Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 77 (2004) 567–573
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Unconditioned and conditioned anxiogenic effects of the cannabinoid receptor agonist CP 55,940 in the social interaction test

Rachel F. Genn[a], Sonia Tucci[a], Eva M. Marco[a], M. Paz Viveros[b,*], Sandra E. File[a]

[a]*Psychopharmacology Research Unit, Centre for Neuroscience, GKT School of Biomedical Sciences, King's College London, Hodgkin Building, Guy's Campus, London SE1 1UL, UK*
[b]*Departmento de Fisiología (Fisiología Animal II), Facultad de Biologia, Universidad Complutense, Ciudad Universitaria, 28040, Madrid, Spain*

Received 30 October 2003; received in revised form 16 December 2003; accepted 17 December 2003

Abstract

In spite of the addictive properties of cannabinoids, under certain circumstances, they can evoke strong anxiogenic and aversive responses in humans and in animal tests of anxiety. Effects of different doses of CP 55,940 (10, 20, and 40 μg/kg) were tested in the low-light, familiar (LF) apparatus test condition of the social interaction test. The 40-μg/kg dose of CP 55,940 significantly decreased the time spent in social interaction, indicating an anxiogenic effect. This dose also had an independent effect of reducing locomotor activity. In rats tested undrugged 24 h after testing with 40 μg/kg, there was a significant anxiogenic effect, indicating conditioned anxiety. The group of rats injected with 40 μg/kg immediately after the social interaction test showed an unexpected significant anxiolytic effect when tested undrugged 24 h later. In an additional experiment, rats were tested in the high-light, familiar (HF) apparatus test condition after 10 or 40 μg/kg, and only those that were tested after 40 μg/kg showed an anxiogenic effect on the test day and a conditioned anxiogenic effect when tested undrugged 24 h later. Once again, those injected with 40 μg/kg after the social interaction test displayed an anxiolytic effect when tested undrugged 24 h later. We provide the first evidence for unconditioned and conditioned anxiogenic-like responses to a cannabinoid agonist in the social interaction test.

Keywords: Anxiety; Classical conditioning; Cannabinoid agonist; CP 55,940; Social interaction test

1. Introduction

The motivation to use marijuana is thought to lie in part on its ability to assuage symptoms of anxiety (Porter and Felder, 2001), but cannabis users report feelings of anxiety and panic reactions (Hall et al., 1994; Hall and Solowij, 1998) in equal measure to those of relaxation and euphoria (Hall et al., 1994). It is possible that the reasons for this lie in bidirectional effects of cannabinoids on anxiety, with low doses having anxiolytic effects and high doses having anxiogenic ones. The data from animal tests provide further evidence of bidirectional modulation of anxiety by the cannabinoid system. Low doses of the cannabinoid receptor agonists, nabilone (Onaivi et al., 1990), CP 55,940 (Genn et al., 2003; Marco et al., in press), and Δ^9-tetrahydrocannabinol (THC) (Berrendero and Maldonado, 2002), induced anxiolytic effects in the elevated plus maze and light–dark crossing tests. In contrast, high doses of the cannabinoid agonist HU-210 produced anxiogenic responses in the defensive withdrawal test (Rodriguez de Fonseca et al., 1996) and enhanced emotional responding to tactile stimulation (Giuliani et al., 2000), whereas mid-high doses of CP 55,940 had anxiogenic effects in the plus maze (Arévalo et al., 2001; Marín et al., 2003; Marco et al., in press).

The cannabinoid receptor agonist CP 55,940 binds to the brain cannabinoid CB_1 receptor with high affinity (Herkenham et al., 1991) and has been shown to be approximately 30 times more potent than THC (Little et al., 1988). However, not all the behavioural effects of CP 55,940 seem to be mediated by the CB_1 receptor inasmuch as the reduction in directed exploration in the holeboard and the anxiogenic effect in the plus maze were not reversed by the CB_1 receptor antagonist SR 141716A (Arévalo et al., 2001; Romero et al., 2002). It has recently been suggested that there is a role for a novel cannabinoid receptor in the mediation of anxiety (Haller et al., 2002) inasmuch as both wild type and CB_1 knockout mice show

* Corresponding author. Tel.: +34-1-3944993; fax: +34-1-3944935.
E-mail address: pazviver@bio.ucm.es (M.P. Viveros).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.12.019

changes in anxiety in the plus maze in response to the functional CB_1 receptor antagonist SR141716A. This antagonist binds to the putative novel cannabinoid-sensitive receptor (Haller et al., 2002), which constitutes a candidate for the mediation of preserved effects of cannabinoids in CB_1 knockout mice (Jarai et al., 1999; Zimmer et al., 1999; Di Marzo et al., 2000; Breivogel et al., 2001; Hajos et al., 2001). It is therefore possible that different cannabinoid receptors may mediate anxiety in different ways. Evidence for an endogenous anxiolytic cannabinoid tone comes from the intrinsic effects of the receptor antagonist SR141716A. This drug has anxiogenic effects in the defensive withdrawal and elevated plus maze tests in rats (Navarro et al., 1997; Arévalo et al., 2001).

The anxiogenic effects of abused drugs may constitute salient stimuli to which drug-associated negative symptoms are classically conditioned. For example, the phenomenon of test-specific conditioned anxiety to an anxiogenic dose of nicotine has been demonstrated in both the social interaction and the elevated plus maze tests (File et al., 2002; Tucci et al., 2002). Other drugs of abuse, such as cocaine (DeVries and Pert, 1998), produce a similar pattern of conditioned anxiety, and this phenomenon has been closely linked to cocaine's abuse potential (Goeders, 1997, 2002).

The purpose of the present experiment was to explore the possible bidirectional effects of CP 55,940 on anxiety in the social interaction test (File, 1992; File and Seth, 2003), as well as whether anxiety could be conditioned to a mid-high dose of the drug. In the social interaction test, which models generalised anxiety disorder, the dependent variable is the time spent in social interaction by pairs of rats, and the anxiety generated by this test can be manipulated by changing the light level and/or the rats' familiarity within the test arena. The greatest level of anxiety is generated when rats are tested in an unfamiliar test arena that is lit by high light [high-light unfamiliar (HU) test condition]. A moderate level of anxiety is generated by testing rats in an arena with which they have been familiarised, but which is lit by high light [high-light familiar (HF) test condition]. The lowest level of anxiety is generated by testing rats in a familiar arena, lit by low light [low-light, familiar (LF) test condition]. Increases in social interaction are indicative of an anxiolytic effect, and decreases indicate an anxiogenic response. To test for possible anxiolytic effects, we used the HU and HF test conditions inasmuch as these conditions generate low levels of social interaction in the control group and are most sensitive to the effects of anxiolytics (File and Hyde, 1978; File, 1980; File and Seth, 2003). To test for possible anxiogenic effects, we used the LF test condition, which generates the highest level of social interaction and is most sensitive to anxiogenic drug effects (e.g., File et al., 1982, 1988). Conditioned anxiogenic effects were explored in both the LF and HF test conditions.

2. Materials and methods

2.1. Animals

Male hooded Lister rats (Charles River, Margate, Kent, UK) weighing 220–250 g were housed singly in 45 × 28 × 20-cm-high cages for 7 days prior to the start of behavioural testing. All the cages were in racks that allowed the rats to see, hear, and smell other rats. Food and water were freely available to all the animals. The room in which animals were housed was lit with dim light and maintained at 22 °C. Lights were on from 0700–1900 h. The experimental procedures carried out in this study were in compliance with the UK Animals (Scientific Procedures) Act 1986 (Home Office Project Licence Number 70/5436).

2.2. Apparatus

The social interaction test arena was a 60 × 60-cm wooden box, with 35-cm walls, and was lit by high light or low light (300 or 30 lux, respectively). A camera was mounted vertically above the arena, and the rats were observed on a monitor in an adjacent room. The time spent in social interaction (sniffing, following, and grooming the partner, and boxing and wrestling) provided the measure of anxiety and was scored by an observer who was blind to the drug treatment. The interruption of infrared beams from photocells mounted in the walls 3.5 cm from the floor provided an automated measure of locomotor activity (File, 1980).

2.3. Drugs and chemicals

The cannabinoid receptor agonist CP 55,940 (Tocris, UK) was dissolved in ethanol (BDH, Poole, UK)–cremophor (Sigma, Poole, UK)–saline (1:1:18) in a volume of 1 ml/kg body weight. Control animals received equal volume injections of the corresponding vehicle.

2.4. Experiment 1: effects of low doses of CP 55,940 in the HU and HF test conditions

On the first test day, rats were allocated to pairs in such a way that members of a pair did not differ in weight by more than 10 g. Pairs of rats were randomly allocated between four groups ($n = 8$ pairs/group): vehicle, CP 55,940 2.5, 5, and 10 μg/kg. Rats were intraperitoneally injected 30 min before testing. Both members of a pair always received the same drug treatment.

The pairs of rats were placed in the test arena, and social interaction was scored for 4.5 min by an observer blind to drug treatment. Social interaction initiated by either member of the pair was scored, resulting in a single score for each pair of rats. Rats were tested between 0900 and 1300 h in an order randomised for drug treatment,

and the test arena was cleaned with a paper towel after each trial. On the first test day, the rats were tested in the HU test condition, and they were then retested the following day, after the same drug treatment, in the HF condition.

2.5. Experiment 2: effects of mid-high doses of CP 55,940 in the LF test condition

The rats were given two familiarisation trials in the test arena on the days preceding the test. Pairs of rats were randomly allocated between four groups ($n = 8$ pairs/group): vehicle, CP 55,940 10, 20, and 40 μg/kg. Rats were intraperitoneally injected 30 min before testing, and both members of a pair received the same dose. An additional group ($n = 8$) was tested undrugged and then injected 5 min after the social interaction test with CP 55,940 (40 μg/kg).

To test for conditioned anxiety, the following groups of rats were retested undrugged 24 h later: vehicle; CP 55,940 (40 μg/kg during testing); and CP 55,940 (40 μg/kg after testing).

2.6. Experiment 3: conditioned anxiety in the HF test condition

To further explore the possibility of a conditioned anxiogenic effect to 40 μg/kg CP 55,940, pairs of rats were randomly allocated between the following four groups ($n = 8$ pairs/group): vehicle; CP 55,940 (10 and 40 μg/kg during testing); and CP 55,940 (40 μg/kg after testing on Day 1). On Day 2, all rats were tested undrugged. The rats were tested under HF test conditions. The purpose of this experiment was twofold. First, we wished to replicate the unexpected anxiolytic effect found in Experiment 2 in the group tested undrugged 24 h after receiving the 40-μg/kg dose after testing on Day 1 and to determine whether this effect could also be observed under high-light test conditions. Second, we wished to determine whether there would be any behavioural consequences when rats were tested undrugged 24 h after testing on Day 1 in the social interaction test with a nonanxiogenic dose of CP 55,940 (i.e., 10 μg/kg). If an anxiogenic effect were found on Day 2 in the 40-μg/kg group and not in the 10-μg/kg group, then this would provide further evidence for a conditioned anxiogenic effect.

2.7. Statistics

The data from Experiment 1 were analysed using a split-plot analysis of variance (ANOVA) with test arena familiarity as the within-subjects variable and drug dose as a between-subjects variable. The data from Experiments 2 and 3 were analysed by one-way ANOVAs with drug treatment as the independent variable. Further comparisons between individual groups were then made with Fisher's least squares difference (LSD) test. In cases where CP 55,940 changed both measures of anxiety and motor activity, analyses of covariance (ANCOVAs) were conducted to determine the independence of changes.

3. Results

3.1. Experiment 1: effects of low doses of CP 55,940 in the HU and HF test conditions

The split-plot ANOVA revealed that there were no effects of drug on the time spent in social interaction or on locomotor activity [$F(3,28) < 1.0$, n.s. in both cases; see Table 1]. Neither were there significant Drug × Familiarity interactions under these conditions on either social interaction [$F(3,28) = 0.6$, n.s.] or locomotor activity [$F(3,28) = 2.6$, n.s.]. However, there were significant effects of familiarity to increase both social interaction [$F(1,28) = 8.3$, $P < .01$] and locomotor activity [$F(1,28) = 18.7$, $P < .01$].

3.2. Experiment 2: effects of mid-high doses of CP 55,940 in the LF test condition

When rats were tested in the LF test condition, CP 55,940 significantly reduced the time spent in social interaction [$F(3,28) = 8.9$, $P < .0005$] and significantly decreased locomotor activity [$F(3,28) = 11.0$, $P < .0001$]. Post hoc tests showed that both these decreases were solely due to the 40-μg/kg dose, and therefore, an ANCOVA was used to determine the independence of these two effects of this dose. Following ANCOVA, both changes remained significant [social interaction, $F(1,13) = 8.1$, $P < .05$; locomotor activity, $F(1,13) = 4.8$, $P < .05$], indicating that CP

Table 1
Effects of low doses of CP 55,940 on social interaction (Experiment 1) and conditioned anxiety to a mid-high dose (Experiment 3, Day 1)

Experiment	Test condition	Dose (μg/kg)	Time spent in social interaction (s)	Locomotor activity (beam breaks)
1	HU	Vehicle	153.3 ± 13.2	235.9 ± 13.8
		2.5	139.4 ± 9.6	218.1 ± 15.6
		5	137.3 ± 18.1	210.1 ± 14.9
		10	131.2 ± 11.7	218.0 ± 18.3
1	HF	Vehicle	164.2 ± 18.3	255.8 ± 28.5
		2.5	164.5 ± 19.3	281.4 ± 17.4
		5	188.4 ± 24.2	218.6 ± 12.3
		10	177.7 ± 13.4	280.3 ± 30.9
3 (Day 1)	HF	Vehicle	114.3 ± 10.3	183.1 ± 25.0
		10	119.9 ± 8.4	161.0 ± 19.5
		40	66.5 ± 10.5 **	73.3 ± 10.2 **

Mean (± S.E.M.) time (s) spent by pairs of rats ($n = 8$ per group) in social interaction and locomotor activity (beam breaks), tested 30 min after interperitoneal injection with vehicle or CP 55,940 in the HU and HF test conditions of the social interaction test. For results of ANCOVA, see text.

** $P < .01$, compared with control group, post hoc tests after ANOVA.

55,940 40 μg/kg had an independent effect on both measures (see Fig. 1).

When rats were retested undrugged in the social interaction test on Day 2, there was a significant difference between the groups in the time spent in social interaction [$F(2,19)=16.9$, $P<.0001$]—see Fig. 2. Post hoc tests showed that the group that had been tested on Day 1 after injection with CP 55,940 (40 μg/kg) spent significantly less time in social interaction compared with the group that had been tested after a vehicle injection on Day 1, thus indicating a conditioned anxiogenic effect. This reduction in social interaction could not be due to a persisting drug effect inasmuch as the rats that had received 40 μg/kg after the social interaction test on Day 1 did not show this effect. Surprisingly, this group also differed significantly from the control group but showed a significant increase in the time spent in social interaction, indicating an anxiolytic effect. There were no group differences in the locomotor scores on Day 2 [$F(2,19)=0.4$, n.s.]—see Fig. 2.

3.3. Experiment 3: conditioned anxiety in the HF test condition

When rats were tested on Day 1 in the HF test condition, CP 55,940 significantly reduced both the time spent in social interaction [$F(2,21)=9.0$, $P<.002$] and locomotor activity [$F(2,21)=9.8$, $P<.0001$]. Post hoc tests showed that in both cases, the only group to differ significantly ($P<.01$) from the vehicle control group was the group tested after injection with CP 55,940 (40 μg/kg)—see Table 1. ANCOVAs were therefore conducted on the scores of these two groups, and in both cases, the effect remained significant [$F(2,20)=3.8$, $P<.05$ for social interaction and $F(2,20)=3.9$, $P<.05$ for motor activity].

When the rats were tested undrugged on Day 2, there was again a significant effect on social interaction [$F(3,28)=12.3$, $P<.01$], and post hoc tests showed that the group previously tested on Day 1 after the 40-μg/kg dose had significantly lower scores than the vehicle control ($P<.05$), whereas the group previously tested on Day 1 after the 10-μg/kg dose did not differ from the vehicle control group (see Table 2). The group that had been given 40 μg/kg after testing on Day 1 had significantly ($P<.01$)

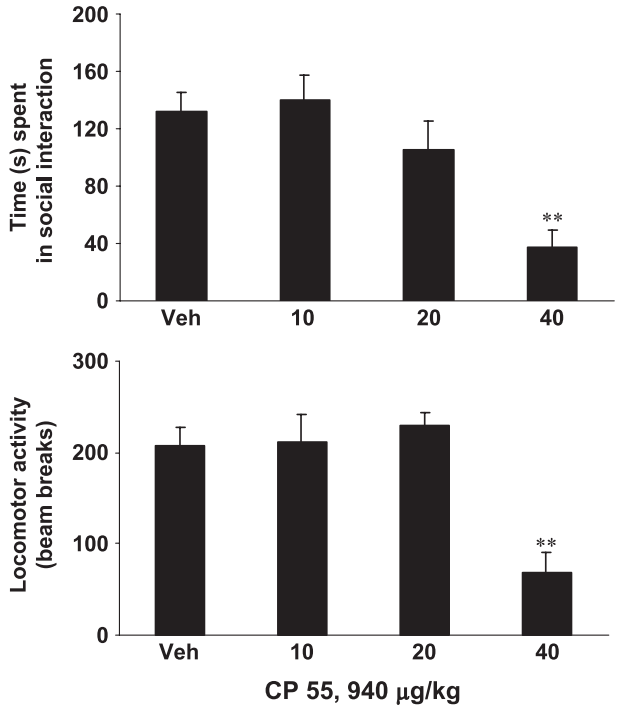

Fig. 1. Effects of CP 55,940 on social interaction in LF condition (Experiment 2). Mean (± S.E.M.) time (s) spent in social interaction (upper panel) and locomotor activity (number of beam breaks; lower panel) after injection with CP 55,940 (10, 20, or 40 μg/kg ip) or vehicle 30 min before testing. ** $P<.01$ compared with vehicle control group.

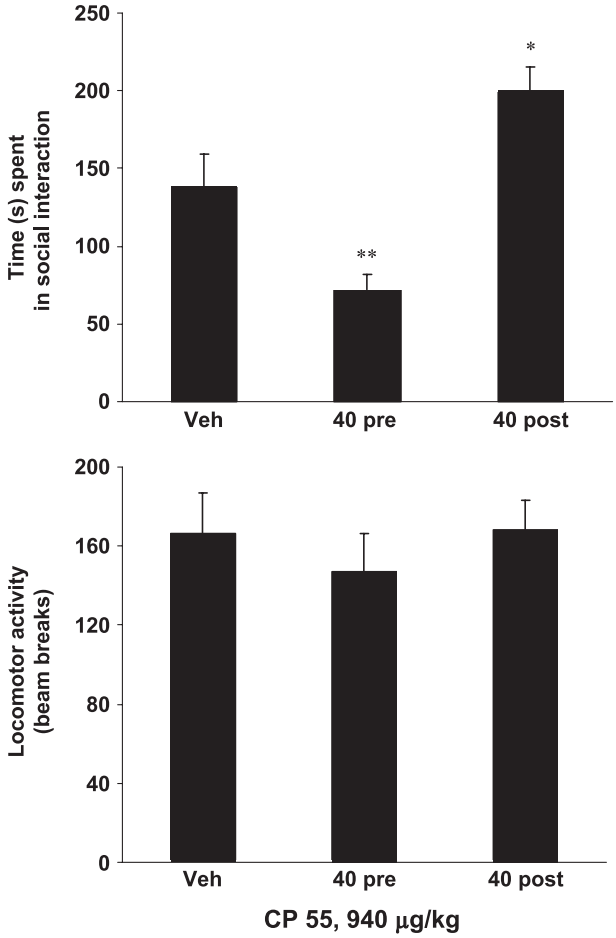

Fig. 2. Effects of CP 55,940 on social interaction in LF condition (Experiment 2). Mean (± S.E.M.) time (s) spent in social interaction (upper panel) and locomotor activity (number of beam breaks; lower panel) by pairs of rats tested undrugged 24 h after CP 55,940 (40 μg/kg ip) or vehicle. Injections were either given 30 min before testing (PRE) or immediately after testing (POST) on the previous day. * $P<.05$, ** $P<.01$, compared with vehicle control group.

Table 2
Conditioned anxiety to a mid-high dose of CP 55,940 (Experiment 3, Day 2)

Group	Time spent in social interaction (s)	Locomotor activity (beam breaks)
Veh	180.3 ± 16.2	183.0 ± 27.7
10 Pre	169.9 ± 6.8	166.9 ± 18.4
40 Pre	129.2 ± 7.5 *	158.0 ± 19.1
40 Post	260.6 ± 25.0 **	237.4 ± 28.1

Mean (± S.E.M.) time (s) spent in social interaction and locomotor activity (beam breaks) of pairs of rats tested undrugged on Day 2 in the HF test condition 24 h after previous testing on Day 1. Veh: Vehicle injection before test on Day 1; 10 Pre: 10 μg/kg CP 55,940 injection before test on Day 1; 40 Pre: 40 μg/kg CP 55,940 injection before test on Day 1; 40 Post: rats tested undrugged on Day 1 but receiving 40 μg/kg CP 55,940 immediately after test.

* $P < .05$ compared with Veh group when all rats tested undrugged on Day 2.

** $P < .01$ compared with Veh group when all rats tested undrugged on Day 2.

higher scores than the vehicle control group on Day 2 (see Table 2). There were no significant effects on locomotor activity on Day 2 [$F(3,28) = 2.2$, n.s.].

4. Discussion

We found no evidence for an anxiolytic effect in the social interaction test following low doses of CP 55,940. Thus, the effects in this test differ from those seen in the elevated plus maze, in which anxiolytic effects have been seen (Genn et al., 2003; Marco et al., in press). There is evidence from factor analysis studies that the two tests measure different states of anxiety (File, 1992) and considerable evidence that they are mediated by different neurobiological pathways (Cheeta et al., 2000; File et al., 1996, 2000). This raises the interesting possibility that the cannabinoid system may be differently involved in different animal tests and in different states of anxiety.

Experiments 2 and 3 revealed for the first time clear anxiogenic effects of a mid-high dose (40 μg/kg) of CP 55,940 in two conditions of the social interaction test. However, as well as decreasing social interaction, this dose also had an independent effect of decreasing locomotor activity. In the social interaction test, social interaction and locomotor activity have been dissociated both statistically and pharmacologically. Amphetamine and caffeine reduced social interaction but increased locomotor activity (File and Hyde, 1979). Adrenocorticotropic hormone (ACTH; File and Clarke, 1980), corticotropin-releasing factor (CRF) (Dunn and File, 1987) and benzodiazepine receptor inverse agonists (File et al., 1984) have been shown to decrease social interaction without a concomitant decrease in locomotor activity. The reductions in social interaction and locomotor activity induced by high doses of nicotine administered intraperitoneally can be separated by ANCOVA. The two effects are also dissociable following administration into dorsal hippocampus, where only social interaction is decreased (File et al., 1998). The present results indicate that the decreasing effects of CP 55,940 on social interaction and locomotor activity can be dissociated statistically by ANCOVA. Future work on the mechanisms underlying the effects of this cannabinoid agonist on social interaction will further clarify if the two effects can be dissociated pharmacologically. In this respect, it is worth mentioning previous results from other behavioural tests. It has been reported that the reduction of motor activity produced by higher doses of cannabinoid receptor agonists is mediated by the CB_1 subtype of cannabinoid receptor. However, the reduction of directed exploration in the holeboard and the anxiogenic effect in the plus maze caused by CP 55,940 were resistant to reversal by the selective CB_1 antagonist SR 141716A (Arévalo et al., 2001; Romero et al., 2002). We have recently shown that the selective κ-opioid receptor antagonist nor-binaltorphimine antagonised the anxiogenic effect of CP 55,940 (75 μg/kg) in the plus maze, but it did not reverse the decrease in holeboard activity induced by the cannabinoid agonist (Marín et al., 2003). Moreover, the 5-HT_{1A} receptor antagonist WAY 100635 attenuated the anxiogenic effect of CP 55,940 (50 μg/kg) in the plus maze and antagonised its reducing effect on exploratory activity in the holeboard. On the other hand, the decrease in rearing (motor activity) induced by CP 55,940 in this latter test was not modified by WAY 100635 (Marco et al., in press). These results support the view that distinct mechanisms subserve the effects of CP 55,940 on motor activity, exploration, and anxiety.

There was evidence from Experiments 2 and 3 that anxiety in the social interaction test could be conditioned to the anxiogenic dose of CP 55,940, an effect similar to that previously seen with nicotine (File et al., 2002). To see an anxiogenic effect when rats were tested undrugged on Day 2, it was necessary for them to have experienced an anxiogenic dose in the social interaction test on Day 1. It was not sufficient to have separate experiences of the 40-μg/kg dose and the social interaction test, and it was not sufficient to have experience of a lower, nonanxiogenic dose in the social interaction test. Further experiments will be needed to determine the mechanisms mediating the conditioned anxiogenic effect of CP 55,940, but CRF has been shown to mediate conditioned anxiety to both nicotine and cocaine (DeVries and Pert, 1998; Tucci et al., 2003).

The anxiogenic effect observed 24 h after testing in the social interaction test was not due to a residual effect of CP 55,940 per se inasmuch as the rats that received CP 55,940 injections after test showed no such effect. The conditioned anxiogenic response was sufficiently strong to overcome the anxiolytic response that resulted in the social interaction test simply from the drug injection 24 h earlier, as evidenced in the group that received drug after testing on Day 1. Perhaps the most important finding of this study was this unexpected clear anxiolytic effect 24 h after a dose of 40 μg/kg, and this

finding was replicated in Experiment 3 and thus was observed under both low- and high-light test conditions of the social interaction test. The two tests of anxiety also differed in this phenomenon inasmuch as no effects were observed in the plus maze in rats that had previously received a home cage experience of CP 55,940 (40 μg/kg) [unpublished data]. The striking anxiolytic effect could have been mediated by an active metabolite of CP 55,940. An incidental report of hyperdipsia and anorexia observed up to 24 h following administration of a dose of 100 μg/kg CP 55,940 gives credence to this idea (McGregor et al., 1996). Inasmuch as, to our knowledge, there have been no explicit attempts to identify and/or measure active metabolites 24 h after acute high doses of CP 55,940, this issue remains unresolved. Although it is noteworthy that changes in receptor number and/or function have been found after chronic administration of CP 55,940 (Rubino et al., 1998), it is less likely that a single injection could produce such significant changes at the receptor level. However, it cannot be assumed that the effects 24 h after a high dose of a cannabinoid agonist would necessarily resemble those 30 min after administration of a low dose.

Our findings of this anxiolytic effect 24 h after CP 55,940 administration are somewhat similar to those of Valjent and Maldonado (2000). They found a conditioned place aversion with 5 mg/kg THC and no effect with 1 mg/kg using a standard protocol. However, if mice received a priming dose of THC in their home cage 24 h before starting the place preference conditioning procedure, they showed a place preference with 1 mg/kg THC and no effect of 5 mg/kg THC. Although the paradigms are very different, there is an important analogy with respect to the present experiment. These authors avoided place aversion (5 mg/kg THC) and revealed place preference (1 mg/kg THC) by preventing the association of the aversive reaction of the animals to the first exposure to the drug, with the apparatus. In our case, an anxiolytic effect of a relatively high dose of CP 55,940 is found 24 h after its administration, if the drug is administered after the first exposure to the apparatus, thus avoiding the association of the short-term anxiogenic effect of the drug with the apparatus, that is, with the context. Therefore, it is not surprising that low doses of CP 55,940 have been associated with both conditioned place preference (20 μg/kg) (Braida et al., 2001) and conditioned place aversion (10 μg/kg) (McGregor et al., 1996), as the timing between injection and pairing plays a large part in the expression of these affective differences (Lepore et al., 1995).

In summary, the present results extend the anxiogenic profile of CP 55,940 to unconditioned effects in the social interaction test and to conditioned anxiety. Further experiments will be necessary to elucidate the underlying mechanisms. In particular, it would be of interest to determine whether κ-opioid and 5-HT_{1A} receptors are involved in the anxiogenic effect in the social interaction test, as has been found for the anxiogenic effect in the plus maze (Marín et al., 2003; Marco et al., in press). It will also be of interest to determine whether activation of CRF receptors underlies the anxiety that can be conditioned to CP 55,940.

Acknowledgements

This study was supported in part by a grant from the CONICIT-ULA, Venezuela.

References

Arévalo C, De Miguel R, Hernández-Tristán R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. Pharmacol Biochem Behav 2001;70:123–31.

Berrendero F, Maldonado R. Involvement of the opioid system in the anxiolytic-like effects induced by delta (9)-tetrahydrocannabinol. Psychopharmacology 2002;163:111–7.

Braida D, Pozzi M, Cavallini R, Sala M. Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience 2001;104:923–6.

Breivogel CS, Griffin G, Di Marzo V, Martin BR. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol Pharmacol 2001;60:155–63.

Cheeta S, Kenny PJ, File SE. Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. Prog Neuropsychopharmacol Biol Psychiatry 2000;24:1053–67.

DeVries AC, Pert A. Conditioned increases in anxiogenic-like behavior following exposure to contextual stimuli associated with cocaine are mediated by corticotropin-releasing factor. Psychopharmacology 1998; 137:333–40.

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, et al. Levels, metabolism, and pharmacological activity of anandamide in CB1 cannabinoid receptor knockout mice: evidence for non-CB_1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. J Neurochem 2000;75:2434–44.

Dunn AJ, File SE. Corticotropin-releasing factor has an anxiogenic action in the social interaction test. Horm Behav 1987;21:193–202.

File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J Neurosci Methods 1980;2: 219–38.

File SE. Behavioural detection of anxiolytic action. In: Elliot JM, Heal DJ, Marsden CA, editors. Experimental approaches to anxiety and depression. New York: Wiley; 1992. p. 25–44.

File SE, Hyde JR. Can social interaction be used to measure anxiety? Br J Pharmacol 1978;62:19–24.

File SE, Hyde JR. A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. Pharmacol Biochem Behav 1979;11:65–9.

File SE, Clarke A. Intraventricular ACTH reduces social interaction in male rats. Pharmacol Biochem Behav 1980;12:711–5.

File SE, Seth P. A review of 25 years of the social interaction test. Eur J Pharmacol 2003;463:35–53.

File SE, Lister RG, Nutt DJ. The anxiogenic action of benzodiazepine antagonists. Neuropharmacology 1982;21:1033–7.

File SE, Lister RG, Maninov R, Tucker JC. Intrinsic behavioural actions of *n*-propyl beta-carboline-3-carboxylate. Neuropharmacology 1984;23: 463–6.

File SE, Mabbutt PS, Walker JH. Comparison of adaptive responses in familiar and novel environments: modulatory factors. Ann NY Acad Sci 1988;525:69–79.

File SE, Gonzalez LE, Andrews N. Comparative study of pre- and post-synaptic 5-HT1A receptor modulation of anxiety in two ethological animal tests. J Neurosci 1996;16:4810–5.

File SE, Kenny PJ, Ouagazzal AM. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. Behav Neurosci 1998;112:1423–9.

File SE, Cheeta S, Kenny PJ. Neurobiological mechanisms by which nicotine mediates different types of anxiety. Eur J Pharmacol 2000;393: 231–6.

File SE, Cheeta S, Irvine EE, Tucci S, Akthar M. Conditioned anxiety to nicotine. Psychopharmacology 2002;164:309–17.

Genn RE, Tucci S, Marco E, Viveros MP, File SE. Anxiolytic and anxiogenic effects of the cannabinoid agonist CP 55,940 in animal tests of anxiety. J Psychopharmacol 2003;17:A27.

Giuliani D, Ferrari F, Ottani A. The cannabinoid agonist HU 210 modifies rat behavioural responses to novelty and stress. Pharmacol Res 2000;41: 47–53.

Goeders NE. A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 1997;22:237–59.

Goeders NE. The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 2002;27:13–33.

Hajos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. Neuroscience 2001;106:1–4.

Hall W, Solowij N. Adverse effects of cannabis. Lancet 1998;352:1611–6.

Hall W, Solowij N, Lemon J. The health and psychological consequences of cannabis use. National drug strategy monograph series, vol. 25. Canberra: Australian Government Publishing Service; 1994.

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF. The effects of genetic and pharmacological blockade of the CB_1 cannabinoid receptor on anxiety. Eur J Neurosci 2002;16:1395–8.

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. J Neurosci 1991;11:563–83.

Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, et al. Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB_1 or CB_2 receptors. Proc Natl Acad Sci U S A 1999; 96:14136–41.

Lepore M, Vorel SR, Lowinson J, Gardner EL. Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. Life Sci 1995;56:2073–80.

Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J Pharmacol Exp Ther. 1988;247:1046–51.

Marco EM, Pérez-Alvarez L, Borcel E, Rubio M, Guaza C, Ambrosio E, et al. Involvement of 5-HT_{1A} receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. Behav Pharmacol [in press].

Marín S, Marco E, Biscaia M, Fernandez B, Rubio M, Guaza C, et al. Involvement of the kappa-opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats. Pharmacol Biochem Behav 2003;74:649–56.

McGregor IS, Issakidis CN, Prior G. Aversive effects of the synthetic cannabinoid CP 55,940 in rats. Pharmacol Biochem Behav 1996;53: 657–64.

Navarro M, Hernandez E, Munoz RM, del Arco I, Villanua MA, Carrera MR, et al. Acute administration of the CB_1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. NeuroReport 1997;8:491–6.

Onaivi ES, Green MR, Martin BR. Pharmacological characterization of cannabinoids in the elevated plus maze. J Pharmacol Exp Ther 1990; 253:1002–9.

Porter AC, Felder CC. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol Ther 2001;90:45–60.

Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GF, et al. Corticotropin-releasing factor (CRF) antagonist [D-Phe^{12}, $Nle^{21,38}$, $C^{\alpha}MeLeu^{37}$] CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. J Pharmacol Exp Ther 1996;276:56–64.

Romero EM, Fernández B, Sagredo O, Gomez N, Urigüen L, Guaza C, et al. Antinociceptive, behavioural and neuroendocrine effects of CP 55,940 in young rats. Dev Brain Res 2002;136:85–92.

Rubino T, Patrini G, Massi P, Fuzio D, Vigano D, Giagnoni G, et al. Cannabinoid-precipitated withdrawal: a time-course study of the behavioral aspect and its correlation with cannabinoid receptors and G protein expression. J Pharmacol Exp Ther 1998;285:813–9.

Tucci S, Cheeta S, Genn RF, Seth P, File SE. Anxiety conditioned to nicotine in the elevated plus-maze is time dependent. Behav Pharmacol 2002;13:615–20.

Tucci S, Cheeta S, Seth P, File SE. Corticotropin releasing factor antagonist α-helical CRF_{9-41}, reverses nicotine-induced conditioned, but not unconditioned, anxiety. Psychopharmacology 2003;167:251–6.

Valjent E, Maldonado R. A behavioural model to reveal place preference to delta 9-tetrahydrocannabinol in mice. Psychopharmacology 2000;147: 436–8.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB_1 receptor knockout mice. Proc Natl Acad Sci U S A 1999;96:5780–5.