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ORIGINAL INVESTIGATION

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CB_1 cannabinoid receptor agonists increase intracranial self-stimulation thresholds in the rat

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Abstract *Rationale:* Addictive drugs have a number of commonalities in animal behavioral models. They lower intracranial self-stimulation (ICSS) thresholds, support self-administration, and produce conditioned place preference (CPP). However, cannabinoids appear atypical as drugs of abuse, since there are controversial data in the literature concerning their reinforcing properties. *Objectives:* The aim of the present study was to examine the effects of cannabinoids on brain reward using the rate–frequency curve shift paradigm of ICSS. *Methods:* Male Sprague–Dawley rats were implanted with electrodes into the medial forebrain bundle (MFB). Rate–frequency functions were deterined by logarithmically decreasing the number of cathodal pulses in a stimulation train from a value that sustained maximal responding to one that did not sustain responding. After brain stimulation reward thresholds stabilized rats received intraperitoneal (IP) injections of the potent CB_1 receptor agonists WIN 55,212-2 (graded doses 0.1, 0.3, 1 and 3 mg/kg), CP 55,940 (graded doses 10, 30, 56 and 100 μg/kg), or HU-210 (graded doses 10, 30, 100 μg/kg). *Results:* With the exception of the highest dose of all cannabinoid agonists tested, which significantly increased the threshold frequency required for MFB ICSS, all other doses of the tested drugs did not affect ICSS thresholds. The CB_1 receptor antagonist SR141716A reversed the actions of WIN 55,212-2 and CP 55,940, but not HU-210. However, the selective CB_1 cannabinoid receptor antagonist AM 251 counteracted the effect of HU-210. Both CB_1 receptor antagonists, at the doses used in the present study, did not affect reward thresholds by themselves. *Conclusions:* The present results indicate that cannabinoid agonists do not exhibit reinforcing properties in the ICSS paradigm, but rather have an inhibitory influence on reward mechanisms. The results suggest that the anhedonic effects of cannabinoids are probably mediated by cannabinoid CB_1 receptors.

Keywords Intracranial self-stimulation · Cannabinoid · Medial forebrain bundle · Reward · WIN 55,212-2 · CP 55,940 · HU-210 · SR141716A · AM 251

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Introduction

Abused substances such as cocaine, amphetamine and morphine, which produce euphoric states in humans, are believed to interact with reinforcement mechanisms by actions on certain brain areas and circuits, which are collectively known as brain reward systems (Di Chiara et al. 1993; Wise 1996, 1998). Since the discovery of self-stimulation, this behavioral paradigm has been used extensively to study the neuroanatomical and neurochemical substrates of reward (Fibiger and Phillips 1986; Wise and Rompré 1989; Wise 1996). The reinforcing effects of brain stimulation are thought to involve the same reward circuitries as the reinforcing effects of addictive drugs (Wise 1980, 1996, 1998). Indeed, drugs with reinforcing properties in animals and addictive potential in humans tend to facilitate the reinforcing effects of brain stimulation in rats, i.e. to lower thresholds for rewarding brain stimulation shifting to the left the function that relates response strength to stimulation strength (for reviews see, Stellar and Rice 1989; Wise 1996, 1998). On the other hand, drugs that antagonize brain stimulation reward increase thresholds for rewarding brain stimulation shifting the function to the right when administered alone, and also block the ability of abused drugs to cause leftward shifts (Fouriezos and Wise 1976; Fouriezos et al. 1978; Franklin 1978; Wasserman et al. 1982; Wise 1982; Gallistel and Karras 1984; Gallistel and Freyd 1987; Rompré and Wise 1989; Wise et al. 1998; Panagis et al. 2000; Harrison et al. 2001). Therefore, the intracranial self-

stimulation (ICSS) paradigm allows clear dissociation and quantification of the reward-potentiating and reward-inhibiting effects of a drug and it is well suited for assessing reward-related drug interactions.

Δ^9-Tetrahydrocannabinol (THC) is the main psychoactive constituent of marijuana, the most widely consumed recreational illicit drug (Adams and Martin 1996). Over the past decade we have seen significant advances in cannabinoid pharmacology by the discovery and characterization of two cannabinoid receptors (Matsuda et al. 1990; Munro et al. 1993) the discovery of endogenous cannabinoid ligands (Devane et al. 1992; Mechoulam et al. 1995; Hanus et al. 2001; Huang et al. 2002; Porter et al. 2002) and the development of new synthetic cannabinoid agonists and antagonists (Compton et al. 1992; Rinaldi-Carmona et al. 1994; Pertwee 1999; Palmer et al. 2002). Thus it is now evident that there exists within the brain a cannabinoid system, which is involved or modulates nociception, motor function, memory and appetite (Chaperon and Thiébot 1998; Ameri 1999; Porter and Felder 2001; Iversen 2003; Tanda and Goldberg 2003; Grotenhermen 2004). Synthetic cannabinoid compounds, that have higher affinities for cannabinoid receptors than THC, can be used to study cannabinoid pharmacology as well as to assess the possible therapeutic potential of cannabinoids. However, the interest in therapeutic applications of cannabinoids has been restrained by the fear of a potentially harmful abuse liability.

The rewarding properties of THC and various cannabinoid agonists in the currently used rodent models are controversial. A number of studies failed to show self-administration of cannabis or THC in rodents or primates (Corcoran and Amit 1974; Harris et al. 1974; Leite and Carlini 1974; Van Ree et al. 1978; Mansbach et al. 1994). However, some reports indicate facilitation of brain stimulation reward (Gardner et al. 1988; Lepore et al. 1996), sustained self-administration (Takahashi and Singer 1979; Tanda et al. 2000; Justinova et al. 2003) and conditioned place preference (CPP) (Lepore et al. 1995; Valjent and Maldonado 2000) by THC in experimental animals. Similarly, sustained self-administration of the selective CB_1 receptor agonist WIN 55,212-2 has been reported in drug naive mice (Martellotta et al. 1998) and rats (Fattore et al. 2001), whereas various CB_1 agonists have been shown to establish both place conditioning (Braida et al. 2001a) and place aversion (McGregor et al. 1996; Sañudo-Pena et al. 1997; Chaperon et al. 1998; Mallet and Beninger 1998; Cheer et al. 2000; Robinson et al. 2003) or taste aversion (Elsmore and Fletcher 1972; Hunt and Amit 1987; Parker and Gillies 1995; McGregor et al. 1996). Arnold et al. (2001) 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 CB_1 receptor antagonist SR141716, in higher doses than those used in the present study, appears to decrease the reinforcing efficacy of the stimulation (Arnold et al. 2001; Deroche-Gamonet et al. 2001). Interestingly, recently we have shown that the CB_1 receptor agonist WIN 55,212-2, in a dose range from 0.1 mg/kg to 1 mg/kg does not affect baseline self-stimulation, but reduces the reinforcing effects induced by cocaine. WIN 55,212-2, also, does not affect brain stimulation reward in rats pre-treated with the drug (Vlachou et al. 2003).

The aim of the present study was to further investigate the influence of the endogenous cannabinoid system on reinforcement processes. In particular, we studied the effects of the selective CB_1 receptor agonists WIN 55,212-2, CP 55,940 and HU-210 on reward, using the ICSS paradigm. Since most of the central mediating effects of THC and cannabinoid agonists are reversed or attenuated by selective CB_1 receptor antagonists, such as SR141716, we also studied the ability of such agents to counteract the effects of CB_1 receptor agonists on brain stimulation reward. Unequivocally, ICSS behavior has the advantage of not being affected by satiation (factor) or dysphoric effects, which are potentially modulated by cannabinoids. On the other hand, since THC and cannabinoid agonists disrupt motor activity/performance capacity in a dose–response manner (Stark and Dews 1980; Chaperon and Thiébot 1998; Romero et al. 2002; Iversen 2003), the use of a rate-free, reward selective measure, like the curve-shift, was requisite. Thus, the effects of the different drugs used on the rewarding efficacy of self-stimulation were inferred using the curve-shift paradigm. This rate-independent method enabled us to distinguish between reward and performance, while allowing quantification of the drug effect (Liebman 1983; Miliaressis et al. 1986; Markou and Koob 1992, 1993).

Materials and methods

Animals and surgery

Male Sprague–Dawley rats (n=140) 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, IM) and xylazine (10 mg/kg, IM). Atropine sulphate (0.6 mg/kg, IM) was injected to reduce bronchial secretion. Moveable monopolar stimulating electrodes (Model SME-01, Kinetrods, Ottawa, Ontario, 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 from a flat skull), according to Paxinos and Watson (1998).

The electrodes consisted of a plastic guiding base and a 0.25 mm diameter moveable stainless-steel wire, which were insulated with Epoxylite except for the conically shaped tip. The anode was an Amphenol pin connected to five 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).

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. During the acquisition phase the animals were trained to self-stimulate for at least 3 consecutive days (1 h daily), using stimulation parameters that maintained near maximal bar-pressing rates. After self-stimulation has been acquired and stabilized for a given pulse frequency, animals were 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. A rate–frequency determination lasted about 45 min. One rate–frequency curve was established daily, for 10–12 days, depending on the period when the self-stimulation indices (i.e. curve shift and threshold measure) were stable.

Drugs

WIN 55,212-2 (Tocris), CP 55,940 (Tocris), HU-210 (Tocris), SR141716A (synthesized by Lilly Research Laboratories) and AM 251 (Tocris) were dissolved into a vehicle solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL and 90% of 0.9% NaCl and injected intraperitoneally (IP) at a volume of 3 ml/kg body weight. The doses of the cannabinoid compounds tested are within the range of doses regularly used in a plethora of functional studies (see, for example, Chaperon and Thiébot 1998).

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. The animals were tested 10 min after the last injection. This time interval has also been used in self-stimulation studies with other drugs of abuse (see, for example, Maldonado-Irizarry et al. 1994; Ranaldi and Beninger 1994; Vlachou et al. 2003), and appears to be critical for the observation of other behavioral and physiological effects of cannabinoids (see original studies in, e.g. Chaperon and Thiébot 1998). In the present study we used a mixed design, i.e. some animals received only one treatment, whereas other animals received all doses for only one drug-treatment tested. In this case, the sequence of injections for the different drug doses was counterbalanced with respect to order and a 3-day period was allowed between injections. As we have observed in a previous study (Vlachou et al. 2003), this period is considered sufficient for the behavior of the animals to return to stable, pre-treatment levels, and not being affected by prior cannabinoid administration, i.e. no carry-over effects of the cannabinoids were detected.

Study 1

Experiment 1: effects of systemically administered WIN 55,212-2 on brain stimulation reward in drug-naive rats Nineteen rats were used. Four of them received all doses of WIN 55,212-2 (0.1, 0.3, 1, 3 mg/kg, IP) or its vehicle in a randomized order, while 15 received only one drug treatment.

Experiment 2: reversal of the action of WIN 55,212-2 by the CB_1 receptor antagonist SR141716A Twenty-three rats were used. Seven of them received all different doses of SR141716A (0, 0.02, 0.3, 1 mg/kg, IP) followed 5 min later by WIN 55,212-2 (3 mg/kg, IP) or its vehicle in a randomized order, while 16 received only one combination of SR141716A and WIN 55,212-2.

Study 2

Experiment 1: effects of systemically administered CP 55,940 on brain stimulation reward in drug-naive rats Twenty rats were used. Five of them received all doses of CP 55,940 (10, 30, 56, 100 μg/kg, IP) or its vehicle, in a randomized order, while 15 received only one drug treatment.

Experiment 2: reversal of the action of CP 55,940 by the CB_1 receptor antagonist SR141716A Fifteen rats were used. Three of them received all different doses of SR141716A (0, 0.02 mg/kg, IP) followed 5 min later by CP 55,940 (100 μg/kg, IP) or its vehicle in a randomized order, while 12 received only one combination of SR141716A and CP 55,940.

Study 3

Experiment 1: effects of systemically administered HU-210 on brain stimulation reward in drug-naive rats Sixteen rats were used. Four of them received all doses of HU-210 (10, 30, 100 μg/kg, IP) or its vehicle, while 15 received only one drug treatment.

Experiment 2: effects of the CB_1 receptor antagonist SR141716A on HU-210 Twenty-six rats were used. Six of them received all different doses of SR141716A (0, 0.02, 0.3, 1, 3 mg/kg, IP) followed 5 min later by HU-210 (100 μg/kg, IP) or its vehicle in a randomized order, while 20 received only one combination of SR141716A and HU-210.

Experiment 3: reversal of the action of HU-210 by the CB_1 receptor antagonist AM 251 Twenty-one rats were used. Three of them received all different doses of AM 251 (0, 1, 3 mg/kg, IP) followed 5 min later by HU-210 (100 μg/kg, IP) or its vehicle in a randomized order, while 18 received only one combination of AM 251 and HU-210.

Data analysis and statistics

Data gathered from pre-injection and post-injection portions of each session were curve fitted and threshold and asymptote estimates were obtained using the Gompertz sigmoid model (Coulombe and Miliaressis 1987):

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

In this equation, α represents the maximum rate (asymptote), whereas *xi* (*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 (effects of cannabinoid agonists alone) or two-way (effects of combined administration of cannabinoid agonists and antagonists) analyses of variance (ANOVA) followed, whenever appropriate, by the LSD test for multiple contrasts.

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%) and trichloroacetic acid (0.5%) in 10% formalin. The brains were then removed and stored in 10% formalin for 3 days, and 2 days in a 30% sucrose solution. 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

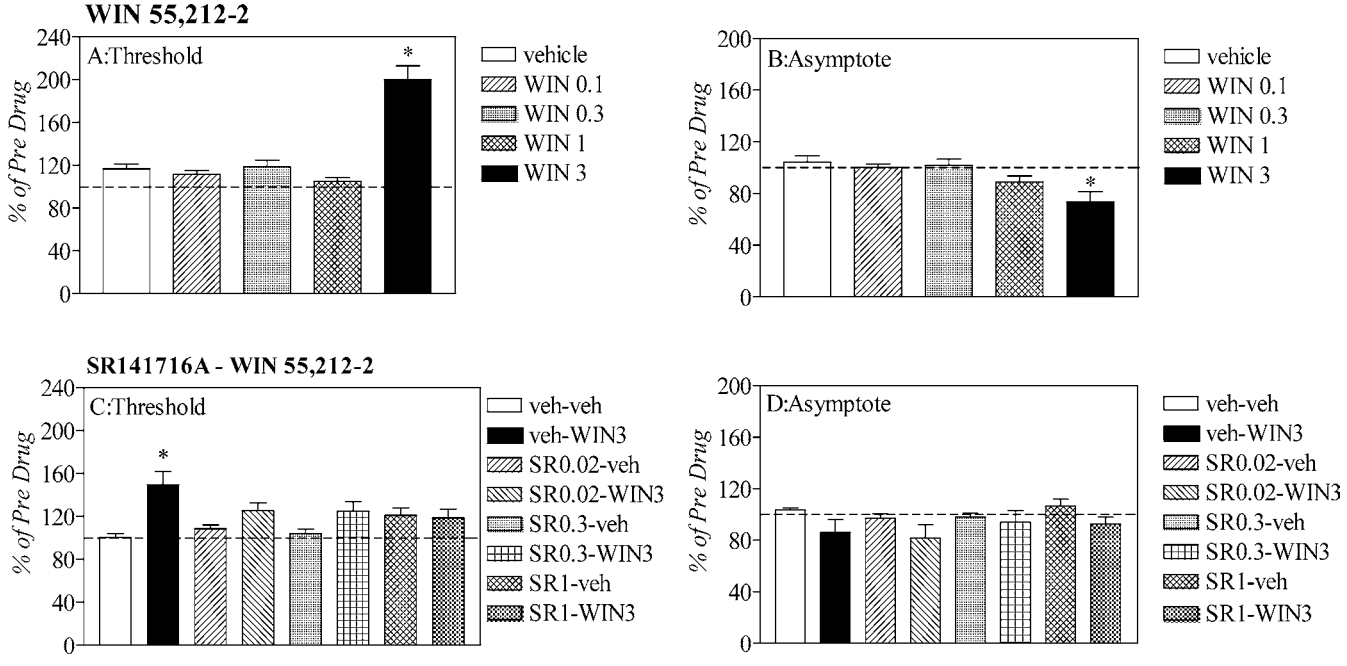

Fig. 1 Changes in self-stimulation threshold **a**,**c** and asymptotic rate **b**,**d** of responding (expressed as percentage of pre-drug values) following WIN 55,212-2 (0, 0.1, 0.3, 1, 3 mg/kg, IP) and SR141716A (0, 0.02, 0.3, 1 mg/kg, IP)+WIN 55,212-2 (0, 3 mg/kg, IP) treatments. *Vertical bars* represent the standard errors of the mean. *Asterisk* signifies an ICSS threshold and asymptote value significantly different from the control condition

which tracks from the electrode were verified to be located in the MFB were included in this study. Electrode tips were examined in all animals tested.

Results

Study 1

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

The changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of the CB_1 receptor agonist WIN 55,212-2 are presented in Fig. 1a, b, respectively. WIN 55,212-2 significantly increased self-stimulation thresholds [$F(4,34)=29.887$, $P<0.05$] and decreased the asymptotic rate of responding [$F(4,34)=5.908$, $P=0.001$]. Post hoc analysis with the LSD test showed that these effects were significant in the group receiving 3 mg/kg WIN 55,212-2. There was a significant increase of the self-stimulation thresholds ($P<0.0001$) and the asymptotic rate of responding ($P=0.001$), compared with the vehicle group.

Experiment 2: reversal of the action of WIN 55,212-2 by the CB_1 receptor antagonist SR141716A

Figure 1c, d present the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of SR141716A or its vehicle and WIN 55,212-2 or its vehicle. WIN 55,212-2 (3 mg/kg) produced an increase in self-stimulation threshold. Administration of SR141716A significantly blocked this effect [$F(7,71)=4.000$, $P<0.05$]. Further analysis with LSD test showed that all three doses of SR141716A (0.02, 0.3, 1 mg/kg) reversed the action of WIN 55,212-2 ($P=0.026$ and $P=0.024$ and $P=0.005$, for the three doses of SR141716A, respectively) compared with the vehicle+WIN 3 treated group. There were no significant differences in the asymptotic rate of responding between the different groups [$F(7,71)=0.370$, $P=0.775$].

Study 2

Experiment 1: effects of systemically administered CP 55,940 on brain stimulation reward in drug-naive rats

The changes of self-stimulation threshold and asymptotic rate of responding after systemic injection of the CB_1 receptor agonist CP 55,940 are presented in Fig. 2a, b, respectively. As it can be seen, CP 55,940 significantly increased self-stimulation thresholds [$F(4,39)=2.985$, $P<0.05$], whereas it did not affect the asymptotic rate of responding [$F(4,39)=2.206$, $P=0.088$]. Post hoc analysis with the LSD test showed that the effects on the self-stimulation threshold were significant in the group receiving 100 μg/kg CP 55,940 ($P=0.008$), compared with the vehicle group.

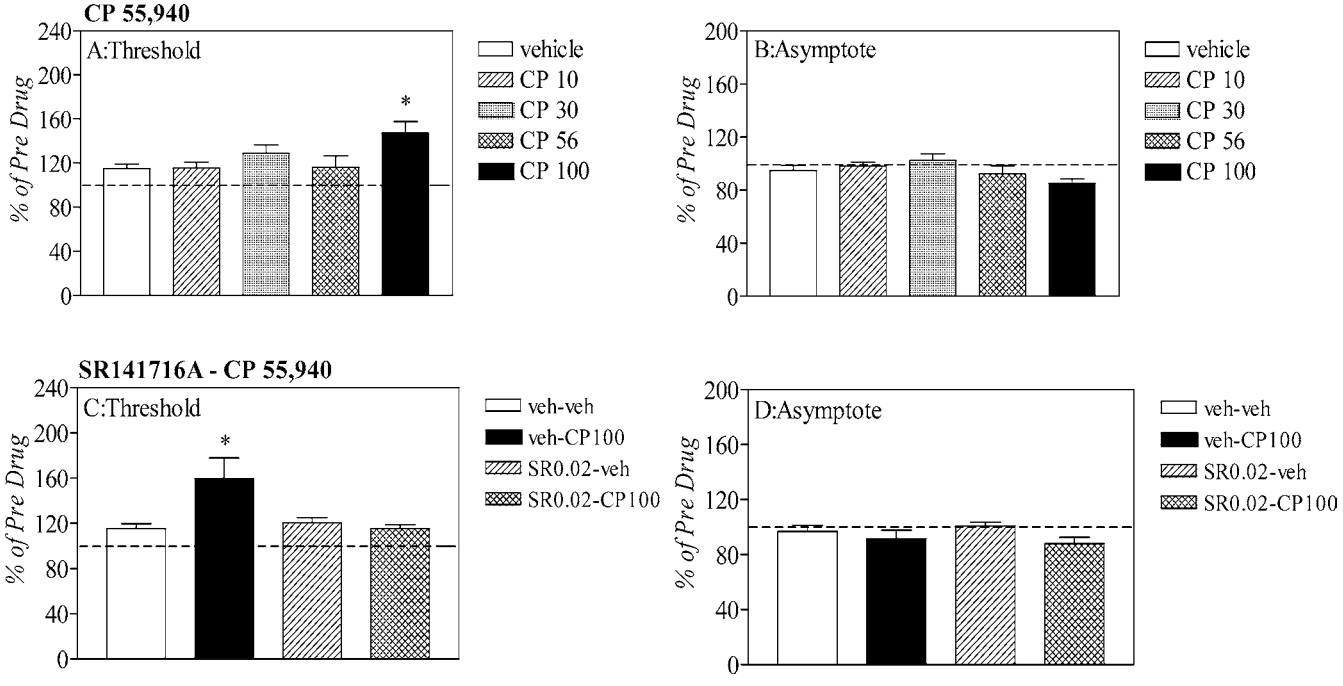

Fig. 2 Changes in self-stimulation threshold **a**,**c** and asymptotic rate **b**,**d** of responding (expressed as percentage of pre-drug values) following CP 55,940 (0, 10, 30, 56, 100 μg/kg, IP) and SR141716A (0, 0.02 mg/kg, IP)+CP 55,940 (0, 100 μg/kg, IP) treatments. *Vertical bars* represent the standard errors of the mean. *Asterisk* signifies an ICSS threshold significantly different from the control condition

Experiment 2: reversal of the action of CP 55,940 by the CB_1 receptor antagonist SR141716A

Figure 2c, d shows the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of SR141716A or its vehicle and CP 55,940 or its vehicle. CP 55,940 produced an increase in self-stimulation threshold. Administration of the potent CB_1 receptor antagonist SR141716A significantly blocked this effect [$F(3,23)=6.412$, $P<0.05$]. More specifically, post hoc analysis with the LSD test showed that there was a significant difference between the vehicle+CP 55,940 treated group and the rest of the groups ($P<0.005$). There were no significant differences in the asymptotic rate of responding between the different groups [$F(3,23)=0.699$, $P=0.413$].

Study 3

Experiment 1: effects of systemically administered HU-210 on brain stimulation reward in drug-naive rats

The changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of the CB_1 receptor agonist HU-210 are presented in Fig. 3a, b, respectively. As it can be seen, HU-210 produced a significant increase in self-stimulation threshold [$F(3,27)=22.313$, $P<0.001$]. Post hoc analysis with the LSD test showed that this effect was significant at the doses of 30 μg/kg and 100 μg/kg ($P<0.005$ and $P<0.0001$, respectively), compared with the vehicle group. HU-210 also produced a significant decrease in the asymptotic rate of responding in the highest dose tested (100 μg/kg) [$F(3,27)=5.117$, $P<0.01$]. Post hoc

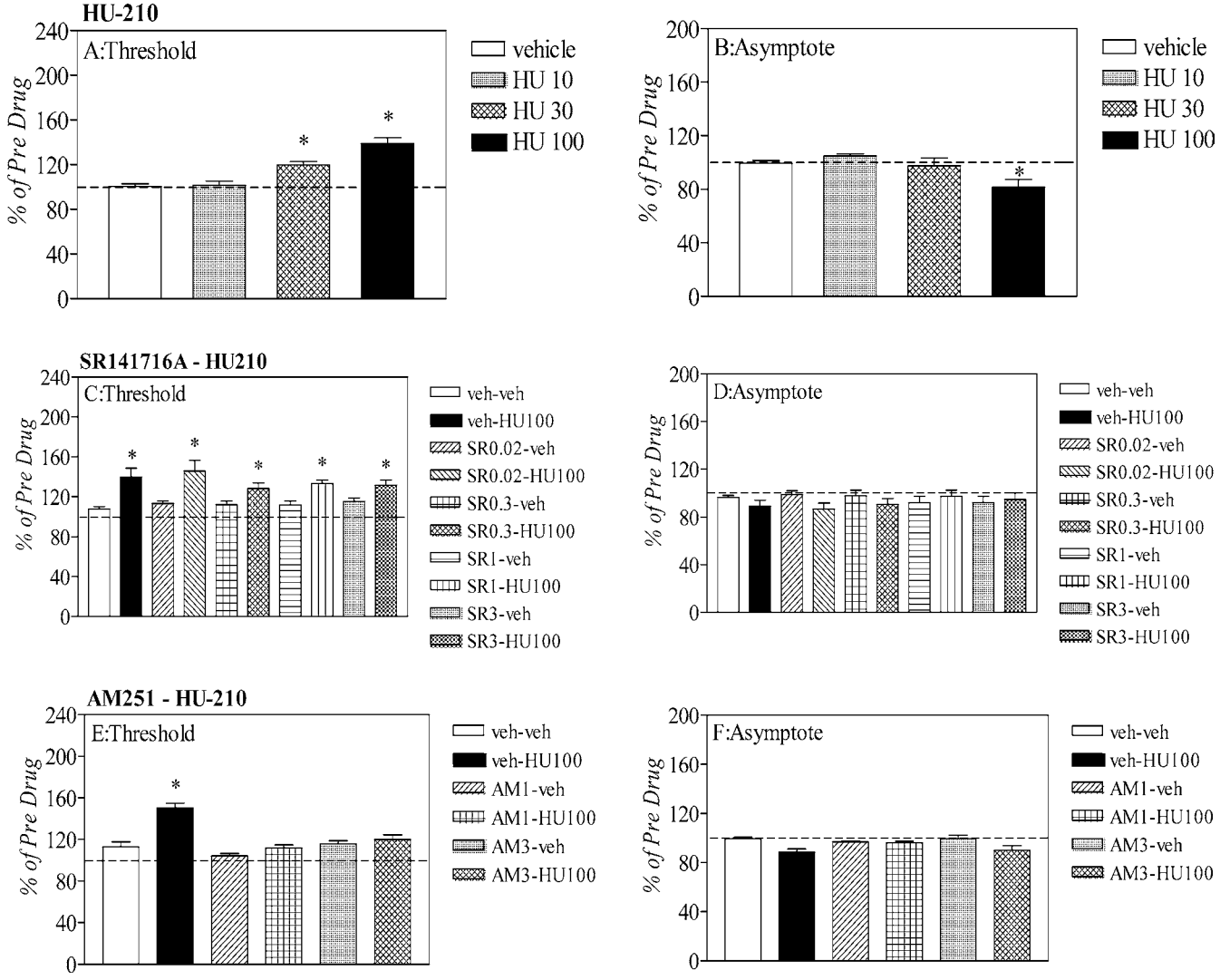

Fig. 3 Changes in self-stimulation threshold **a**,**c**,**e** and asymptotic rate **b**,**d**,**f** of responding (expressed as percentage of pre-drug values) following HU-210 (0, 10, 30, 100 μg/kg, IP) and SR141716A (0, 0.02, 0.3, 1, 3 mg/kg, IP)+HU-210 (0, 10, 30, 100 μg/kg, IP) or AM 251 (0, 1, 3 mg/kg, IP)+HU-210 (0, 100 μg/kg, IP) treatments. *Vertical bars* represent the standard errors of the mean. *Asterisk* signifies an ICSS threshold and asymptote value significantly different from the control condition

analysis showed that the group receiving the highest dose (100 μg/kg) differed significantly from the other three groups ($P<0.01$, compared with the vehicle group).

Experiment 2: effects of the CB_1 receptor antagonist SR141716A on HU-210

Figure 3c, d presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of SR141716A or its vehicle and HU-210 or its vehicle. HU-210 produced an increase in self-stimulation threshold. SR141716A did not block the effect of HU-210 at any of the doses tested [$F(9,79)=1.049$, $P=0.388$], while it did not by itself affect the asymptotic rate of responding [$F(9,79)=1.175$, $P=0.329$].

Experiment 3: reversal of the action of HU-210 by the CB_1 receptor antagonist AM 251

Figure 3e, f shows the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of AM 251 or its vehicle and HU-210 or its vehicle.

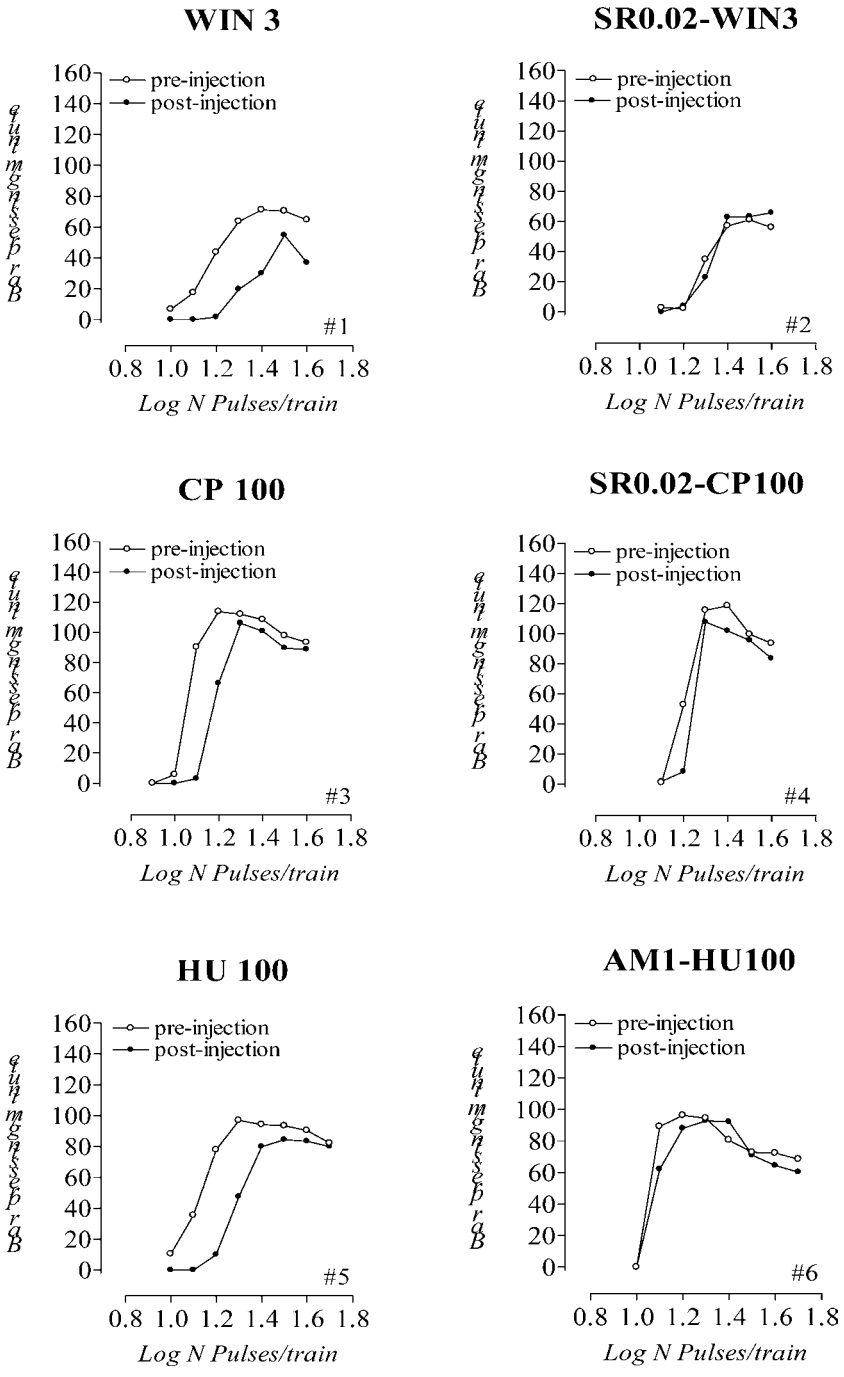

Fig. 4 Rate–frequency functions (rate of lever pressing as a function of stimulation frequency) taken from representative animals for each drug treatment. Each plot represents data from a single animal under pre-drug and drug conditions. Rate–frequency functions were obtained by logarithmically decreasing the frequency of the stimulation pulses from a value that sustained maximal lever pressing to one that failed to sustain lever pressing

HU-210 produced a significant increase in self-stimulation threshold. The potent CB_1 receptor antagonist AM 251 blocked the effect of HU-210 [$F(5,35)=8.958$, $P=0.001$]. Post hoc analysis with the LSD test showed that AM 251 blocked the effect of HU-210 in both doses tested (1 mg/kg and 3 mg/kg) [$P<0.0001$, for both doses], compared with the vehicle+HU-210 treated group, while it did not by itself affect the asymptotic rate of responding [$F(5,35)=3.042$, $P=0.063$].

Figure 4 depicts rate–frequency functions from representative animals obtained before and after antagonist–agonist injections. As indicated in the figure, each of the cannabinoid agonists produced a parallel curve shift to the right, indicating a clear decrease in the rewarding efficacy of the stimulation. On the other hand, we can see that this effect was reversed by co-administration of SR141716A (for WIN 55,212-2 and CP 55,940) or AM 251 (for HU-210).

Discussion

The present series of experiments demonstrates that low doses of CB_1 receptor agonists did not affect the reinforcing efficacy of brain stimulation, whereas higher doses increased brain stimulation reward thresholds and either did not affect or decreased response rates. The later seems to be independent of cannabinoid agonist-induced motor impairment, since changes in threshold current may discriminate between reward and performance (Liebman 1983; Miliaressis et al. 1986; Miliaressis and Rompré 1987; Markou and Koob 1992). For example, Miliaressis and Rompré have shown that motoric factors that produced profound quantitative changes in the response rate of self-stimulation failed to shift the rate–frequency function to any significant degree (Miliaressis and Rompré 1987). Moreover, the self-stimulation threshold procedure applied in the present study allowed determining threshold and response rate separately and concurrently in the same self-stimulation session. It is worth noting, that a decrease in performance is not always associated with an increase in threshold frequency, and more importantly attenuated performance has been observed in association with a decrease in threshold frequency (Panagis and Spyraki 1996). In this study, the higher dose of CP 55,940 increased brain stimulation reward thresholds, without affecting maximal rates of responding. On the other hand, HU-210 increased brain stimulation reward thresholds in the two higher doses tested, while decreased maximal rates of responding only in the highest dose.

The major difference in self-stimulation behavior of the cannabinoid receptor agonists examined in the present study relates to potency, rather than efficacy. Thus, it could be assumed that the behavioral profile of these compounds is mainly determined by the compound's receptor affinity, rather than its intrinsic activity at the CB_1 receptor sites.

According to the results of the present study, classical cannabinoids (i.e. THC-like) like HU-210 as well as non-classical (THC-unlike) cannabinoids like WIN 55,212-2 and CP 55,940 in low doses did not affect ICSS behavior. Interestingly, several studies have suggested that therapeutic effects of cannabinoids occur at low to moderate doses (see, for example, Felder and Glass 1998). The later along with the fact that both the cannabinoid behavioral syndrome (Martin et al. 1991) and the effects of cannabinoids in self-stimulation behavior (present results) become apparent at relatively higher doses, indicates that cannabinoids have a relatively favorable therapeutic window.

The effects of the CB_1 receptor agonists were completely reversed by pretreatment with the CB_1 receptor antagonists SR141716A or AM 251, at doses that did not affect baseline self-stimulation by themselves. This indicates that the inhibitory action of cannabinoid agonists on brain stimulation reward is probably mediated through CB_1 receptor stimulation (see also below).

The present results are consistent with previous studies that showed that the synthetic cannabinoid receptor agonists levonantradol, WIN 55,212-2 and CP 55,940 either did not affect or increased brain stimulation reward thresholds (Stark and Dews 1980; Kucharski et al. 1983; Arnold et al. 2001; Vlachou et al. 2003). However, the present results contradict previous studies by Gardner and colleagues, showing that THC increases the rewarding efficacy of self-stimulation (Gardner et al. 1988; Gardner and Vorel 1998). 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. (1996) 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.

Earlier results on the effect of cannabinoid ligands on self-administration behavior or CPP are also conflicting. WIN 55,212-2 has been shown to establish a robust place aversion in rats and this aversive effect was antagonized by SR141716A (Chaperon et al. 1998). However, two recent studies reported intravenous self-administration of WIN 55,212-2 in mice and rats (Martellotta et al. 1998; Fattore et al. 2001). Interestingly, divergent results on reward and reinforcement of behavior have also been reported with other synthetic cannabinoids (Martellotta et al. 1998; Cheer et al. 2000; Braida et al. 2001a,b).

Without doubt, cannabis is one of the most widely used intoxicants. The pleasurable effects of cannabis preparations in humans might appear to be in disagreement with the present work. Several reasons might explain this difference. First of all, cannabis is more than simply a CB_1 receptor agonist. Indeed, cannabis preparations contain several cannabinoid and non-cannabinoid compounds, which may contribute to the behavioral effects of cannabis (see, for example, Russo and McPartland 1993). Secondly, there may be significant species differences in functions of the cannabinoid system in rodents and humans. Perhaps the recreational use of cannabis by humans reflects more complex sensory and cognitive effects that cannot be experienced by lower animals. This may, also, explain why cannabinoids do not induce the typical pattern of obsessive drug seeking and compulsive drug taking behavior, observed in humans

addicted with typical drugs of abuse, such as cocaine, heroin, alcohol and nicotine.

The fact that AM 251, but not SR141716A, reversed the effect of the THC-like, classical cannabinoid HU-210 is not entirely unexpected. Some previous studies that investigated the interaction of SR141716A and cannabinoid receptor agonists have shown that pretreatment with SR141716A either did not affect or partially blocked the behavioral effects of cannabinoid receptor agonists (De Vry and Jentzsch 2003, 2004; Järbe et al. 2003a,b; Solinas et al. 2003). The discrepancy between the effects of AM 251 and SR141716A in counteracting the effects of HU-210 could be explained in several ways. Firstly, we can assume that SR141716A either affected a novel cannabinoid receptor, the existence of which has been recently suggested (Zimmer et al. 1999; Di Marzo et al. 2000; Breivogel et al. 2001; Haller et al. 2002; Zygmunt et al. 2002; Mo et al. 2004) or it affected processes unrelated to the cannabinoid system. Alternatively, SR141716A may act as a partial or inverse agonist at cannabinoid receptors, although the significance of this action in the observed effects is not readily understood (see also, Bouaboula et al. 1997; Landsman et al. 1997; De Vry and Jentzsch 2003). Prima facie the present results suggest that HU-210 and AM 251, but not SR141716A, share some common binding site domains that are probably related to CB_1 receptors in the brain (see Gatley et al. 1996) and which are involved in mediating brain stimulation responses.

In summary, the present study clearly shows that the cannabinoid agonists WIN 55,212-2, CP 55,940 and HU-210 do not exhibit reinforcing properties in the ICSS paradigm, but rather have an inhibitory influence on reward mechanisms. The results suggest that the anhedonic effects of cannabinoids are probably mediated by cannabinoid CB_1 receptors.

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