,51,52]. Importantly, it has been shown that WIN 55,212-2 decreases intravenous cocaine

self-administration in rats [19]. However, it is worth noting that the synthetic cannabinoid agonist HU 210 provokes relapse to cocaine seeking in rats, after prolonged withdrawal periods [15]. The mechanism regulating cocaine's reinforcing effect might, therefore, be influence by the endogenous cannabinoid system. The precise mechanism by which cocaine and cannabinoids interact remains elusive. Cocaine increases dopaminergic neurotransmission. On the other hand, cannabinoids also affect dopaminergic neurotransmission [24]. The recently reported co-expression of CB_1 receptors with dopamine receptors in forebrain regions [28] raises the possibility that stimulation of cannabinoid receptors may lead to activation of a feedback mechanism that blunts and ultimately neutralizes the perception and expression of the incentive value of positive reinforcers, which in the case of drugs of abuse leads to pharmacological dependence.

The effects of WIN 55,212-2 were completely reversed by pretreatment with the selective CB_1 receptor antagonist SR 141716A at a dose that by itself did not affect cocaine's action. This indicates that the action of WIN 55,212-2 is specificall mediated at the CB_1 receptor level. Interestingly, administration of SR 141716A even at a higher dose (i.e. 0.3 mg/kg) did not significantl affect baseline self-stimulation, although there was a clear tendency for a decrease in the ICSS potentiating effect of cocaine. This dose-dependent effect of SR 141716A could be due to the inverse agonistic properties of the drug [31].

In order to develop tolerance to putative aversive/depressant effects of WIN 55,212-2 that could prevent its reinforcing properties, a group of rats was pretreated for 3 days with WIN 55,212-2 (1 mg/kg, i.p.). However, WIN 55,212-2 did not affect brain-reward thresholds in these animals. Interestingly, neither the asymptotic rate of responding was decreased in that group of animals, as was the case with the naïve animals in the firs experiment. Thus, while acute WIN 55,212-2 reduces the asymptotic rate of responding, repeated exposure results in tolerance to this motor depressant effect. This phenomenon has been also reported by others in the literature (for a review see [9]), and may be due to the reported downregulation of the CB_1 receptors after chronic exposure to the cannabinoid agonist.

In summary, the present study clearly shows that acute stimulation of CB_1 receptors per se does not affect baseline self-stimulation, but reduces the reinforcing effects induced by cocaine. The present results suggest that cannabinoids may interfere with brain-reward systems responsible for the expression of acute reinforcing properties of drugs of abuse, such as cocaine, and provide evidence that the cannabinoid system could be a target of interest for drug discovery and development efforts for the treatment of drug addiction.

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