Life Sciences 70 (2001) 97–108

Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats

Jonathon C. Arnold[a,*], Glenn E. Hunt[b], Iain S. McGregor[a]

[a]*Department of Psychology, University of Sydney, Sydney, New South Wales, 2006, Australia*
[b]*Department of Psychological Medicine, University of Sydney Clinical Sciences Building, Concord Hospital, Concord, New South Wales, 2139 Australia*

Received 23 January 2001; accepted 10 April 2001

Abstract

Lewis rats were trained to self-stimulate the medial forebrain bundle (MFB) using a rate-frequency paradigm. They were then tested for the effects of the cannabinoid receptor agonist CP 55,940, the selective cannabinoid receptor antagonist SR 141716 and the dopamine D_1 receptor antagonist SCH 23390. CP 55,940 (0, 10, 25 and 50 μg/kg i.p.) had no effect on MFB self-stimulation behaviour as assessed by the M_{50}, the stimulation frequency at which half-maximal response rates were obtained. With SR 141716, only a very high dose (20 mg/kg i.p.) caused a significant inhibition of the rewarding efficacy of the stimulation. This was seen as an increase in the M_{50}. All other doses of SR 141716 (0, 1, 3, 10 mg/kg i.p.) were ineffective in modulating the M_{50}. By comparison, a relatively low dose (0.06 mg/kg i.p.) of SCH 23390 caused a large increase in M_{50}. These results indicate a relatively modest influence, if any at all, of exogenous or endogenous cannabinoids on reward-relevant neurotransmission.

Keywords: Cannabinoid; Intracranial self-stimulation; Lewis rat; CP 55,940; SR 141716

Introduction

The phenomenon of medial forebrain bundle (MFB) self-stimulation has provided researchers with a unique insight into the neural and chemical profile of brain reward systems [1]. One interesting aspect of self-stimulation is that drugs with rewarding and addictive properties in humans tend to have a facilitatory effect on self-stimulation in rats. One often

* Corresponding author. Dept of Pharmacology, Faculty of Medicine, The University of Sydney, Sydney, New South Wales, 2006, Australia. Tel.: +61-2-9351-6957; fax +61-2-9351-3868.

E-mail address: arnie@pharmacol.usyd.edu.au (J.C. Arnold)

PII: S0024-3205(01)01366-2

cited example of this is cannabis. Gardner and colleagues have reported that the psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC) (1.5 mg/kg), enhances the rewarding potency of electrical MFB stimulation using a titrating threshold stimulation paradigm [2]. Much later this was repeated using a slightly lower dose of Δ^9-THC (1 mg/kg) using a rate-frequency curve shift method to highlight that this rewarding effect occurred in the absence of performance deficits [3]. Interestingly, the leftward shift observed in the rate-frequency function was small (approximately 0.05 $\log_{10}$ units) in comparison to the leftward shift observed in the rate-frequency function of other drugs of abuse such as amphetamine, MDMA or cocaine (approximately 0.2 – 0.5 $\log_{10}$ units) [4–6].

The most important determinant of the observed rewarding effect of Δ^9-THC on electrical brain stimulation was the use of Lewis strain rats [7]. For other strains such as Sprague-Dawley rats, Δ^9-THC only showed a marginal rewarding effect, whereas in Fischer 344 rats Δ^9-THC had no effect [3]. This finding complements other research by Gardner and colleagues which shows that Lewis rats acutely administered Δ^9-THC show greater dopamine efflux in the nucleus accumbens than Sprague-Dawley rats [8].

Since the discovery of the main psychoactive constituent of cannabis, Δ^9-THC, many Δ^9-THC-like compounds have been synthesised including CP 55,940. CP 55,940 is an important compound as it was used in a tritiated form to give a definitive map of cannabinoid binding sites in the brain [9] which foreshadowed the discovery of functional cannabinoid receptors [10]. CP 55,940, while more potent than Δ^9-THC, has a very similar pharmacological profile. CP 55,940 mimics the effects of Δ^9-THC in numerous behavioural tests [11,12] and fully substitutes for Δ^9-THC when tested in the drug discrimination paradigm [13].

In the current study we assessed the effects of CP 55,940, which has never been previously tested on MFB self-stimulation, to observe whether it has similar rewarding effects to Δ^9-THC [3]. We also examined whether there is a reduction in the rewarding value of MFB stimulation 24 hours after a single injection of CP 55,940. This reduction in the rewarding action of brain stimulation has been suggested to occur 24 hours after acute exposure to Δ^9-THC [14] and might reflect a withdrawal-induced dysphoria that has been documented in the self-stimulation paradigm with other drugs such as nicotine and cocaine [15,16].

There is increasing evidence that the endogenous cannabinoid system plays an important role in mediating the rewarding effects of both drugs of abuse and natural reinforcers. Recent results show that mice with targeted deletion of the CB_1 receptor gene exhibit reduced self-administration of morphine [17]. Further, the selective cannabinoid CB_1 receptor antagonist SR 141716 blocks the acquisition of conditioned place preference to cocaine and morphine in rats [18]. SR 141716 also reduces sucrose, ethanol, and beer consumption in rats suggesting an involvement of cannabinoid systems in appetite and alcohol preference [19–21]. In the present study it was reasoned that if the central endogenous cannabinoid system plays a critical role in the neural substrate of reward, then SR 141716 should have a powerful inhibitory effect on the rewarding efficacy of MFB stimulation. As the role of dopamine in the rewarding impact of electrical brain stimulation is well established [22], the effects of the dopamine D_1 receptor antagonist SCH 23390 on MFB self-stimulation was also assessed.

Methods

Subjects

The subjects were 13 Lewis rats (ARC, Perth, Australia) weighing between 330–380 g at the time of surgery. The rats were singly housed in a temperature controlled room (20 ± 2 °C) with food and water provided *ad libitum.* All testing occurred during the light hours of a 12 h:12 h light/dark cycle.

Apparatus

Two identical operant boxes [25 cm (L) × 25 cm (W) × 30 cm (H)] (Coulbourn Instruments, Allentown, PA) were used. Each perspex box contained a standard response lever that was positioned on one side of the box elevated 3 cm above its grid floor. These individual boxes were located within their own sound attenuating chambers [75 cm (L) × 50 cm (W) × 60 cm (H)]. Lever presses delivered a 500 msec stimulation train of 0.1 msec wide cathodal rectangular pulses. Stimulation was supplied by a programmable stimulator and constant current generator through mercury filled commutators via connecting leads. A PC compatible computer controlled the delivery of stimulation and recorded all behavioural data while a Macintosh™ computer controlled the programmable stimulator to allow a descending series of stimulation frequencies to be automatically presented.

Drugs

The dose range of CP 55,940 (Tocris, Bristol, UK) used (0, 10, 25 and 50 μg/kg) is similar to the doses of Δ^9-THC (1 and 1.5 mg/kg) employed in previous studies by Gardner and colleagues that showed Δ^9-THC to enhance brain stimulation reward [2,3]. This similarity of doses is based on previous binding and behavioural studies which have shown that CP 55,940 is approximately 30 times as potent as Δ^9-THC [13,23]. CP 55,940 was prepared by first dissolving it in absolute ethanol and then diluting this solution in saline and Tween 80 to make a final vehicle solution of 2.5% Tween 80, 2.5% ethanol and 95% saline.

The doses of SR 141716 (Sanofi Recherche, Montpelier, France) used (0, 1, 3, 10 and 20 mg/kg) are consistent with the dose ranges that previous investigators have shown to be effective in modulating appetite and the rewarding effects of drugs of abuse (that is, 1–10 mg/kg) [18–21]. In addition, we included a relatively high dose of SR 141716 (20 mg/kg) as this dose has been shown to have inverse agonist-like effects, such as producing hyperactivity in mice [24]. SR 141716 was prepared by initially dissolving its powder formulation in 100% ethanol. Tween 80 was added to this solution and the ethanol was completely evaporated under a stream of nitrogen gas. The resulting solution was diluted with 0.9% saline to give a final solution containing 1:19 (Tween 80:saline).

SCH 23390 (Research Biochemicals Inc., Natick, MA) was prepared by dissolving it in 0.9% saline to create a solution with a concentration of 0.06 mg/ml. Previous studies have shown that 0.04 mg/kg of SCH 23390 produces a large decrease in the rewarding impact of electrical stimulation of the MFB [22,25,26]. Furthermore, a dose of 0.08 mg/kg has been shown to completely abolish responding for electrical stimulation of the MFB [25].

Identical solutions minus the drugs were used as vehicle solutions. All drugs were injected intraperitoneally (i.p.) in an injection volume of 1 ml/kg.

Surgery

Rats were anaesthetised with an i.p. injection of ketamine (60 mg/kg) and xylazine (9 mg/kg) and stereotaxically implanted with a monopolar electrode and indifferent electrode as described previously [27]. The surface of the skull of the rat was level to the horizontal plane when rats were positioned in the stereotaxic apparatus. The electrode tip was aimed at the lateral hypothalamic level of the MFB using the following stereotaxic coordinates: 2.5 mm posterior to Bregma, 1.6 mm lateral to the midline and 9.7 mm ventral from the superior surface of the skull. Rats were allowed to recover from surgery for at least 3 weeks before testing.

Procedure

Rats were trained to lever press under a fixed-interval (FI) 1 sec schedule of reinforcement with the stimulation frequency and current set at 100 Hz and 150 μA respectively. Once the rats started self-stimulating, currents were manipulated (range = 280 – 600 μA; mean = 347 μA) to yield high rates of responding (70 – 130 responses per min). Rats were then trained using a rate-frequency paradigm [28] involving a descending series of 10 different frequencies, beginning at 200 Hz and finishing at 25 Hz. At the beginning of each series of frequencies rats received 5 priming stimulations over 20 sec. Individual frequencies were available for 2 min before the frequency was decreased by 0.1 $\log_{10}$ units. Once all 10 frequencies had been presented, the procedure was repeated. If the rat did not respond for a period of 90 sec, one forced stimulation was supplied.

The effects of CP 55,940 on brain stimulation reward

In the studies that assess the effects of drugs on intracranial self-stimulation, stable responding is usually viewed as acceptable when the frequency supporting half maximal rates of responding (M_{50}) does not vary beyond 0.1 $\log_{10}$ units over three consecutive days [6,29]. In the current study, rats were trained for three weeks using the rate-frequency paradigm before being tested for the effects of CP 55,940. At this point all rats M_{50} did not vary beyond 0.1 $\log_{10}$ units over three consecutive days. Once responding was stable rats were given a dose of CP 55,940 30 min before the start of testing. Testing proceeded so that a rat received no more than one dose per week. All seven rats were administered doses of CP 55,940 (0, 10, 25 and 50 μg/kg) in a randomised order. The rats were then tested drug-free 24 h after the administration of CP 55,940 to assess whether prior cannabinoid administration decreases the rewarding impact of MFB stimulation.

The effects of SR 141716 and SCH 23390 on brain stimulation reward

In this study, rats were trained for three weeks using the rate-frequency paradigm before being tested for the effects of SR 141716. At this point the M_{50} of rats did not vary beyond 0.03 $\log_{10}$ units over three consecutive days. Rats were then administered a dose of SR 141716 20 min before the start of testing. Testing proceeded so that each rat received no more than one dose per week. All six rats were administered doses of SR 141716 (0, 1, 3, 10 and 20 mg/kg) in a randomised order.

Once testing with SR 141716 was complete, the same rats were tested with SCH 23390. Testing with SCH 23390 occurred over three consecutive days, where the first day served as a baseline, the second as a control day where saline was administered, and the final day as a test where 0.06 mg/kg of SCH 23390 was administered.

Histology

Upon completion of drug testing, rats were killed with a lethal injection of pentobarbitone (120 mg/kg, i.p.). They were then decapitated and their brains were removed. Brains were blocked and placed on a microtome stage before being frozen to –14 °C and sliced into 40 μm sections. Frozen slices were examined under a light microscope to confirm that the electrodes were positioned at the lateral hypothalamic level of the MFB. The tissue was then mounted on slides and left overnight to dry. These were stained with toluidine blue and coverslipped.

Data analysis

Data collected consisted of the number of reinforced responses for each frequency presented. Non-linear regression analyses were performed on these data and the Gompertz equation was used to provide two estimates of responding to enter into statistical analysis: 1) M_{50}, which is the frequency supporting half maximal rates of responding, and 2) Y_{max} which is the maximal or asymptotic response rate [28]. The M_{50} provides an estimate of the rewarding efficacy of MFB stimulation, while the Y_{max} provides an indication of any drug effects on performance [1,28].

M_{50} and Y_{max} data for all drug, vehicle and saline tests were expressed as a percentage change from the drug-free baseline on the previous day. The resultant percentages for each of the two sessions within each test were averaged to give a single percentage change score. Average percentage change scores for M_{50} and Y_{max} were then separately analysed using one-factor ANOVA with drug administration (CP 55,940 or SR 141716 or SCH 23390) treated as a repeated measure.

Individual comparisons between each dose of CP 55,940 (either 10, 25, or 50 μg/kg) and vehicle were constructed using Bonferroni corrections to control the Type 1 error rate [30]. These same comparisons were conducted on data retrieved 24 h following CP 55,940 administration to assess whether prior cannabinoid exposure reduces the rewarding impact of MFB stimulation. In addition, individual planned comparisons between each SR 141716 dose (1, 3, 10, or 20 mg/kg) and vehicle were constructed. Bonferroni corrections were then applied to control for Type 1 error.

Results

All electrode tips were confirmed to be within the boundaries of the MFB at the level of the lateral hypothalamus. A representative photomicrograph of the electrode tip locations can be seen in Figure 1.

CP 55,940 was ineffective in modulating either the rewarding efficacy of MFB stimulation or the maximal rate of responding. This was reflected in no significant changes in M_{50} or Y_{max} (Fig. 2.). Individual contrasts comparing each CP 55,940-treatment group and vehicle revealed that CP 55,940 had no affect on the M_{50} at any dose tested ($Fs < 1.7$). In addition, CP 55,940 had no effect on Y_{max} at any dose tested ($Fs < 2.6$).

Twenty-four hours after the administration of CP 55,940 there was no significant shifts in either M_{50} or Y_{max} (Fig. 2.). Individual contrasts comparing each CP 55,940-treatment group and vehicle revealed CP 55,940 given 24 hours earlier had no affect on the M_{50} at any dose

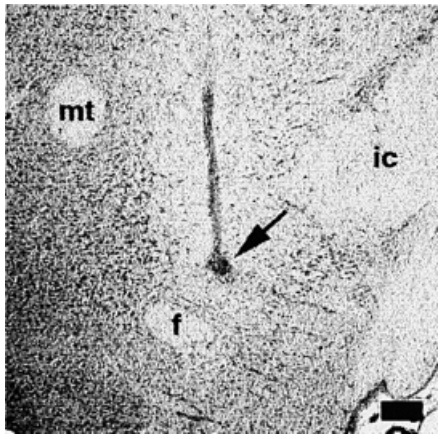

Fig. 1. A photomicrograph of a representative electrode placement approximately 2.5 mm posterior to Bregma. All electrode placements were confirmed to be within the boundaries of the MFB at the level of the lateral hypothalamus. The arrow indicates the placement of the electrode tip. f refers to the fornix, ic to the internal capsule and mt to the mammillothalamic tract. Scale bar = 250 μm.

tested (Fs $<$ 1). In addition, prior CP 55,940 administration had no affect on Y_{max} at any dose tested (Fs $<$ 1).

SR 141716 produced a dose-dependent decrease in the rewarding efficacy of MFB self-stimulation without promoting any significant performance deficit (Fig. 3.). Rate-frequency data for a typical rat are shown in Figure 3. Contrasts comparing each SR 141716 dose to vehicle revealed that only the 20 mg/kg dose significantly increased the M_{50} [$F(1,20) = 26.54$, $p < 0.001$] (a rightward shift of approximately 0.08 $\log_{10}$ units in the rate-frequency function). However, remaining contrasts comparing 1, 3 and 10 mg/kg of SR 141716 separately to vehicle exhibited only a tendency toward significance (Fs = 1.20, 2.37, 5.01 respectively).

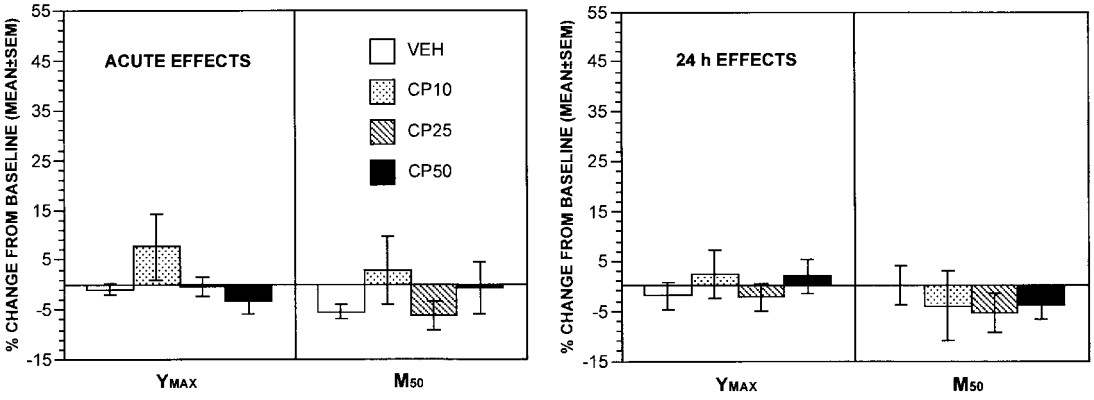

Fig. 2. (left panel) Effects of CP 55,940 [0 (VEH), 10 (CP10), 25 (CP25) and (CP50) 50 μg/kg] on MFB self-stimulation as assessed by Y_{max} and M_{50} (n=7 per group). (right panel) Y_{max} and M_{50} data as measured 24 hours after administration of CP 55,940 (0, 10, 25, 50 μg/kg).

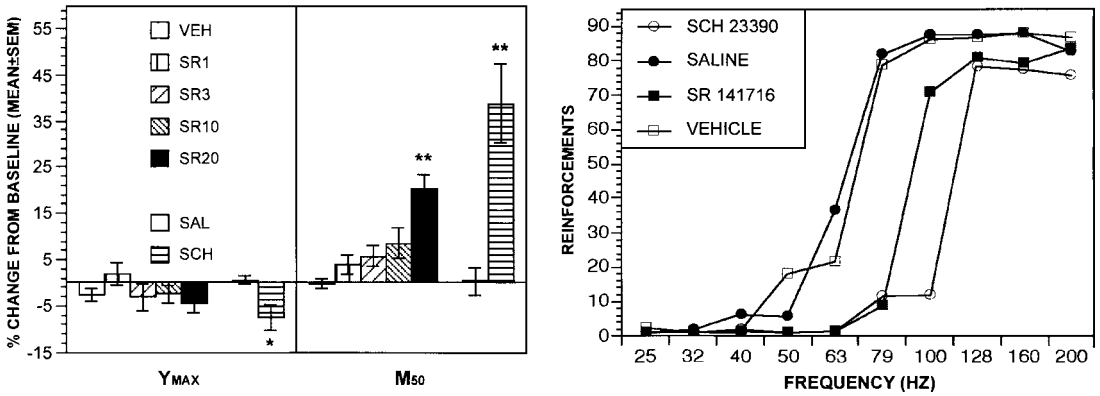

Fig. 3. (left panel) Effects of SR 141716 (0, 1, 3, 10 and 20 mg/kg) and SCH 23390 (0 and 0.06 mg/kg) on MFB self-stimulation as assessed by Y_{max} and M_{50} (n=6 per group). SR 141716 (20 mg/kg) and SCH 23390 (0.06 mg/kg) significantly attenuated the rewarding efficacy of the electrical stimulation as shown by increased M_{50}, while only SCH 23390 significantly reduced Y_{max}: * $p < 0.05$, ** $p < 0.01$. (right panel) Effects of SR 141716 (20 mg/kg) and SCH 23390 (0.06 mg/kg) and their respective vehicles on responding for MFB self-stimulation by a typical rat. Data are expressed as the number of reinforcements obtained per frequency of stimulation.

When first placed in the self-stimulation apparatus it was noticed that rats treated with 20 mg/kg of SR 141716 exhibited clear deficits in coordinated movement and a marked loss of muscle tone. However, these clear behavioural deficits did not impede bar pressing. Thus, no effect of SR 141716 on asymptotic responding was evident, with no significant decreases in Y_{max} for any SR 141716 dose tested (Fs < 1).

As expected, SCH 23390 (0.06 mg/kg) also caused a decrease in the rewarding efficacy of MFB self-stimulation as assessed by M_{50} [$F(1,5) = 23.29$, $p < 0.01$] (a rightward shift of approximately 0.14 $\log_{10}$ units in the rate-frequency function). A one-factor ANOVA directly comparing the inhibitory effects of 20 mg/kg of SR 141716 and 0.06 mg/kg of SCH 23390 on M_{50} indicated a significantly larger inhibitory effect of SCH 23390 [$F(1,5) = 12.61$, $p < 0.05$]. A slight decrease in asymptotic responding was evident in SCH 23390-treated rats, as shown by a significant decrease in Y_{max} [$F(1,5) = 10.99$, $p < 0.05$].

Discussion

The current study reports for the first time the effects of the synthetic cannabinoid receptor agonist CP 55,940 and the selective cannabinoid receptor antagonist SR 141716 on MFB self-stimulation in Lewis rats. CP 55,940 was shown to have no effect on MFB self-stimulation behaviour. Furthermore withdrawal from acute administration of CP 55,940 did not elevate electrical stimulation reward thresholds when measured 24 hours later under drug-free conditions.

The present results are consistent with a previous study which showed that the synthetic cannabinoid receptor agonist, levonantradol, had no effect on electrical brain stimulation thresholds [31]. However, they contradict previous studies by Gardner and colleagues who have shown that Δ^9-THC (1.5 and 1 mg/kg respectively) enhances the rewarding potency of electrical brain stimulation using two different procedures, namely, a titrating threshold stim-

ulation paradigm and a rate-frequency curve shift paradigm [2,3]. Both previous findings by Gardner and colleagues might be seen as complementary to the present finding that CB_1 receptor antagonism causes only a modest inhibitory effect on the rewarding efficacy of self-stimulation in Lewis rats [2,3]. However, the current investigation failed to demonstrate a facilitatory effect of CP 55,940 (10, 25 and 50 μg/kg), a synthetic analogue of Δ^9-THC. Thus, this appears to contradict the findings of Lepore et al. [3] which also used a rate-frequency curve shift paradigm and a dose of Δ^9-THC that is comparable to the doses of CP 55,940 that were used in the current study.

It could be argued that the discrepancy between the findings of the current study and the Lepore et al. [3] study may be due to subtle differences between Δ^9-THC and CP 55,940 in their chemical structures and their effects on intracellular transduction pathways [32]. However, this seems unlikely, given that CP 55,940 has been shown to have the same effects as Δ^9-THC in numerous behavioural assays including tests of locomotor activity, catalepsy, nociception and body temperature [11,12].

An alternative explanation for why CP 55,940 did not modulate reward is based on close scrutiny of the methodology employed by Lepore et al. [3]. First, it is important to note that the magnitude of effect of Δ^9-THC found in their study was very small compared to the effects of other drugs of abuse such as amphetamine, MDMA and cocaine [4–6]. These drugs can reliably shift the rate-frequency function to the left by between 0.2 and 0.5 $\log_{10}$ units [4–6]. However, the Lepore et al. [3] study showed that Δ^9-THC could only shift the rate-frequency function to the left by approximately 0.05 $\log_{10}$ units. Interestingly, this magnitude of effect of Δ^9-THC was smaller than what we, and most other investigators, judge as being stable baseline responding (usually within 0.1 $\log_{10}$ units over consecutive days). Second, Lepore et al. [3] used a very strict criterion of stable responding (within 0.01 $\log_{10}$ units over days), possibly to ensure that the subtle effect of Δ^9-THC could be distinguished from normal baseline variation over days. Therefore, it is possible that in the current study the baseline variation in responding permitted across days masked a possible subtle effect of CP 55,940 on the rewarding impact of electrical stimulation of the MFB.

There was no elevation in brain stimulation thresholds (using the M_{50} estimate of threshold) when measured 24 hours after administration of CP 55,940 (10, 25 and 50 μg/kg). This is inconsistent with a recent report by Gardner and colleagues which showed that administration of a single 1 mg/kg dose of Δ^9-THC elevates thresholds 24 hours later under drug-free conditions [7,14]. Such elevations in reward threshold have been promoted as a model of the depression or dysphoria experienced by human users dependent on a drug when they attempt to abstain from using that drug. In the animal model such elevations in threshold have been reported to occur upon withdrawal from *chronic* administration of a number of different drugs of abuse, including nicotine [15,33], ethanol [34], amphetamine [35–37], cocaine [16,38–40] and morphine [41]. Close scrutiny of these findings shows that such elevations in reward threshold can not be discerned after a single administration of these drugs. Thus, the finding of Gardner et al. [7,14] are questionable, and this underscores the importance of future studies that: 1) attempt to replicate the finding of Gardner et al. [7,14] and; 2) seek to determine whether withdrawal from chronic administration of a cannabinoid receptor agonist, such as Δ^9-THC, can elevate reward thresholds as assessed by the intracranial self-stimulation paradigm.

SR 141716 significantly attenuated the rewarding potency of MFB stimulation, but only at the highest dose tested (20 mg/kg). Further this effect was small in comparison to the effects of the D_1 receptor antagonist SCH 23390. It is notable that in previous studies, much lower doses of SR 141716 (0.1 – 3 mg/kg) have been effective in reducing the rewarding effects of morphine and cocaine and inhibiting appetite for palatable foods and alcoholic beverages [18,20,21]. Thus 3 mg/kg of SR 141716 almost completely blocked operant responding for beer in an animal model of alcohol craving [21] while 0.1 – 3 mg/kg prevented the acquisition of a conditioned place preference to cocaine [18]. The need to use a much higher dose of SR 141716 to affect self-stimulation is possibly explained by Lewis rats being less sensitive to the effects of SR 141716 than other rat strains. To our knowledge this is the first study to administer SR 141716 to the Lewis strain of rat.

The fact that such a high dose of SR 141716 was needed to affect self-stimulation behaviour also raises the possibility that such a dose acts on non-cannabinoid systems. An *in vitro* study showed that SR 141716, at 1 μM concentrations, did not bind to other known receptors including dopamine, opioid, glutamate, serotonin and GABA receptors [42]. However, it is possible that 20 mg/kg of SR 141716 exceeds the concentrations that were used in this *in vitro* study. Therefore, it is possible that the 20 mg/kg dose of SR 141716 also affected non-cannabinoid receptors. To settle this dispute future studies could attempt to reverse the effect of SR 141716 on electrical brain stimulation behaviour using a cannabinoid receptor agonist to show that the effect was selectively mediated by CB_1 receptors.

If it is assumed that the effect of 20 mg/kg of SR 141716 on self-stimulation was selectively mediated by CB_1 receptors, then cannabinoid systems have a relatively minor influence on reward. Both the need to use such a high dose, and the lack of effects observed with lower doses (1, 3 and 10 mg/kg) emphasise this view. One explanation for the effect of SR 141716 in the present study is that it blocks a tonic activation of reward-relevant neural substrates provided by endogenous cannabinoid ligands such as 2-arachidonyl glycerol and anandamide. Consonant with this, Δ^9-THC is known to activate the mesolimbic dopamine pathway, thought to be a critical brain reward substrate [7,14,43]. If a tonic level of endogenous cannabinoid activity in the mesolimbic dopamine system contributes to the rewarding effects of MFB self-stimulation, then antagonising this tone with SR 141716 will presumably reduce the rewarding efficacy of MFB stimulation. One problem with this hypothesis is that SR 141716 has been found to have no effect on dopamine release in the nucleus accumbens shell at doses up to 10 mg/kg [43,44]. However, it is possible that higher doses of SR 141716 may have such an effect.

Another possible explanation for the effects of SR 141716 seen here relies on recent evidence that SR 141716 acts as an inverse agonist at the CB_1 receptor [45,46]. Based on biochemical evidence, SR 141716 appears to have direct effects on receptor-mediated intracellular transduction signaling that are opposite to those seen with cannabinoid receptor agonists [45]. This raises the issue of whether the effects of SR 141716 on self-stimulation reflect a silent inhibition of endogenous cannabinoid tone or whether they rely on an intrinsic effect of SR 141716 on the CB_1 receptor and signal transduction processes. The issue of whether SR 141716 is a competitive antagonist or inverse agonist could be reconciled by future investigations.

Conclusion

The present results suggest that the endogenous cannabinoid system may only play a small role in the neural substrate of brain stimulation reward, if it plays any role at all. This is consistent with other research showing that endogenous cannabinoids have a subtle neuromodulatory role in brain function [47]. In the current study, a large dose of 20 mg/kg of SR 141716 was able to increase electrical MFB stimulation thresholds in Lewis rats. Lower doses (1 – 10 mg/kg) of SR 141716 had no such effect. Future studies may attempt to test whether the effect of 20 mg/kg of SR 141716 is selectively mediated by CB_1 receptors. If the effect is mediated only by CB_1 receptors then the role of the endogenous cannabinoid system in the substrate of MFB stimulation reward is relatively modest at best. This is highlighted by the magnitude of effect of SR 141716 being relatively small in comparison to the effects of the D_1 receptor antagonist SCH 23390. The current study also reported that acutely administered CP 55,940 (10 – 50 μg/kg) had no effect on MFB self-stimulation thresholds. In addition, withdrawal from acute administration of CP 55,940 did not elevate electrical brain stimulation reward thresholds when measured 24 hours later under drug-free conditions.

Acknowledgements

This study was supported by a National Health and Medical Research Council grant to ISM and GEH and an Australian Research Council grant to ISM. In addition, this research was also supported by a postgraduate scholarship awarded to JCA.

References

1. Wise RA. Addictive drugs and brain stimulation reward. Annual Reviews in Neuroscience 1996; 19: 319–40.
2. Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen D, Morrison D. Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. Psychopharmacology 1988; 96: 142–4.
3. Lepore M, Liu X, Savage V, Matalon D and Gardner EL. Genetic differences in delta (9)-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. Life Sciences 1996; 58: PL365–72.
4. Bauco P, Wise RA. Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. Journal of Pharmacology & Experimental Therapeutics 1997; 283: 1160–7.
5. Gallistel CR, Karras D. Pimozide and amphetamine have opposing effects on the reward summation function. Pharmacology, Biochemistry & Behavior 1984; 20: 73–7.
6. Lin HQ, Jackson DM, Atrens DM, Christie MJ, McGregor IS. Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. Brain Research 1997; 744: 351–7.
7. Gardner EL. Cannabinoid transmission and reward-related events. Neurobiology of Disease 1998; 5: 502–33.
8. Chen JP, Paredes W, Lowinson JH, Gardner EL. Strain-specific facilitation of dopamine efflux by delta 9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study. Neuroscience Letters 1991; 129: 136–40.
9. Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. Journal of Neuroscience 1991; 11: 563–83.
10. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Molecular Pharmacology 1988; 34: 605–13.
11. Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. Journal of Pharmacology & Experimental Therapeutics 1988; 247: 1046–51.

12. Mechoulam R, Devane WA, Glaser R. Cannabinoid geometry and biological activity. In: Murphy L, Bartke A Editors. Marijuana/Cannabinoids Neurobiology and Neurophysiology. Boca Raton FL: CRC Press, 1992. pp. 1–33.
13. Gold LH, Balster RL, Barrett RL, Britt DT, Martin BR. A comparison of the discriminative stimulus properties of delta 9-tetrahydrocannabinol and CP 55,940 in rats and rhesus monkeys. Journal of Pharmacology & Experimental Therapeutics 1992; 262: 479–86.
14. Gardner EL. Cannabinoid interaction with brain reward systems. In: Nahas GG, Sutin KM, Harvey DJ, Agurell S Editors. Marihuana and Medicine. Totowa, NJ: Humana Press Inc., 1999. pp. 187–205.
15. Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. Nature 1998; 393: 76–9.
16. Markou A, Koob GF. Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology 1991; 4: 17–26.
17. Ledent C, Valverde O, Cossu C, Petitet F, Aubert LF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 1999; 283: 401–4.
18. Chaperon F, Soubrie P, Puech AJ, Thiebot MH. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. Psychopharmacology 1998; 135: 324–32.
19. Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sciences 1998; 63: PL113–7.
20. Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, Le Fur G. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology 1997; 132: 104–6.
21. Gallate JE, McGregor IS. The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. Psychopharmacology 1999; 142: 302–8.
22. Nakajima S. Subtypes of dopamine receptors involved in the mechanism of reinforcement. Neuroscience & Biobehavioral Reviews 1989; 13: 123–8.
23. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC. Cannabinoid receptor localization in brain. Proceedings of the National Academy of Sciences of the United States of America 1990; 87: 1932–6.
24. Compton DR, Aceto MD, Lowe J, Martin BR. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. Journal of Pharmacology & Experimental Therapeutics 1996; 277: 586–94.
25. Nakajima S, O'Regan NB. The effects of dopaminergic agonists and antagonists on the frequency-response function for hypothalamic self-stimulation in the rat. Pharmacology, Biochemistry and Behavior 1991; 39: 465–8.
26. Nakajima S, Liu X, Loong Lau C. Synergistic interaction of D1 and D2 dopamine receptors in the modulation of the reinforcing effect of brain stimulation. Behavioral Neuroscience 1993; 107: 161–5.
27. Hunt GE, McGregor IS. Rewarding brain stimulation induces only sparse Fos-like immunoreactivity in dopaminergic neurons. Neuroscience 1998; 83: 501–15.
28. Coulombe D, Miliaressis E. Fitting intracranial self-stimulation data with growth models. Behavioral Neuroscience 1987; 101: 209–14.
29. Johnson PI, Stellar JR. Comparison of delta opiate receptor agonist induced reward and motor effects between the ventral pallidum and dorsal striatum. Neuropharmacology 1994; 33: 1171–82.
30. Keppel G. Design and Analysis: A Researcher's Handbook. Englewood Cliffs, NJ: Prentice Hall, 1991.
31. Kucharski LT, Williams JEG, Kornetsky C. The effects of levonantradol on rewarding brain stimulation thresholds in the rat. Pharmacology, Biochemistry and Behavior 1983; 19: 149–51.
32. Bonhaus DW, Chang LK, Kwan J, Martin GR. Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. Journal of Pharmacology & Experimental Therapeutics 1998; 287: 884–8.
33. Watkins SS, Stinus L, Koob GF, Markou A. Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. Journal of Pharmacology & Experimental Therapeutics 2000; 292: 1053–64.

34. Schulteis G, Markou A, Cole M, Koob GF. Decreased brain reward produced by ethanol withdrawal. Proceedings of the National Academy of Sciences of the United States of America 1995; 92: 5880–4.
35. Kokkinidis L, Zacharko RM. Response sensitization and depression following long-term amphetamine treatment in a self-stimulation paradigm. Psychopharmacology 1980; 68: 73–6.
36. Lin D, Koob GF, Markou A. Time-dependent alterations in ICSS thresholds associated with repeated amphetamine administrations. Pharmacology, Biochemistry & Behavior 2000; 65: 407–17.
37. Wise RA, Munn E. Withdrawal from chronic amphetamine elevates baseline intracranial self-stimulation thresholds. Psychopharmacology 1995; 117: 130–6.
38. Baldo BA, Koob GF, Markou A. Role of adenosine A2 receptors in brain stimulation reward under baseline conditions and during cocaine withdrawal in rats. Journal of Neuroscience 1999; 19: 11017–26.
39. Frank RA, Manderscheid PZ, Panicker S, Williams HP, Kokoris D. Cocaine euphoria, dysphoria, and tolerance assessed using drug-induced changes in brain-stimulation reward. Pharmacology, Biochemistry & Behavior 1992; 42: 771–9.
40. Kokkinidis L, McCarter BD. Postcocaine depression and sensitization of brain-stimulation reward: analysis of reinforcement and performance effects. Pharmacology, Biochemistry & Behavior 1990; 36: 463–71.
41. Schulteis G, Markou A, Gold LH, Stinus L, Koob GF. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. Journal of Pharmacology & Experimental Therapeutics 1994; 271: 1391–8.
42. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Letters 1994; 350: 240–4.
43. Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ1 opioid receptor mechanism. Science 1997; 276: 2048–50.
44. Alonso R, Voutsinos B, Fournier M, Labie C, Steinberg R, Souilhac J, Le Fur G, Soubrie P. Blockade of cannabinoid receptors by SR141716 selectively increases Fos expression in rat mesocorticolimbic areas via reduced dopamine D-2 function. Neuroscience 1999; 91: 607–20.
45. Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand JP, Le Fur G, Casellas P. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. Journal of Biological Chemistry 1997; 272: 22330–9.
46. MacLennan SJ, Reynen PH, Kwan J, Bonhaus DW. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. British Journal of Pharmacology 1998; 124: 619–22.
47. Di Marzo V, Melck D, Bisogno T, De Petrocellis L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends in Neurosciences 1998; 21: 521–8.