Enhancement of endocannabinoid neurotransmission through CB₁ cannabinoid receptors counteracts the reinforcing and psychostimulant effects of cocaine

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Abstract

Cannabinoids, in contrast to typical drugs of abuse, have been shown to exert complex effects on behavioural reinforcement and psychomotor function. We have shown that cannabinoid agonists lack reinforcing/rewarding properties in the intracranial self-stimulation (ICSS) paradigm and that the CB₁ receptor (CB₁R) agonist WIN55,212-2 attenuates the reward-facilitating actions of cocaine. We sought to determine the effects of the endocannabinoid neurotransmission enhancer AM-404 (1, 3, 10, 30 mg/kg) on the changes in ICSS threshold and locomotion elicited by cocaine and extend the study of the effects of WIN55,212-2 (0.3, 1, 3 mg/kg) on cocaine-induced hyperlocomotion. AM-404 did not exhibit reward-facilitating properties, and actually increased self-stimulation threshold at the highest dose. Cocaine significantly reduced self-stimulation threshold, without altering maximal rates of responding. AM-404 (10 mg/kg) attenuated this action of cocaine, an effect which was reversed by pretreatment with the selective CB₁R antagonist SR141716A. WIN55,212-2 decreased locomotion at the two highest doses, an effect that was blocked by SR141716A; AM-404 had no effect on locomotion. Cocaine caused a significant, dose-dependent increase in locomotion, which was reduced by WIN55,212-2 and AM-404. SR141716A blocked the effects of WIN55,212-2 and AM-404 on cocaine-induced hyperlocomotion. SR141716A alone had no effect on ICSS threshold or locomotion. These results indicate that cannabinoids may interfere with brain reward systems responsible for the expression of acute reinforcing/rewarding properties of cocaine, and provide further evidence that the cannabinoid system could be explored as a potential drug discovery target for the treatment of psychostimulant addiction and pathological states associated with psychomotor overexcitability.

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Introduction

The endocannabinoid system consists of several recently discovered lipid mediators that produce their effects via interaction with specific cannabinoid binding sites (Rodríguez de Fonseca et al., 2005). Ample evidence indicates that endocannabinoids play a significant modulatory role in the control of motor behaviour, nociception, appetite, cognition, affect and behavioural reinforcement/reward processes (Chaperon and Thiébot, 1999). Most of the behavioural studies have been conducted with direct cannabinoid agonists, which are known for their undesirable psychotropic effects.

Recently, interest has increased for substances that enhance endocannabinoid neurotransmission through inhibition of elimination, and therefore may lack the adverse psychotropic effects induced by direct stimulation of the cannabinoid CB₁ receptor (CB₁R; Huestis et al., 2001). Although some of these endocannabinoid modulators have been examined in
various behavioural paradigms (see e.g. Compton and Martin, 1997; de Lago et al., 2004; Kathuria et al., 2003), only a few studies have examined their possible reinforcing/rewarding properties (Bortolato et al., 2006; Gobbi et al., 2005; Hansson et al., 2007; Vlachou et al., 2006).

Cannabis and compounds that stimulate endocannabinoid neurotransmission may arguably affect the actions of typical drugs of abuse (del Arco et al., 2002; Gallate et al., 1999; Solinas et al., 2005; Vigano et al., 2004; Vlachou et al., 2003). Although interactions with most drugs of abuse have been extensively studied, little is known about the interaction between cannabinoids and psychostimulants, possibly because most psychostimulants, in contrast to other drugs of abuse, affect the mesolimbic dopaminergic terminals (increasing extracellular dopamine concentrations) directly (Lupica and Riegel, 2005). Human and animal studies (De Vries et al., 2001; Foltin et al., 1993; Lukas et al., 1994; Vinklerova et al., 2002) have shown that cannabis enhances cocaine’s euphorogenic and behavioural effects. Inversely, other studies reveal a counteracting effect of cannabinoids on cocaine-induced behaviour-stimulating actions (Chaperon et al., 1998; Fattore et al., 1999; Martin et al., 2000; Parker et al., 2004; Schenk and Partridge, 1999; Vlachou et al., 2003). Inconsistent findings have also been reported with cannabinoids and other psychostimulants, e.g. amphetamine and MDMA (Braida et al., 2005; Lamarque et al., 2001; Muschamp and Siviy, 2002). Interestingly, intracerebroventricular co-administration of the CB₁R agonist CP55,940 and MDMA reduces the mean lever presses for their combined self-administration, compared to self-administration for each drug alone (Braida and Sala, 2002). In this direction, we have recently shown that the CB₁R agonist WIN55,212-2, in a dose that does not affect baseline self-stimulation threshold, reduces cocaine’s reinforcing/rewarding actions (Vlachou et al., 2003).

The psychomotor effects of cannabinoids have attracted interest, since the endocannabinoid system has been implicated in different psychomotor disorders, such as Parkinson’s and Huntington’s disease and Tourette’s syndrome (Pacher et al., 2006). Cannabinoids show a biphasic effect on locomotion, causing hyperactivity in low doses and hypoactivity or even catalepsy in high doses (Drews et al., 2005; Sañudo-Peña et al., 2000; Sulcova et al., 1998). However, our knowledge of the effects of indirect cannabinoid modulators on either spontaneous locomotion or hyperlocomotion induced by psychostimulants remains rather limited.

Accordingly, we investigated the effects of the endocannabinoid neurotransmission enhancer AM-404 (Beltramo et al., 1997) on brain stimulation reinforcement and on cocaine’s reward-facilitating action. To assess the possible involvement of CB₁R in these effects, we also examined whether they could be reversed by pretreatment with the selective CB₁R antagonist SR141716A (Rinaldi-Carmona et al., 1994), since not all of the centrally mediated effects of endocannabinoids occur through CB₁R stimulation. Additionally, we examined the effects of WIN55,212-2 and AM-404 on spontaneous locomotion and cocaine-induced hyperlocomotion, and whether SR141716A could counteract these effects.

Materials and methods

Intracranial self-stimulation (ICSS) studies

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-h light–dark cycle (lights on 08:00 hours) with free access to food and water. The animals were anaesthetized with intramuscular (i.m.) injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulphate (0.6 mg/kg i.m.) was injected to reduce bronchial secretion. Movable monopolar stimulating electrodes (Model SME-01, Kinetrons, Ottawa, ON, Canada) were lowered into the medial forebrain bundle (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 movable 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.

Apparatus and procedures for self-stimulation

One week after surgery, the animals were tested for self-stimulation in an operant chamber made of transparent Plexiglas (25 cm wide, 25 cm deep and 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 lever 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. During the acquisition phase the animals were trained to self-stimulate for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal lever-pressing rates. After self-stimulation had 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 ~0.1 log units. Each frequency was tested within trials of 60 s 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 on the rewarding efficacy of self-stimulation were inferred using the curve-shift paradigm. This method, which appears to have the reward selectivity that is required in psychopharmacological research, enabled us to distinguish between reward and performance, while allowing quantification of the drug effect.

Drugs

Cocaine hydrochloride (National Monopoly for Narcotics, Ministry of Health, Greece) was dissolved in 0.9% NaCl and injected intraperitoneally (i.p.) at a volume of 1 ml/kg of body weight. AM-404 (Tocris Bioscience, Ellisville, MO, USA) and SR141716A (synthesized by Lilly Research Laboratories, Indianapolis, IN, USA) were dissolved into a vehicle solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL and 90% of 0.9% NaCl and injected i.p. at a volume of 3 ml/kg of body weight. The doses of the cannabinoid compounds tested were within the range of doses regularly used in a plethora of functional studies (see e.g. Compton and Martin, 1997; de Lago et al., 2004; Kathuria et al., 2003).

Experimental procedure

Drug testing began for each animal when the function relating lever-pressing rate to pulse frequency (the rate-frequency function) was stable for at least three consecutive days. The criterion for stability was met when the frequency thresholds did not vary by more than 0.1 log unit. 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.

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-d period was allowed between injections. As we have observed in our previous studies, this period is considered sufficient for the behaviour of the animals to return to stable, pre-treatment levels, and not be affected by prior cannabinoid administration.

Experiment 1. Effects of systemically administered AM-404 on brain stimulation reward. Twenty-seven rats were used. Seven of them received all doses of AM-404 (1, 3, 10, 30 mg/kg i.p.) or its vehicle in a randomized order, while 20 received only one drug treatment.

Experiment 2. Effects of the CB₁R antagonist SR141716A on AM-404-induced changes in brain stimulation reward. Fifteen rats were used. Three of them received i.p. SR141716A (0.02 mg/kg) or vehicle followed 5 min later by AM-404 (30 mg/kg) or its vehicle i.p. in a randomized order, while 12 received only one combination of SR141716A and AM-404 (or their respective vehicles).

Experiment 3. Effects of AM-404 on the cocaine-induced lowering of brain reward thresholds. Sixteen rats were used. Four of them received AM-404 (0, 10 mg/kg i.p.) followed 5 min later by cocaine (0, 5 mg/kg i.p.), while 12 received only one combination of AM-404 and cocaine.

Experiment 4. Reversal of the action of AM-404 on the cocaine-induced lowering of brain reward thresholds by pretreatment with SR141716A. Fifteen rats were used. Three of them received all combinations of SR141716A (0, 0.02 mg/kg i.p.) and AM-404 (0, 10 mg/kg i.p.), followed 5 min later by cocaine (0, 5 mg/kg i.p.), while 12 received only one combination of SR141716A, AM-404 and cocaine.

Data analysis and statistics

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 (Coulombe and Miliaressis, 1987):

\[ f(X) = ae^{-e^{b(x-c)}}. \]
In this equation, \( \alpha \) 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 using one-way (effects of cannabinoid agonists alone) or two-way (effects of combined administration of cannabinoid agonists and antagonists) or three-way (effects of combined administration of cocaine, cannabinoid agonists and antagonists) analyses of variance (ANOVA) followed, whenever appropriate, by the LSD test for multiple contrasts. It should be noted that in our design, some animals received all treatments and others received only one (see above). In order to combine these two datasets, for all rats we expressed drug effects as a percentage of pre-injection baseline, which was established independently for each drug or vehicle treatment. Each drug test was thus treated as an independent measure for statistical analysis.

**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 protocol described by Vlachou et al. (2006). The brains were then removed and stored in a solution of 10% formalin until sectioned and stained for verification of the electrode tips. Only the rats in which tracks from the electrode were verified to be located in the MFB were included in this study.

**Spontaneous motor activity**

**Animals**

Male Sprague–Dawley rats \((n=576\) total) weighing 300–350 g were used for all experiments. Animals were housed three per cage under a 12-h light–dark cycle (lights on 08:00 hours) with free access to food and water.

**Assessment of locomotor activity**

Spontaneous motor activity was measured using an activity recording system (Model 7445, Ugo Basile). Each system consists of an animal cage and an electronic unit incorporating a counter and a printer. The rectangular animal cage \((56 \times 56 \times 30 \text{ cm})\) has transparent sides and lid to allow observation. The cage floor has horizontal and vertical infrared sensors. The counter sums up the photocell disruptions, and a printer displays the results at preset intervals. In our studies, a summation of photocell disruptions of locomotor activity and rearing, for each 5-min interval period, during the 1 h observation period was registered. The behavioural testing was performed between 08:00 and 16:00 hours. All animals were gently handled for 15 min each for 1 wk before the beginning of the experimental procedure. Two days before the drug testing each rat was gently handled for 15 min, injected i.p. with saline and habituated to the experimental room for 3 h in the home cage. The rats were also accustomed to the experimental room, for 1 h prior to the experiment. Each animal received only one drug treatment.

**Drugs**

Cocaine hydrochloride (National Monopoly for Narcotics, Ministry of Health, Greece) was dissolved in 0.9% NaCl and injected i.p. at a volume of 1 ml/kg of body weight. WIN55,212-2 (Tocris Bioscience), AM-404 (Tocris Bioscience) and SR141716A (synthesized by Lilly Research Laboratories) were dissolved into a vehicle solution that consisted of 5% dimethylsulfoxide, 5% cremophor EL and 90% of 0.9% NaCl and injected i.p. at a volume of 3 ml/kg of body weight.

**Study 1**

**Experiment 1. Effect of WIN55,212-2 on locomotor activity.** Animals \((n=44)\) were injected with WIN55,212-2 \((0.3, 1, 3 \text{ mg/kg})\) or its vehicle and after 10 min placed in the centre of the chamber for 1 h.

**Experiment 2. Reversal of WIN55,212-2-induced hypolocomotion with the CB\(_1\)R antagonist SR141716A.** Animals \((n=40)\) were injected with SR141716A \((0.02 \text{ mg/kg})\) or its vehicle, and after 5 min with WIN 55,212-2 \((3 \text{ mg/kg})\) or its vehicle, and 10 min later they were placed in the centre of the chamber for 1 h.

**Experiment 3. Effect of WIN55,212-2 on cocaine-induced hyperlocomotion \((5 \text{ mg/kg})\).** Animals \((n=32)\) were injected with WIN55,212-2 \((1 \text{ mg/kg})\) or its vehicle and after 5 min with cocaine \((5 \text{ mg/kg})\) or saline; immediately thereafter the animals were placed in the locomotor activity chambers.

**Experiment 4. Reversal of the action of WIN55,212-2 on cocaine-induced hyperlocomotion \((5 \text{ mg/kg})\) with SR141716A.** Animals \((n=72)\) were injected with...
SR141716A (0.02 mg/kg) or its vehicle and WIN55,212-2 (1 mg/kg) or its vehicle, followed after 5 min by cocaine (5 mg/kg) or saline.

**Experiment 5. Effect of WIN55,212-2 on cocaine-induced hyperlocomotion (10 mg/kg).** Two separate experiments (expts 5 and 6) with a higher dose of cocaine (10 mg/kg) than the dose used for the ICSS experiments (5 mg/kg) were added in the study to ascertain the robustness and the reproducibility of the effects of WIN55,212-2 on counteracting cocaine-induced hyperlocomotion, as cocaine clearly increases locomotion in a dose-dependent manner. Animals (n = 36) were injected with WIN55,212-2 (1 mg/kg) or its vehicle and after 5 min with cocaine (10 mg/kg) or saline.

**Experiment 6. Reversal of the action of WIN55,212-2 on cocaine-induced hyperlocomotion (10 mg/kg) with SR141716A.** Animals (n = 64) were injected with SR141716A (0.02 mg/kg) or its vehicle and WIN55,212-2 (1 mg/kg) or its vehicle, followed after 5 min by cocaine (10 mg/kg) or saline.

**Statistical analysis**

The total locomotor activity and rearing counts (dependent variables) over the 1 h observation period were statistically evaluated by using one-way (effects of compounds alone), two-way (effects of combined administration of two compounds) or three-way (effects of combined administration of three compounds) ANOVA, followed by post-hoc analysis using the LSD multiple comparison test in order to reveal specific differences between groups.

All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1996). Efforts were made in order to minimize the number of animals used and reduce their suffering.

**Results**

**ICSS studies**

**Experiment 1. Effects of systemically administered AM-404 on brain stimulation reward.** The changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of the endocannabinoid neurotransmission enhancer AM-404 are presented in Figure 1(a, c), respectively. AM-404 (1, 3, 10, 30 mg/kg i.p.) significantly increased self-stimulation thresholds at the highest dose tested [F(4, 50) = 4.239, p = 0.005], while it had no effect in the asymptotic rate of responding [F(4, 50) = 1.100, p = 0.367]. Post-hoc analysis with the LSD test showed that these effects on the self-stimulation thresholds were significant at the highest dose tested (30 mg/kg i.p.), compared with the other groups (p = 0.011, p = 0.024 and p = 0.012, respectively) and with the vehicle-treated group (p < 0.001).

**Experiment 2. Effects of SR141716A on AM-404-induced changes in brain stimulation reward.** Figure 1(b, d) presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection...
of SR141716A or its vehicle and AM-404 or its vehicle. Two-way ANOVA showed that AM-404 (30 mg/kg) produced an increase in self-stimulation threshold \( F(1, 20) = 16.467, p < 0.001 \). The administration of SR141716A (0.02 mg/kg) blocked this effect of AM-404 \( F(1, 20) = 7.996, p = 0.010 \). Two-way ANOVA also showed that neither AM-404 (30 mg/kg), nor SR141716A (0.02 mg/kg), nor their combined administration affected the asymptotic rate of responding.

**Experiment 3. Effects of AM-404 on cocaine-induced lowering of brain reward thresholds.** The changes in self-stimulation threshold and asymptotic rate of responding (c, d), following acute i.p. AM-404 (0, 1, 3, 10, 30 mg/kg) or SR141716A (0.02 mg/kg) and AM-404 (30 mg/kg) administration. Vertical bars represent the SEMs. The asterisks (*) signify an intracranial self-stimulation (ICSS) threshold significantly different from the control group: *** \( p < 0.001 \), # \( p < 0.05 \), ### \( p < 0.001 \) compared to the other groups.

Experiment 4. Reversal of the action of AM-404 on cocaine-induced lowering of brain reward thresholds by pretreatment with the CB1R antagonist SR141716A. Figure 2(b, d) presents the changes in self-stimulation threshold and asymptotic rate of responding after systemic injection of SR141716A or its vehicle, AM-404 or its vehicle and cocaine or saline. Three-way ANOVA showed that SR141716A (0.02 mg/kg) \( F(1, 32) = 3.806, p = 0.060 \) and AM-404 (10 mg/kg) \( F(1, 32) = 1.034, p = 0.317 \) had no effect on the self-stimulation threshold per se \( F(1, 24) = 3.867, p = 0.061 \). Pretreatment with AM-404 significantly blocked the facilitation of brain stimulation reward caused by cocaine \( F(1, 24) = 10.252, p = 0.004 \). Two-way ANOVA also showed that neither AM-404 (10 mg/kg), nor cocaine (5 mg/kg), nor their combined administration affected the asymptotic rate of responding.
self-stimulation threshold \( F(1, 32) = 32.291, p < 0.0001 \).

Co-administration of SR141716A and AM-404 had no effect on the self-stimulation threshold \( F(1, 32) = 2.372, p = 0.133 \). Co-administration of AM-404 and cocaine significantly blocked the facilitation of brain stimulation reward produced by cocaine \( F(1, 32) = 5.670, p = 0.023 \). It was found that the CB\(_1\)R antagonist SR141716A (0.02 mg/kg) completely reversed the inhibitory action of AM-404 (10 mg/kg) on the cocaine-induced lowering of brain-reward thresholds \( F(1, 32) = 5.322, p = 0.028 \). Three-way ANOVA also showed that neither SR141716A (0.02 mg/kg), nor AM-404 (10 mg/kg), nor cocaine (5 mg/kg), nor their combined administration affected the asymptotic rate of responding (Figure 3).

**Spontaneous motor activity**

**Study 1**

**Experiment 1. Effect of WIN55,212-2 on locomotor activity.** The effect of WIN55,212-2 on locomotor activity and rearing is shown in Figure 4(a, c), respectively. WIN55,212-2 decreased spontaneous locomotor activity \( F(3, 40) = 3.955, p = 0.015 \). Further analysis with the LSD test showed that this effect was statistically significant at the highest dose tested (3 mg/kg), compared to the vehicle group \( p = 0.002 \). WIN55,212-2 also reduced rearing at all doses tested \( F(3, 40) = 4.231, p = 0.011 \). Further analysis with the LSD test showed that this effect was statistically significant at all doses tested (0.3, 1, 3 mg/kg) compared to the vehicle group \( p = 0.029, p = 0.025 \) and \( p = 0.001 \), respectively.

**Experiment 2. Reversal of WIN55,212-2-induced hypolocomotion with the CB\(_1\)R antagonist SR141716A.** The effect of SR141716A on WIN55,212-2-induced hypolocomotion and rearing is shown in Figure 4(b, d), respectively. Two-way ANOVA showed that WIN55,212-2 decreased spontaneous locomotor activity \( F(1, 36) = 3.831, p = 0.05 \) and rearing \( F(1, 36) = 8.496, p = 0.006 \) at the highest dose tested (3 mg/kg). The CB\(_1\)R antagonist SR141716A (0.02 mg/kg) reversed this effect both on locomotion \( F(1, 36) = 12.250, p = 0.001 \) and rearing \( F(1, 36) = 3.664, p = 0.044 \), while it had no effect by itself.

**Experiment 3. Effect of WIN55,212-2 on cocaine-induced hyperlocomotion (5 mg/kg).** The effect of WIN55,212-2 on cocaine-induced hyperlocomotion and rearing is
presented in Figure 5(a, c), respectively. WIN55,212-2 (1 mg/kg) did not produce any significant change on motor activity. Cocaine (5 mg/kg) produced a statistically significant increase in spontaneous motor activity $[F(1, 28) = 5.030, p = 0.033]$ and rearing $[F(1, 28) = 4.067, p = 0.053]$, compared to the vehicle group. WIN55,212-2 (1 mg/kg) completely blocked this effect on locomotion $[F(1, 28) = 3.820, p < 0.05]$ and rearing $[F(1, 28) = 14.005, p = 0.001]$, while it had no effect per se.

Experiment 4. Reversal of the action of WIN55,212-2 on cocaine-induced hyperlocomotion (5 mg/kg) with SR141716A. Figure 5(b, d) presents the reversal of the
action of WIN55,212-2 on the cocaine-induced hyperlocomotion and rearing, respectively, by SR141716A. Cocaine (5 mg/kg) produced a statistically significant increase in spontaneous motor activity $[F(1, 64) = 110.959, p < 0.0001]$ and rearing $[F(1, 64) = 31.443, p < 0.0001]$. SR141716A and WIN55,212-2 had no effect per se on either locomotor activity or rearing. SR141716A (0.02 mg/kg) completely reversed the effect of WIN55,212-2 on the cocaine-induced hyperlocomotion $[F(1, 64) = 7.596, p = 0.008]$ and increased rearing $[F(1, 64) = 6.182, p = 0.016]$.

Experiment 5. Effect of WIN55,212-2 on cocaine-induced hyperlocomotion (10 mg/kg). The effect of WIN55,212-2 on cocaine-induced hyperlocomotion and rearing is presented in Figure 6(a, c), respectively. Two-way ANOVA showed that cocaine (10 mg/kg) produced a statistically significant increase in spontaneous motor activity $[F(1, 28) = 27.049, p < 0.0001]$, compared to the vehicle group, while it did not affect rearing $[F(1, 28) = 3.604, p = 0.068]$. WIN55,212-2 (1 mg/kg) completely blocked this effect on locomotion $[F(1, 28) = 7.515, p = 0.011]$ and rearing $[F(1, 28) = 4.855, p = 0.036]$, while it had no effect per se $[F(1, 28) = 3.510, p = 0.071]$.

Experiment 6. Reversal of the action of WIN55,212-2 on cocaine-induced hyperlocomotion (10 mg/kg) with SR141716A. Figure 6(b, d) presents the reversal of the action of WIN55,212-2 on the cocaine-induced hyperlocomotion and rearing, respectively, by SR141716A. Cocaine (10 mg/kg) significantly increased spontaneous motor activity $[F(1, 56) = 186.407, p < 0.0001]$ and rearing $[F(1, 56) = 46.455, p < 0.0001]$. SR141716A and WIN55,212-2 had no effect per se on either locomotor activity $[F(1, 56) = 3.141, p = 0.082$ and $F(1, 56) = 2.761, p = 0.102$, respectively] or rearing.
[F(1, 56) = 1.122, p = 0.294 and F(1, 56) = 3.067, p = 0.085, respectively]. SR141716A (0.02 mg/kg) completely reversed the effect of WIN55,212-2 on the cocaine-induced hyperlocomotion [F(1, 56) = 7.170, p = 0.010] and increased rearing [F(1, 56) = 4.855, p = 0.036].

Study 2

Experiment 1. Effect of AM-404 on locomotor activity. AM-404 did not affect significantly locomotion [F(3, 32) = 0.701, p = 0.595] or rearing [F(3, 32) = 0.995, p = 0.408], although there was a tendency for a reduction in both indices at all doses tested, as shown in Figure 7(a, b), respectively.

Experiment 2. Effect of AM-404 (10 mg/kg) on cocaine-induced hyperlocomotion (5 mg/kg). Cocaine (5 mg/kg) produced a statistically significant increase in spontaneous motor activity [F(1, 28) = 14.535, p = 0.001] and rearing [F(1, 28) = 14.141, p = 0.001]. AM-404 (10 mg/kg) significantly blocked this effect on locomotion [F(1, 28) = 5.319, p = 0.029] and rearing [F(1, 28) = 6.974, p = 0.013], as presented in Figure 8(a, c), respectively.

Experiment 3. Reversal of the action of AM-404 (10 mg/kg) on cocaine-induced hyperlocomotion (5 mg/kg) with SR141716A. Cocaine (5 mg/kg) produced a significant increase in motor activity [F(1, 72) = 119.549, p < 0.0001] and rearing [F(1, 72) = 48.882, p < 0.0001]. SR141716A (0.02 mg/kg) did not have any effect on motor activity. There was a statistically significant interaction between AM-404 and cocaine in motor activity [F(1, 72) = 4.846, p = 0.031] and rearing [F(1, 72) = 4.065, p = 0.048]. This was due to the fact that, AM-404 blocked the stimulatory effects of cocaine. SR141716A (0.02 mg/kg) completely reversed the effect of AM-404 on cocaine-induced hyperlocomotion [F(1, 72) = 5.439, p = 0.022] and increased rearing [F(1, 72) = 5.188, p = 0.026], as shown in Figure 8(b, d), respectively.

Experiment 4. Effect of AM-404 (10 mg/kg) on cocaine-induced hyperlocomotion (10 mg/kg). Figure 9(a, d) shows the effect of AM-404 on cocaine-induced hyperlocomotion and rearing, respectively. Cocaine (10 mg/kg) produced a statistically significant increase in locomotor activity [F(1, 28) = 22.912, p < 0.0001] and rearing [F(1, 28) = 12.646, p = 0.001].
Experiment 5. Effect of AM-404 (30 mg/kg) on cocaine-induced hyperlocomotion (10 mg/kg). Figure 9(b, e) shows the effect of AM-404 (30 mg/kg) on cocaine-induced hyperlocomotion (10 mg/kg) and rearing, respectively. Two-way ANOVA showed that cocaine (10 mg/kg) produced a statistically significant increase in locomotor activity \[ F(1,32) = 158.361, p < 0.0001 \] and rearing \[ F(1,32) = 9.333, p = 0.005 \] and AM-404 (30 mg/kg) significantly blocked the hyperlocomotion \[ F(1,32) = 5.381, p = 0.023 \] and rearing \[ F(1,32) = 5.231, p = 0.029 \] induced by cocaine.

Experiment 6. Reversal of the action of AM-404 (30 mg/kg) on cocaine-induced hyperlocomotion (10 mg/kg) with SR141716A. Cocaine (10 mg/kg) produced a significant increase in motor activity \[ F(1,64) = 158.361, p < 0.0001 \] and rearing \[ F(1,64) = 86.983, p < 0.0001 \]. There was a statistically significant effect of cocaine after the administration of SR141716A and AM-404 on motor activity \[ F(1,64) = 4.599, p = 0.036 \] and \[ F(1,64) = 6.600, p = 0.013 \], respectively and rearing \[ F(1,64) = 4.579, p = 0.034 \] and \[ F(1,64) = 5.381, p = 0.024 \], respectively, although it is clearly shown that AM-404 reduced the hyperlocomotor effect of cocaine. SR141716A (0.02 mg/kg) completely reversed the effect of AM-404 on the cocaine-induced hyperlocomotion \[ F(1,64) = 5.153, p = 0.027 \], while there seemed to be no interaction on rearing \[ F(1,64) = 2.742, p = 0.103 \], as shown in Figure 9(c, f), respectively.

Discussion

The present study showed that the endocannabinoid neurotransmission enhancer AM-404 at relatively low doses (1, 3 and 10 mg/kg) did not affect intracranial self-stimulation behaviour, whereas at the highest dose (30 mg/kg) increased brain reward thresholds. These changes were not accompanied by significant changes in asymptotic rates of responding. The anhedonic-like effect of AM-404, observed at the highest dose, was completely abolished by pretreatment with the CB₁R antagonist SR141716A. However, since it is not clear whether AM-404 at the
dose of 30 mg/kg can still selectively prevent anandamide elimination, the observed effects could be due to a non-selective increase of extracellular concentrations of endocannabinoids other than anandamide or due to unselective actions of this drug, such as direct stimulation of the CB₁R, the latter been more probable since AM-404 action was reversed by SR141716A (Dickason-Chesterfield et al., 2006). It is worth noting that in our study SR141716A did not affect ICSS threshold by itself, suggesting lack of a tonic regulation of reward/reinforcement processes via CB₁R. The present results are in agreement with a recent study from our laboratory (Vlachou et al., 2006), in which the endocannabinoid neurotransmission modulators PMSF, URB-597 and OMDM-2, depending on the dose administered, either do not affect or decrease brain reward function. The finding that AM-404 lacks appetitive properties also agrees with other studies, where anandamide and URB-597 do not produce conditioned place preference in male Wistar rats (Gobbi et al., 2005; Mallet and Beninger, 1998). However, it should be mentioned that anandamide and methanandamide have been shown to be self-administered by squirrel monkeys (Justinova et al., 2005) and AM-404 induces conditioned place preference in rats living in an enriched environment (Solinas et al., 2005). These seemingly contrasting results could be attributed to differences in the pharmacological properties and the dose range of the tested compounds, the species and the strain of the animals used or the methods followed; in addition, the route of administration might be a determinant factor in assessing appetitive actions of cannabinoids, as has previously been argued (Vlachou et al., 2003, 2005, 2006, 2007).

Acute administration of AM-404 counteracted the reward-facilitating effect of systemic cocaine at a dose that by itself was ineffective in altering brain stimulation reward thresholds. Cocaine (5 mg/kg) produced a significant reduction in self-stimulation threshold, without altering maximal rates of responding, in agreement with previous studies (Maldonado-Irrizary et al., 1994; Matthews et al., 1996; Panagis et al., 2000; Panagis and Spyraki, 1996; Ranaldi et al., 1997; Vlachou et al., 2003). AM-404 (10 mg/kg) attenuated the reward-facilitating action of cocaine, an effect which was reversed by pretreatment with SR141716A (0.02 mg/kg). The present results are in agreement with a number of studies, indicating interactions of cannabinoids with psychostimulants (Braida et al., 2001; Braida and Sala, 2002; Cadoni et al., 2001; De Vries et al., 2001; Fattore et al., 1999; Gallate et al., 1999; Ghoshland et al., 2002; González et al., 2002a,b; Filip et al., 2006; González-Cuevas et al., 2007; Higuer-Matas et al., 2007; Lamarque et al., 2001; Navarro et al., 2001; Pontieri et al., 2001a,b; Tanda, 2007; Vlachou et al., 2003). We have recently shown that the CB₁R agonist WIN55,212-2 at a dose that does not affect brain stimulation reward per se counteracts the reward-facilitating effects of cocaine (Vlachou et al., 2003). Fattore and colleagues have shown that WIN55,212-2 reduces the intravenous self-administration of cocaine (Fattore et al., 1999), a finding also consistent with the most recent study by Panlilio and colleagues, in which Δ⁹-tetrahydrocannabinol (THC) was found to reduce the self-administration of cocaine under a progressive ratio schedule of reinforcement (Panlilio et al., 2007), and with another recent study by Cippitelli and colleagues, in which AM-404 was found to reduce ethanol self-administration (Cippitelli et al., 2007). On the other hand, it has been found that the potent CB₁R agonist HU-210 induces relapse to cocaine-seeking after a prolonged withdrawal period (De Vries et al., 2001), and CP55,940 does not seem to affect the arousal caused to
Cebus monkeys by \( d \)-amphetamine (Madsen et al., 2006). Moreover, it has recently been shown that CB\(_1\)R knockout mice respond to a lesser extent to cocaine self-administration (Soria et al., 2005; but see Cossu et al., 2001). Conversely, cocaine self-administration does not alter dialysate levels of anandamide or 2-arachidonoylglycerol and SR141716A does not affect cocaine self-administration (Caille et al., 2007). The fact that there appear to be contrasting effects of cannabinoids on the actions of cocaine supports the hypothesis that there is a differential interaction between these two classes of drugs depending on context as well as on the pharmacological and behavioural modalities under study (Chaperon et al., 1998; De Vries et al., 2001; Lesscher et al., 2005; Xi et al., 2007). Although the neurobiological mechanisms underlying this interaction remain unclear, we speculate that, since there is co-existence of CB\(_1\) and dopamine receptors in brain-reward circuits (Hermann et al., 2002), the CB\(_1\)R stimulation could act as a counteracting feedback mechanism for the increased extracellular dopamine concentrations in the nucleus accumbens produced by cocaine and other psychostimulants (see e.g. Centonze et al., 2004; Giuffrida et al., 1999); whether this results in a modified dopamine response remains to be unequivocally shown.

WIN55,212-2 decreased spontaneous locomotor activity at the highest dose tested (3 mg/kg), while AM-404 (3, 10, 30 mg/kg) had no significant effect on motor function per se at any of the doses tested, although there was a tendency for reduction of locomotion at the highest dose administered. SR141716A (0.02 mg/kg) blocked the effects of WIN55,212-2 on spontaneous motor activity. It is well known that psychostimulant drugs, increase spontaneous motor activity (Beninger, 1983; Fung and Lau, 1988; Iwamoto, 1984; Norton, 1973). Studies that have been conducted in the last years with cannabinoids have shown that they have a biphasic effect on neurotransmitter release (Tzavara et al., 2003), as well as on locomotion, causing both hyperlocomotion in low doses (Drews et al., 2005; McGregor et al., 1996; Muschamp and Siviy, 2002; see also, Introduction) and hypolocomotion or even catalepsy at high doses (e.g. Meschler et al., 2001; Rodríguez de Fonseca et al., 1998; Sañudo-Peña et al., 2000). Since the CB\(_1\)R are predominantly located in the basal ganglia and associated structures with the highest density among all other receptors in the brain, their motor effects have been hypothesized to be caused by...
an interplay of actions on the dopaminergic, glutamatergic and GABAergic systems in these brain areas (San˜udo-Pen˜a et al., 1999, 2000). Therefore, the reduction in locomotor activity observed in our studies could be attributed to stimulation of CB1R in the striatum, which increases GABAergic neurotransmission in the globus pallidus or substantia nigra (Maneuf et al., 1997). It could also be due to trans-synaptic stimulation of GABAergic neurons, which project to the substantia nigra and cause reduced stimulation of the nigrostriatal dopaminergic neurons (Gueudet et al., 1995).

Our results are in agreement with previous studies showing that anandamide, THC or CB1R agonists reduce motor function (Darmani, 2001; Ferrari et al., 1999; Romero et al., 2002; Singh et al., 2005; Valjent et al., 2002). However, other studies have shown different results (Drews et al., 2005; Muschamp and Siviy, 2002; Przegali´nski et al., 2005). Przegali´nski and colleagues have shown that administration of 3 mg and 6 mg WIN55,212-2 does not affect locomotion, while in our study there was a clear hypolocomotion and reduced rearing after administration of 3 mg WIN55,212-2. Our results also differ from Muschamp and Siviy (2002), where there was an increase in locomotion with 1 mg/kg WIN55,212-2, although this was shown in Lewis rats and only in the first 15 min of the experimental session. It is probable that the different results could be attributed to different experimental conditions.

Cocaine (5, 10 mg/kg) caused a significant, dose-dependent increase in locomotor activity, which was

Figure 9. Effects of AM-404 (10 and 30 mg/kg) and cocaine (10 mg) on locomotor activity and effect of SR141716A (0.02 mg/kg) on the AM-404 (30 mg)-induced reduction of cocaine’s hyperlocomotion. Histograms represent the photocell disruptions caused by the animals’ locomotion (a–c) and rearing (d–f) (mean ± S.E.M.). The asterisks (*) show a statistically significant effect compared to the vehicle group: * p<0.05, ** p<0.01, *** p<0.001, * p<0.05, ** p<0.01, *** p<0.001 compared to AM10 or AM30+Sal (a, b, d, e), or compared to all other groups (c, f); + p<0.05, compared to the AM10+Coc10 group; +++ p<0.001 compared to the Veh+Coc10 (e) or all the cocaine groups (c, f).
reduced by WIN55,212-2 (1 mg/kg) and AM-404 (10, 30 mg/kg, respectively). AM-404 (10 mg/kg) did not affect the hyperlocomotor effects of cocaine (10 mg/kg), although it did reduce cocaine’s stimulatory action on rearing. A higher dose of AM-404 (30 mg/kg) was found to reduce cocaine (10 mg/kg)-induced hyperactivity. SR141716A (0.02 mg/kg) blocked the effects of WIN55,212-2 and AM-404 on spontaneous motor activity and on the hyperlocomotion induced by cocaine, while it had no effect on motor function per se. The above finding shows that WIN55,212-2 and probably AM-404 exhibited their actions through CB$_1$R stimulation. Ferrari and colleagues have also shown that HU-210 reduces the increased locomotion and rearing caused by cocaine (Ferrari et al., 1999), as it was also recently shown for morphine and alcohol (Hagues et al., 2007). Similarly, Muschamp and Siviy (2002) have shown that acute administration of WIN55,212-2 (1 mg/kg), in Lewis rats inhibits the hyperlocomotion and increased rearing induced by cocaine. Our results also agree with other studies involving THC and its counteracting action on amphetamine-induced hyperlocomotion (Gorriti et al., 1999). Importantly, in a very recent study Rodvelt and colleagues (2007) have shown that WIN55,212-2 attenuates nicotine-induced hyperactivity. It is probable that WIN55,212-2 and AM-404 alter the hyperactivity produced by cocaine through an indirect mechanism, such as alteration in endocannabinoid tone of motor circuits. A possible mechanism could involve a negative feedback mechanism induced by cannabinoids in the striatum, as mentioned above (see Centonze et al., 2004, 2007; Giuffrida et al., 1999). A second alternative mechanism of action of at least WIN55,212-2 could be the inhibition of dopamine transporter, which has been shown in the case of WIN55,212-2 resulting in competition with cocaine that also inhibits the dopamine transporter (Steffens and Feuerstein, 2004).

In conclusion, acute systemic administration of the direct CB$_1$R agonist WIN55,212-2 and the endocannabinoid neurotransmission enhancer AM-404, even at high doses, did not have reinforcing effects, but rather reversed the reward-facilitating effects of cocaine in the ICSS paradigm. Furthermore, the same compounds either did not affect, or (at high doses) decreased locomotor activity. Interestingly, both WIN55,212-2 and AM-404 in doses that did not affect spontaneous motor activity reduced the hyperlocomotion induced by cocaine. These effects are mediated through CB$_1$R stimulation, since they were reversed by pretreatment with the CB$_1$R antagonist SR141716A. Therefore, cannabinoids clearly play a modulatory role in cocaine’s psychostimulant actions. Based upon the present findings, we speculate that drugs increasing the cannabinergic tone could be further tested as a potential treatment for various aspects of psychostimulant addiction and psychomotor excitability. In this regard, it is worth-noting that Tzavara et al. (2006) have recently shown that endocannabinoids acting through vanilloid receptors reduce hyperdopaminergia-related hyperactivity in a relevant genetic animal model. Thus, endocannabinoid neurotransmission modulators appear to offer the potential of a dual receptor action through both CB$_1$R and non-CB$_1$R counteracting overt psychomotor stimulation without affecting basal function and with seemingly low overall psychotropic liability.

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Statement of Interest

None.

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